C. Barut Rankin
1991

ATOLL RESEARCH BULLETIN

THE TWIN CAYS MANGROVE ECOSYSTEM, BELIZE:
BIODIVERSITY, GEOLOGICAL HISTORY,
AND TWO DECADES OF CHANGE
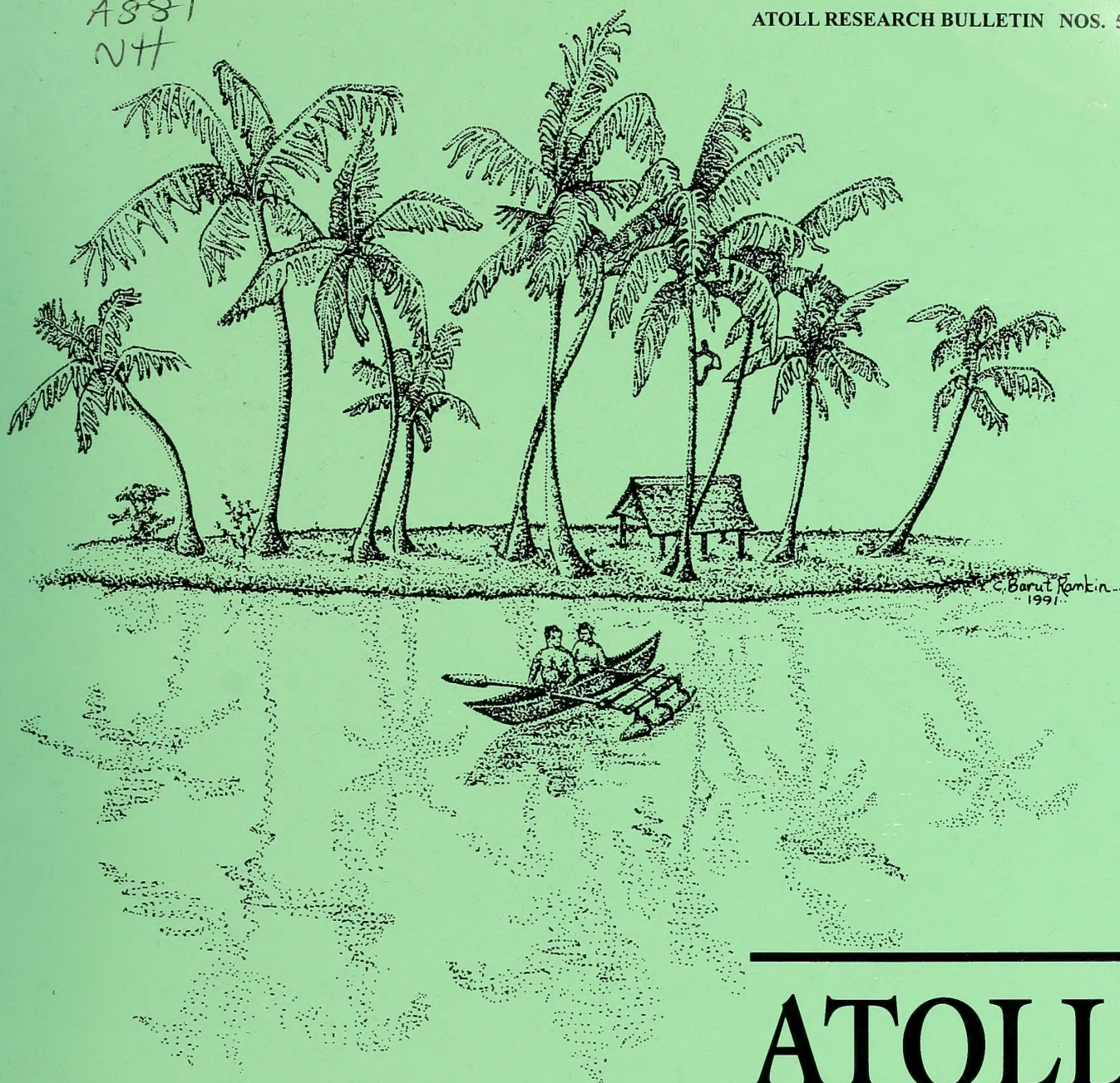
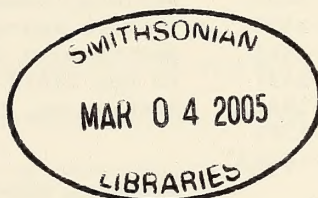EDITED BY

IAN G. MACINTYRE, KLAUS RÜTZLER, AND
ILKA C. FELLER

ATOLL RESEARCH BULLETIN

NOS. 509-530

THE TWIN CAYS MANGROVE ECOSYSTEM, BELIZE: BIODIVERSITY,
GEOLOGICAL HISTORY, AND TWO DECADES OF CHANGE

EDITED BY

IAN G. MACINTYRE, KLAUS RÜTZLER, AND ILKA C. FELLER

ACKNOWLEDGMENT

All statements made in papers published in the Atoll Research Bulletin are the sole responsibility of the authors and do not necessarily represent the views of the Smithsonian nor of the editors of the Bulletin.

Articles submitted for publication in the Atoll Research Bulletin should be original papers in a format similar to that found in recent issues of the Bulletin. First drafts of manuscripts should be typewritten double spaced and can be sent to any of the editors. After the manuscript has been reviewed and accepted, the author will be provided with a page format with which to prepare a single-spaced camera-ready copy of the manuscript.

ATOLL RESEARCH BULLETIN

Nos. 509–530

Participants of the conference on The Twin Cays, Belize, Mangrove Ecosystem in Ft. Pierce, Florida, 14–17 December 2003. Front row, from left: Michael Lang, Samantha Chapman, Michelle Nestlerode, Anja Schulze, Ian Macintyre, Maria Faust (standing), Ana Signorovich, Amy Erickson, Janie Wulff (standing), Samantha Joye, Mary Parrish. Back row, from left: Ray Feller, George Venable, Molly Ryan, Mary Rice, Klaus Ruetzler, Valerie Paul, Cristina Diaz, Ed Buskey, Will Davis, Bjorn Tunberg, Scott Taylor, Woody Lee, Candy Feller, Susan Richardson, Raphael Ritson-Williams, Wilfrid Rodriguez, David Krayesky. (Photo, Julie Piraino.)

PREFACE

For 22 years the Smithsonian Caribbean Coral Reef Ecosystems Program (CCRE) has been conducting a comprehensive study of a mangrove community on the barrier reef of Belize, Central America, in an area known as Twin Cays. Established in 1982, the Twin Cays mangrove program was originally known as the Smithsonian Western Atlantic Mangrove Program (SWAMP). A first review of its work—titled The Twin Cays Mangrove, Belize, and Related Ecological Systems—took place in November 1988. Although the proceedings appeared only in an internal progress report, they created a new platform for scientific collaboration and exchange and, more important, provided the impetus that enabled SWAMP to evolve into the vibrant CCRE program of today.

In December 2003, a second review was undertaken at a conference in Fort Pierce, Florida, organized by staff of the Smithsonian National Museum of Natural History (NMNH, Washington, D.C.), Environmental Research Center (SERC, Edgewater, Maryland), and Marine Station (SMS, Ft. Pierce). This bulletin is dedicated to some of the results of that conference, titled The Twin Cays Mangrove Ecosystem, Belize: Biodiversity, Geological History, and Two Decades of Change.

The two-day Ft. Pierce colloquium was convened by Klaus Ruetzler (NMNH), Ilka Feller (SERC), and Ian Macintyre (NMNH) and co-hosted by Valerie Paul (SMS). Its 35 participants included Smithsonian staff and collaborators from scientific institutions throughout the country; some collaborators from overseas sent in contributions. Talks, posters, and discussions centered on the systematics and ecology of microbes, algae, invertebrates, and fishes. Marine and terrestrial microbe, plant, protozoan, and animal taxa were strongly represented, notably dinoflagellates, algae, ciliates, foraminiferans, placozoans, sponges, gnathostomulid and sipunculan worms, crustaceans, insects, bryozoans, asicidans, and fishes. Other topics discussed were the geological development of the mangroves, geomorphological changes over the two decades, ecological impact of sediments, tidal flow, and anthropogenic stress, chemical defense mechanisms, scientific illustration of communities, and aspects of data management.

Conference participants along with visiting NMNH Director Cristián Samper, Associate Director Sue Fruchter, and Smithsonian Institution Marine Science Network Coordinator Michael Lang also toured the Smithsonian Marine Station and attended a special reception at the Smithsonian Marine Ecosystems Exhibit. These events and discussions brought into sharper focus the continuing contributions of the Twin Cays program.

Unfortunately, owing to limitations of time and space, the present volume is unable to cover those contributions in entirety. However, many aspects of the work—such as recent advances in the discovery of new taxa, in scientific understanding of distributional patterns, and in studies in ecology and ecophysiology—will no doubt soon appear elsewhere. All in all, the Twin Cays program remains a milestone in Caribbean mangrove research. It will stimulate discussion and new perspectives for many years to come. In view of the noticeable environmental stress and degradation in our Caribbean coastal communities, it is essential to periodically set baselines against which future species and habitat surveys can be measured.

The Editors
Washington, D.C.
September 2004

MODERN SEDIMENTARY ENVIRONMENTS, TWIN CAYS, BELIZE,
CENTRAL AMERICA

BY

IAN G. MACINTYRE, MARGUERITE A. TOSCANO, AND GREGOR B. BOND
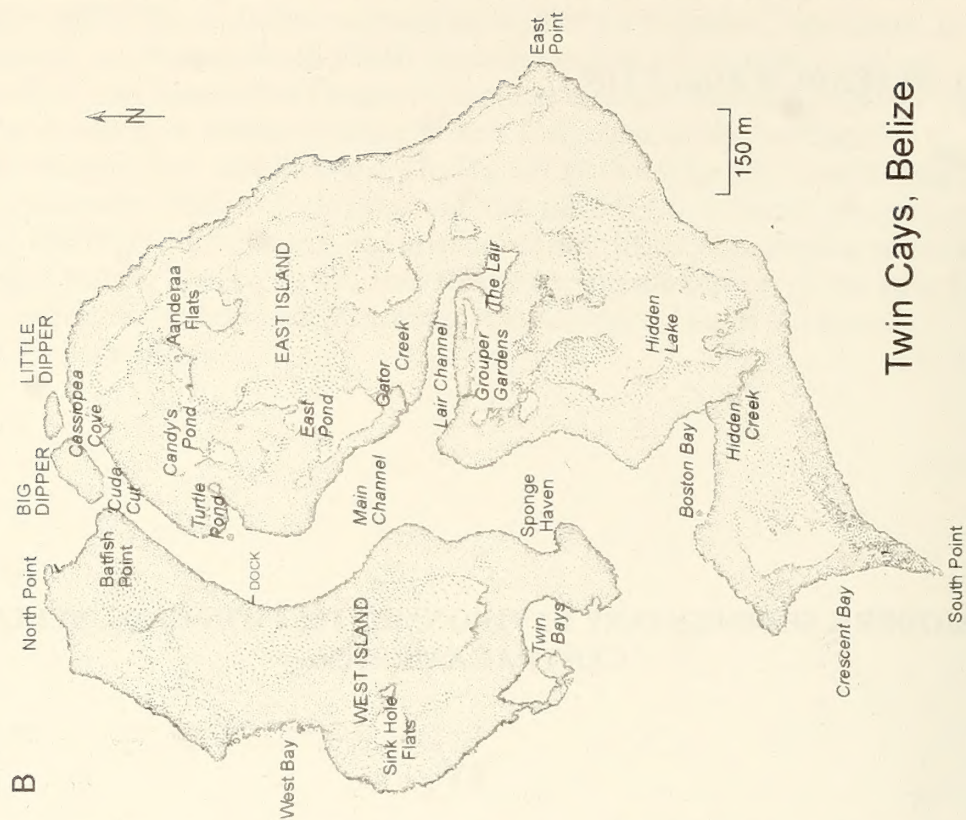
Figure 1. Twin Cays, Belize. A) Aerial photograph montage assembled from photographs supplied by David R. Stoddart in 1979. B) Map with place names introduced in the 1982 survey and by later workers.

MODERN SEDIMENTARY ENVIRONMENTS , TWIN CAYS, BELIZE, CENTRAL AMERICA

BY

IAN G. MACINTYRE[1], MARGUERITE A. TOSCANO[1], AND GREGOR B. BOND[2]

ABSTRACT

The mapping of environmental settings on and around Twin Cays was completed in 1982 with the aid of aerial photographs. A total of three mangrove-island and five marine depositional environments were identified. Size analyses of surface samples collected from 120 marine sites indicate that the higher percentages of mud are found mostly in protected areas between the two islands and in deeper locations. In contrast, the very sandy sites were found in nearshore shallow flats and isolated offshore shoals where winnowing action has removed the finer fraction. *Halimeda* and molluscs dominate the sand-size fraction.

INTRODUCTION

The continental shelf off Belize is about 250 km long and 15-50 km wide (Purdy et al., 1975). It consists of an Outer Barrier Platform that supports a variety of patch reefs and mangrove islands and is bordered along its eastern edge by a fringing reef off Ambergris Cay that extends southward into the longest Caribbean barrier reef (Smith, 1971). Shoreward, this shallow platform gives way to the Shelf Lagoon that to the north is 20-25 km wide and less than 15 m deep but deepens to the south to more than 15 m and reaches widths of more than 40 m before expanding into the Gulf of Honduras (Rützler and Macintyre, 1982).

Our study site at Twin Cays is located in the Central Province (Burke, 1982) which extends from Gallows Point Reef to Gladen Spit (Purdy et al., 1975). This province is characterized by long sections of uninterrupted barrier reef behind which numerous patch reefs and mangrove islands occur on the shallow platform lagoon.

In the Central Province, terrigenous sediments occur in the Shelf Lagoon and are dominantly quartz sand and terrigenous clay. This is in marked contrast to the carbonate sands and muds that characterized the Outer Barrier Platform (Purdy et al., 1975). Purdy et al. (1975) included the Twin Cays site in their carbonate *"Halimeda"* facies (p.25).

The purpose of this study is to provide a more detailed understanding of the distribution patterns of sediments surrounding Twin Cays and in the channel that separates the two islands.

---

[1] Department of Paleobiology, P.O. Box 37012, National Museum of Natural History, Smithsonan Institution, Washington, DC 20013-7012.
[2] Hydroenvironmental Technologies, Inc., P. O. Box 25073, Chicago, IL 60625

METHODS

In 1982, I. G. Macintyre and W. T. Boykins mapped the major marine and subaerial surface depositional environments for Twin Cays and the surrounding area with the aid of aerial photographs (Fig. 1). During this mapping, a total of 120 surface sediment samples were collected (Fig. 2) from the marine environments. Size analyses were carried out in all but the very organic-rich samples collected in the channel areas. Samples were wet-sieved at the Texas A&M Geosciences laboratory (mesh sizes 2 mm, 1 mm, 0.5 mm, 0.25 mm, 0.125 mm, and <0.063 mm) (Bond, 1988). The mud fractions were then sent to the Sedimentology Laboratory at Smithsonian Institution and separated into silt (63 mm - 4μ) and clay (<4μ) size fractions by decantation. Percentages for each size fraction were calculated for all samples (Table 1). Samples estimated to be composed of >50% organics were not sieved. These have been omitted from Table 1.
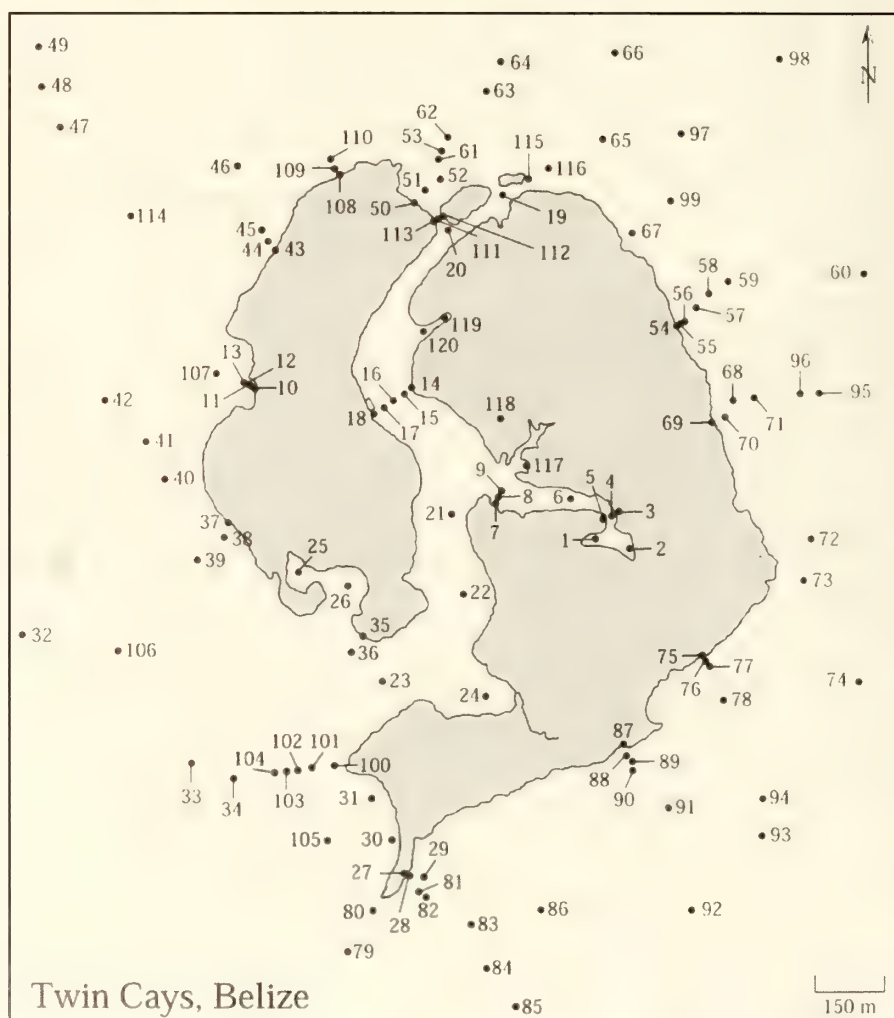
Figure 2. Map of Twin Cays with numbered sediment sample locations.

3

Three thin sections, in the coarse, medium, and fine sand ranges, were made from a sediment sample representing each major marine depositional environments as determined in the field from aerial and boat-based reconnaissance. Thin sections were point-counted to a minimum of 320 points per slide. Loose grains of the very coarse sand and granule size distributions were also counted for these samples, with either the entire set of grains being counted or a subsample if more than 600 grains were present.

RESULTS

Three emergent and five submerged surficial sedimentary environments were characterized based on field and aerial reconnaissance of sediments and vegetation cover (Fig. 3).

Figure 3. Depositional environments map of Twin Cays based on 1982 field observations.

4

Emergent Environments

Mangrove cover consists of thick-to-moderate density of either mixed red and black mangroves or monospecific red mangroves along the outer margins of the islands, closest to water. Mangroves create an *in situ* peat substrate with leaf mats, which intertongue with carbonate sands at the shoreline.

Sparse mangrove cover is found in shallow ponds and other interior portions of the islands. Scattered red mangrove development occurs in the ponds, while a mixture of red and black mangroves grows around the ponds and extends into interior regions. Sediment on pond bottoms consists of flocculated mud cover on a thick peat base, with scattered *Halimeda* sp. and *Thalassia testudinum*. The forest mat is a peat base with leaf litter layers and a mixture of remains of other succulent plants.

Mangroveless environments occur near the large shallow pond in the interior of the island. The bottom is covered with a thin layer of flocculated mud over a peat base. Scattered molluscs occur as well as rare dwarf red mangrove trees.

Submerged Environments

The "Mud bottom" environment occurs in both the interior channels of Twin Cays and in the protected leeward lagoon near the island. Mud is described as "thixotropic" and/or flocculent. Patchy *Thalassia testudinum*, *Halimeda* and *Penicillus* occur on the mud bottom. Callianassid shrimp burrows and the upside-down jellyfish, *Cassiopeia xamachana,* are common (Fig. 4). Along channel shorelines, the mud bottom is replaced by a *Halimeda* sand bottom adjacent to mangrove prop roots surrounded by *Halimeda* mats. The oyster *Crassotrea rhizophorae* also commonly encrusts prop roots, giving rise to adjacent shell hash. Mangrove leaf mats cover some muddy areas. In the extremely restricted Lair Channel the mud bottom is very rich in organic content (Fig. 5).

The "Thalassia on Mud" bottom environments consist of muddy or soft sand-mud bottoms with either a patchy or extensive *Thalassia testudinum* cover, both in open deeper (~>3m) areas and in channels (Fig. 6). The *Thalassia* has longer blades, typical for deeper areas. Scattered *Halimeda* and *Penicillus* grow within the *Thalassia* areas. *Caulerpa* sp., *Diadema* and Callianassid shrimp burrows are common. This depositional environment has a wide depth range (0.5 - 7.5 m; avg. 3.5 m).

The "Thalassia on Sand" environment occurs on the windward side of Twin Cays and is characterized by a compact sandy or sandy mud bottom densely covered by *Thalassia testudinum,* with *Syringodium filiforme*, *Penicillus* sp., *Halimeda opuntia* (forming large mounds), *Diadema*, other echinoids, *Porites furcata* and Callianassid shrimp mounds (Fig. 7). The sediments in some places contain shell hash layers or overlie a peat base. Sand blowouts are sandy depressions in these *Thalassia* beds that have been eroded during severe storm activity. Water depths range from 0.0 to 2.5 m (avg. 1.2 m).

The *"Sand with Thalassia"* bottom is generally a shallow water (0.1 - 3.9 m; avg. 1.7 m) environment (Fig. 8) trending E-W across the northern coast of Twin Cays. Smaller areas occur at South Point and in small shallow patches within the deeper *"Thalassia*

on Mud" environment on the leeward western side of the islands (Fig. 3). The bottom consists predominantly of hard, compact, rippled sand. Varying densities of the seagrasses *Thalassia, Syringodium,* and *Halodule* occur with the algae *Halimeda opuntia, Penicillus, Laurencia* and branching *Neogoniolithon strictum.* Corals include *Manicina areolata, Porites divaricata, Porites furcata, Porites astreoides, Siderastrea radians,* branching *Cladocora arbuscula,* and rare occurrences of *Acropora cervicornis. Diadema* are numerous as are Callianassid shrimp burrows. Other echinoids occur, as do gorgonians.

Figure 4. "**Mud bottom**" depositional environment in north end of the channel separating the West and East Islands. Note scattered *Halimeda* and the upside-down jellyfish *Cassiopeia xamachana* (arrow).

Figure 5. Organic-rich mud bottom of the Lair Channel.
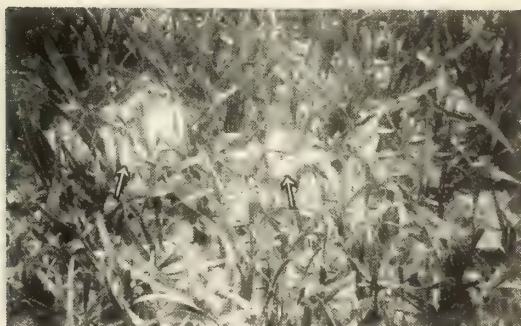
Figure 6. "*Thalassia* on Mud" depositional environment along the leeward (western) lagoonal side of West Island, Twin Cays. Note long *Thalassia* blades and Callianassid shrimp burrows (arrows).
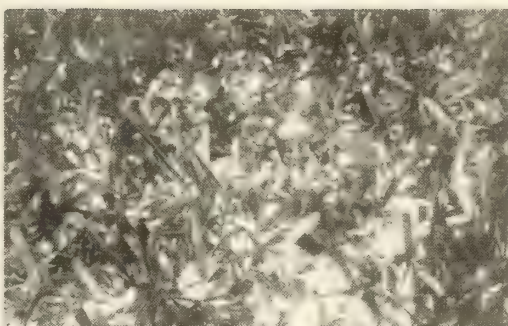
Figure 7. "*Thalassia* on Sand" depositional environment along the windward (eastern) shoreline of East Island, Twin Cays.
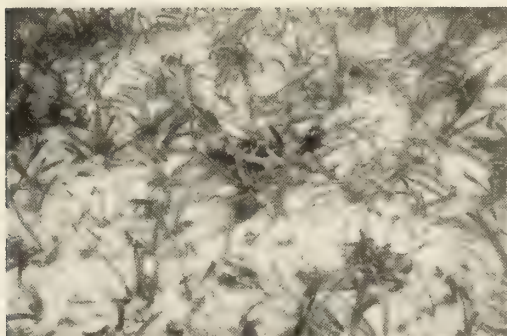
Figure 8. "**Sand with *Thalassia***" depositional environment along the northern side of Twin Cays (also near South Point). Note the *Porites furcata* coral colony and black *Diadema* urchin in the center of the

Surface Samples

A plot of the sediment size analyses (Table 1) on a triangular size/nomenclature diagram (Folk, 1961) was placed on a map (Fig. 9) to define three basic sediment grain-size categories: sand (>90 %), dominantly sand (50-89%) with some silt and clay, and finally silt and clay size with sand (<50%).

Table 1. Sediment size analysis (wet sieved) from data of Bond (1988). S = Sand, Z = silt, M = mud, C = clay. sM = sandy Mud, zM = silty Mud, mS = muddy Sand, sC = sandy Clay, zC = silty Clay, etc. *Th* = *Thalassia*.

Sample No.	Depth (m)	%Sand	%Silt	%Clay	Sediment	Environment
7	1.0	96.1	2.0	2.0	S	Mud
8	1.5	40.5	40.0	20.0	sM	*Th* on Mud
11	0.5	93.0	3.0	5.0	S	Mud
13	0.9	40.4	40.0	20.0	sM	*Th* on Mud
15	0.5	16.3	69.0	15.0	sM	*Th* on Mud
16	1.2	20.3	29.0	51.0	sM	*Th* on Mud
17	2.9	25.2	27.0	48.0	sM	*Th* on Mud
19	2.6	84.0	9.0	7.0	mS	Sand w/ *Th*
20	3.2	64.0	14.0	22.0	mS	*Th* on Mud
21	4.3	29.2	23.0	48.0	sC	*Th* on Mud
22	4.5	28.1	24.0	48.0	sC	*Th* on Mud
23	5.2	34.1	21.0	45.0	sC	*Th* on Mud
25	1.0	41.3	18.0	41.0	sC	*Th* on Mud
26	1.9	67.0	10.0	23.0	mS	*Th* on Mud
27	0.0	100.0	0.1	0.1	S	*Th* on Sand
28	0.0	100.2	0.1	0.1	S	*Th* on Sand
29	0.5	98.0	0.1	0.1	S	*Th* on Sand
30	1.0	97.0	2.0	1.0	S	Sand w/ *Th*
31	1.6	88.0	1.0	1.0	S	Sand w/ *Th*
32	2.2	95.0	3.0	2.0	S	Sand w/ *Th*
33	1.8	99.0	0.1	1.0	S	Sand w/ *Th*
34	7.0	55.0	15.0	30.0	cS	*Th* on Mud
35	0.7	89.0	3.0	8.0	(m)S	Sand w/ *Th*
36	2.4	76.0	19.0	5.0	zS	Sand w/ *Th*
37	0.1	91.3	2.0	7.0	S	Sand w/ *Th*
38	1.0	98.0	1.0	1.0	S	Sand w/ *Th*
39	2.8	58.0	11.0	31.0	cS	*Th* on Mud
40	1.7	96.0	2.0	2.0	S	Sand w/ *Th*
41	2.9	59.1	15.0	26.0	cS	*Th* on Mud
42	1.8	98.0	1.0	1.0	S	Sand w/ *Th*
44	1.3	98.0	1.0	1.0	S	Sand w/ *Th*
45	1.7	96.0	2.0	2.0	S	Sand w/ *Th*
46	2.5	96.0	2.0	2.0	S	Sand w/ *Th*
47	2.6	94.0	3.0	3.0	S	Sand w/ *Th*
48	7.5	45.0	12.0	43.0	cS	*Th* on Mud
49	2.8	92.0	5.0	3.0	S	Sand w/ *Th*
51	0.9	94.0	5.0	1.0	S	Sand w/ *Th*
52	2.8	83.0	8.0	9.0	mS	Sand w/ *Th*
53	1.1	94.0	6.0	0.1	S	Sand w/ *Th*
54	0.35	96.0	3.0	1.0	S	*Th* on Sand
55	1.0	94.0	3.0	3.0	S	*Th* on Sand
56	0.9	64.0	22.0	14.0	mS	*Th* on Sand
57	0.7	81.0	12.0	7.0	mS	*Th* on Sand
58	0.9	92.0	4.0	4.0	S	*Th* on Sand
59	1.4	88.0	8.0	4.0	(m)S	*Th* on Sand

60	2.6	98.0	1.0	1.0	S	*Th* on Sand
61	0.9	79.0	14.0	7.0	mS	Sand w/ *Th*
62	1.4	100.0	0.1	0.1	S	Sand w/ *Th*
63	2.8	86.0	9.0	5.0	mS	Sand w/ *Th*
64	6.3	34.0	49.0	18.0	sZ	*Th* on Mud
65	0.8	94.0	4.0	2.0	S	Sand w/ *Th*
66	3.0	93.0	3.0	4.0	S	Sand w/ *Th*
67	2.0	73.0	20.0	7.0	zS	*Th* on Sand
68	1.4	92.0	5.0	3.0	S	*Th* on Sand
69	0.25	80.0	16.0	4.0	zS	*Th* on Sand
70	1.0	94.0	4.0	2.0	S	*Th* on Sand
71	1.5	39.0	41.0	20.0	sZ	*Th* on Sand
72	2.5	97.0	2.0	1.0	S	*Th* on Sand
73	0.9	92.0	6.0	4.0	S	*Th* on Sand
74	1.8	83.0	11.0	6.0	zS	*Th* on Mud
75	0.4	98.0	1.0	1.0	S	*Th* on Sand
76	1.4	86.0	10.0	4.0	mS	*Th* on Sand
77	1.7	94.0	4.0	2.0	S	*Th* on Sand
78	1.05	82.0	10.0	8.0	mS	*Th* on Sand
79	1.8	91.0	5.0	4.0	S	Sand w/ *Th*
80	0.9	84.0	9.0	7.0	mS	Sand w/ *Th*
81	1.4	79.0	10.0	11.0	mS	*Th* on Sand
82	1.7	73.0	16.0	11.0	mS	*Th* on Sand
83	1.5	90.0	6.0	5.0	S	Sand w/ *Th*
84	2.9	80.0	13.0	8.0	mS	Sand w/ *Th*
85	7.0	77.1	19.0	4.0	zS	*Th* on Mud
86	2.0	92.0	4.0	4.0	S	Sand w/ *Th*
87	0.3	100.1	0.1	0.1	S	*Th* on Sand
88	1.8	94.0	3.0	3.0	S	*Th* on Sand
89	1.5	60.0	19.0	21.0	mS	*Th* on Sand
90	0.75	82.0	10.0	8.0	mS	*Th* on Sand
91	2.1	80.0	11.0	9.0	mS	*Th* on Sand
92	5.5	63.0	23.0	14.0	mS	*Th* on Mud
93	5.5	42.0	42.0	16.0	sZ	*Th* on Mud
94	2.3	87.0	6.0	7.0	mS	*Th* on Mud
95	1.7	96.0	2.0	2.0	S	*Th* on Sand
96	1.5	82.0	11.0	7.0	mS	*Th* on Sand
97	1.85	99.0	0.1	1.0	S	*Th* on Sand
98	4.0	77.0	14.0	9.0	mS	*Th* on Mud
99	1.05	95.0	4.0	1.0	S	*Th* on Sand
100	0.75	93.0	4.0	3.0	S	Sand w/ *Th*
101	0.9	96.0	2.0	2.0	S	Sand w/ *Th*
102	1.4	91.0	5.0	4.0	S	Sand w/ *Th*
103	1.75	98.0	1.0	1.0	S	Sand w/ *Th*
104	2.35	83.0	7.0	10.0	mS	Sand w/ *Th*
105	3.2	53.0	21.0	26.0	mS	*Th* on Mud
106	5.5	40.0	39.0	20.0	zS	*Th* on Mud
107	2.1	61.0	31.0	8.0	zS	*Th* on Mud
108	0.2	101.2	0.1	0.1	S	Sand w/ *Th*
109	0.65	92.0	3.0	2.0	S	Sand w/ *Th*
110	1.15	87.0	8.0	6.0	mS	Sand w/ *Th*
111	3.9	87.0	7.0	6.0	mS	Sand w/ *Th*
112	2.5	43.0	25.0	32.0	sM	*Th* on Mud
113	3.2	87.0	5.0	8.0	mS	Sand w/ *Th*
114	2.4	95.0	2.0	2.0	S	Sand w/ *Th*
115	1.35	96.0	2.0	2.0	S	*Th* on Sand
116	0.7	98.0	1.0	1.0	S	*Th* on Sand
120	2.0	17.2	53.0	30.0	sM	Mud

8

Figure 9. Map showing the sediment grain-size size characteristics at each location based on Folk's (1961) nomenclature illustrating the percentage of sand for each sample. Compare to depositional environment map (Figure 3).

Thin Sections

Particle types that were point-counted included: *Halimeda* plates, bivalves, gastropods, miliolid foraminifera, soritid foraminifera, rotilid foraminifera, echinoderm fragments, coral fragments, coralline algae, crustacean fragments, tunicate spicules, alyconarian spicules, diatoms, fish teeth and quartz grains (Bond, 1988). Point count data (percentages of various grain types) of samples (33, 34, 48, 63, 72, 94, 96, 113) representing the depositional environments (Table 2) indicate that *Halimeda* is the dominant skeletal component in the thin sections forming the primary skeletal grain in the sand size range and in the granule class. Both bivalve and gastropod shell fragments are the next most abundant grain type, followed by foraminifera. The combined compositional percentages of *Halimeda* and molluscs correlate closely to the percent sand in the samples, except in two mud-dominated samples. Other constituent grains include, in the coarsest size fractions, molluscs, miliolid foraminifers and echinoderm fragments, with bivalves in certain cases being locally dominant. Foraminifera tend to be miliolids in the sand size fractions and in the finer grained fractions rotilid and soritid foraminifera become increasingly abundant. Echinoderm fragments form no more than three percent of any thin section point count.

Table 2. Thin section point count data – sedimentary constituents (after Bond 1988). % Sand is from Table 1. The % Sand correlates to the total of % *Halimeda* + % Molluscs in all cases except samples 34 and 48, which are located in muddy areas and have higher percentages of molluscs, foraminifera and other components. *Th* = *Thalassia*.

Sample No.	% *Halimeda*	% Molluscs	% Forams	% Other	% Sand	Environments
33	78.7	14.4	2.0	4.9	99.0	Sand w/ *Th*
34	41.2	25.4	14.9	18.5	55.0	*Th* on Mud
48	47.4	25.4	11.0	16.2	45.0	*Th* on Mud
63	67.4	21.7	5.2	5.7	86.0	Sand w/ *Th*
72	80.9	12.7	3.5	2.9	97.0	*Th* on Sand
94	78.4	10.3	5.8	5.5	87.0	*Th* on Sand
96	73.5	15.9	4.3	6.3	82.0	*Th* on Sand
113	60.9	26.7	3.1	9.3	87.0	Sand w/ *Th*

DISCUSSION AND CONCLUSIONS

A total of three emergent mangrove and five marine depositional environments occur on and around Twin Cays (Fig. 3). In marine depositional environments surrounding Twin Cays, sediment analyses (Table 1) and depositional environments mapped in Figure 3 indicate a generally sand-dominated series of bottom types distinguished by their windward or leeward locations, water depths, and the density of aquatic vegetation. An extensive study of the distribution of submerged macroalgae and seagrasses at Twin Cays was conducted by Littler et al. (1985). The sheltered channels of Twin Cays apparently contained lower species diversity (dominated by productive filamentous algae) and greater total cover than the more exposed bay community, where *Halimeda* and *Thalassia* provided the dominant cover. Lower levels of physical

10

disturbance, lack of herbivory and greater availability of recycled nutrients in channels near mangroves account for the greater incidence of filamentous algae than in the wave-exposed bay environments (Littler et al., 1985). Nearshore windward depositional environments ("*Thalassia* **on Sand,**" Fig. 7) consist of sandier (*Halimeda*-rich) sediments with a denser cover of *Thalassia testudinum* than in leeward and offshore environments. The deeper windward offshore area is classified as "*Thalassia* **on Mud**" (Fig. 6), a depositional environment that extends into the main channel between East and West Islands, as well as across much of the western (leeward) shelf. Areas of "**Sand with** *Thalassia*" (Fig. 8) occur in patches on the western shelf, at South Point and along the northern edges of the islands. The "**Sand with** *Thalassia*" depositional environment contains a lower density of *Thalassia* than the "*Thalassia* **on Sand**" areas along the windward coast. The main muddy areas are limited to the W-E trending channel into the interior of East Island. Sediment samples 1-6 obtained from the inner reaches of this channel were judged to be too organic-rich for sediment analysis.

A comparison of the sediment grain-size patterns in Figure 8 with the marine depositional environments map in Figure 3 indicates that the mud-rich areas are mostly limited to the low-energy sites in the Twin Cays channels and to the deeper "*Thalassia* **on Mud**" depositional environment. The muddy sands also tend to be found in more protected sites off the coasts and in the deeper "*Thalassia* **on Sand**" areas. Finally, the very sandy samples are generally limited to the shallow well-winnowed and compact bottoms of the "**Sand with** *Thalassia*" environment.

The *Halimeda* sand-dominated sediments of the Twin Cays area are consistent with Purdy et al.'s (1975) designation of a large *Halimeda* facies for this area of the Barrier Reef Platform lagoon. Our sediment analyses allowed us to differentiate Purdy et al.'s (1975) general *Halimeda* facies into the three distinct environments described above based on the percentage of sand (Fig. 9). The dominance of mud-size sediment in the protected channel areas has persisted for thousands of years as illustrated in the Holocene cross-sections in Macintyre et al. (this volume).

The other muddy environment ("*Thalassia* **on Mud**"), consisting of mud with a rich cover of *Thalassia,* occurs in the deeper (2-7 m) areas of the windward coast, all through the central channel between the Islands, continuing outward along the bay side (Fig. 3). The sandy areas ("**Sand with** *Thalassia*;" "*Thalassia* **on Sand**"), directly adjacent to the shorelines, consist of shallow (0-4 m) flats of compact sand with scattered *Thalassia*. Again, the Holocene sections (Macintyre et al., this volume) indicate that over the 8,000 cal-year mangrove-peat history of Twin Cays, these lagoonal *Halimeda*-rich sands transgressed into the mangroves, sometimes for thousands of years. *Halimeda* dominated the sedimentation, while *Rhizopora mangle* formed peat and both maintained the buildup with sea-level rise over the Holocene.

Along the north coast of Twin Cays, the "**Sand with** *Thalassia*" depositional environment meets a shoreline of eroding peat and mangroves, similar to the north coast of nearby Tobacco Range (Macintyre et al., 1995). This erosion is likely due to the destructive N-NE winter wave climate. In contrast, the windward southern and eastern shores consist of mangroves that are prograding over lagoonal sediments, as reflected in the elongate shape of Eastern Island and the lobes of sandy deposits surrounding South Point.

REFERENCES

Bond, G.B.
  1988. Sedimentology and Holocene stratigraphy of a carbonate mangrove buildup, Twin Cays, Belize, Central America. Unpublished MSc thesis. Texas A&M University, College Station, Texas.108p.

Burke, R.B.
  1982. Reconnaissance study of the geomorphology and benthic communities of the outer Barrier reef Platform, Belize. *In:* Rutzler, K. and I.G. Macintyre, eds., *The Atlantic Barrier Reef Ecosystem at Carrie Bow Cay, Belize 1, Smithsonian Contributions to the Marine Sciences* No. 12: 509-526, Smithsonian Press, Washington, DC.

Folk, R.L.
  1961. *Petrology of Sedimentary Rocks.* Austin Texas, Hemphill's 154p.

Littler, M.M., P.R. Taylor, D.S. Littler, R.H. Sims, and J.N. Norris
  1985. The distribution, abundance, and primary productivity of submerged macrophytes in a Belize barrier-reef mangrove system. *Atoll Research Bulletin* 289, 20p.

Macintyre, I.G., M.M. Littler, and D.S. Littler
  1995. Holocene history of Tobacco Range, Belize, Central America. *Atoll Research Bulletin* 430, 18p.

Macintyre, I.G., M.A. Toscano, R.G. Lighty, and G.B. Bond.
  2004. Holocene history of the mangrove islands of Twin Cays, Belize, Central America. *Atoll Research Bulletin* (this issue).

Purdy, E.G., W.C. Pusey III, and K.F. Wantland
  1975. Continental shelf of Belize – regional shelf attributes. *In*: Wantland, K.F. and W.C. Pusey, eds., *Belize Shelf – Carbonate Sediments, Clastic Sediments, and Ecology, American Association of Petroleum Geologists,* Studies in Geology 2: 1-39.

Rützler,K., and I.G. Macintyre
    1982. The Atlantic Barrier Reef Ecosystem at Carrie Bow Cay, Belize, 1 Structure
            and Communities. *Smithsonian Contributions to the Marine Sciences* 12,
            539p, Smithsonian Press, Washington, DC.
Smith, F.G.W.
    1971. *Atlantic Reef Corals*. Miami, Florida. University of Miami Press 164 p.

HOLOCENE HISTORY OF THE MANGROVE ISLANDS OF TWIN CAYS,
BELIZE, CENTRAL AMERICA

BY

IAN G. MACINTYRE, MARGUERITE A. TOSCANO,
ROBIN G. LIGHTY, AND GREGOR B. BOND
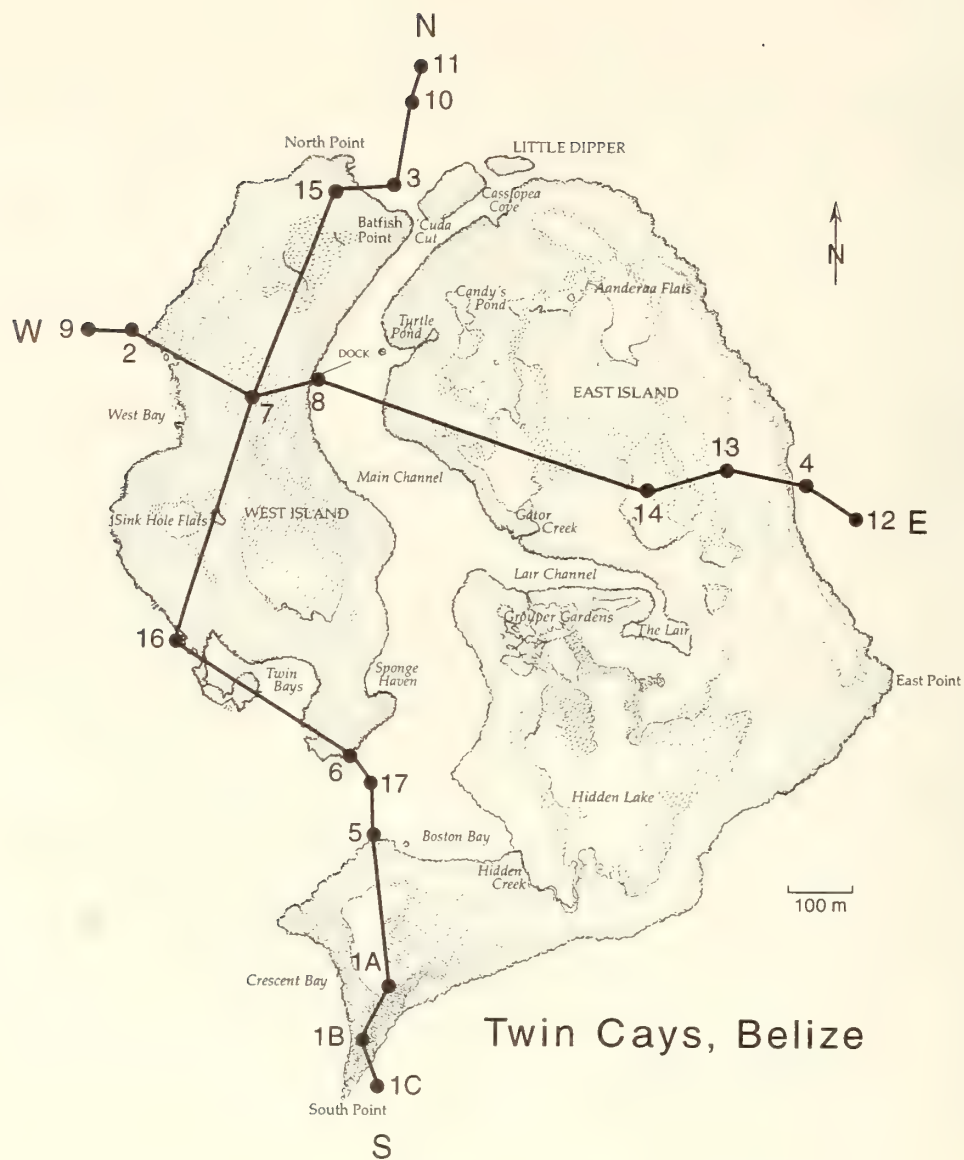
Figure 1. Location Map of Twin Cays showing Main Channel, N-S and E-W cross sections and core locations.

HOLOCENE HISTORY OF THE MANGROVE ISLANDS OF TWIN CAYS, BELIZE, CENTRAL AMERICA

BY

IAN G. MACINTYRE[1], MARGUERITE A. TOSCANO[1], ROBIN G. LIGHTY[2], AND GREGOR B. BOND[3]

ABSTRACT

Nineteen vibracores collected along north/south and east/west transects across Twin Cays, Belize indicate that these two mangrove islands have been formed by accumulations of mangrove peat and sediment that are almost 9 m thick. Mangrove communities were established on a Pleistocene limestone substrate about 8,000 calendar (cal) years ago and kept pace with the late Holocene transgressing seas. At several stages lagoonal sands invaded the mangrove communities and commonly were subsequently overgrown by the mangroves. The initiation of the main channel that separates these two cays was established early in the history of these islands and appears to be related to the topography of the underlying limestone. Radiocarbon dates of peat and sediment from this study were calibrated and plotted with respect to mean sea level and were shown to be in agreement with the corrected western Atlantic sea-level curve (Toscano and Macintyre, 2003).

INTRODUCTION

Mangrove communities were rapidly established on the Pleistocene surface of the Belize Barrier Reef Platform after it was flooded about 8,000 cal years ago by the rising seas of the Holocene Transgression (Purdy, 1974; Purdy et al., 1975; Ebanks, 1975; Halley et al., 1977; Shinn et al., 1982; Macintyre et al., 1995). The majority of these mangrove communities subsequently were drowned on the deeper Pleistocene surfaces and covered by calcareous lagoonal deposits. In the south-central area of the Belize Barrier Reef Platform, however, mangrove communities that were established on high relief on the underlying Pleistocene limestone kept pace with the rising seas and formed very thick accumulations of peat, particularly at Tobacco Range (Macintyre et al., 1995) and Twin Cays.

---

[1] Department of Paleobiology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560
[2] 3414 Walnut St., Camp Hill, PA 17011
[3] Hydroenvironmental Technologies, Inc. P.O. Box 25073, Chicago IL 60625

We investigated the Holocene history of the mangrove islands of Twin Cays and show the changing patterns of development of these islands as they kept pace with advancing sea level. In addition, calibrated radiocarbon dates on mangrove peat, mangrove wood and *Halimeda* sand are used to demonstrate that the Toscano and Macintyre (2003) corrected sea-level curve for the western Atlantic is valid for the Belize area.

PREVIOUS WORK

Purdy (1974) was the first to report radiocarbon dates for peat samples taken from sediment cores collected in the inner Southern and Northern Shelf Lagoons. As he was mainly interested in establishing the rates of deposition of sediment accumulations, he did not report the water depths of his core sites. We recently obtained this information from him (E.G. Purdy, November 2002) and have discovered that peat samples ranging from depths of 1 m to 24 m yielded uncalibrated ^{14}C dates of 5,600 ± 140 yrs BP to 10,075 ± 210 yrs BP.

Thin accumulations of basal mangrove peat overlying a terrigenous clay deposit on the eroded Pleistocene limestone surface have been reported at Ambergris Cay (about 130 km north of Twin Cays) where they were patchy in distribution and undated (Ebanks, 1975). Similar thin peat deposits were found around Boo Bee Patch Reef in the Southern Shelf Platform Lagoon, 3 km south of Twin Cays, where they were only 0.3 m thick and yielded an uncalibrated ^{14}C date of 8,780 ± 100 yrs BP at a depth of 18 m below sea level (Halley et al., 1977).

Shinn et al. (1982) sampled peat from vibracores collected along a transect that started at Carrie Bow Cay and extended west to the mainland. The peat was generally at the base of five cores. On the Barrier Reef Platform they were less than 1 m thick at depths of 9.5 m and 16 m below sea level. These peats were not ^{14}C-dated, but the three vibracores collected in the Southern Shelf Lagoon encountered peat shallowing shoreward at depths below sea level of about 15 m, 7 m and 5 m. A total of five ^{14}C dates for these peats ranged (uncalibrated) from 8,808 ± 600 yrs BP to 2,861 ± 190 yrs BP.

Some of the thickest accumulations of mangrove peat ever reported were documented in vibracores and in samples taken with Macauly and Davis soil-sampling probes along a northwest-to-southeast transect across Tobacco Range (Macintyre et al., 1995). Almost all of the sections sampled were entirely mangrove peat, both from the island and offshore. This peat, which was established on terrigenous basal mud on Pleistocene limestone, was up to 10 m thick and gave a maximum uncalibrated ^{14}C date of 6,920 ± 100 yrs BP. We are now reporting that Twin Cays, which is only 5 km south of Tobacco Range, has very similar thick accumulations of mangrove peat overlying Pleistocene limestone.

METHODS

Field Work

A total of 19 vibracores were collected along north/south and east/west transects across Twin Cays (Fig. 1). The first 6 cores were collected by a team led by R.G. Lighty in 1984. In the following year, I.G. Macintyre's group collected an additional 13 cores. Continuous sediment and peat sections were vibracored using 9 m-long, 7.6 cm-diameter aluminum core tubes, each fitted with a core catcher (Fig. 2). Offshore work was relatively easy compared with the movement of this heavy equipment into the interior (Fig. 3).

Figure 3. Crossing Hummingbird Pond (between cores 13 and 14 on Figure 1) with vibracore unit. The tripod can be seen at site 14 in the background.

Figure 2. Collecting vibracore 16 off the west coast of Twin Cays with the aid of a Zodiac (inflatable boat).

Core recovery and depth of penetration were recorded at each site so that corrections could be made for sample compaction (primarily of peat) that occurred during coring. In addition, a record was made of the elevation of each core site with respect to mean sea level. Cores were left in their aluminum tubes, cut in 3 m sections, capped and shipped for laboratory analyses.

Radiocarbon dates were determined by the former Smithsonian Institution Radiocarbon Laboratory, using the Libby half-life of 5568 years; 95% of the activity of the National Bureau of Standards oxalic acid was used as the modern standard. The peat samples were studied under the microscope to remove rootlets that appeared noncontemporaneous, and all carbonate material was removed by acid pretreatment.

4

Radiocarbon Date Calibration

Uncalibrated ^{14}C dates on intertidal mangrove peat (*Rhizopora mangle* and related species) were determined using the Libby ^{14}C half life of 5568 years (rather than the true ^{14}C half life of 5730 years) and neither analyzed nor corrected for ^{13}C; thus they are not valid calendar ages. Standard ^{14}C dates therefore are not consistent with the more accurate temporal framework available from high-precision U-Th-dated coral records for tracking sea level.

Calibration data, which account for temporal variations in atmospheric ^{14}C/^{12}C over time, are now widely used to arrive at the true (calendar) ages of radiocarbon-dated samples (Stuiver et al., 1998a; b). For peat samples, correction for ^{13}C and atmospheric calibration shifts the original ^{14}C data to older values, with the offset increasing as the samples get older (Bard et al., 1990). It is important to calibrate ^{14}C data from intertidal mangrove peat deposits because they are believed to accumulate in a specific facies having a well-defined relationship to tidal range and hence to sea level (e.g. Scholl et al., 1969; Robbin, 1984; Boardman et al., 1988; Digerfeldt and Hendry, 1987). As such, mangrove peats can provide essential upper-limit information for the actual position of sea level above the elevation of contemporaneous coral-reef crest growth.

None of the mangrove samples presented and referenced in this paper had originally been analyzed for ^{13}C fractionation. Peat dates were calibrated with the CALIB program (Stuiver and Reimer, 1993) as nonconventional radiocarbon ages using a ^{13}C$_{PDB}$ value of 27±0.2‰ (Smith and Epstein, 1971) and the atmospheric calibration dataset A (CALIB Manual version 4.1; Stuiver and Reimer, 1993; Stuiver et al., 1998 a; b).

Toscano and Macintyre (2003) synthesized and calibrated all available peat and coral ^{14}C age data to construct a sea-level curve for the western Atlantic. This curve included the peat samples taken from Twin Cays cores and discussed herein, as well as 23 peat samples from the Tobacco Range group of mangrove islands in the Central Belize Lagoon (Macintyre et al., 1995). This calibrated coral-peat sea-level curve is presented in relation to the Twin Cays data to understand the sea-level history of the Central Belize Lagoon.

RESULTS

Subsurface Facies

The two cross sections based on vibracore transects (Figs. 4 and 5) indicate that mangroves were established on an elevated section of the erosional surface of the Pleistocene limestone substrate (Macintyre and Toscano, this volume) about 8,000 cal years ago. The maximum thickness of the mangrove peat is about 9 m, which is slightly less than that of the peat sections recovered from Tobacco Range (Macintyre et al., 1995), which is about 5 km north of Twin Cays.
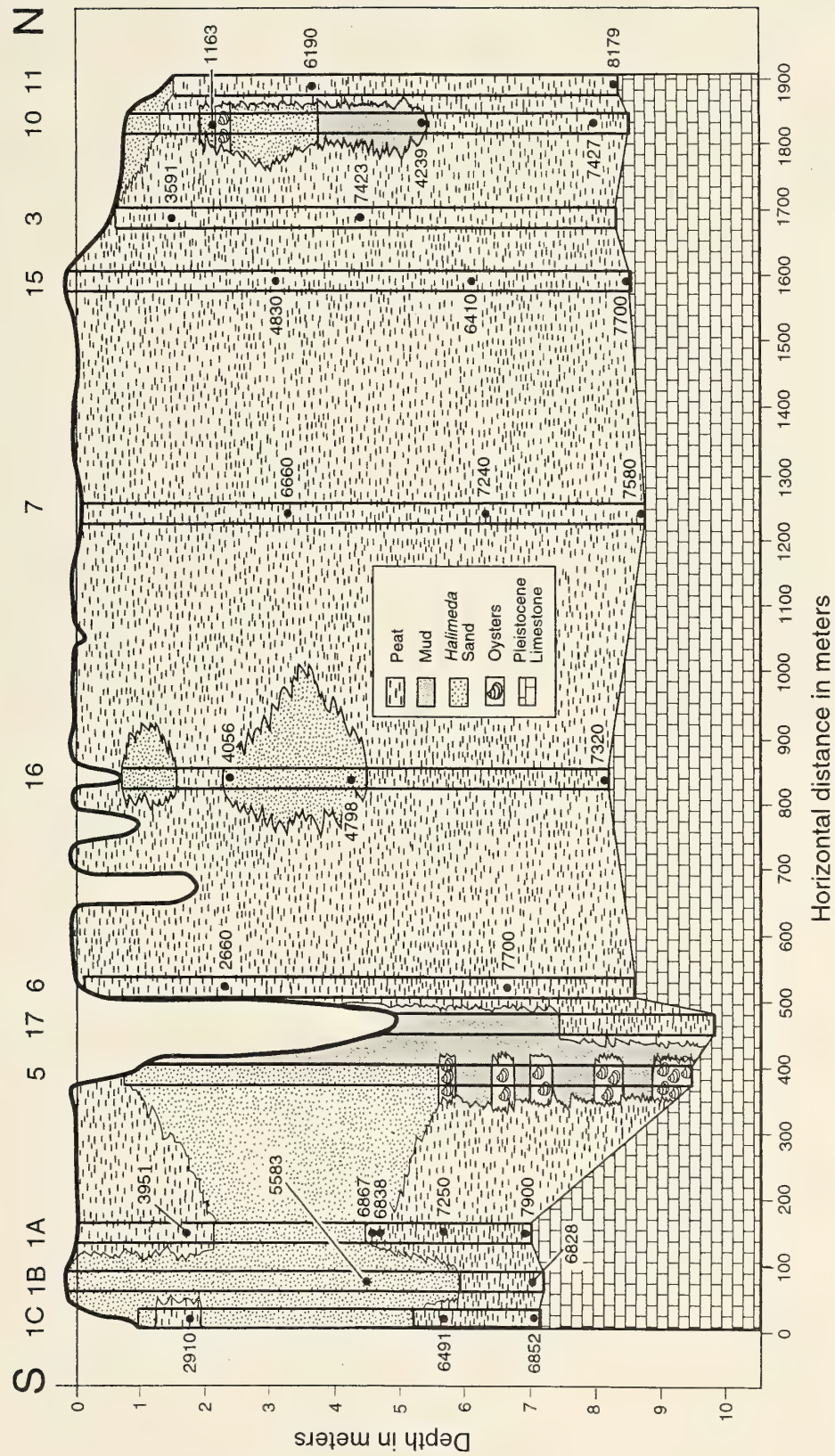
Figure 4. N-S vibracore cross section across Twin Cays, showing distribution of dominant mangrove facies and locations of radiocarbon-dated samples. All ages are in calendar (cal) years.
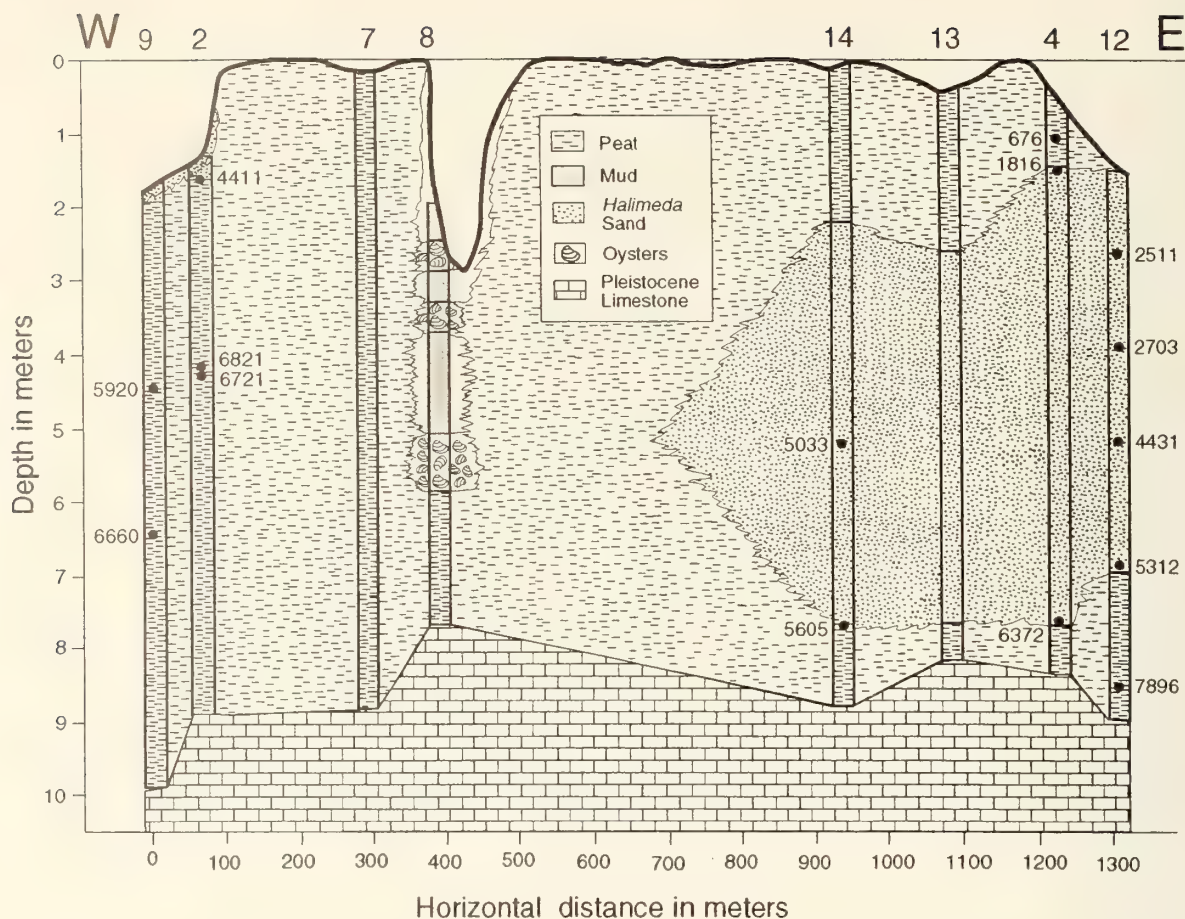
Figure 5. W-E vibracore cross section across Twin Cays, showing distribution of dominant mangrove facies and locations of radiocarbon-dated samples. All ages are in calendar (cal) years.

Four basic facies were recognized in the Twin Cays cores:

Mangrove Peat Facies. The mangrove peat, like the peat from Tobacco Range (Macintyre et al., 1995), was composed of two types that are related to stages of peat preservation. The uppermost sections were dominated by well-preserved "broad-fibered peat", which ranged in color from brown to reddish brown and generally had a spongy texture of coarse-fibered roots and rootlets. In contrast, the "fine-fibered peat" was usually found in lower sections of the cores and ranged in color from dark brown to black and consisted of a pasty dense texture of fine fibers. This decomposed early peat usually lacked identifiable plant remains. All stages of alteration of peat were found along with a common intermixing with both sand and mud deposits.

<u>*Halimeda*</u> *Sand Facies.* This facies consisted of a calcareous sand dominated by *Halimeda* grains in varying amounts of carbonate mud matrix. Molluscs were common and consisted of a wide variety of bivalves and gastropods. Other constituent grains included branching *Porites* fragments, echinoid spines, and worm tubes.

Mud Facies. These grey calcareous muds contained scattered grains of molluscs, including bivalves and gastropods, *Halimeda* plates and echinoid spines. The mud section found in Core 10 differed significantly from the other muds. It contained mostly benthic foraminifer tests with traces of gastropods, bivalves, and echinoid spines, and was probably a mangrove pond deposit.

Oyster Bed Facies. This facies, which is always found interbedded with the mud facies, was dominated by oyster valves (*Isognomon alatus*) with minor amounts of other molluscs, *Halimeda* grains and barnacles.

Calibrated Radiocarbon Dates

Calibration of ^{14}C dates from Twin Cays peat, wood, and *Halimeda* sand samples (Table 1) pushes the ages back by 100 to 900 years over the approximately 700-8,200-year time frame. Toscano and Macintyre (2003) presented similar calibration age shifts for their larger western Atlantic coral and peat database.

Plots of calibrated radiocarbon dates of Twin Cays samples with respect to mean sea level are shown in Figure 6. The sea-level curve in Figure 6 is the western Atlantic sea-level curve based on the combined calibrated coral and peat dataset of Toscano and Macintyre (2003). This curve keeps most of the Twin Cays mangrove peat and wood samples above it and the subtidal *Halimeda* sands below.

As can be seen in Figure 6, a few subtidal *Halimeda* sand samples plot above this curve and probably represent transported deposits. Two peat dates that plot slightly below this curve can likely be explained by younger root contamination (age shift) or compaction that occurred during coring (elevation shift). The elevations of peat dates well above the curve are more problematic as discussed at length by Toscano and Macintyre (2003).

Age calibration provides an improved temporal component to the curve; however, elevation issues still exist in the large (up to 4 meters) vertical range of the peat samples. Figures 4 and 5 (cross sections across depositional environments) indicate that several mangrove environments exist across these islands. As these facies likely shifted over time, vertical sections in particular cores may not contain consistent peat compositions throughout. Given the lack of identifiable macroscopic remains in the peat, the assumption that intertidal *Rhizopora mangle* dominates all vertical sections is not verifiable. Unfortunately, the actual elevation range of the modern surface environments is less that 0.5 m (this study as well as Tobacco Range; Macintyre et al., 1995) making it difficult to assume that the elevation range of the geologic peat samples in the sea-level curve is related to topographic changes in facies preserved in the cores. In addition, the elevation of wood samples higher in the section with the elevated peats might be an indication that a number of peats represent preserved canopy-level deposits. Another possibility includes the need to factor out tidal or storm-surge variability from the elevations, which is not possible to quantify in a geologic context. Lastly, glacio-hydro-isostatic 3D earth model predictions (Lambeck et al., 2002) may eventually provide some downward correction which is site-specific; however, the 4 m elevation range of the peat database may also be indicative of age errors possibly caused by contamination of peat by older carbon from pre-Holocene substrate and/or terrestrial sources.

8

Table 1. Calibrated radiocarbon dates for Twin Cays samples plotted in Figures 6 and 8. Type MP is Mangrove Peat, W is Wood, HS is *Halimeda* Sand.

Location	Sample Type	Core ID	Elevation, m MSL	^{14}C Date yrs BP	±	CalBP Years
Twin Cays	MP	1A-128	-1.76	3655	80	3951
Twin Cays	MP	1A-355	-4.59	6065	75	6867
Twin Cays	MP	1A-350	-4.73	6030	105	6838
Twin Cays	MP	1B-360	-7.06	6070	110	6828
Twin Cays	MP	1C-60	-1.77	2835	130	2910
Twin Cays	MP	1C-425	-5.76	5745	150	6491
Twin Cays	MP	1C-555	-7.19	6040	145	6852
Twin Cays	MP	2-15	-1.55	3960	95	4411
Twin Cays	MP	2-210	-4.25	5915	90	6721
Twin Cays	MP	3-90	-1.53	3400	105	3591
Twin Cays	MP	3-260	-4.50	6520	160	7423
Twin Cays	MP	4-55	-1.05	800	70	676
Twin Cays	MP	6-200-216	-2.31	2490	60	2660
Twin Cays	MP	6-620-640	-6.75	6920	110	7700
Twin Cays	MP	7-250-270	-3.30	5870	100	6660
Twin Cays	MP	7-500-520	-6.35	6310	90	7240
Twin Cays	MP	7-693-713	-8.71	6730	90	7580
Twin Cays	MP	9-200-220	-4.45	5210	90	5920
Twin Cays	MP	9-350-370	-6.43	5870	90	6660
Twin Cays	MP	11-190-210	-3.71	5420	70	6190
Twin Cays	MP	15-250-270	-3.08	4270	80	4830
Twin Cays	MP	15-500-520	-6.15	5670	80	6410
Twin Cays	MP	15-690-709	-8.49	6920	90	7700
Twin Cays	MP	16-630-644	-8.12	6450	90	7320
Twin Cays	W	1A-420	-5.70	6340	60	7250
Twin Cays	W	1A-518	-7.02	7080	110	7900
Twin Cays	W	2-204	-4.18	6050	100	6821
Twin Cays	HS	1B-237	-4.58	4815	55	5583
Twin Cays	HS	4-95	-1.48	1810	40	1816
Twin Cays	HS	10-115-125	-2.20	1190	90	1163
Twin Cays	HS	12-90-100	-2.63	2390	70	2511
Twin Cays	HS	12-190-200	-3.89	2490	100	2703
Twin Cays	HS	12-290-300	-5.15	3920	70	4431
Twin Cays	HS	14-490-500	-5.20	4380	70	5033
Twin Cays	HS	16-138-148	-2.39	3620	70	4056
Twin Cays	HS	16-300-310	-4.27	4150	80	4798

8

Figure 6. Calibrated age data from peat, wood, and *Halimeda* sand taken from Twin Cays cores in relation to the corrected western Atlantic sea-level curve of Toscano and Macintyre (2003).
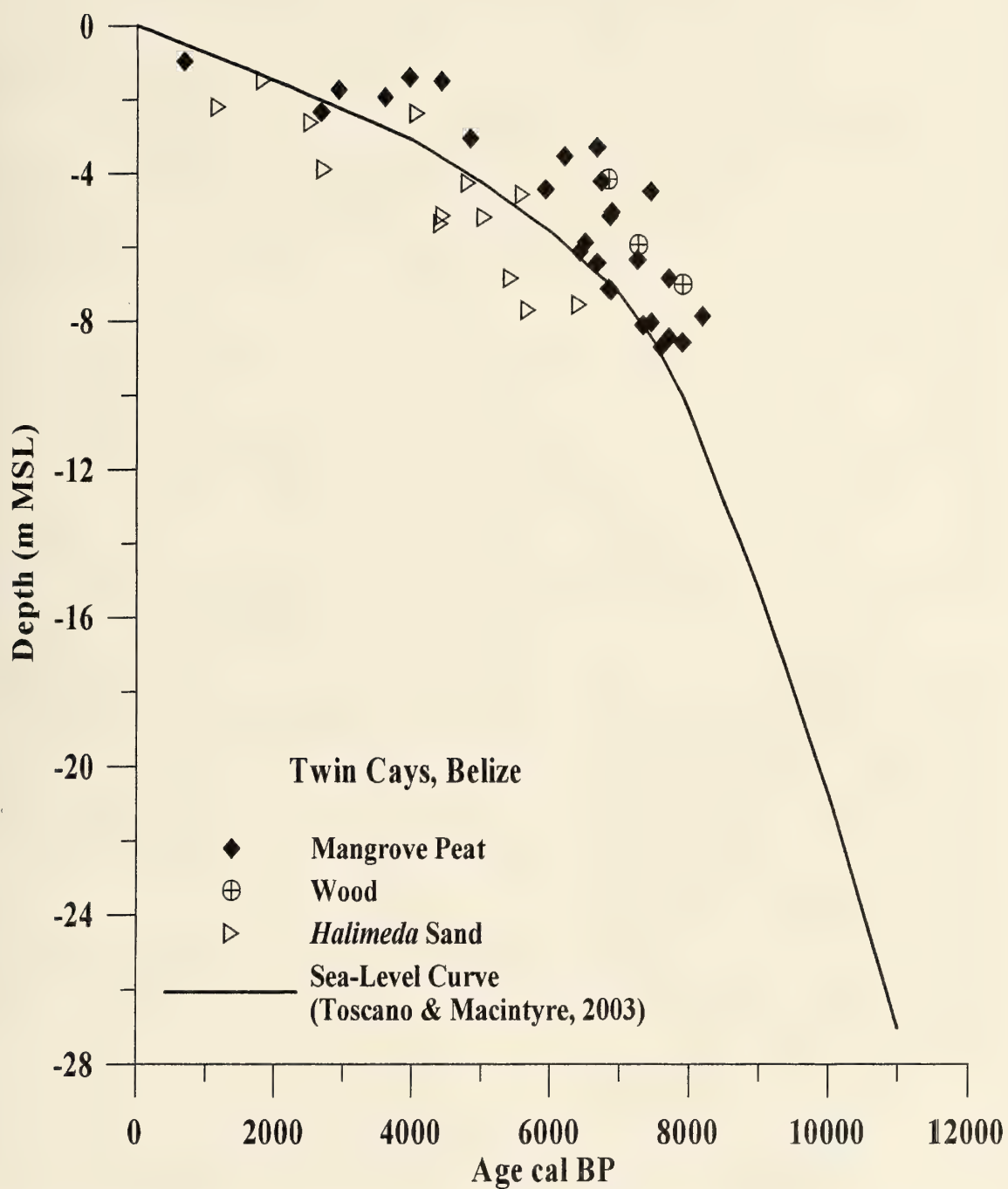
DISCUSSION

Holocene History

It can be seen on the two cross sections (Figs. 4 and 5) that mangrove peat at Twin Cays started to accumulate about 8,000 cal years ago on an erosional Pleistocene limestone surface at depths of 9 to 10 m below present sea level. Unlike Tobacco Range (Macintyre et al., 1995), where basal clay deposits were found in both vibracore and sediment-probe samples, this residual soil deposit was only recovered at the base of Core 11 at Twin Cays. This material, which represents the soil that accumulated on the exposed Pleistocene limestone, also was reported below peat deposits in northern Belize (High, 1975; Ebanks, 1975) and Belizean offshore atolls (Gischler, 2003). Basal clays were either lost during the coring on Twin Cays or washed off the Pleistocene surface prior to the establishment of the mangrove communities.

Oysters are commonly found in large quantities on mangrove roots exposed at the edges of channels or ponds (Fig. 7). Therefore, the presence of interlayered mud and oyster beds (Cores 5 and 17) above the Pleistocene surface in the main channel at the southern section of Twin Cays (Fig. 4) indicates that this channel existed during most of the Holocene history of these islands. In this area the position of the channel appears to be related to a topographic low point in the underlying Pleistocene limestone surface. At the north end of this S-N cross section (Fig. 4), the interlayered mud, sand, and oysters in the middle portion of Core 10 suggests the existence of a mangrove pond that was eventually closed off by mangroves and later submerged by rising sea level.

Further north on the E-W cross section (Fig. 5), the main channel (seen in Figure 4, cores 5 and 17) developed or extended into that area about 2,000 years later, independent of the Pleistocene substrate relief (Fig. 5, core 8).

Major transgressions of lagoonal *Halimeda* sands occurred around 5,000 to 6,000 cal years ago off both the east and south coasts (Figs. 4, 5). This sand was overgrown by mangrove communities off the east coast about 2,000 cal years ago but off the south coast the sand has maintained its position and presently forms South Point (Fig. 1). Off both the west and north coasts, similar but considerably smaller incursions of lagoonal *Halimeda* sands have occurred at later dates, around 4,000 to 5,000 cal years ago. This indicates that the north and west

Figure 7. Modern *Isognomon alatus* (oyster) growth on exposed mangrove roots at Twin Cays analagous to the oysters cored in tidal channel deposits. Field drawing by Mary Parrish (2002).

coasts of the smaller island (Figs. 1, 4, 5) have had more stable mangrove development during the formation of these islands than have the east and south coasts of the larger island. This could be related to more exposure to hurricane damage and the resultant retreat of mangroves off the seaward-facing southern and eastern coasts.

Holocene Sea-Level History of Belize

Our calibrated sea-level database consisting of ^{14}C-dated mangrove peat and associated *Halimeda* sands from Twin Cays basically agrees with the Toscano and Macintyre (2003) corrected western Atlantic sea-level curve (Fig. 6). However, a publication by Gischler and Hudson (2004) introduced a sea-level curve for Belize that is considerably elevated above the western Atlantic sea-level curve. This contrast in
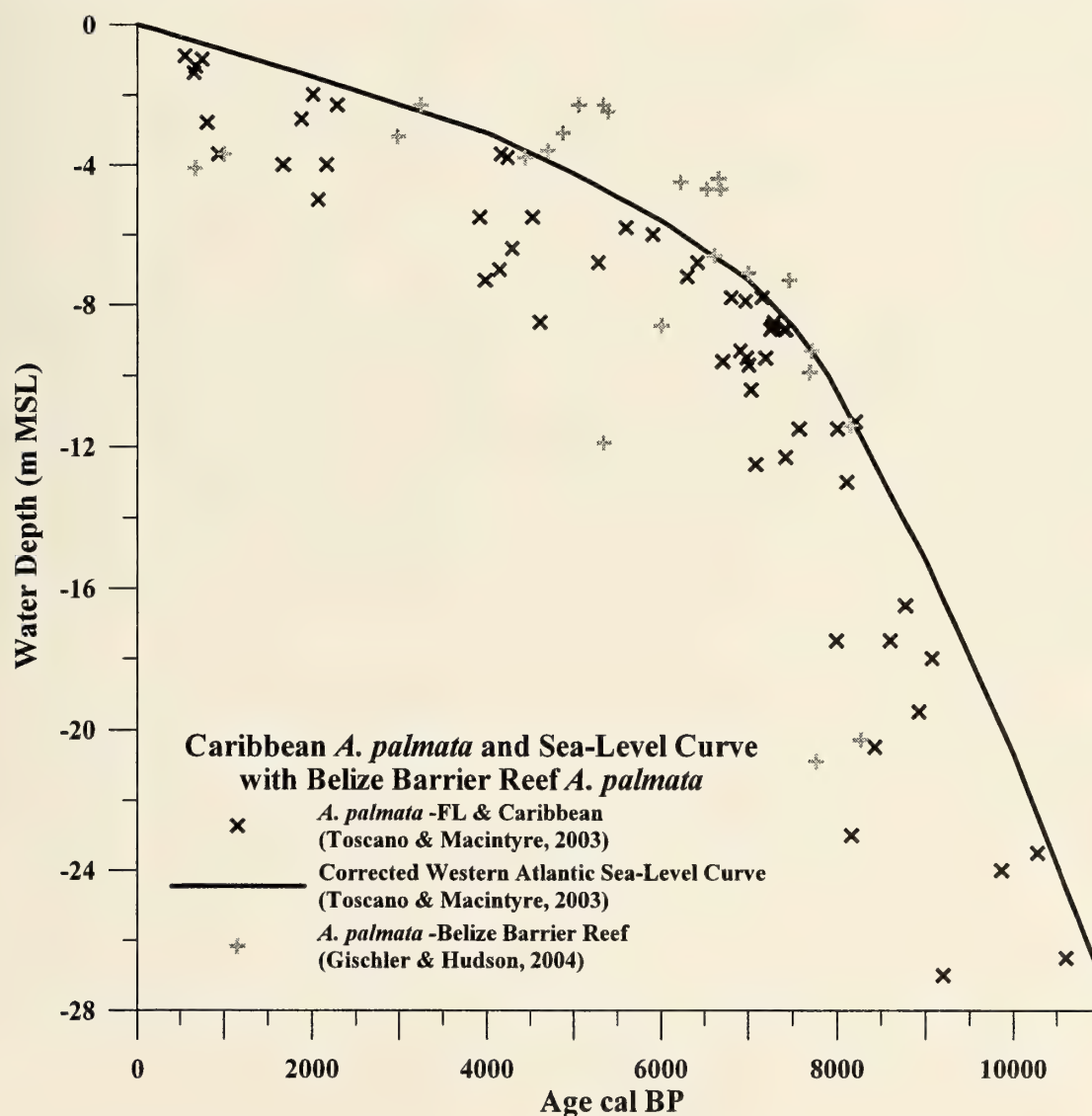
Figure 8. Western Atlantic sea-level curve (Toscano and Macintyre, 2003) with plots of calibrated age dates of *A. palmata* used in the construction of this curve and calibrated dates of *A. palmata* from Gischler and Hudson (2004).

our understanding of Belizean sea-level history over the last 10,000 years is based, for the most part, on a number of *Acropora palmata* dates that plot well above the western Atlantic sea-level curve.

In Figure 8 we show the western Atlantic sea-level curve with plots of the 51 calibrated *A. palmata* samples that were used in the construction of this curve (Toscano and Macintyre, 2003). We have also plotted the 23 *A. palmata* dates from Gischler and Hudson (2004). Although as many as 13 of their dates from the shallow-water *A. palmata* agree with the western Atlantic curve, those contrast with 10 plots (forming two distinct clusters and 1 outlier) that are located significantly above the curve (Fig. 8). With the overall history along the Belize barrier-reef complex being one of subsidence progressively increasing southward (Purdy, 1974; Choi and Ginsburg, 1982; Choi and Holmes, 1982; Lara, 1993; Purdy et al., 2003), and the fact that many of Gischler and Hudson's (2004) coral dates agree with (lie on or below) the western Atlantic curve, it is not possible to relate the elevated dates to tectonic uplift. In addition, the elevated, clustered coral plots are geographically limited largely to three cores (BBR2, BBR10, BBR11) taken on a Pleistocene high on the Belize shelf, according to Gischler and Hudson's (2004) cross section (their Figure 2). In addition, these three cores contain a high percentage of cemented coral debris. The age range represented by the corals on this Pleistocene high (-7m to –5 m MSL) is too old when compared with the depths of the same age range of samples taken from the other cores, both to the north and the south of the high area (- 21m to -7m). Given that Gischler and Hudson (2004) state that they are unable to determine if these corals are in place, we suggest that the 10 elevated *A. palmata* are corals that have been transported during multiple episodes of severe storm activity and concentrated on the topographic high. The high area containing these cores is also located in a "window" between the south ends of Turneffe and Lighthouse atolls and the north end of Glovers atoll; therefore it is open to direct wave action. This pattern of coral storm deposits plotting well above previous sea levels is well demonstrated in cores from the Holandes Cay storm ridge off the Caribbean coast of Panama (Macintyre et al., 2001).

The same situation holds true for an earlier manuscript on the Holocene history of the reefs on atolls off Belize (Gischler and Hudson, 1998). In this case six radiocarbon-dated *A. palmata* samples plotted well above the western Atlantic sea-level curve (Toscano and Macintyre, 2003), in contrast to the 10 other coral dates that plot in agreement with (i.e. on or under) this sea-level curve. As with the Belize Barrier Reef samples, the elevated *A. palmata* samples from the offshore atolls all came from cores containing "well-cemented grainstone-rudstone" (p. 338), which is indicative of high wave energy deposits. As expected, these cores were collected on the windward sides of the atolls, all sites where modern storm deposits are accumulating---most recently the extensive storm ridge system that formed on the windward side of Glover's Reef after Hurricane Mitch passed over this atoll in 1998 (Macintyre, personal observation, 1999).

Other authors have also presented elevated sea-level curves for Belize, but these were based on very few and highly questionable elevated peat dates. Davies and Montaggioni (1985) went so far as to suggest an early attainment of near-present sea levels (less than 2 m below present sea level) about 5,000 to 6,000 years ago with a

slow continuous rise to present sea level (p. 497). However, in a later figure they show their Belize sea-level curve catching up with present sea level 5,000 years ago and compared it to sea-level patterns recorded in the Pacific (p. 504). Likewise, the sea-level curve for Belize presented by Westphall (1986) shows a sharp rise in sea level until reaching about -2 m approximately 5,000 years ago followed by a gradual rise to present sea level.

The comprehensive western Atlantic sea-level curve effectively combines calibrated data from Caribbean-wide peats (including Twin Cays data), and the shallow-water coral *Acropora palmata*, which bracket and define this sea-level curve (Toscano and Macintyre, 2003). We therefore do not accept any premise suggesting that Belize has had a significantly different isostatic history in comparison to the rest of the western Atlantic, particularly because the sea-level curve proposed by Gischler and Hudson (2004) places most of the Twin Cays peat at unrealistic depths below sea level. As our Twin Cays radiocarbon dates of mangrove peat and subtidal *Halimeda* sand also closely bracket this western Atlantic curve, we propose that this sea-level curve represents the valid late Holocene history of sea-level rise in Belize.

CONCLUSIONS

The 19 vibracores collected along two transects across Twin Cays reveal that these islands were initiated as mangrove communities about 8,000 cal years ago. These mangrove communities accumulated at rates that allowed them to keep pace with the rising late Holocene seas. There were several periods when lagoonal *Halimeda* sands replaced the mangroves, but in most areas the mangroves reestablished over those sand deposits. The main channel that separates the two cays was established early in the history of the islands and was probably related in part to the relief of the underlying Pleistocene limestone.

When calibrated radiocarbon dates from Twin Cays mangrove peat, wood, and *Halimeda* sands are plotted with respect to mean sea level, they establish a record for the rise of sea level in Belize for the last 8,000 cal years that agrees with the Toscano and Macintyre (2003) sea-level curve for the western Atlantic. Other sea-level curves proposed for Belize (Davies and Montaggioni, 1985; Westphall, 1986; Gischler and Hudson, 2004) are elevated above this western Atlantic curve and are considered invalid because they are based in large part on problematic peat and coral data.

ACKNOWLEDGMENTS

14

was a staff member. He and his student, Greg Bond, described the cores and Bond included a limited description of the history of Twin Cays in his Masters Thesis (Bond, 1987), which was originally intended to be a study of the surface sediments around Twin Cays. This is an effort to present a comprehensive account of this work that was largely supported by Smithsonian funds and facilities. We present, for the first time, core logs and cross-sectional reconstructions that are corrected for compaction and calibrated radiocarbon dates for both the peat and carbonate samples. We thank Eberhard Gischler for comments on the manuscript. We especially thank Mary Parrish for her art work and graphics and W.T. Boykins for text and photographic layouts. (CCRE Contribution Number 676).

REFERENCES

Bard, E., B. Hamelin, R.G. Fairbanks, and A. Zindler
  1990. Calibration of the ^{14}C timescale over the past 30,000 years using mass spectrometric U-Th ages from Barbados corals. *Nature* 345:405-410.
Boardman, M.R., A.C. Neumann, and K.A. Rasmussen
  1988. Holocene sea-level in the Bahamas, in Mylroie, J. (ed), *Proceedings, 4th Symposium on the Geology of the Bahamas*. San Salvador, Bahamian Field Station, pp 45-52.
Choi, D.R., and R.N. Ginsburg
  1982. Siliclastic foundations of Quaternary reefs in the southernmost Belize lagoon, British Honduras. *Geological Society of America Bulletin* 93:116-126.
Choi, D.R., and C.W. Holmes
  1982. Foundations of Quaternary reefs in south-central Belize Lagoon, British Honduras. *American Association of Petroleum Geologists Bulletin* 66:2663-2671.
Davies, P. J., and L. Montaggioni
  1985. Reef growth and sea-level change: the environmental signature. *Proceedings, Fifth International Coral Reef Congress* 3:477-511.
Digerfeldt, G., and M.D. Hendry
  1987. An 8,000-year Holocene sea-level record from Jamaica: implications for interpretation of Caribbean reef and coastal history. *Coral Reefs* 5:165-169.
Ebanks, W.J., Jr.
  1975. Holocene carbonate sedimentation and diagenesis, Ambergris Cay, Belize, in Wantland, K. F. and W. C. Pusey III (eds), *Belize Shelf – Carbonate Sediments, Clastic Sediments, and Ecology. American Association of Petroleum Geologists*, Studies in Geology 2:1-39.
Gischler, E.
  2003. Holocene lagoonal development in the isolated carbonate platforms off Belize. *Sedimentary Geology* 159:113-132.

Gischler, E., and J.H. Hudson
    1998. Holocene development of three isolated carbonate platforms. Belize, Central America. *Marine Geology* 144:333-347.
Gischler, E., and J.H. Hudson
    2004. Holocene development of the Belize Barrier Reef. *Sedimentary Geology* 164:223-236.
Gischler, E., and A.J. Lomando
    2000. Isolated carbonate platforms of Belize, Central America: sedimentary facies, late Quaternary history and controlling factors. Pages 135-146 in E. Insalaco, P.W. Skelton, and T. Palmer (eds), *Carbonate Platform Systems: Components and Interactions.* Special Publication, Geological Society London, vol. 178.
Halley, R.B., E.A. Shinn, J.H. Hudson, and B. Lidz
    1977. Recent and relict topography of Boo Bee patch reef, Belize. *Proceedings, 3rd International Coral Reef Symposium*, Miami, Florida 2:29-35.
High, L.R., Jr.
    1975. Geomorphology and sedimentology of Holocene coastal deposits, Belize, in Wantland, K. F. and W. C. Pusey III (eds), *Belize Shelf – Carbonate Sediments, Clastic Sediments, and Ecology. American Association of Petroleum Geologists*, Studies in Geology 2:1-39.
Lambeck, K., Y. Yokoyama, and T. Purcell
    2002. Into and out of the Last Glacial Maximum: sea-level change during Oxygen Isotope Stages 3 and 2. *Quaternary Science Reviews* 21:343-360.
Lara, M.E.
    1993. Divergent wrench faulting in the Belize southern lagoon: implications for Tertiary Caribbean plate movements and Quaternary reef distribution. *American Association of Petroleum Geologists Bulletin* 77:1041-1063.
Macintyre, I.G., P.W. Glynn, and R.S. Steneck
    2001. A classic Caribbean algal ridge, Holandes Cays, Panama: an algal-coated storm deposit. *Coral Reefs* 20:95-105.
Macintyre, I.G., M.M. Littler, and D.S. Littler
    1995. Holocene history of Tobacco Range, Belize, Central America. *Atoll Research Bulletin,* 430 pp.
Purdy, E. G.
    1974. Karst-determined facies patterns in British Honduras: Holocene carbonate sedimentation model. *American Association of Petroleum Geologists Bulletin* 58:825-855.
Purdy, E.G., E. Gischler, and A.J. Lomando
    2003. The Belize margin revisited. 2. Origin of Holocene antecedent topography. *International Journal of Earth Sciences (Geol Rundsch)* 92:552-572.
Purdy, E.G., W.C. Pusey III, and K.F. Wantland
    1975. Continental shelf of Belize – regional shelf attributes, in Wantland, K. F. and W. C. Pusey III (eds), *Belize Shelf – Carbonate Sediments, Clastic Sediments, and Ecology. American Association of Petroleum Geologists*, Studies in Geology 2:1-39.

Robbin, D.M.
　　1984. A new Holocene sea-level curve for the upper Florida Keys and Florida reef
　　　　tract, in Gleason PJ (ed), Environments of south Florida, present and past.
　　　　Miami Geological Society, pp 437-458.
Scholl, D.W., F.C. Craighead, and M. Stuiver
　　1969. Florida submergence curve revisited: its relation to coastal sedimentation rates.
　　　　Science 163:562-564.
Shinn, E.A., J.H. Hudson, R.B. Halley, B. Lidz, D.M. Robbin, and I.G. Macintyre
　　1982. Geology and sediment accumulation rates at Carrie Bow Cay, Belize.
　　　　Smithsonian Contributions to the Marine Sciences No.12:63-75, Washington,
　　　　DC: Smithsonian Institution Press.
Smith, B.N., and S. Epstein
　　1971. Two categories of $^{13}C/^{12}C$ ratios for higher plants. *Plant Physiology* 47:380-
　　　　384.
Stuiver, M., and P.J. Reimer
　　1993. Extended ^{14}C database and revised CALIB radiocarbon calibration program.
　　　　Radiocarbon 35:215-230.
Stuiver, M., P.J. Reimer, and T.F. Braziunas
　　1998b. High-precision radiocarbon age calibration for terrestrial and marine samples.
　　　　Radiocarbon 40:1127-1151.
Stuiver, M., P.J. Reimer, E. Bard, J.W. Beck, G.S. Burr, K.A. Hughen, B. Kromer, F.G.
McCormac, J. v. d. Plicht, and M. Spurk
　　1998a. INTCAL98 Radiocarbon age calibration 24,000 - 0 cal BP. *Radiocarbon* 40:
　　　　1041-1083.
Toscano, M.A., and I.G. Macintyre
　　2003. Corrected Western Atlantic sea-level curve for the last 11,000 years based on
　　　　calibrated ^{14}C dates on *Acropora palmata* framework and intertidal mangrove
　　　　peat. *Coral Reefs* 22:257-270.
Westphall, M.J.
　　1986. *Anatomy and history of a ringed-reef complex, Belize, Central America.*
　　　　Masters thesis, University of Miami, Coral Gables, Florida, 135 pp.

ATOLL RESEARCH BULLETIN

NO. 511

THE PLEISTOCENE LIMESTONE FOUNDATION BELOW TWIN CAYS,
BELIZE, CENTRAL AMERICA

BY

IAN G. MACINTYRE AND MARGUERITE A. TOSCANO
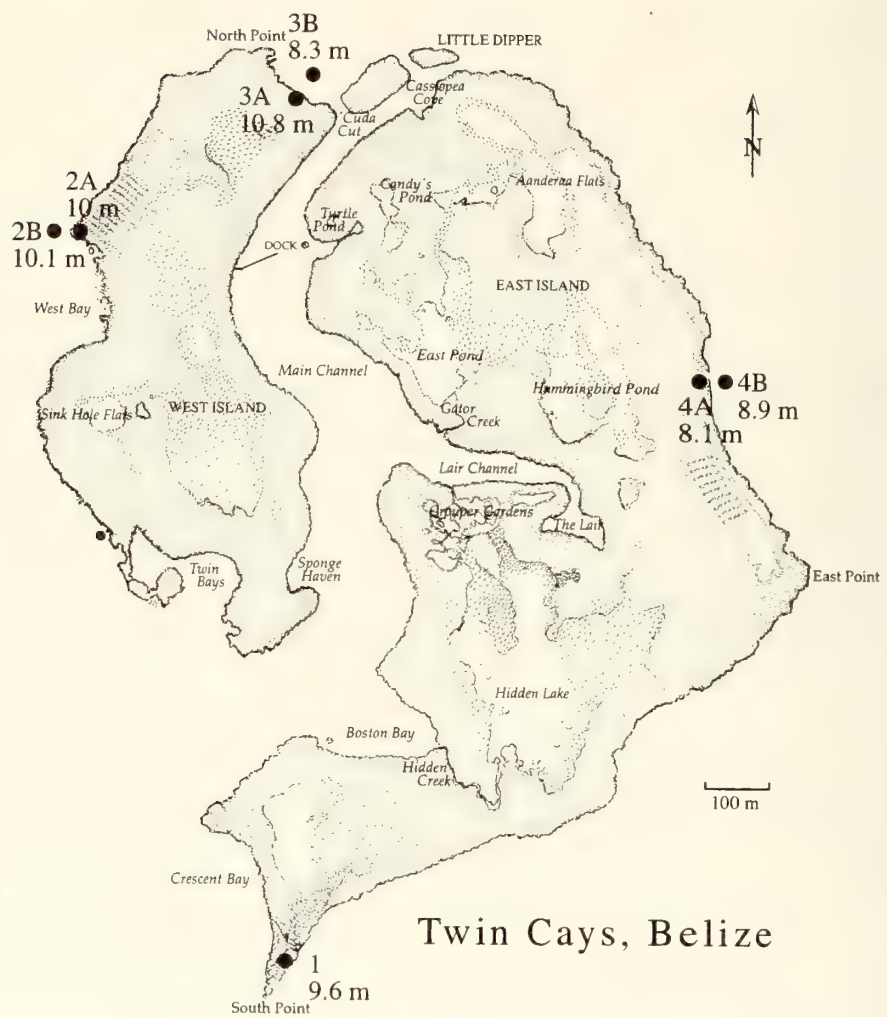
Figure 1. Location map of Twin Cays showing location of drill holes and depth of Pleistocene surface below mean sea level.

THE PLEISTOCENE LIMESTONE FOUNDATION
BELOW TWIN CAYS, BELIZE, CENTRAL AMERICA

BY

IAN G. MACINTYRE and MARGUERITE A. TOSCANO

ABSTRACT

Seven core holes drilled around the perimeter of Twin Cays, Belize, penetrated the upper 1.58 m of the Pleistocene limestone, upon which 9m of Holocene mangrove peat and lagoonal sediments have accumulated. Despite extensive subaerial alteration, the cores reveal both a branching *Porites* facies and a *Thalassia*/sediment facies. X-ray diffraction analyzes indicate that all of this limestone has altered to calcite. The overall pattern consists of a mottled chalky limestone with numerous rhizolith tubules, which are scattered throughout a dense partially leached limestone. Over 70,000 years of subaerial exposure of this limestone has resulted in an extensive diagenetic alteration assisted by roots from a terrestrial plant system.

INTRODUCTION

Twin Cays, like Tobacco Range, Belize is a mangrove cay in the south-central area of the Belize Barrier Reef Platform. Since 8000 cal years BP, nine meters of Holocene mangrove deposits accumulated in this area of relief on the underlying Pleistocene limestone substrate and kept pace with rising sea level (Toscano and Macintyre, 2003; Macintyre et al., 2004).

Geologic studies of Pleistocene units underlying the Holocene reefs and mangroves of the Belize shelf include both broad-based, regional investigations (Purdy, 1974; Tebbutt, 1975; Choi and Ginsburg, 1982; Choi and Holmes, 1982; Lara, 1993) as well as incidental data obtained during Holocene reef studies (Halley et al., 1977; Shinn et al., 1982). Extensive subaerial exposures of Pleistocene limestones occur at the northern end of the Belize barrier reef complex, particularly at Ambergris Cay (18°N; Tebbutt, 1975; Macintyre and Aronson, 1997) where the Northern Belize Lagoon is shallow (<8 m) and further north along the Yucatan Peninsula. Structural investigations (Dillon and Vedder, 1973) revealed that the Pleistocene surface dips progressively to the south. As a result, south of the Belize River (approximately 17.5°N) Pleistocene limestone exposures or islands are not found above sea level in contrast with the exposures in northern Belize and the numerous Pleistocene islands throughout the wider Caribbean.

Department of Paleobiology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560

Structural Investigations of the Belize Shelf

The Campeche Bank became submerged during the Tertiary Period as the Yucatan block tilted northward. As a result, NNE-trending normal faults paralleling the eastern coast of Belize created a series of multiple-fault block ridges that controlled the alignment of coastal and shelf features including the Belize Barrier Reef and atolls. The Ambergris Cay Shoreline trend, Turneffe-Chinchorro trend, and Glovers-Lighthouse trend (from west to east respectively) have been maintained by continued extensional faulting and subsidence during the Cenozoic (Dillon and Vedder, 1973).

Purdy (1974) used seismic profile and core data to indicate that Holocene reef distribution is controlled by the underlying Pleistocene karst erosion surface, which varies from subdued relief on the northern shelf to high relief (tower karst) on the southern shelf. Halley et al. (1977) and Shinn et al. (1982) encountered Pleistocene reef and lagoonal mudstone deposits beneath cored Holocene sections approximately 20 km south of Twin Cays at Boo Bee Patch Reef (14 to 21 m below sea level) and Carrie Bow Cay (16.2 m below sea level). Both these studies cited evidence that the Holocene reefs and associated islands were formed on top of relict Pleistocene topographic highs including fossil reefs. Choi and Ginsburg (1982) interpreted from seismic reflection profiles and cores that the Quaternary carbonate section on the shelf in the southernmost Belize Lagoon (about 50 km south of Twin Cays) appears to be founded on elevations of siliclastic fluvial-deltaic coastal plain topography incised during early Pleistocene lowstands. Choi and Holmes (1982) interpreted reef distribution patterns in the south-central Belize lagoon as having been controlled by the fluvial geomorphology. Lara (1993) recognized the imperfect association between Holocene sedimentation patterns and long-lived troughs (forming channels) and ridges (acting as templates for carbonate deposition). She suggested that much older, deeper structural trends have influenced the locations of reefs and major channels and deep water in the Belize Southern Lagoon.

Age Dating of Pleistocene Deposits

Szabo et al. (1978) dated detrital corals from upper Pleistocene beach ridge deposits at 2 m to 10 m above present sea level along the northeastern tip of the Yucatan Peninsula (Cancun and Cozumel) north of Belize. The corals were inferred to have been reworked from reef facies seaward of the beach ridges. Six mineralogically pristine samples yielded an average alpha-spectrometric age of 122,000 ± 2000 yrs, with ^{230}Th and ^{231}Pa concordancy in one of the samples. Because the elevation range of the deposits is similar to those of other late stage 5 reef complex deposits on stable margins of the Caribbean (Florida: Broecker and Thurber, 1965; Hoffmeister and Multer, 1968; Coniglio and Harrision, 1983; Bahamas: Chen et al., 1991), the Northern Yucatan Peninsula was inferred to have been tectonically stable over the last 130 kyrs.

Gischler et al. (2000) dated seven Pleistocene coral samples from the upper 5 meters of Pleistocene section from cores in the offshore, isolated carbonate platforms seaward of the Belize barrier reef at Lighthouse Reef, Turneffe Islands, and Glovers Reef. One additional sample was taken from a subaerially exposed (+1 m) nearshore reef in

northern Belize at Ambergris Cay (Ambergris Cay Shoreline trend; Dillon and Vedder, 1973). The latter was correlative, at 128.28 ka ± 1.33 kyrs, to Szabo et al.'s (1978) last interglacial dates from Cancun and Cozumel, while the deeper offshore samples ranged from 124.99 ka to 280.30 ka. In contrast to Szabo et al.'s (1978) virtually pristine aragonitic samples, only three of Gischler et al.'s (2000) seven samples contained greater than 97% aragonite. Two samples contained 86% and 89% aragonite and one contained only 5% aragonite. Recrystallization to low-Mg calcite excludes parent U from the calcite lattice thus concentrating Th and biasing the sample ages anomalously older. The sample from the +1m exposure at Ambergris Cay contained 98% aragonite. Its Substage 5e (128 ka) age was associated with a relatively reasonable initial $^{234}U/^{238}U$ activity ratio of 1.157 $^o/_{oo}$, which is just outside the accepted error range for the initial $^{234}U/^{238}U$ ratio in modern seawater (1.14-1.15 $^o/_{oo}$). Because elevated $^{234}U/^{238}U$ values are also known, like recrystallization, to be indicative of anomalously old analytical ages (Gallup et al., 1994), none of Gischler et al.'s (2000) deeper samples cored from the offshore platforms, all of which have $^{234}U/^{238}U_{init}$ values ranging from 1.157-1.71 $^o/_{oo}$, can be considered reliable age indicators of last interglacial highstand deposition. Despite these acknowledged issues with the accuracy of the dates, Gischler et al. (2000) concluded that the shallow-water limestones underlying the offshore atolls were deposited during the +6m highstand which peaked at 125 ka, and suggest that their current depth range of –4 m to –9 m is indicative of differential subsidence and variation in intensity of karst processes during the subsequent glacial period.

Sedimentary Facies Interpretations

Tebbutt (1975) described the geology, petrography and diagenesis of four facies in the exposed Pleistocene limestones on Ambergris Cay along the seaward edge of the continental shelf of northernmost Belize. Samples were obtained above sea level from discontinuous outcrops. Facies were distinguished by faunal content into reef crest (I), back reef (II), outer, middle and inner shelf (III a, b, c), and mudbank (IV) categories. The three shelf/lagoon subfacies (III a, b, c) were most extensive and consisted of mottled pelmicrites or biopelmicrites with varying amounts of coral, mollusks, *Halimeda*, and ooids. With the exception of the middle shelf zone, which consisted of ooids/ooliths in the Pleistocene, Pleistocene facies were faunally similar to analogous Holocene/modern environments in Belize, and retained some of the original aragonite and magnesium calcite skeletal mineralogy despite long-term exposure in the vadose zone. The Mudbank facies (IV), analogous to *Thalassia* beds in the modern system, may be most similar to the subsurface Pleistocene sediments encountered in this study. Halley et al. (1977) indicated that the Pleistocene mudstone found in three off-reef cores at Boo Bee Patch Reef was identical to typical Holocene lagoonal sediments of the Belizean lagoons. These mudstones showed evidence of subaerial exposure and subsequent burial by terrigenous clays (Halley et al., 1977).

Early descriptions of root casts in Quaternary limestones by Darwin (1895) preceded numerous reports of these organosedimentary structures (see Ward, 1975; Klappa, 1979; 1980; Esteban and Klappa, 1983; Bain and Foos, 1994). Northrop (1890)

4

introduced the term "rhizomorphs" to describe these calcareous root structures, which were later termed "rhizocretions" by Kindle (1923, p. 631). However, despite the many reports of calcareous root casts, very little detailed attention was paid to these important paleoecological features until Klappa (1980) introduced a new term "rhizoliths" (p. 613) and described five basic types of rhizolith structures. Bain and Foos (1994) described a range of sedimentary structures that resulted from subaerial exposure, including rhizoliths, pedotubules, and calcified root hairs in Bahamian limestones, and documented the diagenetic environment created by root penetration and water migration. We will demonstrate that the Pleistocene limestone beneath Twin Cays exhibits the basic features of rhizolith structures described by Klappa (1979; 1980) and Bain and Foos (1994). It is therefore an excellent example of extensive diagenetic alteration of a well-lithified limestone by roots of a terrestrial plant system.

METHODS

In March and April of 1981 a total of seven core holes were drilled around the perimeter of Twin Cays (Fig. 1) with a hand-operated hydraulic drill (Macintyre, 1975; 1978). One hole was drilled on South Point and two holes, one onshore and the other a short distance offshore, were drilled along the east, west and north coasts (Fig. #2, a, b). Only peat debris and molluscan shell hash were recovered above the Pleistocene surface. On encountering the Pleistocene limestone, however, the core recovery was excellent, commonly reaching close to, or 100, percent (Table 1).

Figure 2. Drilling at site of core hole 2. A) Collecting a core on land. B) Collecting a core offshore.

Table 1. Core Hole Data for Seven Drill Sites, Twin Cays, Belize.

Borehole Number and Location	Core Number	Core Interval (m)	Recovery (m)	Recovery (%)	Core Top	Pleistocene Surface Elevation (m MSL)
#1 South Point	1	0-1.5	0	0	+0.4	-9.6
	2	1.5-10.0	0	0		
	3	10-11.6	1.57	98		
#2A NW Coast	1	0-10.5	0	0	+0.5	-10.0
	2	10.5-11.7	0.9	75		
	3	11.7-12.5	0.44	41		
#2B Offshore 38m from #2A	0*	0-9.4				
	1	9.4-10.6	0.83	69	-0.7	-10.1
#3A N Coast	0*	0-11.2				
	1	11.2-12.4	0.68	57	+0.4	-10.8
#3B Offshore 46m from #3A	0*	0-7.6				
	1	7.6-8.5	0.81	90	-0.7	-8.3
#4A E Coast	0*	0-8.2				
	1	8.2-8.3	0.10	100	+0.1	-8.1
	2	8.3-8.75	0.45	100		
	3	8.75-9.0	0.19	76		
#4B Offshore 40m from #4A	0*	0-7.8				
	1	7.8-9.4	1.58	98.8	-1.1	-8.9

*Holocene peat and unconsolidated *Halimeda* sands washed from core during drilling; not recovered.

Mineralogic analyses were carried out by standard X-ray diffraction techniques (Goldsmith and Graf, 1958; Milliman, 1974), using a Sintag X-ray diffractometer with Cu K radiation, a Peltier detector, and zero-background quartz mounting plates.

A total of five petrographic thin sections were prepared to study diagenetic textures and small samples of the extensively altered patches were mounted on aluminum stubs, gold plated, and examined with a Leica-440 scanning electron microscope.

We were very interested in establishing the age of the Twin Cays Pleistocene limestone by U-Th radiometric dating. However, our X-ray diffraction analyses of well-preserved coral samples all yielded pure calcite values (Fig. 3). This complete alteration of the original aragonite in all corals left us with no valid material to date.

6
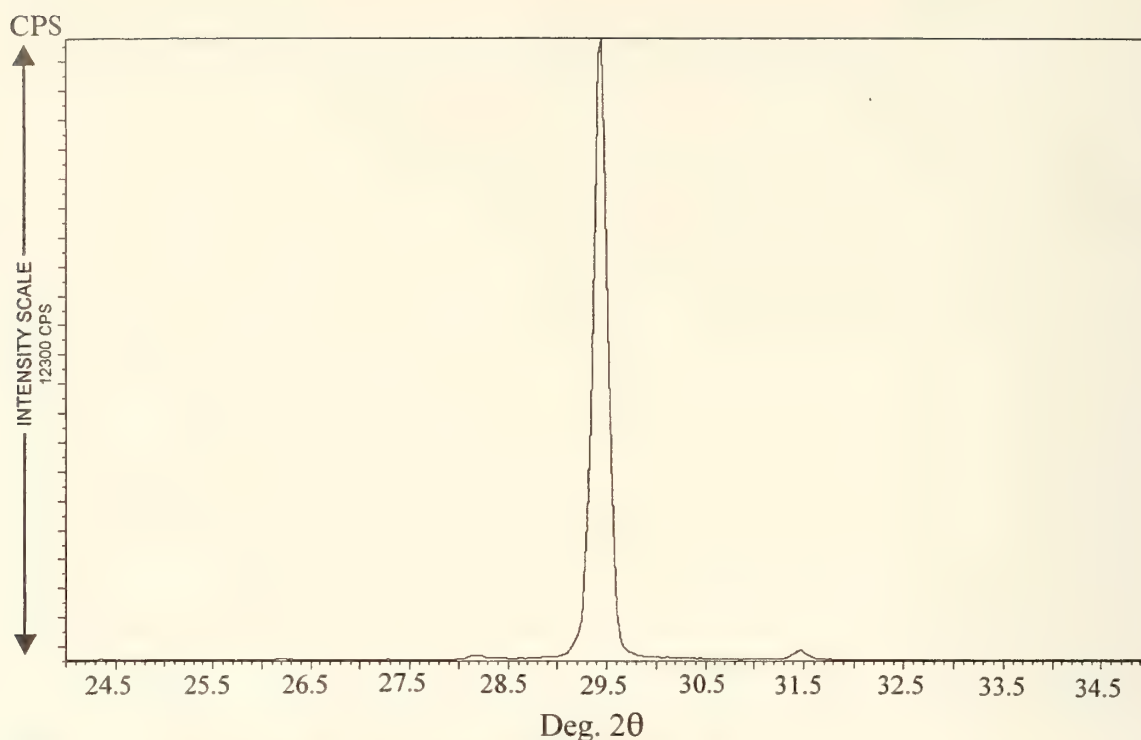
Figure 3. X-Ray diffractogram of a coral *Montastrea annularis* from core hole 2B showing complete recrystallization of biogenic aragonite to calcite. This and all other specimens analyzed were virtually completely recrystallized and unsuitable for radiometric dating.

RESULTS

Core Data

As can be seen in Figure 4, despite the high degree of diagenetic alteration with some loss of the skeletal record, there are two basic reef facies recognizable in these cores. A branching *Porites* facies consists of *Porites* branches in a microcrystalline matrix (Fig. 5). Other corals scattered throughout this facies include *Montastraea annularis* complex species, *Montastraea cavernosa,* and *Manicina areolata.* Molluscs, including both bivalves and gastropods, are common, often as leached casts as are well preserved echinoderm fragments.

The second molluscan facies consists basically of molluscan fragments, mostly bivalves but some gastropods, in various stages of preservation, in a dense microcrystalline matrix. Echinoid fragments are also well preserved in this facies. Corals scattered throughout this facies include *Manicina areolata* and some *Porites astreoides* (Fig. 6).

Figure 4. Map of Twin Cays core sites with core logs. Depths above cores in meters indicate depth of top of Pleistocene below mean sea level. Depths in cores marked in cm are depths below the top of the Pleistocene section. Darker lines mark core intervals.

8
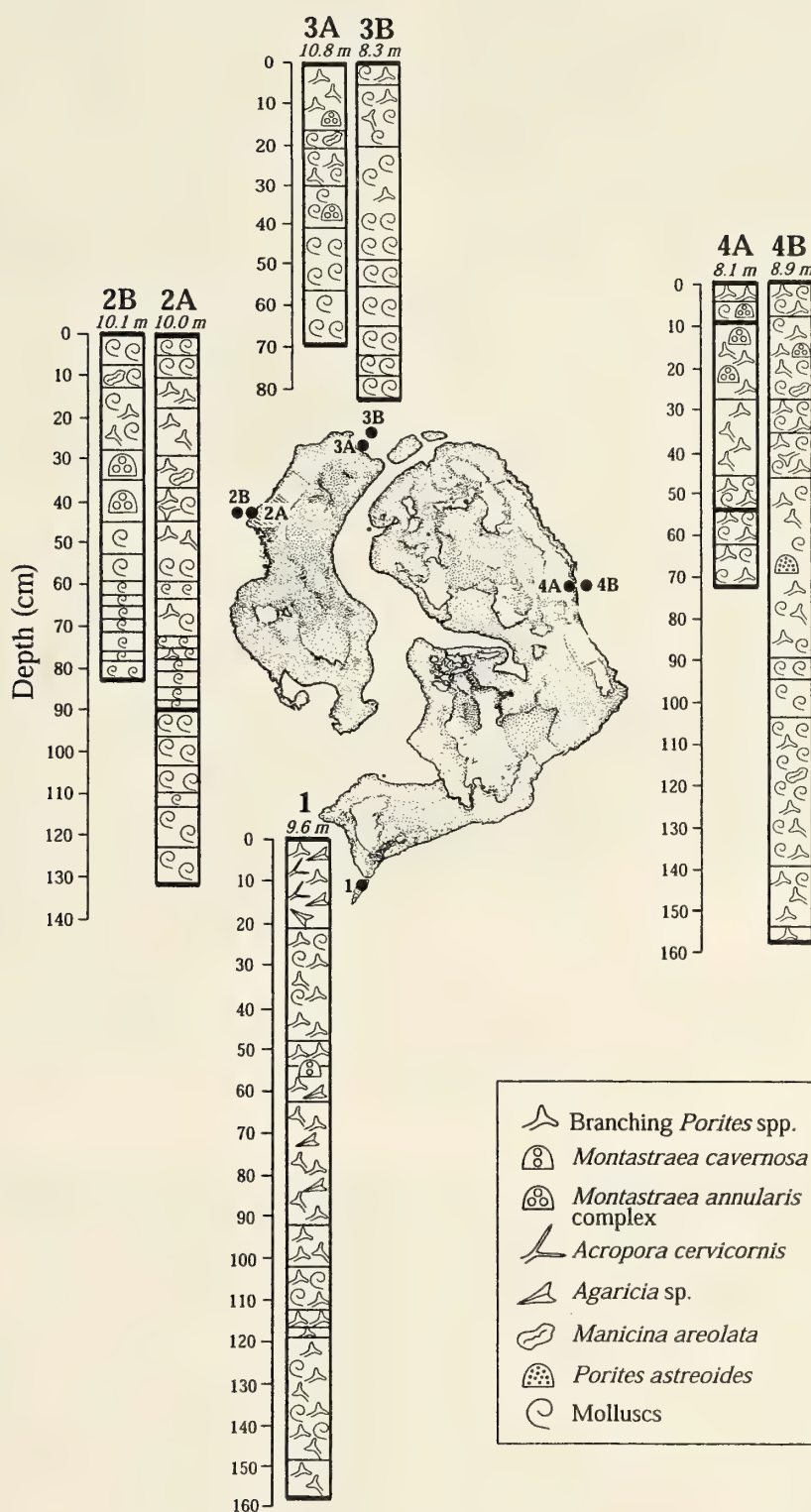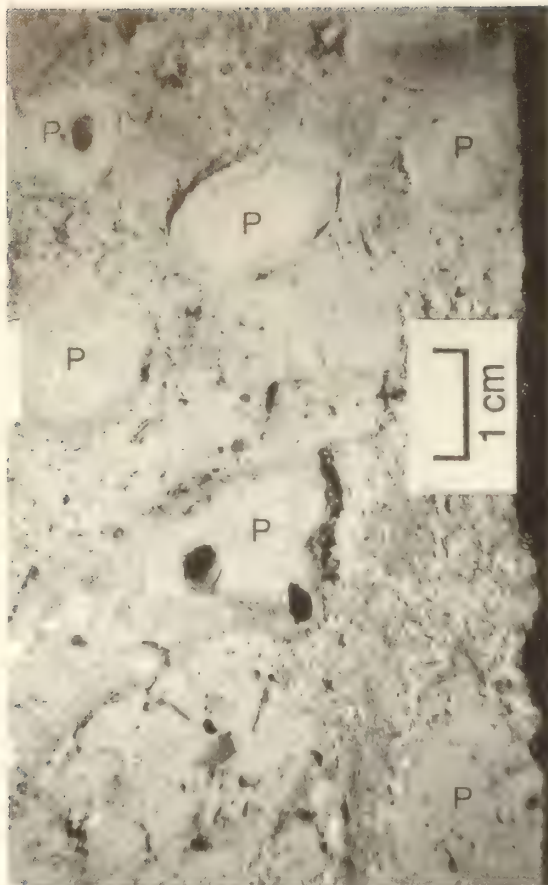
Figure 5. Core photo showing *Porites* (each marked with "P") in muddy matrix.
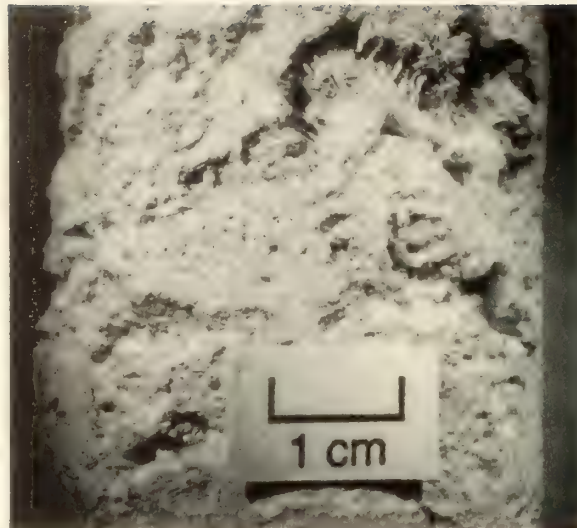
Figure 6. Core photo showing leached and recrystallized *Manicina areolata* in muddy matrix.

All of the cored samples collected in this study have been extensively altered in a subaerial environment. Most samples exhibit a mottled pattern of dense light gray microcrystalline limestone with random patches of chalky light brown-to-buff micrite containing a network of open unoriented tubules. These tubules range in diameter from 0.5 mm to 0.25 (Fig. 7) and are the only readily visible evidence of organic remains in these highly altered chalky patches. Coral, molluscan, and echinoid skeletons were found in various degrees of leaching in the dense grey areas. This extensive alteration, including the chalky patches, extends down to the base of the longest core of 1.58 m collected in Core Hole 4B (Figs. 1, 4).
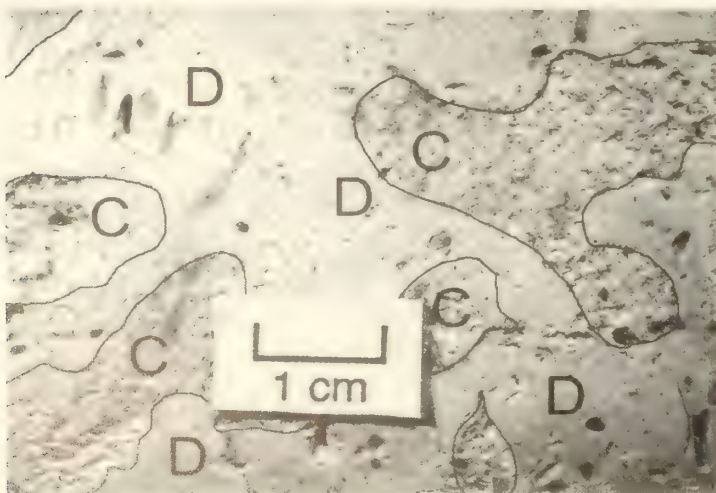
Figure 7. Close-up of core showing contact between dense (D) light gray microcrystalline limestone and chalky (C) light brown-to-buff micrite with unoriented tubules.

Thin Section Analysis

In the thin sections, the chalky areas consist of clotted microcrystalline (30 ηm to 4 ηm) to submicrocrystalline (< 4 mm) calcite with numerous 0.5 mm to 0.25 mm diameter tubules. Micro-tubules 5 ηm to 10 ηm in diameter radiate around the larger tubules (Fig. 8). Most of the skeletal grains in this very porous chalky material are leached out to form molds or are extensively recrystallized to blocky calcite crystals. Recognizable skeletal grains include coral, benthic foraminifera, molluscs, *Halimeda*, echinoid spines and worm tubes. Original Mg-calcite skeletal material such as echinoid spines and benthic foraminifera are generally well preserved. The contact between the chalky tubule calcite and the dense calcite is usually gradual, commonly leaving isolated remnants of the dense calcite surrounded by chalky calcite (Fig. 9). The tubules are also found penetrating the dense calcite and skeletal fragments, such as corals, at these contacts.
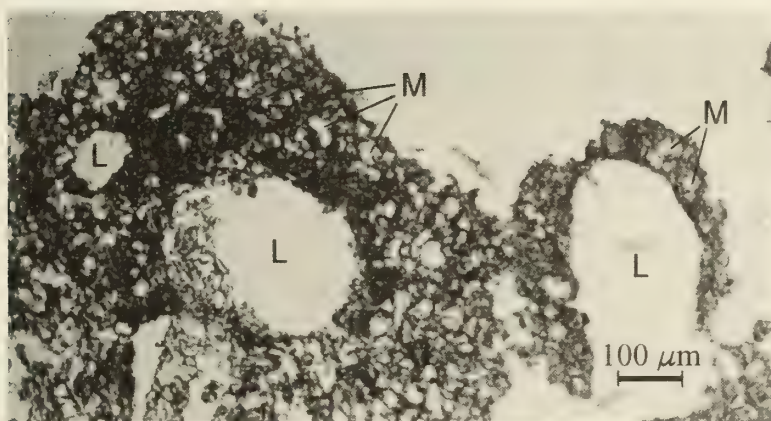
Figure 8. Thin section of core showing clotted microcrystalline to submicrocrystalline chalky calcite with tubules. Micro-tubules (M) radiate from larger tubules (L).

In the dense calcite the skeletal fragments are generally better preserved but there is still extensive leaching of this skeletal material, which results in scattered porosity and fossil molds (Fig. 10). Recognizable skeletal material is identical to that found in the chalky areas with the original Mg-calcite skeletons being the best preserved (Fig. 9).
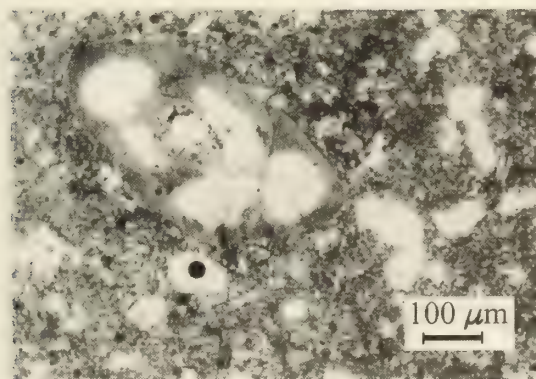
Figure 9. Thin section of core showing gradational contacts between chalky and dense limestone with preserved high-Mg calcite skeletal material (foraminifera).
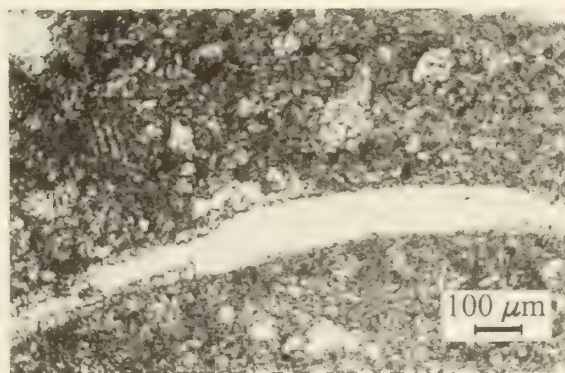
Figure 10. Cross section/mold of leached pelecypod shell in dense limestone.

Scanning Electron Microscopy

Scanning electron microscope (SEM) observations reveal that the chalky calcite crystals range in size from > 4 ηm to 50 ηm (Fig. 11 a, b). These crystals are most commonly anhedral micrite but, in some places, well developed platy calcite (Fig. 11a; Klappa, 1980) and random needle crystals (Fig. 11b; Supko, 1970) can be observed.
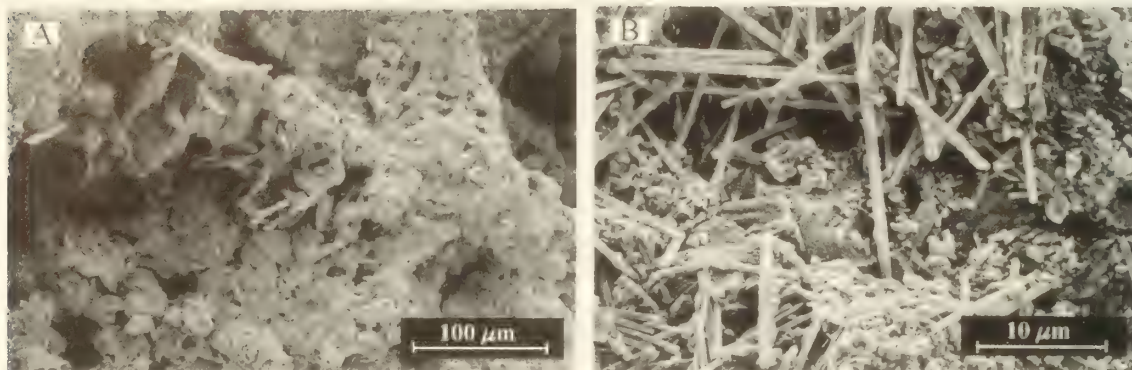
Figure 11. A) SEM image of chalky area showing well-formed platy calcite crystals. B) SEM image of chalky area showing random needle or whisker calcite crystals.

The diameters of the associated tubules vary widely from 5 ηm to 0.5 mm. As seen in thin sections, microtubule casts and molds are closely associated with the larger tubules (Fig. 12). In addition, these tubules commonly have a smooth lining or a secondary rim of dentate calcite crystals, or can be almost completely filled with euhedral crystals. As can be seen in Figure 13, tubules are sometimes found preserved within tubules. The tubules show no preferred orientation and penetrate the chalky calcite in winding random pathways. At contact with the dense calcite areas there is distinct decrease in the density of tubules but some do penetrate the dense areas (Fig. 14). In some cases calcified plant remains, consisting of a root epidermis with radiating root hairs, were found preserved within tubules (Fig. 15).
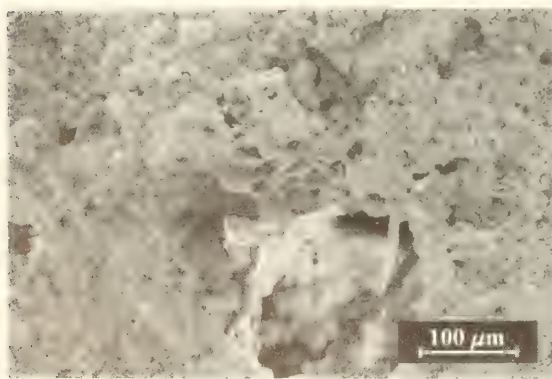
Figure 12. SEM image showing microtubules associated with a larger tubule in a chalky area.
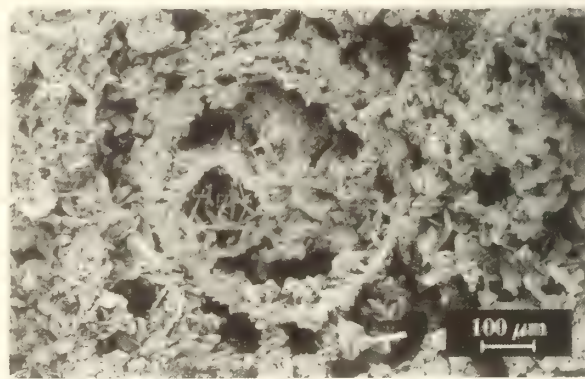
Figure 13. SEM image showing a cross section of a tubule, in which smaller tubules and needle calcite crystals are preserved. Platy calcite crystals surround the large tubule.
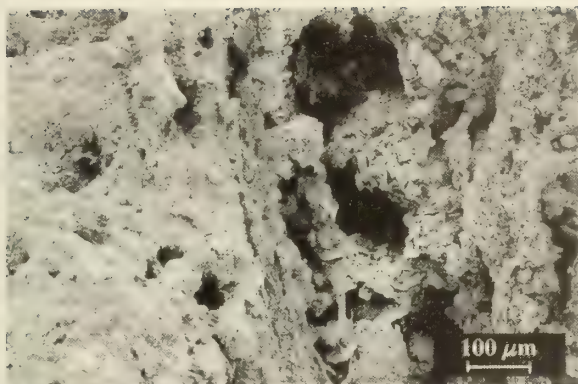
Figure 14. SEM image showing an area of dense limestone adjacent to an area of chalky calcite. The dense limestone contains fewer tubules than the chalky limestone.
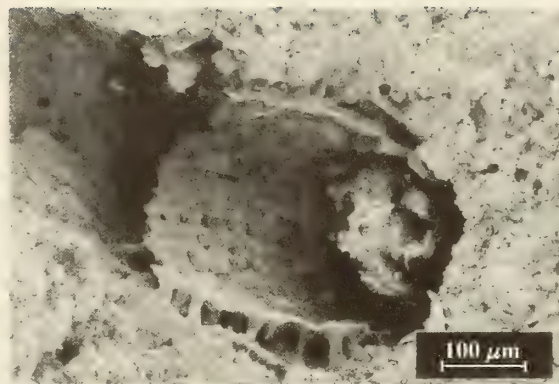
Figure 15. SEM image showing a calcified root epidermis with radiating root hairs.

DISCUSSION

The two reef facies (Fig. 4) consisting of branching *Porites* and scattered molluscs, both in a matrix of fine skeletal material in microcrystalline to submicrocrystalline calcite, are readably distinguishable despite the extensive subaerial recrystallization of this limestone. The branching *Porites* facies is characteristic of lagoonal environments and is well illustrated by the extensive accumulations of branching *Porites* in sandy mud and muddy sand in the Bahia Almirante off the Caribbean coast of Panama (Aronson et al. in prep). The "molluscs in muddy sand with occasional corals", particularly *Manicina areolata,* represent the *Thalassia* community that is still common to the Belize lagoons (e.g. Macintyre et al., 2000). Thus although this Pleistocene limestone has been greatly altered and much of the original texture lost in our cores, it appears that the Holocene mangroves of Twin Cays became established on a Pleistocene substrate formed by lagoonal accumulations of branching *Porites* mounds and *Thalassia* beds, when this substrate was flooded by the Holocene Transgression.

The most striking diagenetic feature in the alteration of this Pleistocene limestone is the widespread mottled chalkification (Constanz, 1989; Fig. 7) associated with a complex tubule pattern (Fig. 12). This appears to be related to the boring activity of a terrestrial plant community. The gradual contact between dense and chalky patches, which appeared to have similar lithology and tubule borings in dense areas, indicates a post-lithification alteration by a penetrating root system that has resulted in extreme recrystallization forming porous chalky patches in an original dense lagoonal limestone.

This Twin Cays altered limestone is an excellent example of what Klappa (1980, p. 613) termed "Rhizoliths — organosedimentary structures resulting in the preservation of roots of higher plants, or remains there of, in mineral matter." This is supported by the presence in this limestone of most of the characteristics that Klappa associates with rhizoliths including root molds, root petrifactions such as calcified epidermis, root tubules around root molds, and encrusted root tubules.

What is so impressive in the Twin Cays limestone is the extensive "rhizomicritization" (Klappa, 1980, p. 628) where solution and reprecipitation have converted a once dense lagoonal limestone into a porous chalky micrite with abundant root and rootlet molds and casts. This limestone, which had been subaerially exposed for a period of 70,000 years or more, was affected by root penetration to at least one and a half meters (our maximum core penetration). Root systems produced such extensive diagenetic alteration that no original Mg-calcite or aragonite remains.

Tebbutt (1975) reported a very similar tubule texture in the Pleistocene limestone of Ambergris Cay in northern Belize. He described this texture as a "puzzling vesicular structure" (p. 311). Interestingly, this texture was most pronounced in his lagoonal "Mudbank" facies, which is very similar to our lagoonal facies. He did not recognize these structures as rhizoliths and suggested that the tubules were primary features "incorporated in the mud" (p. 311) and goes on to speculate on a crustose coralline algal or encrusting foraminifera origin. Only a short distance north, Ward (1975) described rhizoliths, including root-hair sheaths (p. 520), in the carbonate eolianites of northeastern Yucatan Peninsula. Bain and Foos (1994) described the features of vegetative alteration of subaerially exposed late Pleistocene dune, beach and subtidal carbonates on San Salvador Island, Bahamas. Rootlet penetration into the Bahamian limestones resulted in anastomosing pedotubules, alveolar texture consisting of rootlet pores separated by thin micrite walls, and calcified root hairs ($20\mu m$ diameter).

The greatest challenge in fully documenting the geologic history of this deposit is the lack of mineralogically pristine coralline material for high precision U-Th dating. Existing age data from other studies in Belize (Gischler et al., 2000) are problematic and unreliable. Without unequivocal dates, we cannot determine the definitive age of the deposit, the sea-level elevation during its deposition, or provide tectonic subsidence calculations. However, another possibility may be suggested if we assume minimal subsidence similar to the Bahamas and Florida (Toscano and Lundberg, 1999). Using the depth of the top of the deposit (-8 or more meters) and its location well offshore of potential 125 ka-aged deposits (expected to be exposed onshore, above present sea level), we can suggest a correlation to mineralogically pristine Pleistocene reef deposits from –9 to –21 m at the edge of the Florida Keys shelf. Corals forming these reefs were U-Th dated by Toscano and Lundberg (1999) and represent the final reef deposits of the last interglacial period (substage 5b and 5a, 95-79 kyrs BP). These reefs accreted well offshore of the 125 ka Florida Keys as a result of lowered sea levels (-7 m maximum) from 95-80 ka. The maximum sea-level estimate of –7 to –9 m is constrained by the elevation of concurrent speleothem (cave flowstone) growth in the Bahamas (Li et al., 1989). The undated Pleistocene shelf facies underlying Holocene mangroves of Twin Cays occurs over the same depth range as the Florida reef deposits and may have been deposited during the final substage of the last interglacial period, then subaerially exposed when sea level fell after 79 ka (Toscano and Lundberg, 1999), assuming no major subsidence. The deposits dated by Gischler et al. (2000) may also be correlative to latest stage 5 and a lower than present sea level.

CONCLUSIONS

1) The mangrove islands of Twin Cays were established on a lagoonal Pleistocene limestone consisting of branching *Porites* mounds and *Thalassia* beds.

2) This limestone has been totally altered to calcite with no evidence of original aragonite or Mg-calcite. As a result, the age of this limestone could not be reliably established by high-precision U-Th dating techniques. Based on previous work in Florida and the Bahamas, this deposit may best correlate with the outlier reefs of 95-79 ka age (Toscano and Lundberg, 1999; Li et al., 1989), which occur at similar water depths. This correlation may also be applicable to Gischler et al.'s (2000) samples from the offshore atolls, barring any major Pleistocene shelf faulting or significant subsidence.

3) The extensive subaerial diagenetic alteration of this limestone for a period of 70 kyrs by the penetration of terrestrial plant roots has resulted in widespread chalkification and resultant loss of original petrographic characteristics.

4) The presence of root molds, casts, tubules, and petrifications along with extensive chalky micritization in this altered limestone classifies it as an ideal example of rhizoliths.

ACKNOWLEDGEMENTS

REFERENCES

Bain, R., and A.M. Foos
  1994. Carbonate microfabrics related to subaerial exposure and paleosol formation. Pages 19-27 in R. Rezak and D.L. Lavoie (eds). *Carbonate Microfabrics*. Springer-Verlag.
Broecker, W.S., and D.L. Thurber
  1965. Uranium-series dating of corals and oolites from Bahamian and Florida Key limestones. *Science* 149:58-60.
Chen, J.H., H.A. Curran, B. White, and G.J. Wasserburg
  1991. Precise chronology of the last interglacial period: ^{234}U-^{230}Th data from fossil coral reefs in the Bahamas. *Geological Society of America Bulletin* 103:82-97.

14

Choi, D.R., and R.N. Ginsburg
    1982. Siliclastic foundations of Quaternary reefs in the southernmost Belize Lagoon,
        British Honduras. *Geological Society of America Bulletin* 93:116-126.
Choi, D.R., and C.W. Holmes
    1982. Foundations of Quaternary reefs in south-central Belize Lagoon, Central
        America. *American Association of Petroleum Geologists Bulletin* 66:2663-
        2681.
Coniglio, M., and R.S. Harrision
    1983. Facies and diagenesis of late Pleistocene carbonates from Big Pine Key,
        Florida. *Bulletin of Canadian Petroleum Geology* 31:135-147.
Constanz, B.R.
    1989. The primary surface area of corals and variations in their susceptibility
        to diagenesis. Pages 53-75 in J.H. Schroeder and B.H. Purser (eds). *Reef
        Diagenesis*. Springer-Verlag, Berlin.
Darwin, C.
    1844. *Geologic observations on the volcanic islands and parts of South American
        visited during the voyage of HMS Beagle. New York,* D. Appleton, 648 pp.
Dillon, W.P., and J.G. Vedder
    1973. Structure and development of the continental margin of British Honduras.
        Geological Society of America Bulletin 84:2713-2732.
Esteban, M., and C.F. Klappa
    1983. Subaerial exposure. Pages 1-96 in P. A. Scholle, D. Bebout, and C.H. Moore
        (eds). *Carbonate Depositional Environments*. American Association of
        Petroleum Geologists Memoir 153.
Gallup, C.D., R.L. Edwards, and R.G. Johnson
    1994. The timing of high sea levels over the past 200,000 years. *Science* 263:796-
        800.
Gischler, E., A.J. Lomando, J.H. Hudson, and C.W. Holmes
    2000. Last interglacial reef growth beneath Belize barrier and isolated platform reefs.
        Geology 28:387-390.
Goldsmith, J.R., and D.F. Graf
    1958. Relation between lattice constants and composition of the Ca-Mg carbonates.
        American Mineralogist 43:84-101.
Halley, R.B., E.A. Shinn, J.H. Hudson, and B. Lidz
    1977. Recent and relict topography of Boo Bee Patch Reef, Belize. Proceedings,
        Third International Coral Reef Symposium Miami 2 (Geology):29-35.
Hoffmeister, J.E., and H.G. Multer
    1968. Geology and origin of the Florida Keys. *Geological Society of America
        Bulletin* 79:1487-1502.
Kindle. E.M.
    1923. Range and distribution of certain types of Canadian Pleistocene concretions.
        Geological Society of America Bulletin 34:609-648.

Klappa, C.F.
  1979. Calcified filaments in Quaternary calcretes: organo-mineral interactions in the
        subaerial vadose environment. *Journal of Sedimentary Petrology* 49:955-968.
Klappa, C.F.
  1980. Rhizoliths in terrestrial carbonates: Classification, recognition, genesis, and
        significance. *Sedimentology* 27:613-629.
Lara, M.E.
  1993. Divergent wrench faulting in the Belize Southern Lagoon: Implications
        for Tertiary Caribbean plate movements and Quaternary reef distribution.
        American Association of Petroleum Geologists Bulletin 77:1041-1063.
Li, W.-X., J. Lundberg, A.P. Dickin, D.C. Ford, H.P. Schwarcz, R. McNutt, and
D. Williams
  1989. High-precision mass spectrometric uranium-series dating of cave deposits and
        implications for paleoclimate studies. *Nature* 339:534-536.
Macintyre, I.G.
  1975. A diver-operated hydraulic drill for coring submerged substrates. *Atoll
        Research Bulletin* 185:21-26.
Macintyre, I.G.
  1978. A hand-operated submersible drill for coring reef substrata. Pages 75-80 in
        D.R. Stoddart and R.E. Johannes (eds). *Coral Reefs: Research Methods.*
        UNESCO Monographs on Oceanographic Methodology No. 5, Paris.
Macintyre, I.G. and R.B. Aronson
  1997. Field Guide to the Reefs of Belize. *8th International Coral Reef Symposium* 1:
        203-222, Panama City, Panama.
Macintyre, I.G., I. Goodbody, K. Rutzler, D.S. Littler, and M.M. Littler
  2000. A general biological and geological survey of the rims of ponds in the major
        mangrove islands of the Pelican Cays, Belize. Pages 13-34 (no. 467) in I.G.
        Macintyre and K. Rutzler (eds). *Natural History of the Pelican Cays, Belize.*
        Atoll Research Bulletin.
Macintyre I.G., M.A. Toscano and R.G. Lighty
  2004. Holocene history of the mangrove islands of Twin Cays, Belize, Central
        America. *Atoll Research Bulletin* 510:1-16.
Milliman, J.D.
  1974. *Marine Carbonates.* New York, Springer Verlag, 375p.
Northrop, J.I.
  1890. Notes on the Geology of the Bahamas. *Transactions, New York Academy of
        Sciences* 10:4-22.
Purdy, E.G.
  1974. Karst-determined facies patterns in British Honduras: Holocene carbonate
        sedimentation model. *American Association of Petroleum Geologists Bulletin*
        58:825-855.

16

Shinn, E.A., J.H. Hudson, R.B. Halley, B. Lidz, D.M. Robbin, and I.G. Macintyre
    1989. Geology and sediment accumulation rates at Carrie Bow Cay, Belize. Pages 63-
        75 in K. Rutzler and I.G. Macintyre (eds.). *The Atlantic barrier reef ecosystem*
        at Carrie Bow Cay, Belize 1, Structure and Communities. Smithsonian
        Contributions to Marine Sciences 12.

Supko, P.R.
    1970. Whisker crystal cement in a Bahamian rock. Pages 143-149 in O.P. Bricker
        (ed), *Carbonate Cements*. Johns Hopkins University Press, Baltimore.

Szabo, B.J., W.C. Ward, A.E. Weidie, and M.J. Brady
    1978. Age and magnitude of the late Pleistocene sea-level rise on the eastern Yucatan
        Peninsula. *Geology* 6:713-715.

Tebbutt, G.E.
    1975. Paleoecology and diagenesis of Pleistocene limestone on Ambergris Cay,
        Belize. Pages 297-331 in K. F. Wantland and W.C. Pusey (eds.). *Belize shelf*
        – carbonate sediments, clastic sediments, and ecology. American Association of
        Petroleum Geologists Studies in Geology 2.

Toscano, M.A., and J. Lundberg
    1999. Submerged Late Pleistocene reefs on the tectonically-stable southeast Florida
        margin: High-precision geochronology, stratigraphy, resolution of Substage 5a
        sea-level elevation, and orbital forcing. *Quaternary Science Reviews* 18:753-
        767.

Toscano, M.A., and I.G. Macintyre
    2003. Corrected western Atlantic sea-level curve for the last 11,000 years based
        on calibrated ^{14}C dates from *Acropora palmata* framework and intertidal
        mangrove peat. *Coral Reefs* 22:257-270.

Ward, W.C.
    1975. Petrologic diagenesis of eolianites of northeastern Yucatan Peninsula, Mexico.
        Pages 500-571 in K. F. Wantland and W.C. Pusey (eds.). Belize shelf
        – carbonate sediments, clastic sediments, and ecology. *American Association of*
        Petroleum Geologists Studies in Geology 2.

THE AQUATIC ENVIRONMENT OF TWIN CAYS, BELIZE

BY

KLAUS RÜTZLER, IVAN GOODBODY, M. CRISTINA DIAZ,
ILKA C. FELLER, AND IAN G. MACINTYRE

Red mangrove *(Rhizophora mangle)* hanging roots are the primary solid substrata along mangrove channels at Twin Cays and their vertical orientation assures optimal protection from silting. Sponges and ascidians make up most of the biomass of epibionts. Sponges shown include *Lissodendoryx* cf. *isodictyalis* (bluish gray), *Tedania ignis* (orange massive), *Scopalina ruetzleri* (orange encrusting), and *Haliclona manglaris* (turquoise); the ascidians are *Distaplia corolla* (deep orange knobby structures), which also overgrow sponges. (Photo, L. M-Penland).

THE AQUATIC ENVIRONMENT OF TWIN CAYS, BELIZE

BY

KLAUS RÜTZLER[1], IVAN GOODBODY[2], M. CRISTINA DIAZ[3], ILKA C. FELLER[4], AND IAN G. MACINTYRE[5]

ABSTRACT

The rich fauna and flora of Twin Cays off southern Belize were explored and compared with coral and turtle-grass habitats of the surrounding Belize lagoon and the nearby barrier reef. Among the many subtidal habitats found in these cays, some 20 stations were routinely sampled to study the composition of plankton and benthos, sediment and peat bottoms, and to investigate the parameters that determine distribution. The work also focused on distribution patterns, animal behavior, and community development over geological time scales. Each station is examined for its particular properties, including topography, substratum types, environmental parameters, and predominant organisms and communities, particularly the sessile benthos.

INTRODUCTION

Twin Cays off the southern coast of Belize are so named because a wide tidal channel splits the roughly oval mangrove island into two unequal parts. These islands are the closest mangrove development to Carrie Bow Cay and the Smithsonian's Carrie Bow Marine Field Station, less than 4 km to the northwest. They are among hundreds of mangrove cays perched on the leeward top of the Belize barrier reef platform (Plate 1a). We call this type of tidal forest "island mangrove," to distinguish it from "mainland mangroves," which fringe the continental shores, including the tidal mouths of freshwater rivers and creeks (Rützler and Feller, 1996) and are awash by full-oceanic-salinity seawater. By contrast, mainland mangroves are subjected to a salinity gradient ranging from freshwater to fully oceanic, caused by coastal runoff. The absence of freshwater at Twin Cays' marine environment (except during periods of heavy rains), combined with

[1] Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560-0163.

[2] Department of Life Sciences, University of the West Indies, P.O. Box 12, Kingston 7, Jamaica.

[3] Museo Marino de Margarita, Boca del Rio, Peninsula de Macanao, Nueva Esparta, Venezuela.

[4] Smithsonian Environmental Research Center, Smithsonian Institution, Edgewater, MD 21037

[5] Department of Paleobiology, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560-0125.

the area's small tidal range accounts for the extensive and diverse development of marine habitats in these islands.

Twin Cays (16° 49' 48" N, 88° 06' 11" W; at their center) are part of the Stann Creek District and are situated 15.5 km due 81° (ENE) of Sittee Point, the nearest mainland, and 20 km due 142° (SE) from Dangriga, the nearest town. The islands crown the N-S directed barrier-reef carbonate platform which at that location is 8 km wide and 0-7 m (average 2.5 m) deep. To the west of Twin Cays, the platform extends for about 5 km where its depth drops rapidly to about 20 m, the average for the trough-like main lagoon, including the Inner Channel that is a protected shipping lane for large vessels. The lagoon has a sediment bottom covered mainly by seagrass, *Thalassia testudinum*. About 2 km to the east of Twin Cays, the reef platform is delineated by the intertidal barrier-reef-crest. From there, the reef slopes rapidly, within a distance of 400 m, toward the continental dropoff (Rützler and Macintyre, 1982).

Barring unusual currents and storms, the lagoon water west of Twin Cays is influenced by mainland runoffs; the water east of the islands has open-ocean bluewater characteristics that are enhanced by a series of three 600 to 800 m wide and 5 to 8 m deep breaks through the barrier reef, South Water, Carrie Bow, and Curlew cuts. Within the perimeter of the cays, topography, water depth, extent and kind of vegetation, and seasonal and meteorological events determine habitat- and water quality parameters, including substratum composition and inclination, suspended and settled sediments, water level and flow velocity, temperature, salinity, turbidity, dissolved substances, and nutrients.

In the following sections we summarize and illustrate the habitat characteristics of a number of tidal and subtidal locations throughout Twin Cays that served as sites for collecting, observation, illustration, and experimentation of sessile organisms for many researchers over the years since 1984 (see also, Calder, 1991a, b; Diaz et al., 2004; Goodbody, 2004a; Littler and Littler, 2000; Littler et al., 2004; Ott and Bright, 2004; Parrish and Ryan, 2004; Richardson, 2004; Winston, 2004; Wulff, 2004).

STUDY AREA AND METHODS

Twin Cays actually consist of four fully established mangrove islands. To identify the various topographic features, they were labeled by newly invented place names (no traditional local designations existed) suggested by participants of the Smithsonian Caribbean Coral Reef Ecosystems (CCRE) program (Fig. 1). The largest cay is East Island; it measures 52.1 ha in area, is 1400 m in maximum length, and 674 m at its widest point, just south of its center. East Island is nearly cut in half by the west-east oriented, 16 to 58 m wide Lair Channel, separating a northern and southern portion. Both include in-shore ponds, lakes, and tidal mudflats. The northern section contains Candy's, East, and Hummingbird ponds and Aanderaa Flats; the southern part is distinguished by Hidden Lake and Boa Flats. West Island, the second largest cay and nearly a smaller mirror image of the former, extends over 21.5 ha and measures 895 x 377 m. It is separated from East Island by the s-shaped Main Channel which tapers from 136 m width at the south entrance into Twin Cays to 8 m at the northern exit. West Island includes the tidal West

Figure 1. Map of Twin Cays based on aerial photographs taken in 2002, with station numbers (grid coordinates used in this survey (map, M. K. Ryan).

Pond and Sinkhole Flats; its northward extension is formed by two islets, Big Dipper (0.5 ha, 132 x 64 m) and Little Dipper (0.2 ha, 65 x 39 m). Several smaller islets, or, rather, isolated stands of mangrove trees, developed and vanished in different places over the past 20 years of our study, for instance, the one just outside Turtle Cove (Figs. 1, 7).

The total shoreline of Twin Cays (excluding interior ponds, lakes, and mudflats) is 8.1 km long, more than half of that made up by the inshore mangrove fringe, the rest

directly exposed to the open Belize lagoon. The tide is micro-tidal and of mixed semidiurnal type, with a mean range of 15 cm (Kjerve et al., 1982); maximum fluctuations have a range of 40 cm. Water surface temperature (lagoon at Carrie Bow Cay) averages 25° C in February, 30° C in August, with recorded extremes of 23.5°–31.5°; air temperature minima and maxima differ only slightly, 22.5°–34.5° C (Koltes et al. 1998). Predominant wind direction during October through February is from the northwest, the rest of the year it is northeast to east. Monthly preciptation is lowest between March and May (0–25 mm), highest between June and November (100–480 mm) (Rützler and Ferraris, 1982; Koltes et al., 1998). Salinity in the open lagoon is fully oceanic, 35–36‰. Reactive silica content of interstitial water from sediments collected off Twin Cays (South Point) reaches 1.2–1.5 mg SiO_2/l (as compared with 0.3–0.4 mg around Carrie Bow Cay), with terrigenous particulate silica (87–92%) dominating the sediment fine fraction, ≤0.25 mm (0% at Carrie Bow) (Rützler & Macintyre, 1978).

Station numbers were assigned from an arbitrary grid (mesh-size ca. 135 m) superimposed on a 1985 version of a Twin Cays map (Fig. 1). Large-scale measurements and water-depth values outside Twin Cays were taken from nautical chart 28167 (U. S. Defense Mapping Agency, Hydrographic/Topographic Center, Washington, DC; 1984 edition). Topographic measurements at Twin Cays were based on a map from aerial and satellites images (Rodriguez and Feller, 2004).

DESCRIPTION OF STATIONS

These descriptions are presented in sequence from north to south and west to east. Station numbers are based on number-letter combinations from the aforementioned grid (Twin Cay locations are covered by the ranges 4–14 and E–M). Estimates of prevailing environmental conditions are expressed as follows: L=light exposure; C=current flow (+ is strong, ± moderate, – low); T=temperature and S=salinity. These latter parameters are not mentioned if they are normal (close to open-lagoon water conditions) but are listed as "stressful range" (with measured range values where available) if they vary beyond the fluctuations of the open lagoon.

Sta. 4 I, Little Dipper (Figs. 1, 2; Plate 1b)

Location and Topography. South shore of the islet, facing Main Channel.
Habitats. Mixture of free hanging roots and roots embedded in substratum. Sandy bottom with turtle grass *(Thalassia testudinum).*
Depth. 1.5 m.
Environmental Conditions. L: +; C: +.
Sediment. Fine-grained sand.
Communities. Algae *(Caulerpa, Halimeda, Dictyota)* and a modest number of sponges on roots *(Lissodendorys* cf. *isodictyalis, Tedania ignis)* and peat bank *(Haliclona mucofibrosa).*

Figure 2. Aerial view of Twin Cays looking southeast. Little Dipper (sta. 4I), Big Dipper, and 'Cuda Cut (5Ha) (left to right) are in the foreground, Cassiopea Cove (5I) is seen directly behind Big Dipper; Batfish Point South (5Hb) is in the Main Channel, turning right after passing 'Cuda Cut (photo, D. Littler).

Figure 3. Low-altitude aerial view of 'Cuda Cut and Batfish Point (background) (sta. 5Ha; photo, D. Littler).

Sta. 5 Ha, Batfish Point—'Cuda Cut (Figs. 1, 3, 4; Plates 1, 2a)

Location and Topography. The north coast of West Island, from its northeast corner (Batfish Point) to North Bay, opposite Big Dipper (the passage between the two
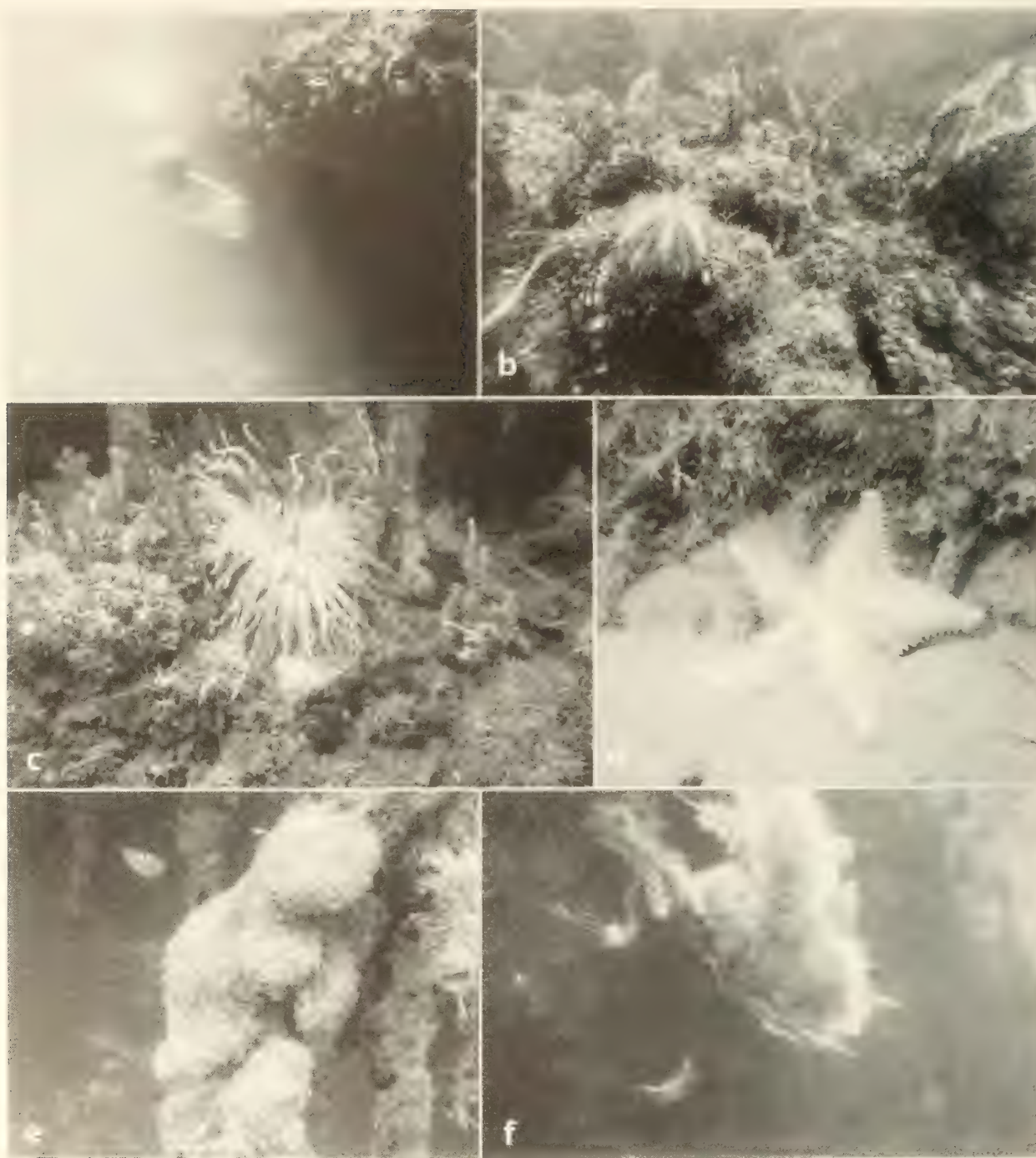
Figure 4. Underwater views of habitats of Batfish Point–'Cuda Cut: *a,* diver pushing plankton net along peat-bank undercut; *b,* peat bank shoulder covered by *Halimeda* algae, with sea urchin *(Lytechinus)* in foreground (photo, C. Clark); *c,* peat bank wall with *Halimeda* and sea anemone, *Condylactis; d,* starfish *Oreaster* at base of peat bank; *e,* mangrove *(Rhizophora)* root covered by filamentous cyanobacteria *(Lyngbya); f,* detached cyanobacteria forming floats, lifted to the surface by trapped photosynthetic air bubbles.

Islands; 'Cuda Cut, was named for its abundance of schooling barracudas). Outward (north) of North Bay, the water is very shallow (<0.5 m) because of sand deposits covered by turtle grass.

Habitats. The southern shore of the passage has an intertidal peat bank protruding over a curved or nearly vertical peat wall, 3 m tall. The wall is recessed 1–3 m from the distal edge of the bank, thus creating an extensive cave habitat. Red mangrove is anchored on top of the bank, its roots protruding here and there from the otherwise smooth or somewhat pitted, compact peat; stilt roots hanging over edge of the bank.

Depth. 3–4 m.

Environmental Conditions. L: + to –; C: +.

Sediment. Sand with mollusk fragments; very fine sand and soft, carbonate mud toward the center of the channels, extensively worked by crustacean burrowers.

Communities. Peat banks are covered by tufts of algae and hydroids, coralline algae, calcified green alga *(Halimeda),* and a number of encrusting or cushion-shaped sponges *(Scopalina ruetzleri, Spongia tubulosa)* and ascidians *(Diplosoma* sp.) and populated by seaurchins *(Lytechinus variegatus).* Sea anemones *(Condylactis gigantea, Bartholomea annulata)* and sabellid polychaetes are anchored in fissures and depressions of the peat wall. The most common encrusting sponges on the dark cave wall and ceiling peat substrata are the orange *Scopalina ruetzleri* and the yellow *Amorphinopsis* sp. and *Clathrina* cf. *coriacea.* Other abundant species in this habitat are *Chondrilla nucula, Clathria venosa, Mycale citrina, M. microsigmatosa,* and *Haliclona mucofibrosa,* and sheet-like forms of ascidians, including *Diplosoma listerianum, D. glandulosum, Lissoclinum abdominale* and *Didemnum conchyliatum.* Crisp white patches of sulfur-fixing bacteria *(Beggiatoa)* are conspicuous on the peat wall near the muddy bottom. Ascidians with a higher profile tend to be confined to hanging roots, as are fleshy macro-algae, and massive sponges such as *Lissodendoryx* cf. *isodictyalis, Mycale laxissima* (also large-growing and common on fully light-exposed peat walls), and *Tedania.* Many of the light-exposed sponges are covered (as observed in August 2004) by rusty-red veils of cyanobacteria *(Schizotrix* spp.). The sediment bottom near the banks is barren except for sporadic algae and seagrass but the Main Channel floor off Batfish Point is covered by stands of *Thalassia* seagrass, and *Halimeda* and *Caulerpa* algae interspersed with characteristic mounds and holes indicating the presence of burrowing decapods *(Glypturus, Alpheus).* Numerous specimens of the benthic jellyfish *(Cassiopea xamancha)* and of the starfish *Oreaster reticulatus* are also regularly encountered. Exiting north into and beyond North Bay one commonly encounters an encrusting black sponge *(Artemisina melana)* covering coral rubble and dead gorgonians.

Sta. 5 Hb Batfish Point South (Figs. 1, 2; Plate 2b,c)

Location and Topography. Main Channel west, northeast shore of West Island just south of Batfish Point.

Habitats. Intertidal, root-consolidated peat-bank with long adventitious red-mangrove roots covered mainly by sponges hanging in front of the bank. About 20 m toward the south, the peat bank decreases in height to about 0.5 m.

Depth. 1–3.5 m.

Environmental Conditions. L: + to –; C: + (wind-generated water flow through the cuts to the open lagoon).

Sediment. Sand with mollusk fragments and *Halimeda* chips.

Communities. Encrusting or low-growing algae, sponges, and ascidians on the bank, like at 'Cuda Cut (Sta. H 5A); ascidian numbers decrease with diminishing waterflow toward the south. Seagrass *(Thalassia)* appears where the current through the cuts slows and sediment accumulates on the bottom. Opposite across the channel (East Island), cool water entering through 'Cuda Cut stimulates a rich community of algae, sponges *(Mycale microsigmatosa, Lissodendoryx, Tedania),* and colonial ascidians *(Didemnum* spp., *Botrylloides nigrum).*

Sta. 5 I, Cassiopea Cove (Figs. 1, 2; Plate 2d)

Location and Topography. North end of Main Channel, a small bay on East Cay, opposite Big Dipper islet.

Habitats. Soft sedimentary bottom with *Thalassia* seagrass. Mangrove stilt-roots along the shore.

Depth. 0.5–2 m.

Environmental Conditions. L: +; C: +.

Sediment. Fine-grained sand and carbonate mud. Median grain diameter of the sand fraction has a range of 94–268 μm, the mud fraction (<63 μm) is 17–33% and the organic content 5.4–9.7% (Dworschak and Ott, 1993). The mud fraction tends to become resuspended during high winds, causing high turbidity.

Communities. Large population of the benthic upside-down jellyfish *Cassiopea xamancha.* Sediment mounds and funnel-shaped holes among stands of algae *(Penicillus, Halimeda)* indicate the presence of burrowing decapods *(Alpheus* spp., *Glypturus acanthochirus).* Stilt or hanging prop roots are covered by algae and a few ascidians *(Perophora)* but much of the epifauna tends to become smothered by whitish flock from resuspended and resettling fine sediment.

Sta. 5 K, East Island, Northeast Coast (Figs. 1, 5)

Location and Topography. Northernmost part of the highly exposed outer shoreline that faces northeast.

Habitats. Mangrove fringe with *Rhizophora* stilt roots hanging freely, or (further to the southeast) firmly embedded in sandy substratum. About 30-40 m offshore, a shallow sandbank covered by turtle grass *(Thalassia)* parallels the coast and separates it from the extended turtle grass meadows of the lagoon.

Depth. 0.5-1 m

Environmental Conditions. L: +; C: +.

Sediment. Medium to coarse sand with ample *Halimeda* chips.

Communities. Algal turf (bostrychietum) and a few ascidians *(Didemnum)* on roots; *Halimeda* algae and *Thalassia* seagrass covering the bottom.
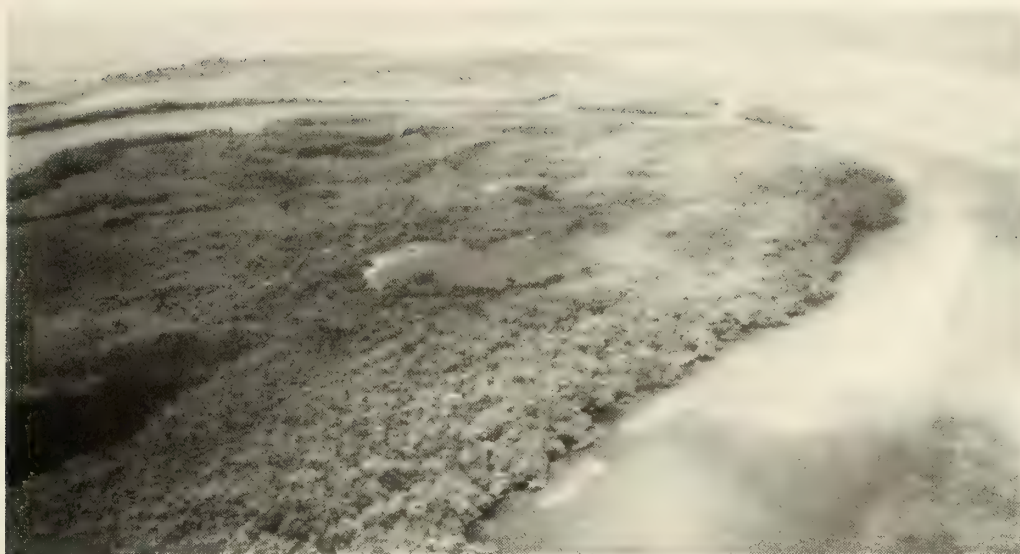
Figure 5. Low-altitude aerial photograph of Northeast coast looking northwest (photo D. Littler).

Sta. 6 G, Main Channel Northwest (Figs. 1, 6, 7)

Location and Topography. Western shore of the Main Channel, along the coast of West Island between the Dock and Batfish Point.

Habitats. Steep and strongly eroded peat bank (1.5 m tall), with overhanging red-mangrove roots of low density protruding in 2–3 m distance from the bank. *Thalassia* seagrass sparsely covering the bottom of the channel which at this location is 55 m wide, 1.5–2 m deep.

Depth. 2–3 m.

Environmental Conditions. L: +; C: + (wind-generated waterflow through the cuts to the open lagoon).

Sediment. Abundance of fine sediments that tend to be resuspended by heavy boat traffic through the channel; it covers most substrata and ebibionts.

Communities. Algal fuzz composed of calcareous red algae *(Jania* cf. *adhaerens)* and cyanobacteria *(Lyngbya, Schizothrix* spp.), fleshy green algae *(Caulerpa verticillata)* and red algae *(Acanthophora spicifera),* sponges *(Lissodendoryx, Tedania, Scopalina ruetzleri, Haliclona implexiformis, H. manglaris, Spongia tubifera),* and a few ascidians *(Didemnum* spp.) on the roots. Sponges are partly covered by rusty red cyanobacteria *(Schizothrix* spp.) and leafy greens (cf. *Anodyomene).* On the peat bank, one finds among other sponges the encrusting *Clathria venosa;* the sea urchin *Lytechinus variegatus* is abundant. Near the Dock there is an abundance of solitary ascidians *(Phallusia nigra, Microcosmus exasperatus),* which are rare elsewhere at Twin Cays.

Swimming across the channel one encounters large mounds caused by burrowing crustaceans, and *Cassiopea* jellyfish among the turtlegrass. The opposite bank looks very much like its western counterpart but the roots are closer to shore, algae are more common than sponges, and there is more deposited fine sediment. Algae include cyanobacterial mats, *Caulerpa,* and *Halimeda.* Most sponges belong to *Lissodendoryx,*

Figure 6. Mangrove roots at Main Channel Northwest: *a,* root covered by sponges, purple *Haliclona implexiformis* (tubes) and orange *Scopalina ruetzler* (encrusting); *b,* mangrove oysters, *Isognomon alatus.*

Tedania, and *Hyrtios;* they alternate with clusters of mangrove oysters *(Isognomon alatus)* and alga-like fuzz of the bryozoan *Zoobotryon verticillatum* (Winston, 2004). Because a counter-current creates pockets of calm water, there are accumulations of light-green floats of cyanobacterial mats composed mainly of filamentous *Lyngbya* sp.

Sta. 6 H, Turtle Cove and Turtle Pond (Figs. 1, 7; Plate 2e)

Location and Topography. A deeply cut bay 30 m wide and 45 m long in the northwest shoreline of East Island. Its entrance from the Main Channel is partly blocked by a small mangrove patch that developed on a sandbank during the past 15 years (25 m diameter in 2003). About 100 m south of the entrance, sand deposits account for a very shallow shoreline covered by seagrass and lined by long hanging roots. The cove extends toward the northeast through a narrow, shallow channel (2–3 m wide, 31 m long), which ends in a deeper (2 m) pond, 16 x 12 m in diameters. Garbage dumping in Turtle Pond has interrupted research at this location for several years.
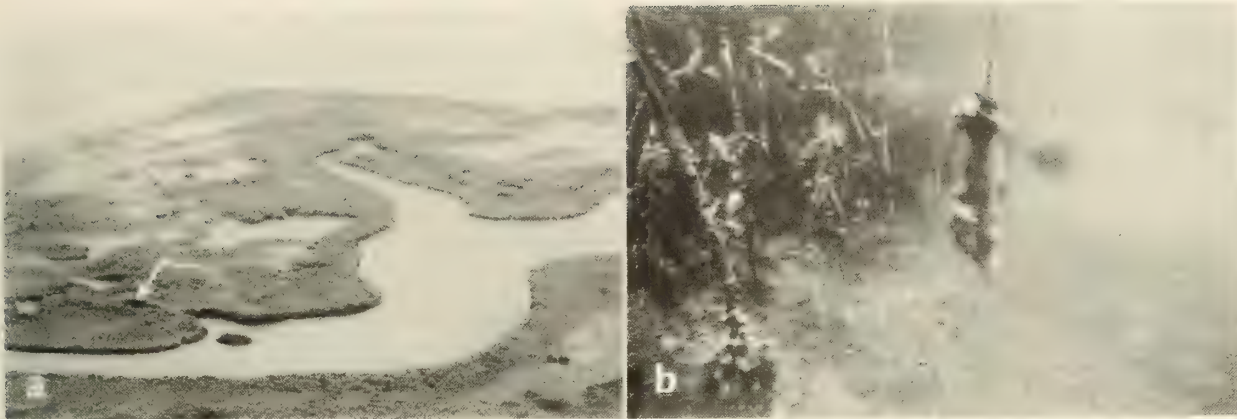
Figure 7. Habitats near the northern Main Channel: a, aerial photograph looking southeast (across Main Channel Northwest, 6G), onto Turtle Cove and Turtle Pond (6H, arrow), which are connected by Turtle Creek; Candy's Pond (6I) is to the far left; b, underwater view of Turtle Creek (photo, M. Carpenter).

Habitats. Dense bed of seagrass *(Thalassia)* at the entrance to the cove; otherwise muddy bottom. Ample free-hanging *Rhizophora* roots from overhanging trees that line cove, channel, and pond. (Roots at this location were measured to grow 0.2–0.4 mm per day.)

Depth. 1–2 m.

Environmental Conditions. L: –; C: –; T, S: stressful range (connection with nearby inshore Candy's Pond).

Sediment. Fine, organics-rich mud.

Communities. Intertidal parts of the roots are covered by algal fuzz and clusters of mangrove oysters *(Isognomon alatus).* Subtidal zones support brown cyanobacteria, algae *(Ulva* sp., *Caulerpa racemosa),* small sponges *(Haliclona implexiformis, Biemna* sp.) and, toward the tips of roots, an abundance of ascidians *(Perophora regina, Didemnum conchyliatum, Eudistoma olivaceum)* competing with serpulid worms and a few sponges *(Haliclona curacaoensis).* Owing to current patterns and the deposit of suspended sediments and other matter (such as loose seagrass leaves left from manatee feeding), roots on the south flank of the cove are less densely colonized by epibionts than the north side. Long-bladed *Thalassia* seagrass predominates the shallow bank just south of Turtle Pond where hanging mangrove roots have a poorly developed epibiont community with a few sponges and anemones. Patches of lower intertidal peat banks are covered by *Halimeda* algae.

Sta. 6 I, Candy's Pond (Figs. 1, 7; Plate 2f)

Location and Topography. An inshore pond on East Island, 65 m from the Main Channel, accessible from Turtle Pond through a narrow, mangrove-overgrown canal, 1.5 m wide, 0.5 m deep, and 45 m long. This is actually a double pond, two equal-sized lagoons of about 200 m² area joined by a narrow neck of water. Only the northern pond is being considered here, which is elongate, 50 m long, and 8–30 m wide.

Habitats. Rhizophora roots. Muddy bottom of decaying plant materials.

Depth. 1–1.5 m.

Environmental Conditions. L: +; C: −. T, S: stressful range; 34°, 37‰ (highest values recorded, May, 1985). Strongly impacted by heavy rains and periods of evaporation over the surrounding swamp.

Sediment. Very loose organic flock, up to 1 m deep.

Communities. Abundance of ascidian *Eudistoma olivaceum,* known for its high stress tolerance, and *Didemnum.* Sponges are rare (mainly *Haliclona implexiformis).* Small sabellid polychaetes *(Bispira melanostigma),* hydroids (cf. *Myrionema),* and a bryozoan *(Bowerbankia,* which always seems to grow near the water surface) are fairly common. The only fishes observed were mangrove snapper and small barracuda. Bacterial mats cover areas of the muddy bottom.

Sta. 7 E, West Bay (Figs. 1, 2, 8; Plate 3c)

Location and Topography. A recess of the outer (western, lagoon-ward) shoreline of West Island. The bay measures about 175 by 45 m, with a ragged shoreline.

Habitats. Rhizophora mangrove lines the fringe, with stout roots firmly anchored in coarse sand along the shallow (0.5 m) coastal zone of West Island. The bay bottom is made up by a variety of coral rock rock, shell (bivalve, conch shells), and sand covered by calcareous and fleshy algae and patches of turtle grass. A barren sandy apron extends from close to shore several hundred meters outward into the lagoon at the southern part of the bay.

Depth. 0.2–3.5 m.

Environmental Conditions. L: +; C: +.

Sediment. Poorly sorted but mainly coarse sand and gravel, patches of medium fine sand.

Communities. The fauna and flora of West Bay are rich, possibly benefiting from nutrient input from the adjacent mangrove, lack of very fine sediments, and a moderate temperature-salinity regime. Mangrove roots support crustose coralline and *Halimeda* algae, algal turfs, and a reef-like fauna of a few sponges *(Clathria venosa, Mycale laevis, Niphates* sp.), coral *(Porites porites and P. atroides, Millepora complanata),* and serpulid worms. The bay floor is covered by a variety of algae *(Halimeda, Penicillus, Rhipocephalus, Caulerpa), Thalassia* and *Halodule* seagrass, clusters of bivalves and coral *(Manicina areolata)* with stands of octocoral, and numerous sea urchins *(Lytechinus variegatus, Clypeaster rosaceus),* and the occasional batfish *(Ogcoephalus).* Cushion-shaped and branching sponges occur on rock (e.g., *Amphimedon viridis, A. compressa, Niphates erecta),* ascidians are rather rare except for a few small patches on seagrass blades (didemnids, *Ecteinascidia minuta)* and a small burrowing form buried around *Thalassia* roots *(Pyura munita).*

Sta. 7 G, Main Channel West, the Dock (Figs. 1, 9)

Location and Topography. West coast of Main Channel (central eastern shore of West Island). The Dock itself, a wooden structure, was built by CCRE Program participants to facilitate access to the swamps of West Island; its submerged pilings are fouled mainly by algae and sponges and are used as support for various experimental setups (settling frames, tide and temperature probes).

Figure 8. Habitats at West Bay: *a*, sieving sand samples in front of the mangrove fringe bordering the bay; *b*, Turtle grass surrounding sand patches with unattached solitary coral, *Manicina;* b, coral *(Porites)* and hydrocoral *(Millepora)* growing on *Rhizophora* stilt roots that are firmly embedded in sand.

Habitats. Dense seagrass, *Thalassia,* comes up to the fringe where there is a low peat bank and roots are covered by sponges. Fallen trees and driftwood are stranded in places and entangled among the prop roots.

Depth. 0.5–1 m.

Environmental Conditions. L: ±; C: ±.

Sediment. Carbonate mud with patches of *Halimeda* chips.

Communities. Fleshy and calcareous algae *(Caulerpa, Halimeda, Jania),* sponges *(Lissodendoryx* cf. *isodictyalis, Chondrilla nucula, Clathria venosa, Tedania ignis, Clathrina* cf. *coriacea),* and mangrove oysters *(Isognomon)* cover the roots. Juvenile fishes are common in protected areas where floating *Sargassum* seaweed is often trapped, particularly in spring. *Thalassia* seagrass covers the bottom.

14

Figure 9. Main Channel West, the Dock: *a,* floating *Sargassum* seaweed entangled among *Rhizophora* roots (photo, M. Carpenter); *b,* tall *Thalassia* seagrass populated by epibionts (photo, L. M-Penland).

Figure 10. View over 'Gator Creek near its entrance into East Pond.

Sta. 8 I, 'Gator Creek (Figs. 1, 10; Plates 3d–f, 4a)

Location and Topography. East Island, starting at the northern flank of the entrance to Lair Channel, and meandering north to East Pond.

Habitats. Rhizophora mangrove roots, peat bank, mud bottom covered by decaying mangrove litter.

Depth. 0.5 m (entrance) 1.5 –3 m.

Environmental Conditions. L: –; C: +. T, S: stressful range, due to tidal drainage of water (heated-hypersaline, or cooled-brackish, depending on the weather) derived from the large, shallow East Pond.

Sediment. Organic mud; patches of *Halimeda* chips.

Communities. The Lair channel bottom slopes up to the creek's entrance where *Thallassia* seagrass becomes dense in 0.5 m depth and interspersed with some algae, mainly *Caulerpa racemosa.* Mollusk and polychaete egg cases crowd the muddy areas. Near the mouth of the creek along the north-shore of Lair Channel, there are clusters of mangrove oyster *(Isognomon)* on the roots, as well as ascidians *Eudistoma olivaceum* and *Didemnum conchyliatum,* the latter a hardy species tolerant of a wide range of environmental extreme (see Goodbody, 2004a) and often found on root tips and at the leaf bases of *Thalassia* seagrass. Algae *(Caulerpa)* and big sponges grow on the mangrove roots flanking the entrance *(Tedania, Lissodendoryx).*

The channel is rich in cyanobacterial mats and tufts, algae *(Halimeda, Caulerpa),* sponges, hydroids, anemones *(Aiptasia tagetes, Condylactis gigantea),* and juvenile fishes hiding among the epibionts. Common sponges on mangrove roots and peat bank include *Lissodendoryx, Biemna, Tedania, Tethya* cf. *actinia, Cinachyrella apion* (mainly on peat walls), *Dysidea etheria, Dysidea* sp. (a large, undescribed bluish gray form also found in Hidden Creek), *Haliclona implexiformis, H. curacaoensis, H. manglaris, H. twincayensis, H. tubifera, H. magnifica* (on peat walls), and *Chalinula molitba.* Sponges are also found loose among the mangrove litter of the channel floor *(Lissodendoryx, Biemna),* together with low-growing algae *(Avrainvillea),* or buried in organic sediment flock (*H. magnifica*). Bryozoans *(Zoobotryon verticillatum, Amathia vidovici)* and small colonies of ascidians *(Eudistoma olivaceum)* are co-occurring with sponges on roots and along the peat wall lining the creek. A conspicuous population of a telestinid gorgonian with brilliant white polyps *(Carijoa riisei),* and two color morphs of *Tedanis ignis* (red-orange and orange-red) with opposite color-morph *Parazoanthus swiftii* (orange-red and red orange) covering their surfaces were observed near the outer one-third distance into the creek in 1984. Four years later (1988), these organisms had disappeared, except for a few small colonies of the telestid, which reappeared in larger numbers by 1992 but were not noted during a survey in 2004. The *Tedania-Parazoanthus* population was never seen again since the original observation.

Sta. 9 I Grouper Gardens (Figs. 1, 11; Plate 4)

Location and Topography. A cluster of at least six interconnected ponds just south of the Lair Channel, which can be entered through a north-south-directed creek located 100 m to the east of the Main Channel. The Lair channel bottom outside Grouper Gardens is 2 m deep and sparsely covered by turtle grass. It slopes up to 0.5 m toward the

16

Figure 11. Grouper Gardens, underwater view along a connecting channel between two ponds, with free-hanging, heavily colonized mangrove roots.

creek where *Thalassia* becomes tall and dense. There are patches of *Halimeda* sand and healthy populations of this alga crowd the nearby *Rhizophora* roots. The creek at its entrance is relatively deep (3 m), but quickly shallows where it connects to the first pond (0.5 m). Most ponds and passages between them are 0.3 m or less deep and difficult to explore by swimming.

Habitats. Vertical peat walls and overreaching hanging stilt roots, soft muddy bottom with seagrass and, particularly in the remote ponds, thick stands of seaweed *(Avrainvillea).*

Depth. 0.1–3 m.

Environmental Conditions. L: +; C: ±.

Sediment. Fine sand with *Halimeda* chips; peat and detritic mud cover the bottoms of ponds and connecting canals.

Communities. The mangrove roots outside the entrance to Grouper Gardens are covered mainly by *Halimeda opuntia triloba.* Just inside, the roots and peat walls are overgrown by sponges *(Tedania, Lissodendoryx, Haliclona implexiformis, H. tubifera, Spongia tubulifera, Calyx podatypa, Geodia papyracea),* more *Halimeda* algae, algal turfs (including intertidal bostrychietum), hydroids, anemones *(Bartholomea annulata, Condylactis gigantea, Aiptasia tagetes),* and a few colonies of the ascidian *Eudistoma olivaceum.* Extensive reddish veils covering epibions and streamer-like strands (up to 2 m long) formed by cyanobacteria *(Lyngbya* spp.) were noted during the month of August (2004).The astrophorid sponge *Geodia papyracea* there has periodically been overcome by stress-related disease involving its own cyanobacterial symbionts (Rützler, 1988). The bottom is covered by stands of turtle grass, *Thalassia,* and patches of *Halimeda* and other algae. The encrusting orange ascidian *Didemnum conchyliatum* is common and often attached to seagrass blades. Short, 1 m deep tidal channels connect the ponds, which average 0.5 m in depth. Mangrove roots in the current flow here support clusters of mangrove oysters *(Isognomon),* a diverse population of sponges *(Spongia tubulifera,*

Haliclona curacaoensis, H. manglaris, Biemna caribaea, Dysidea etheria, Clathrina cf. *coriacea),* hydroids, and algae *(Caulerpa verticillata).* The bottom of the shallow ponds consists mostly by stands of tall green algae *(Avrainvillea longicaulis* f. *laxa)* and supports populations of *Cassiopea* jellyfish and *Tridachia* nudibranchs. Some sponges *(Haliclona spp., Lissodendoryx)* that for some reason had lost their solid root substrate are surviving despite being buried in deep detritus.

Sta. 9 J, Lair Channel (Figs. 1, 12; Plates 4a, 5a–c)

Location and Topography. Second-largest channel (after the Main Channel, from where it originates) cutting west–east into East Island and blind-ending in a pond, known as The Lair. The channel is about 260 m long, widest at its mouth (56 m), and gradually narrows to 14 m (neck of the Lair channel), where it makes a sharp turn to the south and, after 40 m more, opens into a terminal pond, The Lair. Along the flanks of the Lair's neck are extensive peat-bank undercuts, dark cave-like features suggesting that the Lair was once connected to the open lagoon and that water run more briskly through the channel than now, eroding its banks. The undercuts occur along both the west and east banks of the Lair Channel neck, undercutting the root-peat bank horizontally to 0.5–3. Along the western shore the caves are deepest where the Lair neck first turns south, at the east bank they are best developed near the entrance point into The Lair proper, further supporting the idea that they were washed out by current rather than other mechanisms of erosion. Cave ceiling clearance at the entrance of undercuts is 0.4–1.4 m, tapering to 0.3–0.8 m near the rear peat wall.

Habitats. Fringe of hanging *Rhizophora* roots, particularly along the south (west) shore, muddy bottom with sparse seagrass and macrophytes restricted to shallow (<1m) areas and patches of coarse *Halimeda*-chip sand. *Rhizophora*-root reinforced peat undercuts and caves with sediment-free walls and ceiling and detritus-rich, pudding-like mud bottom. The center channel bottom is bare mud with *Cassiopaea* jellyfish, no seagrass.

Depth. 0.8–1.9 m (fringe); to 2–3 m (channel bottom).

Environmental Conditions. L: + to –; C: ± to –. T, S: stressful range, due to tidal drainage of water from surrounding shallow ponds and flats. Particularly affected are sessile communities (algae, sponges, ascidians) on shallow, bank-side roots because water flowing from the flats may differ by several degrees temperature and per-mille salinity from the channel ambient conditions (total range measured, 27°–36° C and 30–34‰ , in May 1985, February and April 1990, and April 1991).

Sediment. Calcareous and detrital mud stabilized by mucuous components; patches of fine sand and *Halimeda* chips. Suspended fine sediments, mainly detritus, were observed to flow out of the creek at ebb tide (February, 1988).

Communities. Thick sponge clusters on roots at both sides of the channel *(Lissodendoryx, Tedania, Spongia tubulifera, Hyrtios proteus, Amorphinopsis* sp., *Biemna caribea, Haliclona curacaoensis, H. manglaris, H. implexiformis, Mycale* spp.). Also, bunches of green algae *(Halimeda, Caulerpa)* and alga-like soft bryozoans *(Zoobotryon),* and ascidians *(Perophora bermudensis)* grow hanging into the water flow.

The light-exposed peat banks are covered by algae *(Halimeda, Caulerpa, Anodyamene),* sponges *(Lissodendoryx, Amorphinopsis, Biemna, Spongia obtusa),*

18

cyanobacterial webs *(Lyngbya)*, and anemones *(Aiptasia, Bartholomea)*. Colorful small to medium-large sponge crusts or cushions populate the dark cave- or overhang-ceilings of the Lair Channel neck where there is no competition from algae (yolk yellow *Biemna* and *Amorphinopsis*, red *Tedania*, greenish *Haliclona manglaris*, pink *H. implexiformis*, sulfur-yellow *Clathrina)*, along with the white calcareous tubes of serpulid polychaetes, patches of the soft filmy cream-colored ascidian *Diplosoma*, and bright-white accumulations of bacteria, *Beggiatoa*.

Here and there, the mud bottom is covered by cyanobacterial mats (see Joye & Lee, 2004) but otherwise is bare except for jellyfish *(Cassiopea)*, mollusk egg cases, and a few protruding sponge fistules (for instance *Lissodendoryx)*; these sponges seem to have fallen off stilt roots that decayed after death of the tree or from borer's action (Kohlmeyer et al., 1995) and survive on and in the mud substratum. Turtle grass occurs only in small patches the shallow fringe areas and is often populated by anemones *(Aiptasia)*.

Near drainage points of interior swamp water into the Lair Channel, large clusters of mangrove oyster, *Isognomon*, appear as well as dense populations of the ascidians *Perophora* spp., suggesting that nutrient-rich water and particulate matter become available; swarms of the mysid *Mysidium columbiae* are also seen concentrated near these drainage locations. Another ascidian common in this community is *Distaplia corolla*, which settles preferentially on the shaded side of free-hanging roots but was all but eliminated (at least to a depth of 0.3 m below low-tide level) during a massive mortality event in early 1986, possibly by unusually warm, hyposaline water draining off the swamp, or by exposure at a very low tide.

Extensive microbial mats harboring a rich community of dinoflagellates, protozoans, and small invertebrates develop in protected (calm-water) locations and may become dislodged by the buoyancy of photosynthetic oxygen bubbles and float to the surface ("floating muck") until oxygen is consumed or dissipated and causes the mat to sink back to the bottom (Faust and Gulledge, 1996). Dense growth of *Thalassia* seagrass covers the light exposed parts of the channel bottom west of the Lair neck. The turtle grass blades are extensively colonized by different color morphs of the ascidian *Diplosoma glandulosum*.

Sta. 9 K, The Lair (Figs. 1, 13; Plate 4a)

Location and Topography. This terminal pond of Lair Channel is elongate and oriented parallel to the channel, but perpendicular to the orientation of the neck that make a sharp bend to the south before entering. It is 100 m long, 12 m wide at its west end, 45 m at its east end.

Habitats. Rhizophora mangrove roots along the margins. Soft, flocculent detritus bottom.

Figure 12 (opposite). Lair Channel: *a*, view along the channel from one of its taller surrounding trees; *b*, cave-like peat undercut at the western flank of the channel's neck; *c*, seagrass along the shallow fringe populated by sea anemones *(Aiptasia)*; *d*, algae-like bryozoans *(Zoobotryon)* on roots; *e*, detached sponges *(Lissodendoryx)* surviving partially buried in the detrital bottom of decomposed mangrove litter; a sediment-covered spherical sponge *(Cinachyrella)* is attached to the peat bank to the right.

20
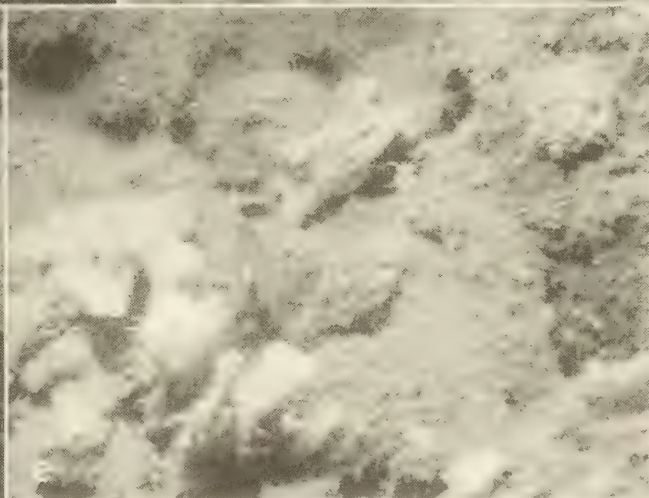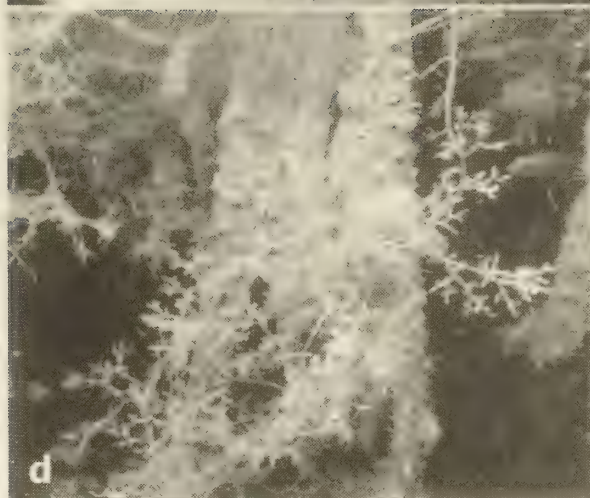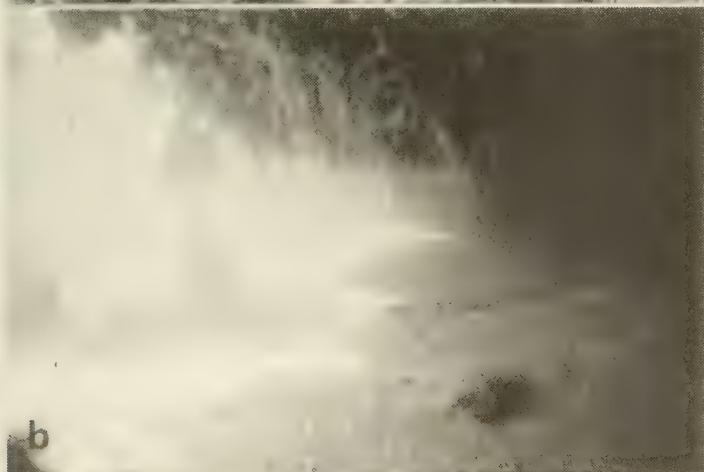
Figure 13. The Lair: *a,* detritus bottom in 2 m depth; the structure in the center is a square experimental frame with settlement plates that became partly buried after one year of exposure; *b,* garbage dumped in May 1990 (photo, M. Carpenter).

Depth. 2 m, at center.

Environmental Conditions. L: + to ±; C: –. T, S: stressful range (see Lair Channel, above). A series of measurements during high and low tide (September 2003) were in the range of 30.5°–33° C temperature and 35.7–36.7‰. Nutrient levels measured in subtidal sites (0.5 m) are highest in the Lair, lowest in the Main Channel stations and locations in direct water exchange with the channel (Sponge Haven North, Sponge Haven South, Hidden Creek entrance). Comparative values (high: low, in January 2003, were: 1.2: 0.2 µMol/l phosphate; 5.5: 1.4 µMol/l ammonium; 0.4: 0.1 µMol/l nitrite, and 1.3: 0.4 µMol/l nitrate. Garbage dumping prevented research for a number of years but was recently discontinued.

Sediment: fine detritus, mainly from decomposed plant material.

Communities. Not as rich as root communities observed elsewhere. Mangrove oysters (*Isognomon*) are common, as well as members of the ascidian family Perophoridae (see Goodbody, 2004a). A few sponges *(Mycale microsigmatosa, Haliclona tubifera)* and anemones *(Aiptasia)*. Algae *(Halimeda, Caulerpa)* and 5 to 8 cm mucuous balls (polychae egg cases) populate the bottom. Cyanobacterial mats are abundant (see 9 J above).

Sta. 10 G, Twin Bays (Figs. 1, 14; Plate 5d)

Location and Topography. A shallow double bay just west of the southern tip of West Island. The outer bay (80 m long x 90 m wide) has a 60 m wide mouth that opens toward the southwest into the main lagoon, to the left (north of the Main Channel entrance. The triangular inner bay (60 x 20 m) is connected by a 10 m wide passage. Regrettably, by 1989 garbage dumping had made Twin Bays unsuitable for research and was excluded as a research site pending future developments.

Habitats. A low (0.5 m) peat bank along the inner margin of the bays with overhanging red-mangrove roots that do not quite touch the bottom. Sandy bottom covered with sparse *Thalassia* seagrass, denser seagrass at the entrance.

Depth. 0.2–1 m.

Environmental Conditions. L: +; C: ± (protected form prevailing north-east winds).
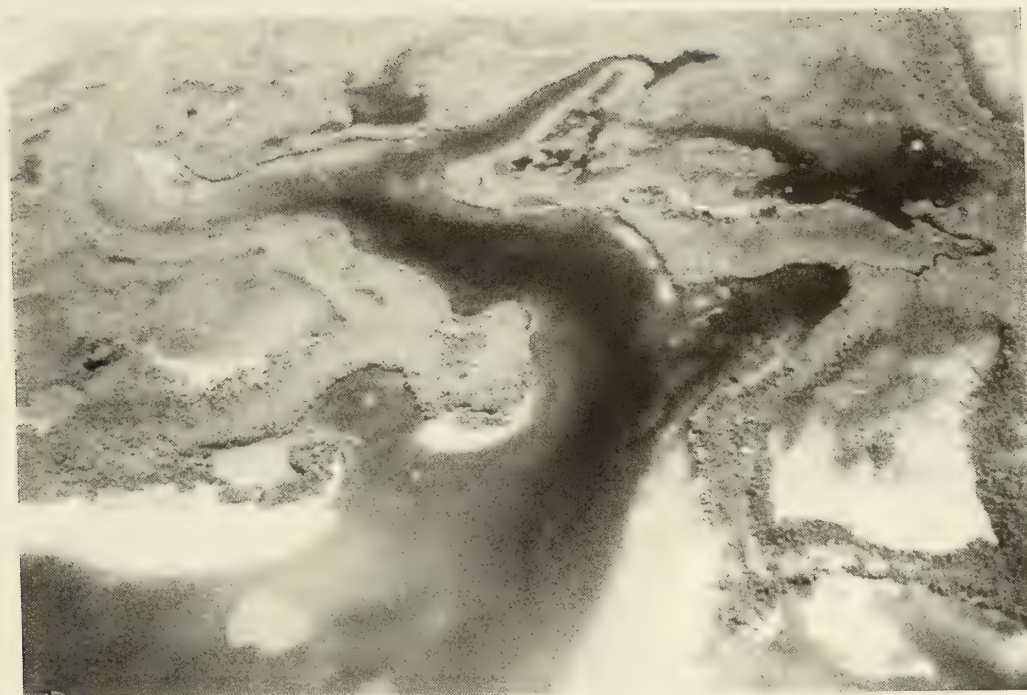
Figure 14. Twin Bays are the first to the left (west) off the Main Channel entrance, viewed from the south (photo, M. Carpenter).

Sediment. Fine calcareous sand and mud.

Communities. Few sponges on the roots, some overgrown by a large population of an ascidian with two color morphs, *Distaplia corolla* (see Goodbody, 2004a). This species was (in 1984) particularly abundant at the western margin of the bank, close to the entrance to the inner bay. Other ascidians are the orange encrusting *Didemnum conchyliatum,* which is widespread and highly tolerant to environmental stress and often overgrows the root tips of mangrove, and the solitary *Phallusia nigra* and *Microcosmus exasperatus,* both characteristic of mildly eutrophic conditions (Goodbody, 2004b). On the bottom of the inner bay margin, specimens of the scyphozoan jellyfish *Cassiopea frondosa* and coral *Manicina areolata* were common.

Sta. 10 H, Sponge Haven (Figs. 1, 15; Plate 5e)

Location and Topography. Shallow, open bay near the southeast tip of West Island, facing the Main Channel. The opening is 65 m, directed eastward, recess is 25 m from the West Island shoreline, at that location. The shoreline is made up of a low (0.2–0.5 m) peat bank with numerous free-hanging and anchored roots in front; this feature extends around the point south of the bay, where the peat bank is even more eroded and recessed and roots hang free and clear of sediments. In the back of the bay there are accumulations of fine-sediment close in and under the roots; along the entrance

22

Figure 15. Sponge Haven South, fringe seen from the Main Channel looking south toward the channel entrance.

there is a shallow longitudinal bank supporting tall seagrass. The northern flank of Sponge Haven ("Sponge Haven North," see Diaz et al., 2004) is also exposed to fine sediment and to accumulations of detached seagrass leaves, floating cyanobacterial mats, and other debris. The coastline of the southern point ("Sponge Haven South," facing the Main Channel Entrance) has clean lagoon water prevailing along the fringe.

Habitats. Hanging and anchored *Rhizophora* roots, peat bank with some undercuts; sandy and seagrass-covered bottom.

Depth. 0.5–1 m.

Environmental Conditions. L: + to ±; C: + (clockwise water circulation, counter the predominant north-south flow in the Main Channel). Measurements taken during high and low tide (September 2003) ranged from 30.4° to 32.1° C in temperature and 35.2 to 35.6‰ salinity.

Sediment. Fine calcareous sand and mud.

Communities. Highly diverse sponge fauna on roots, particularly along Sponge Haven South, including the very common *Geodia papyracea, Clathria venosa, Tedania ignis, Halichondria magniconulosa, H. manglaris, H. mucifibrosa, Calyx podatypa, Hyrtios proteus, Spongia tubifera,* and a few specimens of large *Ircinia strobilina.* Specimens of the sea urchin *Echinometra lucunter* abound along the bank. Many roots are covered by anemones *(Aiptasia)* and mangrove oysters *(Isognomon)* and, in clear water without much sediment loading, by *Halimeda* sp. Algae, the coral *Porites,* sabellid worms, and the ascidians *Diplosoma listerianum* and *Distaplia corolla.* The orange *Scopalina ruetzleri* encrusts large areas of the peat bank and undercuts. Red and green fleshy algae are common on hanging roots along with clumps of cyanobacteria *(Lyngbya* spp.). Extended patches of long-leaved *Thalassia* seagrass with a rich epifauna (hydroids, didemnids, stinging anemone *Bunodeopsis antillensis)* border the fringe and extend into the Main Channel; burrowing alpheid crustaceans abound in sand.

Sta. 10 I, Main Channel Southeast (Figs. 1, 16)

Figure 16. Main Channel Southeast, characteristic community of *Halimeda* algae with sea anemone *Batholomea*.

Location and Topography. West coast of East Island, facing the Main Channel, across from Sponge Haven. It is a gently curved, shallow fringe with low but steep and eroded peat bank that is pitted and exposes old *Rhizophora* roots.

Habitats. Peat bank, some mangrove stilt roots in shallow water, sand banks with *Thalassia* seagrass.

Depth. 0.2–0.5 m.

Environmental Conditions. L: +; C: +. A surface layer of brown swamp-water runoff was noted in places.

Sediment. Fine calcareous sand.

Communities. Halimeda occurs in dense patches on the peat, along with a few sponges *(Mycale microsigmatosa, Haliclona tubifera)* and sea urchins *(Diadema antillarum).* Shallow *Rhizophora* roots are covered by the bostrychietum algal community and other algae, anemones *(Condylactis, Bartholomea),* coral *(Diploria strigosa),* and sporadic ascidians *(Microcosmus exasperatus).* Extended beds of *Thalassia* with long and dense grass blades are populated by juvenile fishes and in places disturbed, indicating that manatees had grazed there.

Sta. 11 G, Main Channel Entrance (Figs. 1, 14, 17; Plate 5f)
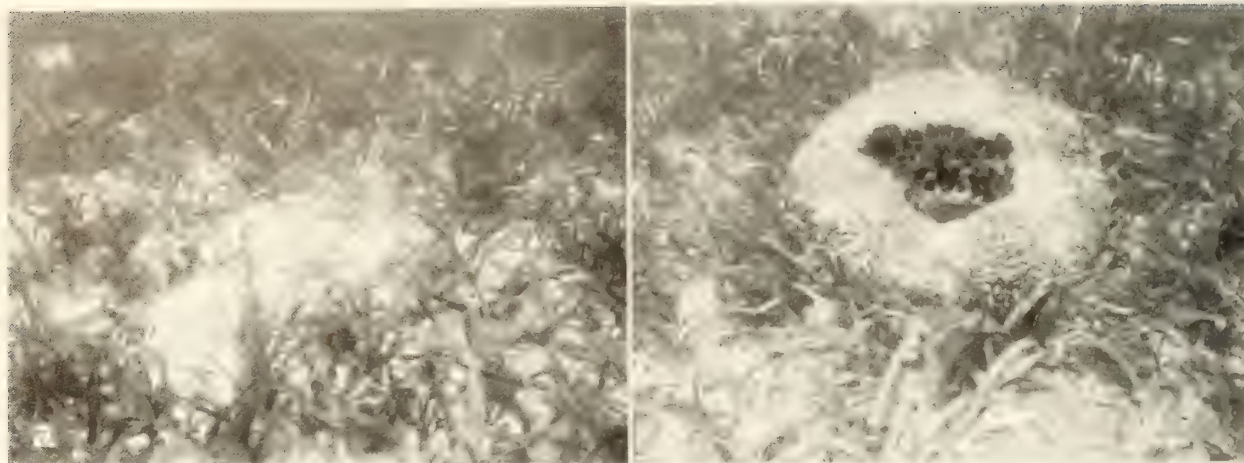
Figure 17. Underwater images of habitats at Main Channel entrance: *a,* turtle grass alternating with sand mounds produced by the burrowing of shrimp *Glypturus; b,* large sponge (60 cm across) among turtle grass, *Spheciospongia,* is the reef-like center of a community of endobiotic invertebrates.

Location and Topography. The funnel-shaped mouth of Main Channel that separates West and East islands and faces west, and some open-lagoon bottom just in front (west) of it. Width of the Channel mouth is about 110 m.

Habitats. Mini-reefs composed of clusters of coral, sponge, algae, and octocoral and large loggerhead sponges *(Spheciospongia vesparium)* with associated epi- and endofauna. Firm sandy bottom with dense stands of *Thalassia* seagrass to the north (West Island), a bare sand apron along the southern shore (East Island).

Depth. 0.5–3 m.

Environmental Conditions. L: +; C: + (open lagoon conditions, but exposure modest because of orientation away from the predominant northeast winds).

Sediment. Medium-grained sand-size fraction (267–426 μm median diameters), with mud-size (<63 μm) fraction 9–21%, organic content 3.9–4.6% (Dworschak and Ott, 1993).

Communities. The mini-patch reefs are formed by coral, *Porites porites* and *P. astreoides* (the latter often adorned by the colorful crowns of sessile polychaetes, *Spirobranchus),* and by sponges *(Spheciospongia vesparium, Tectitethya crypta),* bound together by algae, sponges *(Lissodendoryx colombiensis, Clathria schoenus, A. erina, Hyrtios proteus, Aplysina fulva),* and gorgonians. Associated with these reefs are seagrass *(Thalassia, Halodule),* several algae *(Caulerpa, Halimeda, Rhipocephalus, Udotea),* and anemones *(Condylactis).* Seagrass *(Thalassia, Syringodium)* growing between rubble and conch hides a number of sponges *(Tedania ignis, Amphimedon compressa, Ircinia felix),* sea urchins *(Lytechinus),* and, here and there, specimens of the bivalve *Pinna carnea* and large, erect leathery tubes occupied by the polychaete *Eunice aphroditois.* In sandy areas, small (<10 cm) mounds decorated with pieces of shell indicate the presence of a burrowing shrimp *(Glypturus acanthochirus).* Divers or swimmers in this area are regularly attacked by biting but otherwise harmless isopods, *Rocinela signata.* Along the east bank of the Entrance are shallow, anchored *Rhizophora* roots with *Halimeda* algae.

Sta. 12 I, Hidden Creek (Figs. 1, 18; Plate 6)

Location and Topography. The creek originates from deeply cut but shallow Boston Bay at the southern end of East Island. The mouth of the bay opens into the Main Channel. Hidden Creek starts at the opposite end and meanders first east, then south, and finally turning and running north, enters the large and shallow Hidden Lake. The creek is

Figure 18. Hidden Creek and Lake: *a,* low-altitude aerial view of Hidden Creek looking west; Hidden Lake is to the right, Boston Bay to the back; in the background one can see the Main Channel with Twin Bays (left) and Sponge Haven (right); *b,* detail of the creek; *c,* entrance into the lake.

a constant 3–4 m wide and 2–3 m deep (shallower toward its end), and about 20 m long. Hidden Lake covers an area of over 3 ha and is on average less than 0.5 deep (depth depending on tide).

Habitats. Peat banks and undercuts, hanging *Rhizophora* roots, soft sandy bottom rich in detritus and covered by varying density of seagrass. The bottom of Boston Bay near the Creek's entrance is covered by turtle grass; the seafloor of Hidden Lake consists of soft detritus deposited on firm peat floor.

Depth. 0.5–3 m.

Environmental Conditions. L: + to –; C: + (with each tidal change). T, S: stressful range, due to tidal drainage of water from Hidden Lake that may be hot and hypersaline after a period of exposure to full sun, or cold and brackish after heavy rains. Water drains from nearby Boa Flats (in the south) into Boston Bay and Hidden Creek as well. During the cold season (January, February), the lake can approach the air temperature of a cold night, whereas the deeper lagoon acts as a buffer. Measurements by thermistor recorder just inside the Hidden Creek entrance show a combined maximum temperature range of 18.5°–30° C (January 1990, February 1998) and 28°–37° C (August 1989, 1999). Salinity range at the same location measured by refractometer (April 1993, February 1998, August 1999) ranged from 22 to 38‰ (subsurface), depending on rainfall and direction and timing of tidal current flow.

There were several observations of strong exposure to suspended sediments that may enter through tidal flow from either Hidden Lake or Boston Bay; some of these events may have been caused by boat traffic, a rare but not unusual occurrence.

Sediment. Bottom samples taken at the entrance in 1.3 m depth, and 10 m into the creek (1.9 m) consisted of fine sand (70% less than 2 mm diameter), with larger *Halimeda* chips and shell fragments and a substantial amount (17% dry weight) of detritus (plant material). A similar sample taken near the mangrove fringe in Boston Bay, just outside the creek entrance, revealed mostly decaying plants (detritus) with a small amount (11%) of carbonate chips and shell. Samples included just trace amounts of broken sponge spicules despite the close proximity of large sponge populations.

Communities. *Thalassia* at the Boston Bay entrance is heavily overgrown by filamentous cyanobacteria *(Lyngbya)*, anemones *(Bunodeopsis)*, bryozoans *(Zoobotryon)*, and ascidians *(Didemnum)*. Algae, a great variety of sponges, sabellid polychaetes, and ophiuroids on the peat banks. Roots dominated by sponges and anemones *(Aiptasia, Bartholomea, Condylactis)*, also *Halimeda* and *Caulerpa* algae, hydroids, ascidians, and clusters of mangrove oyster *(Isognomon)* in the intertidal. Common sponge species on the peat bank are *Cinachyrella apion, Biemna caribea, Scopalina ruetzleri, Hyrtios proteus*, and *Clathrina*. On the roots we find *Spongia tubulosa. S. pertusa, Geodia papyracea*, very large specimens of *Lissodendoryx, Scopalina, Biemna caribea, Mycale magniraphidifera, Tedania, Amorphinopsis* sp., *Halichondria* cf. *magniconulosa, Haliclona implexiformis, H curacaoensis*, and *Dysidea* sp. Clusters of bivalve *(Isognomon)* shells in the lower intertidal zone offer additional substratum to small and encrusting sponges (e.g., *Halisarca, Clathrina, Sycon)*, hydroids, and ascidians *(Ecteinascidia minuta, Styela canopus).* During the early 1980s, the green alga *Halimeda* was nearly as prominent as sponges but the population has since diminished. Some algae *(Udotea, Halimeda, Caulerpa)* are scattered over the bottom and sponges *(Haliclona magnifica)* and sabellid polychaetes are embedded in the soft sediment and attached to

the bases of algae in protected side branches and near the entrance into Hidden Lake. Sponges that have fallen from broken or decayed mangrove roots are surviving partially buried in the muddy bottom (for instance, *Lissodendoryx, Haliclona permollis*) and *Udotea* algae were observed supporting small invertebrate settlers (e.g., the ascidian *Botryllus tuberatus*). Polychaete and mollusk egg cases are commonly encountered on the muddy bottom among decayed mangrove litter.

Sta. 13 G, Crescent Bay (Figs. 1, 19)

Figure 19. Aerial photograph of south Twin Cays looking toward the northwest; Crescent Bay (sta. 13G) is behind South Point (occupied by a ranger station of the South Water Cay Marine Reserve); in the center foreground is Southeast Coast (13I).

Location and Topography. A wide (>200 m), gently curved bay located at the southwest end of East Island, just south of the entrance to Main Channel and open toward the west; its southern limit is South Point.

Habitats. Short *Rhizophora* roots along the margin with bostrychietum fuzz but no large invertebrates, anchored in sand. Sandy bottom with seagrass; a large, bare, near-shore sandy patch to the north.

Depth. 0.2–2 m.

Environmental Conditions. L: +; C: + (fully exposed to west and southwest winds); water flow at ebb-tide from the adjacent Boa Flats may affect the organisms living close to the mangrove margin).

Sediment. Fine to medium-grained calcareous sand, rich in *Halimeda* chips.

Communities. Seagrass *Thalassia* and *Halimeda* algae, with solitary coral *(Manicina areolata)* and bivalves *(Pinna carnea, Atrina* sp.). Sandy bottom with traces (mounds) of burrowing shrimps.

Sta. 13 I, East Island, Southeast Coast (Fig. 1, 19)

   Location and Topography. South exposed shore (open lagoon)extending east
from South Point.
   Habitats. Short, stout *Rhizophora* roots anchored in sand along the fringe. Sandy
bottom with dense *Thallassia* seagrass and algae *(Halimeda, Penicillus, Rhipocephalus),*
very similar to other peripheral sites such as 5 K, 13 G, but not as diverse as the more
protected West Bay (7 E).
   Depth. 0.2–1.5 m.
   Environmental Conditions. L: +; C: + (fully exposed to southerly and south-
easterly winds).
   Sediment. Fine to medium-sized calcareous sand; coarser *Halimeda* chips.
   Communities. Mangrove roots are populated mainly by bostrychietum and some
encrusting hydrocoral *(Millepora)* but no large invertebrates. Turtlegrass and *Halimeda*
algae, sponge clusters attached to dead shell, particularly *Hyrtios proteus* and
Amphimedon erina. The only location in the Carrie Bow area where the symbiotic sponge
(with filamentous cyanobacteria) *Hyrtios violacea* is common (Rützler, 1990).

DISCUSSION

   This account of subtidal localities in the Twin Cays mangrove concentrates on
sessile fauna (sponges and ascidians, in particular) because these organisms are excellent
indicators of long-term environmental conditions and community health. Algae too are
highly diverse in mangroves (Norris & Bucher, 1982; Littler and Littler, 1997; Littler et
al., 2004) but occur in different ecological niches and are more tolerant of environmental
changes. Algae in fact are powerful competitors for the limited supply of substratum
space and seem to flourish during the warmer summer months, overgrowing epifauna on
stilt roots.The habitats described represent diverse topographic features that are unique to
mangrove islands and are influenced by a wide range of environmental parameters, from
near-oceanic quality of outer coasts to the life-restricting conditions of inshore ponds.
   Mangroves are forests composed of a diverse collection of salt-tolerant plants, but
in the subtidal realm only one species has direct impact on the marine community: the red
mangrove *(Rhizophora mangle).* Growth of these trees over thousands of years resulted
in an accumulation of peat that is consolidated, eroded, and shaped by various physical
and biological processes (Macintyre et al. 1995; McKee and Faulkner, 2000; Macintyre et
al., 2004).

Primary Substrata

   Availability of solid surfaces distant from sediment bottoms is the key to the
existence of most sessile shallow-water invertebrates. In the mangrove, hard substrata are
provided mainly by the stilt roots of *Rhizophopra,* many of which hang freely into the
water if they are young and where the bottom is too deep to reach, for instance along
deep-cut tidal creeks. Second in importance are the vertical surfaces of eroded peat banks

lining canals and ranging from 0.2 m to nearly 3 m in height. Peat banks are eroded by past or present water currents that have also created deep undercuts and caves where they were forced to bend. Most of the Main Channel's west coast is lined by peat banks where the water flows fastest. High walls are found along 'Gator Creek (station 8I), Grouper Gardens channel (9I), and Hidden Creek (12I). Cave-like undercuts occur at Batfish Point (5Ha) where currents from the open lagoon are the strongest. Undercut caves also occur at the neck of Lair Channel (9J), where water is quite stagnant but flow conditions may have been different in the past when there was a connection to the open lagoon.

Under good conditions, mangrove adventitious roots grow several centimeters per year and therefore constantly provide new substrata for settlement. On the other hand, roots or entire trees may die, causing substrata to rot and leaving the entire epibiont community to sink into the muddy bottom. Most sessile invertebrates become smothered, but a few sponges (notably species of *Haliclona, Lissodendoryx,* and *Ircinia)* were observed buried in detritus and healthy for at least three years. Some developed fistulose structures, presumably for the increase of surface area to compensate for the restricted water and nutrient flow into the incurrent canal system of the sponge.

Secondary Substrata

Although *Rhizophora* roots and peat are the primary substrata for sessile mangrove organisms, secondary space becomes available as the community develops. One of the organism that provide new substrata are the bivalve *Isognomon alatus,* the mangrove oyster, a species that develops clusters of individuals, often near the water surface at the top of a hanging root. Although adjacent shells may be in close juxtaposition, two species of ascidians regularly make use of the shells as a settlement surface. These are the colony-forming *Ecteinascidia minuta* and the solitary species *Styela canopus* (Goodbody, 2004a). Other colonial ascidians, such as *Diplosoma listerianum* and *Didemnum conchyliatum,* and sponges (for instance, *Biemna caribaea, Haliclona curacaoensis, H. manglaris, H. tubulifera,* and *Clathrina* cf. *coriacea),* are more common on the outside of a bivalve cluster but still penetrate the intershell space. Sponges too provide biological surfaces for secondary settlement space, particularly *Spongia tubulifera,* which is always overgrown by other sponges and by algae, ascidians, hydroids and serpulid polychaetes. Other important members of the benthic community such as turtlegrass (*Thalassia)* and calcareous green algae (*Halimeda)* regularly provide secondary space, particularly for hydroids, *Didemnum,* anemones, such as the small but strongly stinging *Bunodeopsis antilliensis, Aiptasia tagetes,* and foraminiferans (Richardson, 2004).

Tides

One condition that determines the existence and structure of subtidal mangrove epifauna is tidal range. Twin Cays, like most parts of the Caribbean, has a small range (mean 20 cm), thus keeping habitats permanently submerged except for an upper, near-surface transition zone to the intertidal populated by species with some resistance to short-term exposure to dessication (Rützler, 1995). In two recorded El Niño years, tides

of 30 cm below mean low during noon hours caused mass mortalities of all organisms in the uppermost subtital zone.

Light

Most organisms in this shallow-water ecosystem are adapted to strong illumination, but in some locations light, or the lack of it, determines the community structure. Photosynthetically active radiation determines most notably the distribution and density of seagrass, *Thalassia,* which is not found on deep channel bottoms with turbid water (for instance, part of Lair Channel, 9J; Lair, 9K). It is also sparse or missing in narrow creeks shaded by overhanging trees and flooded by (brown-colored) water high in tannin content as it returns from the swamp (Gator Creek, 8I; Hidden Creek, 12I) (Calem and Pierce, 1993). Algae and cyanobacteria are more tolerant and successfully compete with sessile animals for free space. Exceptions are peat overhangs and caves, and some recessed peat banks under a dense canopy, where light levels are very low. Sponges flourish under these conditions, without competing algae and (on cave ceilings) free of sediment. Although no unique cryptic species were found, the sponge diversity per area is highest in caves. One cream-colored ascidia *(Diplosoma)* was also found to be a common member of the cave community.

Temperature

Because much of the water in the mangrove occurs in shallow lakes and ponds, it is directly subjected to heating and cooling of the atmosphere. Tidal signature and timing strongly influences the temperature regime. Since the ponds are too shallow and muddy to support large populations of sessile animals, the effect of temperature change is mainly noticeable in the channels where water moves with each tidal cycle and is very hot when the ponds empty after noon hours of full sunshine in summer, very cold following a chilly night in winter. Swamp water also drains sideways into the channels, flowing across the peat banks and noticeable because of its tannin-brown color. The presence of large populations of filter feeders such as sponges and ascidians near the outflow of swamp water indicates high contents in nutrients (dissolved and particulate, mainly as bacterioplankton) overriding the limiting effects of temperature fluctuations. Measurements were taken on numerous occasions but unfortunately, long-term in situ recordings of temperature together with correlated parameters (tide, salinity, rainfall, dissolved organics) are still missing.

Temperature changes in one tidal cycle during winter (January–March) can vary from 18° to 32° C (ocean mean temperature, 26° C); during summer (July–September), they fluctuate from 28° to 41° C (ocean mean, 29° C).

Salinity

Normal seawater in the area has a salinity of 35–36‰. At Twin Cays, the range was measured after heavy overnight rains when ebb tide drained the swamp in the early morning. Samples along the main channel had lower salinity of 21‰ at the surface, 23‰ at 50 cm depth. The same phenomenon has been recorded from the Port Royal, Jamaica,

mangroves where it was demonstrated that if the low salinity water goes too deep it causes heavy mortality among sessile communities in which most organisms lack mechanisms for osmotic control (Goodbody 1961).

During some tidal exchanges after heavy rains, freshwater tends to form the top layer although it is colder by several degrees than the denser but warmer seawater. Intertidal and upper subtidal communities are strongly affected by this phenomenon, as reflected by extensive mortality after such conditions occurred repeatedly.

Sediments

Fine calcareous sand and mud, that is, rich in organics, make up the bottom of the Main and Lair channels and support stands of seagrass *(Thalassia, Halodule)* and algae *(Halimeda, Penicillus, Avrainvillea)*, large populations of upside-down jellyfish *(Cassiopea),* and an ubiquitous burrowing crustacean endofauna that forms characteristic mounds, deposits from its tunneling activity (Dworschak and Ott, 1993). Despite the plant cover and consolidation by mucuous substances derived from bacteria, cyanobacterial mats, fecal pellets, and other sources, the sediment is easily disturbed and resuspended by storms, manatee feeding, and boat traffic. The wake of passing boats may also indirectly cause resuspension of sediments by moving thickly populated free-hanging roots with growth tips close to the bottom. Because most mangrove epibionts are filter-feeders with limited capability to clear themselves from sediment cover, these disturbances may result in burial and smothering. Sponges and ascidians are only successful in these mangroves because many of the hard substrata (mangrove roots, peat banks) have vertical orientation, keeping siltation to a minimum. Nevertheless, increased boat traffic through the channels in recent years may have contributed to a decline in populations, particularly of the rich sponge fauna in Sponge Haven (10H; Diaz et al., 2004). Habitats with anchored roots and strong currents are mostly found along the perimeter of Twin Cays (stations 5 K, 7 E, 11 G, 13 G, 13 I).

The bottoms of inshore ponds and creeks are equally soft but primarily made up by flock of fibrous, broken-down plant materials. As already mentioned above, some sponges are able to flourish being buried in this substratum.

Water Flow

Storms resuspend fine sediments but tidal and wind-generated currents help clear exposed substrata and bring nutrient- and bacteria-rich water from the interior of the swamp to the sessile benthos that is either filter or particle feeding. Water flow is most constant in the main channel because prevailing winds push water either from north or south and create conditions close to those of nearby coral reefs. Some narrow and long creeks connecting the outer lagoons to interior ponds ('Gator Creek 8 I; Hidden Creek, 12 I) display the strongest currents within the system (20 cm/sec were measured) but depend on tidal signature and often combine the beneficial factor of strong flow, washing silt away from settlers, with less desirable poor (hot, cold, high or low salinity, tannin-rich) water quality. The wake of passing boats my indirectly cause resuspension of fine sand and mud by moving heavily populated free-hanging roots with growth tips near the bottom, thus affecting the epibionts both by wave action and by sedimentation.

Biotic Factors

Diversity and species composition are influenced not only by abiotic factors but also by differences in ecological interactions and recruitment history. Transplant experiments and new artificial substrata make it possible to highlight these parameters for sponge communities (Wulff, 2004; Ruetzler, in preparation).

Algae and cyanobacteria affect invertebrate epibionts through space competition at settlement as well as in later stages of community development. During the winter months, large quantities of floating *Sargassum* seaweed enter the Main Channel from the north and prevailing winds push them against the western shore of the channel. Wave or boat wash causes the weed to move up and down along the roots in such a manner as to have an abrasive action damaging or destroying elements of the epibiont community, as was demonstrated by newly marked colonies of the ascidian *Diplosoma glandulosum* (July 1984), many of which were lost or damaged by the following February.

A more serious threat to many epibionts, particularly filter-feeding sponges and ascidians, is the seemingly increasing presence of filamentous cyanobacteria, which tend to overgrow roots and everything settling on them. One common kind develops thick, hair-like filaments of tan, brown, to red color and covers substrata as entangled tufts or clumps, or forms strands that hang into the current like streamers, up to 15 cm wide and 2 m long (for instance, in Grouper Gardens, 9I; August 2004). The main species incorporated in these clusters are *Lyngbya polychroa* and *Lyngbya* sp. Dislodged strands may accumulate as thick masses forming bright green floats (bleached from full exposure to sun light) after they drifted into calm-water bays, such as the cove outside Turtle Pond. Another kind is commonly found as rust-red heavy drapes coating roots, sponges and other surfaces on mangrove roots and is composed mainly of *Schizothrix tenerrima* and *Schizothrix* sp. Their filaments are shorter and more fragile than *Lyngbya* and flake off and disperse easily. This phenomenon was already recorded in 1986 (May) when growths of this organism took over the lowest part of roots that were otherwise occupied by the ascidian *Eudistoma olivaceum*. Today, cyanobacterial accumulations seem ubiquitous throughout Twin Cays, at least during the summer months (July–September), and it remains to be investigated whether it is related to the increase of dissolved organic substances in the lagoon water. An ongoing project attempts to determine what environmental parameters (in particular, nutrient levels, temperature, current flow) stimulate growth of cyanobacteria and to what degree sponge and other sessile invertebrates are affected by microbial competitors and pathogens (Diaz et al., 2004). Shifts in community composition of physiologically sensitive epibionts such as sponges may be useful indicators of long-term environmental health.

Pollution caused by human activities and rapidly increasing with economic development has particularly severe consequences in mangroves because as a tidal community they are affected by both terrestrial and aquatic stresses. Live trees make up the framework and principal components of the community and if lost they cannot be replaced in our life time, maybe never once the substratum buildup is washed away. Effects of clear-cutting are discussed in another contribution (Rodriguez and Feller, 2004). Peat and hanging and anchored roots are the principal solid substratum for sessile organisms and hence the pillars of the rest of the marine community, as we have

demonstrated after examining the effects of an oil spill in another Caribbean mangrove (Rützler and Sterrer, 1970).

Unfortunately, many people still believe that mangroves are areas of decay and therefore suitable dumping grounds for garbage. This notion has caused severe damage to the Twin Cays mangrove community, not to mention setbacks in ongoing research in places such as Turtle Pond (6H), The Lair (9K), and Twin Cays (10K). Repeatedly over the years, these secluded locations were apparently considered good hiding places and, unlike the more spectacular nearby coral reefs not affected by unsightly appearance, were subjected to excessive release of nutrients from kitchen waste and heavy metals from batteries, or the smothering action of large plastic bags.

ACKNOWLEDGMENTS

REFERENCES

Calder, D.R.
1991a.   Associations between hydroid species assemblages and substrate types in the mangal Twin Cays, Belize. Canadian Journal of Zoology 69:2067-2075.
1991b.   Abundance and distribution of hydroids in a mangrove ecosystem at Twin Cays, Belize, Central America. Hydrobiologia 216/217:221-228.
Calem, J.A., and J.W. Pierce
1993.   Distributional control of seagrasses by light availability, Twin Cays, Belize, Central America. *Atoll Research Bulletin* 387:1-13.
Diaz, M.C., K.P. Smith, and K. Rützler
2004.   Sponge species richness and abundance as indicators of mangrove epibenthic community health, Twin Cays, Belize. *Atoll Research Bulletin* 518:1-18.
Dworschak, P.C., and J.A. Ott
1993.   Decapod burrows in mangrove-channel and back-reef environments at the Atlantic barrier reef, Belize. *Ichnos* 2:277–290.
Faust, M.A., and R.A. Gulledge
1996.   Population structure of phytoplankton and zooplankton associated with floating mangrove detritus in a mangrove island, Twin Cays, Belize. *Journal of Experimental Marine Biology and Ecology* 197:159–175.
Goodbody, I.
1961.   Mass mortality of a marine fauna following tropical rains. *Ecology* 42: 150–155.
2004a.   Diversity and distribution of ascidians (Tunicata) at Twin Cays, Belize. *Atoll Research Bulletin* 524:1-19.

2004b.   The ascidian fauna of Port Royal, Jamaica, I: Harbor and mangrove dwelling species. *Bulletin of Marine Science* (in press).

Hajdu, E., and K. Rützler
1998.   Sponges, genus *Mycale* (Poecilosclerida: Demospongiae: Porifera), from a Caribbean mangrove and comments on subgeneric classification. *Proceedings of the Biological Society of Washington* 111: 737–773.

Joye, S.B., and R.Y. Lee
2004.   Benthic microbial mats: Important sources of fixed nitrogen and carbon to the Twin Cays, Belize, ecosystem. *Atoll Research Bulletin* 528:1-24.

Kohlmeyer, J., B. Bebout, & B. Volkmann Kohlmeyer
1995.   Decomposition of mangrove wood by marine fungi and teredinids in Belize. *Marine Ecology* 16: 27-39.

Koltes, K.H., J.J. Tschirky, and I.C. Feller
1998.   Carrie Bow Cay, Belize. In: Kjerfve B., ed., *CARICOMP—Caribbean Coral Reef, Seadrass and Mangrove Sites*. UNESCO, Paris, pp. 79–94.

Littler, D.S, and M.M. Littler
2000.   *Caribbean reef plants*. Off-Shore Graphic, Washington D.C.

Littler, M.M., D.S. Littler, and B.L. Brooks
2004.   Extraordinary mound-building forms of *Avrainvillea* (Bryopsidales, Chlorophyta): Their experimental taxonopmy, comparative functional morphology and ecological strategies. *Atoll Research Bulletin* 515:1-26.

Macintyre, I.G., M.M. Littler, and D.S. Littler
1995.   Holocene history of Tobacco Range, Belize, Central America. *Atoll Research Bulletin* 430:1-18.

Macintyre, I.G., M.A. Toscano, R.G. Lighty, and G.B. Bond
2004.   Holocene history of the mangrove islands of Twin Cays, Belize, Central America *Atoll Research Bulletin* 510:1-16.

McKee, K.L., and P.L. Faulkner
1999.   Mangrove peat analysis and reconstruction of vegetation history at the Pelican Cays, Belize. *Atoll Research Bulletin* 468:45–58.

Ott, J., and M. Bright
2004.   Sessile ciliates with bacterial ectosymbionts from Twin Cays, Belize. *Atoll Research Bulletin* 516:1-7.

Parrish, M., and M.K. Ryan
2004.   Art in the SWAMP: Using field illustrations to prepare drawings of mangrove communities at Twin Cays, Belize. *Atoll Research Bulletin* 530:1-9.

Richardson, S.L.
2004.   Seasonal variation in epiphytic Foraminifera biotas from *Thalassia* seagrass habitats, Twin Cays, Belize. *Atoll Research Bulletin* 517:1-39.

Rodriguez, W., and I.C. Feller
2004.   Mangrove landscape characterization and change in Twin Cays, Belize, using aerial photography and Ikonos satellite data. *Atoll Research Bulletin* 513:1-23.

Rützler, K.
1988.   Mangrove sponge disease induced by cyanobacterial symbionts: failure of a primitive immune system? *Diseases of Aquatic organisms* 5: 143–149.

1990.   Associations between Caribbean sponges and photosynthetic organisms. In: K. Rützler (ed.), *New Perspectives in Sponge Biology,* Smithsonian Institution Press, Washington, D.C., pp. 455–466.

1995.   Low-tide exposure of sponges in a Caribbean mangrove community. *Marine Ecology* 16: 165-179.

Rützler, K., M.C. Diaz, R.W.M. van Soest, S. Zea, K.P. Smith, B. Alvarez, and J. Wulff
2000.   Diversity of Sponge Fauna in Mangrove Ponds, Pelican Cays, Belize. *Atoll Research Bulletin* 476:229-248.

Rützler, K., and C. Feller
1988.   Mangrove swamp communities. *Oceanus* 30(4):16-24.

1996.   Caribbean mangrove swamps. *Scientific American* 274 (3):94-99.

Rützler, K., and J.D. Ferraris
1982.   Terrestrial environment and climate. In: K. Rützler and I.G. Macintyre (eds.), The Atlantic Barrier Reef Ecosystem at Carrie Bow Cay, Belize 1: Structure and Communities, pp. 77–91. *Smithsonian Contributions to the Marine Sciences* 12.

Rützler, K., and I.G. Macintyre
1978.   Siliceous sponge spicules in coral reef sediments. *Marine Biology* 49: 47-159.

1982.   Habitat distribution and community structure of the barrier reef complex near Carrie Bow Cay. In: K. Rützler and I.G. Macintyre (eds.), The Atlantic Barrier Reef Ecosystem at Carrie Bow Cay, Belize 1: Structure and Communities, pp. 9-45. *Smithsonian Contributions to the Marine Sciences* 12.

Rützler, K. and W. Sterrer
1970.   Oil pollution: Damage observed in tropical communities along the Atlantic seaboard of Panama. *Bioscience* 20:222–224.

Weerdt, W. de, K. Rützler, and K.P. Smith
1991.   The Chalinidae (Porifera) of Twin Cays, Belize, and adjacent Waters. *Proceedings of the Biological Society of Washington* 104: 189–205.

Winston. J. E.
2004.   Bryozoans from Belize. *Atoll Research Bulletin* 523:1-14.

Wulff, J.
2004.   How sponge species assemble on mangrove roots, Twin Cays, Belize. *Atoll Research Bulletin* 519:1-10.

PLATES

Plate 1. Aerial views and habitats of northern Twin Cays: *a,* The archipelago looking southeast toward the barrier reef and some of its cays (left to right, South Water Cay, Carrie Bow Cay, and Curlew Bank; *b,* northern shore and channels; *c,* peat undercut and hanging roots at Batfish Point; *d,* peat wall with sponge; *e,* hanging root covered by fire sponge *(Tedania ignis); f,* gray ascidian, *Diplosoma glandulosum; g,* orange sponge *(Scopalina ruetzleri)* on root.

Plate 1

Plate 2. Sessile organisms and habitats of northern Twin Cays: *a, Mycale laxissima,* a sponge typical for the banks of 'Cuda Cut; *b, Botrylloides nigrum,* a common ascidian at Batfish Point; *c,* common encrusting sponge, *Mycale microsigmatosa*; *d,* filamentous cyanobacteria, *Schizothrix* spp., coating many sponges, such as *Lissodendoryx; e,* peat wall in the rear of an undercut showing patch of sulfur-fixing bacteria, *Beggiatoa; f,* vertical view of mud bottom at Cassiopea Cove showing the upside-down jellyfish that gave the cove its name (yellowish-green disks), algae (fluffy green balls), *Penicillus,* and fine sand with openings of tunnels made by burrowing shrimps *(Glypturus, Alpheus).*
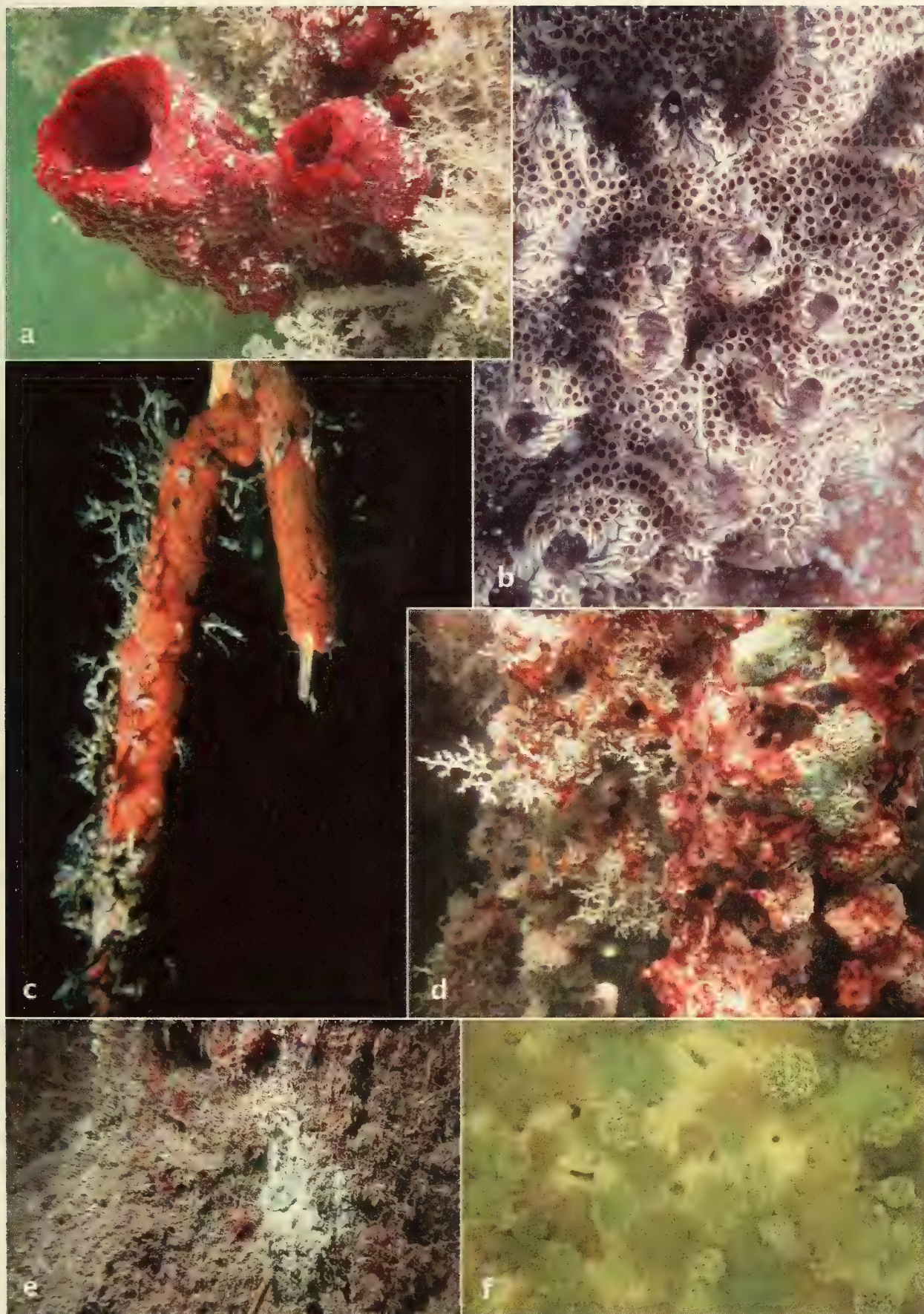
Plate 2

Plate 3. Sessile invertebrates and views of habitats in ponds and bays of northern Twin Cays: *a,* ascidians, *Perophora regina* on a mangrove root at the type locality, Turtle Pond; *b,* typical loose organic-flock bottom in Turtle Pond, with decaying mangrove leaves and covered by a bacterial matt; *c,* West Bay, mini-reef cluster among turtlegrass consisting of green calcareous *Halimeda* algae and sponge *(Clathria schoenus),* visited by a sea urchin *(Lytechinus); d,* 'Gator Creek, the yellow sponge *Cinachyrella apion,* ornamented by porocalices (cups containing pores and oscula) and tiny buds, is typical for the peat wall lining the deep channel; *e,* mollusk egg case on the channel's soft bottom of mangrove litter; *f, Haliclona magnifica,* a sponge with long oscular tubes is common on the peat bank and buried in detritus bottom of 'Gator Creek, its type locality.
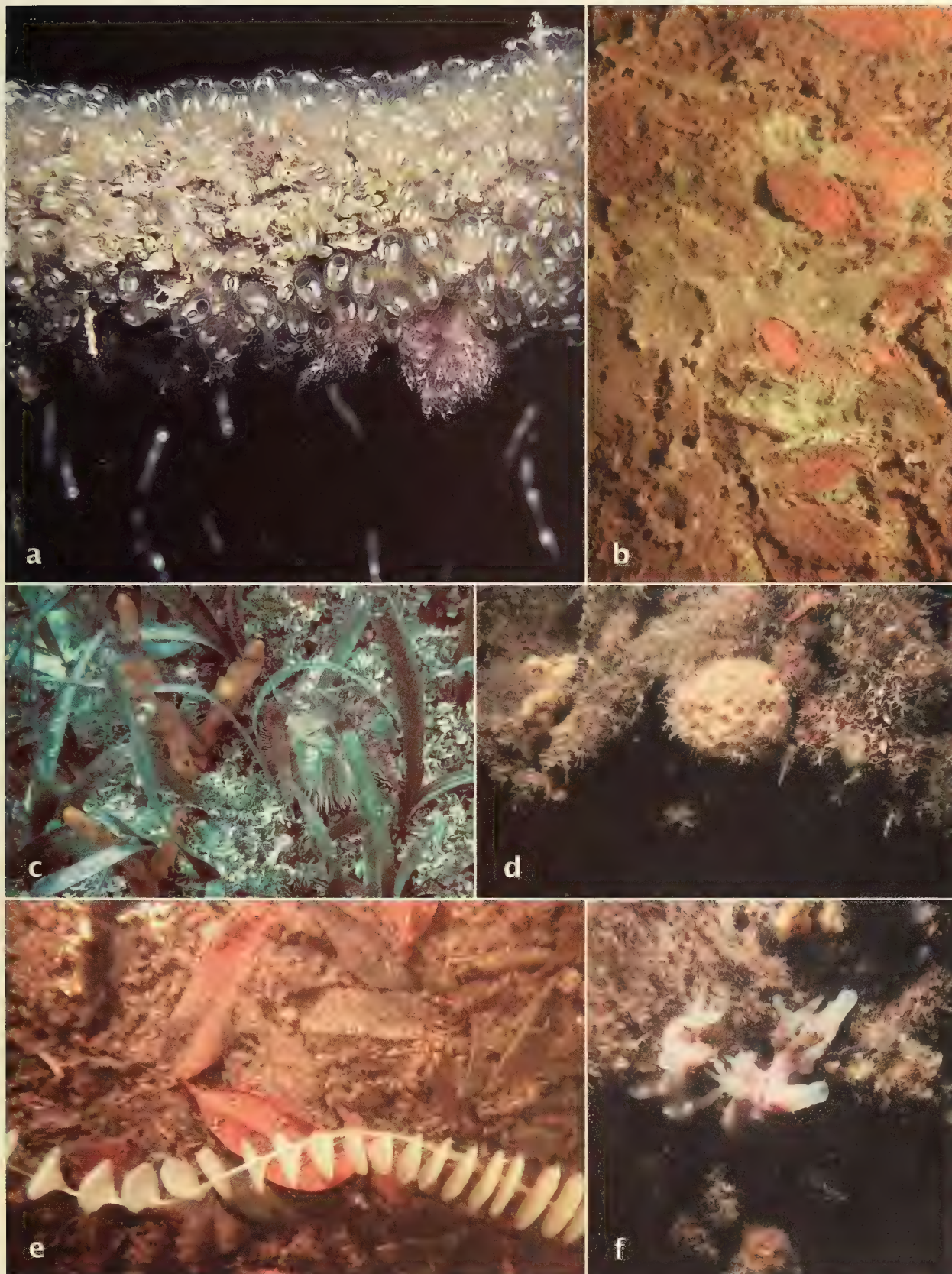
Plate 3

Plate 4. Lair Channel and associated biotopes: *a,* bird's eye view of Lair Channel, leading from the Main Channel eastward (right) through the channel's neck into The Lair; 'Gator Creek branches off at the channel's mouth, toward the north (top left); Grouper Gardens, a cluster of small ponds, is entered through a narrow passage cutting into the opposite (south) bank; *b,* calcified algae, *Halimeda,* and fire sponge, *Tedania* line the entrance to Grouper Gardens; *c,* bunches of mangrove oysters *(Isognomon)* grow on many roots along the canals connecting ponds and serve as a secondary substratum for small sponges, sea anemones, serpulid worms, and ascidians; *d,* in the back of Grouper Gardens entrance sponges, such as this *Calyx podatypa,* may be threatened by thick layers of filamentous cyanobacteria *(Lyngbya),* which develop in masses during the summer months (July to September); *e,* the bottom of Grouper Gardens ponds is overgrown by tall *Avrainvillea* algae which provide shelter for invertebrates, such as this opisthobranch, *Tridachia.*

45
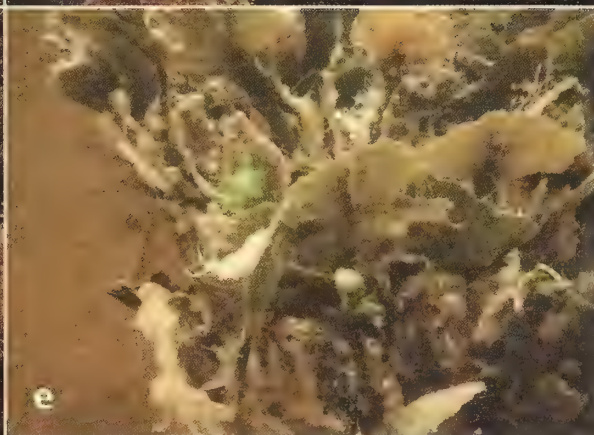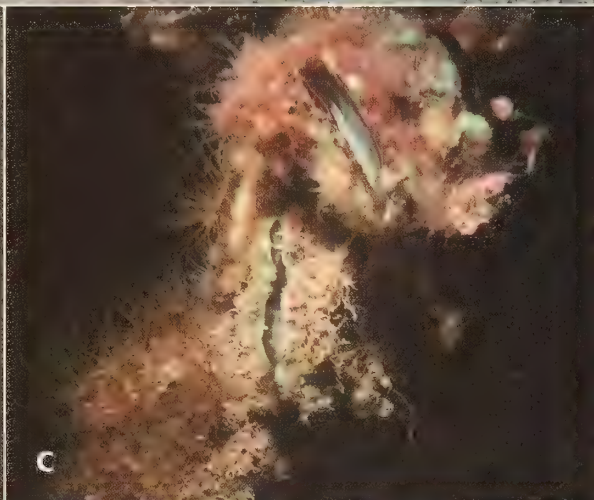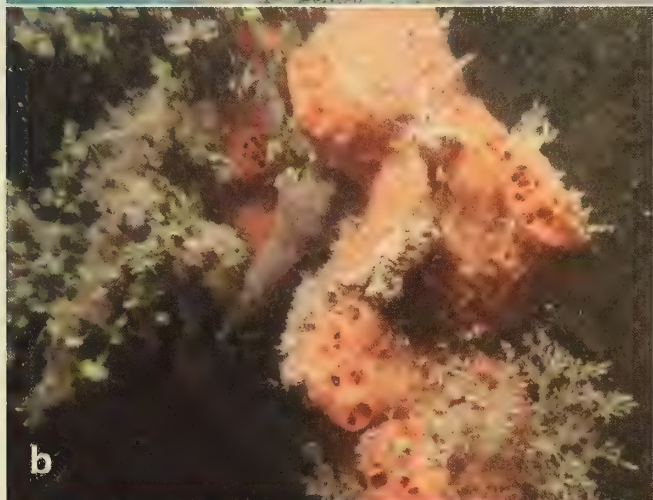
Plate 4

Plate 5. Habitats of south Twin Cays locations: *a,* mangrove root at Lair Channel neck; the older root (right) is covered by the sponges *Haliclona implexiformis* (violet) and *Biemna caribea* (yellow); the sprout is overgrown by *Clathria venosa* (gray) and, on the new tip, turquoise *H. manglaris; b,* dark ceiling of peat cave covered by sponges, with mangrove root sprouting from above; sponges include *H. implexiformis* (pinkish violet), *H. curacaoensis* (light gray), *H. manglaris* (turquoise), *Clathrina* cf. *coriacea* (yellow); c, same habitat as *b* above, soft, cream colored ascidian crust, *Diplosoma* sp.; *d,* ascidian *Distaplia corolla,* a prominent colonizer of mangrove substrata at Twin Bays (sta. 10G); *e,* Sponge Haven fringe (sta. 10H) with researchers displaying a mangrove root heavily populated by sponges; *f,* Main Channel entrance, mini-reef among *Thalassia* seagrass composed of fire sponge *(Tedania ignis)* agglutinating calcified algal *(Halimeda)* thalli.

Plate 5

Plate 6. Hidden Creek habitats and common organisms: *a,* mangrove root hanging over peat-bank undercut overgrown by sponges, *Chalinula molitba* (purple), *Lissodendoryx* (greenish gray), and *Tedania* (orange red); *b,* seagrass *Thalassia* at Boston Bay entrance heavily overgrown by filamentous cyanobacteria, *Lyngbya; c,* turtlegrass blades covered by ascidian, *Didemnum conchyliatum; d,* corroded peat bank with partially exposed mangrove roots covered by sponge, *Biemna caribea; e,* anemones, *Aiptasia tagetes* on roots; *f,* unidentified sabellid worms; *g,* Hidden Lake bottom with upside-down jellyfish, *Cassiopea.*
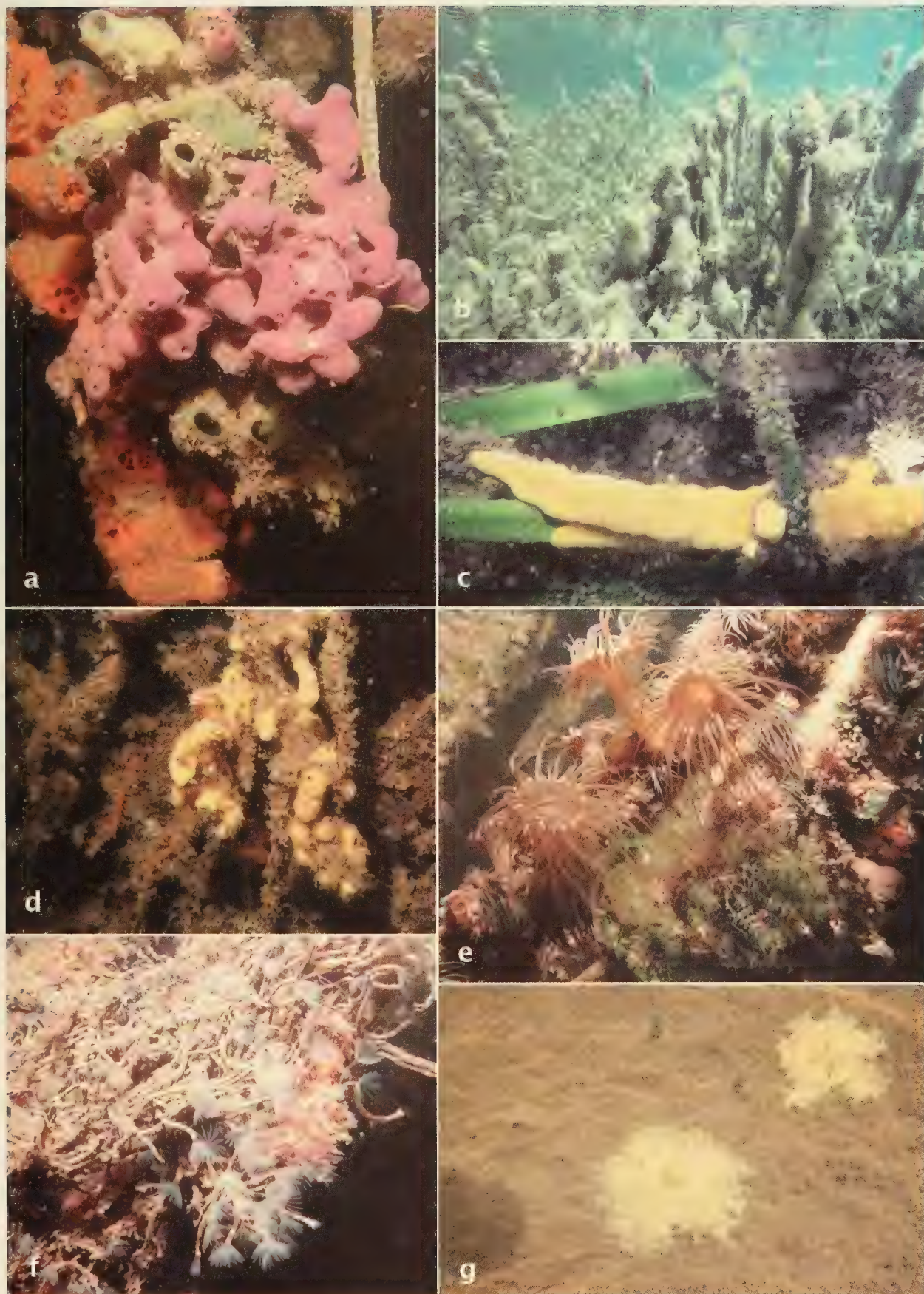
Plate 6

MANGROVE LANDSCAPE CHARACTERIZATION AND CHANGE
IN TWIN CAYS, BELIZE USING AERIAL PHOTOGRAPHY
AND IKONOS SATELLITE DATA

BY

WILFRED RODRIGUEZ AND ILKA C. FELLER
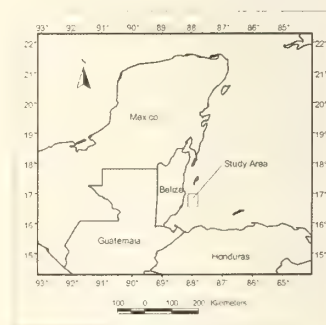
Figure 1. Twin Cays archipelago as imaged from IKONOS-2 December 16, 2003.

MANGROVE LANDSCAPE CHARACTERIZATION AND CHANGE IN TWIN CAYS, BELIZE USING AERIAL PHOTOGRAPHY AND IKONOS SATELLITE DATA

BY

WILFRID RODRIGUEZ[1] AND ILKA C. FELLER[1]

ABSTRACT

We used aerial black-and-white and color photography, in conjunction with multispectral IKONOS satellite imagery, to classify mangrove vegetation and to characterize the spatial distribution of deforestation from 1986 to 2003 at Twin Cays, an intertidal mangrove archipelago located in the Mesoamerican Barrier Reef ecosystem in Belize, Central America. The classification map consists of seven classes and 29 subclasses that reflect the present (up to 2003) condition of mangrove forests (e.g., species, growth status, and deforestation) in the island. Land cover change analysis during this 15-year period showed a 52% increase in deforestation of mangrove communities across the archipelago, the creation of numerous survey lines, and the disappearance of parts of the fringe zone. The vegetation map presented in this study will help us develop spatial relationships at the plot and landscape scales between mangrove growth patterns and biogeochemical, nutrient cycling processes, and hydrological data in follow up studies. Our results could also be used by natural resource managers as a decision-making tool for sustainable management of mangrove tropical ecosystems in the Caribbean and other regions.

INTRODUCTION

The ecological importance of mangrove forests in tropical areas has long been recognized (Chapman, 1969; Blasco, 1988 a,b). Mangrove forests are a characteristic feature of coastal shorelines and reef ecosystems of the tropics and subtropics; their root systems (prop roots and pneumatophores) stabilize the sediment, dampen wave energy, provide habitat and shelter for numerous organisms, and form the base of the nearshore marine foodweb (Vicente et al., 1993).

A partial regional inventory (Snedaker, 1993) estimated that the five species (*Avicennia germinans* L. Stearn, *Cornocarpus erectus* L., *Laguncularia racemosa* L., *Gaertn.* F., *Pelliciera rhizophorae* Triana and Planchon, and *Rhizophora mangle* L.) that form the mangrove flora of the Intra-Americas Sea occupy an area of approximately 3.2 million hectares or ~15% of an estimated total world area of mangroves of 22 million hectares. Snedaker (1993) also showed that in Belize, island and land area of mangrove forests has been estimated as 75,000 ha (Fisheries Unit Laboratory, Belize City), 100,000

[1]Smithsonian Environmental Research Center, PO Box 28, Edgewater, MD 21037

ha (Klaus Rützler, Smithsonian Institution, Washington), and 244,000 ha (Oscar Rosado, Ministry of Natural Resources, Belmopan).

In Central America and the Caribbean, deforestation practices for farming and development purposes have been the direct cause of destruction of mangrove communities (Ibrahim and Hashim, 1990; Ramirez-Garcia et al., 1998; Chauvaud et al., 2001; Sanchez-Azofeifa et al., 2003). Clearing of mangrove forests continues up to the present throughout the tropics, and it is especially worrisome when it happens in peat-based islands like Twin Cays (Macintyre et al., 1995) along the Belizean reef system. With that type of geomorphology human pressures to develop the landscape would only bring rapid ecological degradation.

In the Mesoamerican Barrier Reef ecosystem along the Belize coast, research carried out over approximately two decades has focused on the biodiversity, nutrient cycling, biogeochemistry, and sensitivity of mangrove forests existing in tidal islands like Twin Cays (e.g., Feller, 1995; Rützler and Feller, 1996; McKee et al., 2002; Feller et al., 2003 a,b,c). Woodroffe (1995) conducted a vegetation classification of Twin Cays based on seven categories of land cover (e.g., unvegetated flat, *Rhizophora* scrub, *Rhizophora* thicket, *Rhizophora* woodland, *Avicennia* woodland, *Avicennia* open woodland with *Rhizophora* scrub, and dead *Rhizophora*). His classification was an important first step but it was not detailed enough to discriminate mangrove status in relation to hydrology, nutrient availability, and disturbance. Characterization and quantification of the spatial heterogeneity of these processes are needed to understand the history of mangrove growth and to predict the impact of future landscape changes to these sensitive ecosystems.

As our understanding of a number of ecological processes (i.e., nutrient cycling, hydrology) in island mangrove forests in the Caribbean evolves, there is a need for an accurate and detailed characterization and monitoring of land use/cover status, and land-cover change. Land use refers to human influences and productive aspects of the landscape, while land cover describes the biophysical attributes of the surface. Land cover is a fundamental parameter that provides a descriptive definition of the biomes present at a location. It is regarded as the most important aspect of global change affecting ecological systems (Vitousek, 1994). Land cover also has significant effects on basic ecological processes, including biogeochemical cycling, global warming (Skole and Tucker, 1993; Penner, 1994) and soil erosion, which affects land use sustainability (Douglas, 1999). Land cover is also estimated to be the most important variable affecting biodiversity (Chapin et al., 2000; August et al., 2002). Human induced land-cover changes are significant and are more relevant to change detection than natural causes (Running et al., 1999).

Use of remotely sensed data has been fundamental in the quantification of land-cover at different scales. The recent deployment of satellite sensors such as IKONOS (Space Imagining Corp., Thornton, CO, USA) launched in 1999 provides the finest spatial resolution publicly available from space – 1-m panchromatic and 4-m multispectral (blue, green, red, and near-infrared (Table 1)) – with high radiometric fidelity and geometric accuracy (Dial et al., 2003; Zanoni and Goward, 2003). The ease of its integration with other geographical digital data (i.e., aerial photography) within a Geographic Information System (GIS) presents an ideal platform to advance study of the biocomplexity of mangrove communities in the Caribbean. Classification of tropical

ecosystems using high-resolution, remotely-sensed images is a cost-effective method to update and quantify land-cover distributional patterns and spatio-temporal changes in island ecosystems due to natural or anthropogenic pressures including deforestation, the dynamics of clearing, abandonment, regrowth and re-clearing.

Our main objectives in this study were to: 1) characterize the spatial distribution of mangrove species as it relates to growth, tidal influence, and human disturbance using high spatial resolution (1-m) aerial photography and IKONOS satellite data; and 2) quantify land-cover changes resulting from deforestation over a 15-year period from 1986 to 2003. An important goal of this study was to determine the current state (until December 2003) of mangrove forests in Twin Cays. That information is critical in the spatial integration of hydrological and biogeochemical data and their relationship to mangrove growth. The new functional classification presented in this manuscript will also serve as the principal reference data for a subsequent image classification of IKONOS satellite imagery based on computerized spectral pattern recognition.

Table 1. IKONOS instruments characteristics.

Band	Electromagnetic Spectrum	Wavelength (μm)	Ground Resolution (m)
1	BLUE	0.45 - 0.52	4
2	GREEN	0.52 - 0.60	4
3	RED	0.63 - 0.69	4
4	Near IR	0.76 - 0.90	4
Panchromatic		0.45 - 0.90	1

METHODS

Study Area

This study was conducted in Twin Cays, a peat-based archipelago of off-shore mangrove islands just inside the crest of the barrier reef of central Belize, approximately 12 km from the mainland. Most of the 75 ha of land mass at Twin Cays is in two islands, East Island and West Island. The geology of the island consists of a carbonate substrate made up of a dense limestone formed by finger corals and mollusk fragments overlaid by 8 to 12 m of peat that has accumulated over the past 8000 years (Macintyre et al., 1995). Twin Cays receives no terrigenous inputs of freshwater or sediments. The shoreline gradient is intertidal but physiognomically varied and interrupted by tidal creeks that bring water into the interior area of the island flats and shallow ponds. The vegetation is dominated by *Rhizophora mangle* L. (red mangrove), *Avicennia germinans* (black mangrove), and *Laguncularia racemosa* (white mangrove). Forests here are characterized by a pronounced tree-height gradient, which parallels other gradients such as productivity and tidal flushing. The tree-height gradient can be subdivided into three zones. From the sea to landward, the seaward-most zone is a narrow fringe of uniformly tall red mangrove

4

trees (5-6 m). Next is a transition zone (2-4 m tall), where all three mangrove species are present, followed by a zone of uniformly stunted, dwarf red mangrove trees (~1.5 m), which form vast stands in the interior of the islands. The Smithsonian Institution's Marine Field Station on nearby Carrie Bow Cay, approximately 5 km from the study site, provided laboratory, living accommodations, and logistical support during the fieldwork (Rützler and Feller, 1996). Staging of equipment and boat support were also provided at the Smithsonian's research station.

Data and Image Processing

Characteristics of the digital data used in this study are presented in Table 2. The 1986 black-and-white photo (photographed by Royal Air Force) and 2003 color photo (photographed by Ilka C. Feller) were scanned, saved in tagged image file format (tiff) and registered to the satellite images. Satellite data included 1-m multispectral IKONOS images for the years 2001 and 2003.

Image preprocessing of satellite imagery consisted of radiometric and geometric corrections, and spectral enhancement prior to classification and change detection analysis. For geometric correction of both aerial and satellite data, we used the image-to-image registration method with the IKONOS 2001 image as reference. All images were georeferenced to the Universal Transverse Mercator (UTM) coordinate system, and spatial resolution was maintained at 1 m. The root-mean-square error between scene coregistrations was maintained at ± 0.5 pixels to avoid misregistration problems. Because of image warping and other inconsistencies found in the aerial photography data that prevented their perfect overlay on the new satellite data, we used a rubber-sheet model with approximately 320 Ground Control Points (GCPs) in their coregistration process. Spectral enhancement was done on both IKONOS images to help us in the creation of boundary polygons and vegetation classification; they included: Principal Component Analysis (PCA), Normalized Difference Vegetation Index (NDVI), natural color, and transformation of red, green, and blue (RGB) values to intensity, hue, and saturation (IHS) values (ERDAS 2003). Image processing was done with ERDAS Imagine 8.6 software (ERDAS Atlanta, GA, USA). All data were integrated into a GIS for storage, update and subsequent geographic analysis.

Land-Cover Classification

To inventory and map mangrove forests and other land cover types, it is necessary to classify remotely-sensed images into land cover categories or types. This is called thematic mapping of land-cover "themes". A number of classification schemes have been developed that can incorporate land-use and/or land-cover data obtained by interpreting remotely-sensed data. The U.S. Geological Survey Land Use/Land Cover Classification System (Anderson et al., 1976; USGS, 1992) is a resource-oriented (land-cover) scheme with eight main categories. This system contrasts with various human activity (land-use) oriented systems, such as the Standard Land Use Coding Manual (Jensen et al., 1983) or the U.S. Fish and Wildlife Service (Cowardin et al., 1979) system, which is widely used to characterize wetland systems based on hydrological, soil, and vegetation characteristics.

Classification of land-cover types in Twin Cays began with on-screen visual-interpretation and digitization of mangrove and landscape features from aerial photography taken in March 2003 and was finalized with new thematic information extracted from satellite data from December 2003 in which the latest deforestation on East Island was clearly visible (Fig.1) . The 2003 classification map was used as baseline data to classify the 1986 black-and-white image into general land cover classes (i.e., forest, ponds, etc.). Reference or ancillary data consisted of ground-truthing done for two weeks in August 2003, oblique aerial photos taken in March 2003, satellite data from 2001, and expert knowledge. Classification was accomplished using ArcInfo 8.3 (ESRI, Redlands, USA) and ERDAS Imagine 8.6 software. Classification of satellite images based on computer data categorization (spectral analysis) will be presented in a second manuscript.

Table 2. Characteristics of the satellite imagery and aerial photography used in the study.

Image	Latitude*	Longitude*	Acquisition date	Multi-spectral Files
IKONOS	16.9294	-88.1555	13 December 2003	R,G,B,NIR bands
IKONOS	16.9295	-88.1548	13 September 2001	R,G,B bands
Aerial Photo	Without coordinates		15 March 2003	Color (R,G,B)
Aerial Photo	Without coordinates		Year 1986	Black and White

Notes: * Lower left corner of the image scene, negative longitude for west. R, G, B, NIR refers to
red, green, blue, and near infrared spectral bands. Spatial resolution was 1-meter.
Coordinate system for all images: Universal Transverse Mercator (UTM), North hemisphere,
Zone number 16, Datum WGS84.

Change Detection Analysis

Two methods of change analyses were performed in this study. The first involved a simple comparison of selected land cover classes to quantify their overall percent change from 1986 to 2003. The second method of change detection is called post-classification comparison (Singh, 1986; Jensen, 1996). The advantages of this technique are that it provides 'from-to' information for each pixel and does not require data normalization (e.g., reduction of interscene variability resulting from differing atmospheric conditions, radiation incidence angle, and detector disparity) because the two dates are classified separately (Singh, 1989). However, this method does propagate error from the initial land cover maps- the accuracy of the resulting change map is dependent on the accuracy of the land cover maps used to create it (Jensen, 1996).

With five vegetation/land cover categories (U, D, B, S, F) a matrix of dimension 5 x 5 is formed, producing a total of 25 possible combinations of 'from-to' change classes (Table 3). Since we were specifically interested in quantifying changes in forest cover

6

only five combinations were included in the analysis: undisturbed-forest to deforested (UD), undisturbed-forest to beach erosion (UB), undisturbed-forest to survey lines (US), deforested to undisturbed-forest (DU), and deforested to beach erosion (DB). Because creation of survey lines on the landscape entails some deforestation, we decided to merge deforested and survey lines classes for change analysis.

Table 3. Matrix of all possible "from-to" change themes that could be formed with five land cover categories used in the classification of aerial photography from 1986 and IKONOS satellite imagery from 2003. Each row and column combination represents one type of land cover change from 1986 to 2003. For example, class UD is the change from an undisturbed mangrove forest in 1986 to a deforested area in 2003.

From (row): 1986 To (column): 2003	Undisturbed forest	Deforested	Beach erosion	Survey line	Lost fringe
Undisturbed forest	UU	UD	UB	US	UF
Deforested	DU	DD	DB	DS	DF
Beach erosion	BU	BD	BB	BS	BF
Survey line	SU	SD	SB	SS	SF
Lost fringe	FU	FD	FB	FS	FF

Note: since beach erosion and survey lines were not in evidence in the 1986 data, only undisturbed and deforested classes were considered in the "from-to" change analysis. In this study the change classes of interest included: UU, UD, UB, US, DU, DD, and DS. Shaded classes were not considered for analysis.

RESULTS

Land-Cover Classification

Using satellite and aerial photography data we were able to characterize with a high level of detail the status of mangrove forest communities in Twin Cays. The resource-oriented classification scheme developed in our study was primarily based on forest structure characteristics (e.g., height, density, and growth) and tidal-flow influences on mangrove vegetation. Classification consisted of seven general classes (Level I) and 29 subclasses (Level II) (Table 4 and Fig. 2).

A breakdown of level II classes showed 78% of the total landscape in Twin Cays, during the year 2003, characterized by six land cover types which included: (1) *R. mangle* dwarf with 25%; (2) mixed woodland with 15%; (3) fringe with 13%; (4) open pond dwarf with 10%; (5) clearcut areas with 8%; and (6) *R. mangle* floc zones (e.g. areas of decaying algal material) with approximately 7% (Table 5). Although deforested land did not make up a large percentage of the total area of Twin Cays, a recent land clearing event where 21,335 m^2 (see zone LC-2 in Fig. 2) of mangrove were cut between March and December in 2003 suggests that deforestation events will likely continue into the future.

Other thematic classes with an important ecological spatial-temporal component include algal mats, diebacks, hydrology-zone trees and relic fringe. Feller et al. (2003 a, c) have related these zones to nutrient cycling and biogeochemical processes influencing mangrove growth in Twin Cays.

Land-Cover Change

The spatial distribution of anthropogenic disturbance at Twin Cays during 1986 and 2003 are shown in Figures 3 and 4. An overall comparison of five land cover classes during this 15-year period showed a 52% increase in deforestation of mangrove communities across the archipelago, a 6% decrease in undisturbed mangrove forests, as well the creation of survey lines and the disappearance of parts of the fringe zone (Table 6).

Results from the overall comparison do not quantify land-cover changes occurring from one particular class to another, information that is necessary because change could be natural and/or anthropogenic. Post-classification change detection analysis quantifies those 'from-to' land cover categories changes. A selected number of these more detailed changes are presented in Table 7, and their spatial distribution is shown in Figure 5. Results for the undisturbed-forest category showed that on a percentage basis, change from undisturbed forest to other categories was relatively small, i.e., 90% of forest in 1986 remained undisturbed in 2003. Change to deforested areas reached 8%, beach erosion 0.3 %, and losses in mangrove forests in the fringe zone (rim of the island) about 1 %. Results for the deforested category showed and interesting result for the change from deforested to undisturbed-forest (DU) (i.e., forest regeneration). This change represented a 46% regeneration of deforested mangrove areas computed from the 1986 data.

Changes in the total area of Twin Cays showed increases of mangrove forest in the fringe zone totaling 12,850 m^2, most of if found on the southwest section of West Island (Fig. 5). Nevertheless, this gain in mangrove vegetation was somewhat offset by fringe losses in East Island that ranged from 4,762 m^2 to 7,968 m^2. The higher value was the result of including beach erosion in the fringe-loss category.

Table 4. Classification scheme, based on forest structure and tidal flow influences, used to categorize mangrove vegetation in Twin Cays, Belize.

Level I	Level II	Criteria
1. *Avicennia germinans* forest	1.1 *A. germinans* basin	Tall, straight-trunked *A. germinans*, dense *Batis maritima* understory, 4-7 m tall, not permanently flooded, flushing varies seasonally.
	1.2 *A. germinans* dwarf	Dominated by *A. germinans*, with few *R. mangle* intermixed, 1.4 < 1.5 m tall.
	1.3 *A. germinans* orchard	Widely spaced trees, little understory, spreading canopies, 3-4 m tall, not permanently flooded, flushing varies seasonally.
	1.4 *A. germinans* scrub	Sparse and slow growth, lots of dead wood, along moribund zones, not permanently flooded, flushing varies seasonally.
	1.5 *A. germinans* woodland	Dense stands of *A. mangle* in the interior of the island, 2-3 m tall
	1.6 Regenerating *A. germinans*	New growth, dense, small trees, intermixed *R. mangle* and *L. racemosa*, 2-3 m tall.
	1.7 Lone *A. germinans* tree	Large trees (5-6 m tall) standing alone.
2. *Rhizophora mangle* forest	2.1 *R. mangle* dwarf	*R. mangle* stand in shallow ponded areas in the interior, ≤1.5 m tall.
	2.2 *R. mangle* floc zone	Pure *R. mangle* stand adjacent to floc accumulating areas along ponds in the interior of the island, vigorous, fast growing, flooding varies.
	2.3 *R. mangle* woodland	Dense stands of *R. mangle* in the interior of the island, 2-3 m tall
	2.5 Lone *R. mangle* tree	Large trees (5-6 m tall) standing alone.
	2.6 Fringe	Pure *R. mangle* stand along water's edge around the periphery of the islands, 4-7 m tall, not permanently flooded but flushed daily.
	2.7 Relic fringe	Remnants of old area *R. mangle* fringes where water flow once existed.
	2.8 Moribund	Typically adjacent to floc zone tree, lots of dead branches and trees, dominated by *R. mangle*, 2-3 m tall, not permanently flooded, flushing varies seasonally.

Level I	Level II	Criteria
3. Mixed forest	3.1 *R. mangle/A. germinans* dwarf	Mixed dwarf stand, but usually dominated by *R. mangle*, typically dense, often with *Distichlis spicata* understory, ≤1.5 m tall.
	3.2 Mixed woodland	*R. mangle* dominated, *A. germinans* & *L. racemosa* mixed, 2-4 m tall, not permanently flooded, flushing varies seasonally.
4. Agriculture	4.1 Planted coconut trees	Deforested areas, filled and used for planting schemes.
5. Water	5.1 Ponds	Unvegetated, relatively deep, soft bottom.
	5.2 Open pond dwarf	Sparse vegetation, shallow, firm bottom, *Batophora oerstedi* subtidally on roots and peat.
	5.3 Algal mats	Alongside ponds, thick crust on surface of deep floc layer.
6. Barren	6.1 Beach	Sandy areas along the shoreline.
	6.2 Clear-cuts	Deforested areas without mangrove trees, herbaceous vegetation only.
	6.3 Eroded shoreline	Areas where deforestation of fringe accelerated the effect of wave erosion.
	6.4 Dieback	Patches of dead mangrove trees, usually adjacent to floc accumulation areas,
7. Other	7.1 Experimental trees	Phosphorus fertilized dwarf trees, vigorous growth, 2-5 m tall.
	7.2 Hydrology zone trees	Trees alongside tidal channels, increased flushing, vigorous growth.
	7.3 Saplings	Young trees alongside floc accumulation area, vigorous growth.

10

Table 5. Thematic classes identified on the 2003 classification map of Twin Cays, Belize. Their corresponding areas, relative importance (% of total area) and perimeters were quantified with a geographic information system (GIS).

Land Cover Classification (Level II)	Area (m^2)	Percent of Total (%)	Perimeter (m)
Algal Mats	3,214.8	0.43	1,295.4
A. germinans Basin	5,842.0	0.78	656.0
A. germinans Dwarf	1,495.0	0.20	216.0
A. germinans Orchard	12,033.0	1.61	1,686.0
A. germinans Scrub	22,805.0	3.05	4,074.0
A. germinans Woodland	180.0	0.02	68.0
Beach	484.0	0.06	168.0
Clearcut	59,146.4	7.90	4,472.8
Coastal Scrub	2,101.0	0.28	484.0
Dieback	3,006.0	0.40	984.0
Eroded Shoreline	2,819.0	0.38	548.0
Experimental Trees	2,363.0	0.32	1,214.0
Fringe	99,667.3	13.31	21,808.6
Hydrology Zone Trees	4,034.0	0.54	880.0
Lone *A. germinans*	1,713.0	0.23	496.0
Lone *Laguncularia*	117.0	0.02	58.0
Lone *R. mangle*	2,667.0	0.36	1,132.0
Mixed Woodland	115,102.3	15.38	15,092.2
Moribund *R. mangle*	3,124.2	0.42	705.9
Open Pond Dwarf	73,305.2	9.79	9,107.4
Planted Coconuts	8,949.0	1.20	758.0
Pond	28,588.0	3.82	2,800.0
Regenerated *A. germinans* Forest	8,174.0	1.09	676.0
Relic Fringe	4,426.9	0.59	803.2
R. mangle Dwarf	189,006.2	25.25	21,638.6
R. mangle Floc Zone	49,882.0	6.66	12,570.0
R. mangle Woodland	24,222.0	3.24	6,072.0
R. mangle / *A. germinans* Dwarf	19,166.0	2.56	2,500.0
Saplings	995.0	0.13	464.0
Total	748,628.4		113,428.2

10

Table 6. Comparison of areas under different land cover during two periods, 1986 and 2003.

Land Cover Class	1986 Area (m^2)	Percent of Total Area (%)	2003 Area (m^2)	Percent of Total Area (%)	Percent Change in Land Cover 1986 - 2003
Undisturbed forest	615,493	82	578,205	77	-6
Deforested	30,349	4	63,547	8	+52
Beach erosion	0	0	3,339	0.4	+100
Survey lines	0	0	2,119	0.3	+100
Lost fringe	0	0	4,791	1	+100

Note: Percent change = $((A_{2003} - A_{1986}) / A_{2003}) \times 100$

Table 7. Post-classification change detection analysis using deforested and survey line classes as cover. "From-to" change analysis determined change from two land-cover classes in 1986 (e.g. undisturbed and deforested) to undisturbed, deforested, beach erosion, and lost fringe categories in 2003.

Year 1986	Year 2003			
	Undisturbed forest	Deforested	Beach erosion	Lost fringe
Undisturbed forest (m^2)	551,376	51,322	2,034	3,704
Percent (%)	90	8	0.3	1
Deforested (m^2)	14,103	14,058	1,305	720
Percent (%)	46	46	4	2

Note: Areas are given in m^2. Percent values are based on 1986 total areas (Table 6).

Year 1986	Year 2003			
	Undisturbed (U)	Deforested (D)	Beach erosion (B)	Lost fringe (F)
Undisturbed (U)	653,269	51,322	2,034	3,704
Percent	91.06	7.15	0.28	0.52
Deforested (D)	14,103	14,058	1,305	720
Percent	46.47	46.32	4.30	2.37

Note: Areas are given in square meters. Percent values are based on 1986 areas.

DISCUSSION

"Habitat fragmentation is the most serious threat to biological diversity and is the primary cause of the present extinction crisis" (Wilcox and Murphy, 1985). Habitat fragmentation causes the fracture of ecosystem processes, it hinders dispersal and movement of species, and it also increases invasion from generalists into the interior of isolated habitats. Spatio-temporal processes in mangrove forest communities have not been studied extensively. Spatial patterns play an important part in plant community dynamics. These patterns reflect a complex series of interactions of past-and-present events and form the basis for future states of growth and condition (Herben et al., 2000).

Land Cover Classification

We have used multitemporal and multispectral data to classify and characterize the landscape in Twin Cays archipelago. The high number of vegetation themes (e.g., 29 land-cover classes) used in the classification presented in this study were the result of an effort-intensive methodology based on visual-interpretation, on-screen digitization, ground-truthing, and expert knowledge. The next step in our analysis will be to classify satellite data based on computerized spectral-pattern recognition using the present classification results as training polygons, reducing processing time and providing accurate characterization of other areas along the Mesoamerican Barrier Reef and the coast of Belize.

The land-cover classification developed in this study is part of the basic digital database needed in the development of a comprehensive spatially-explicit biogeochemical model of mangrove forests at the field and landscape scales (i.e., distribution of Net Primary Productivity (NPP) of mangrove forests). Such quantitiative information can be important in sustainable resource management and climate change studies.

Land Cover Change

In change detection analysis, each natural and anthropogenic change in the landscape has a characteristic cause and effect. It is important to inventory all of the forms of change. The natural changes often dictate human action, and form the basis for much environmental research. The human change is important to inventory to determine the long term effects to undisturbed mangrove forests. This, in turn, will help interested parties to define the kind of policy that encourages intelligent use of mangrove forest resources and protects their valuable contribution to the overall health of the Belizean Reef ecosystem.

Land-cover change in Twin Cays has been dramatic during a 15-year period and it is clear that it can occur within very short periods of time as it was found from imagery taken within a 9-mo interval in 2003. The impact of this type of deforestation on mangrove net primary productivity and other biogeochemical- and nutrient-cycling processes in Twin Cays is still unknown. The series of aerial photographs and satellite images from 1986, 2001, and 2003 revealed that the changes in the Twin Cays' landscape were mainly attributable to anthropogenic activity. Additional oblique photographs of the islands taken in 1991 are our first records of survey lines in the island's interior which were cut to mark the boundary lines of at least seven leases to land at Twin Cays. These lines remain clearly visible in the subsequent aerial photographs and satellite images and have been quantified in the study presented here (Fig.5). In some instances, the areas delineated by the survey lines were later deforested. In addition, two of the clear-cut areas were filled with bottom materials dredged from adjacent waters. Change detection analysis showed that 6% of the landmass at Twin Cays had gone from undisturbed to disturbed. However, observations while ground-truthing these data suggest that hydrological changes caused by the cutting of mangroves have magnified the impact of the disturbance. For example, newly formed tidal channels have developed along the survey lines and have altered the water-flow patterns into the interior of the island. Thus, they provide a more rapid and frequent renewal of ocean water to interior ponds. Wright et al. (1991) showed that prior to these survey tracks, tidal exchange in these areas was extremely restricted. We observed that the growth pattern of dwarf forest along these survey lines had changed in response to the altered hydrological regimes. As a result, we created a vegetation class, hydrology-zone trees, to describe the distinctive stands of vigorously growing trees found along these newly formed tidal creeks. Studies are continuing to investigate the relationship of the hydrologic changes, tidal flushing, nutrients, and mangrove growth characteristics. Experimental studies at Twin Cays have recently documented a spatial N- to P-limitation gradient across the tree-height gradient, with fringe trees N-limited and dwarf trees P-limited (Feller et al., 2003a). Fertilization with N or P also altered nutrient concentrations of the mangrove tissue. Within 5 yrs, P-fertilized dwarfs were transformed into tall, vigorous trees, similar in growth, nutrient content, and architecture to similar fringe trees located in the hydrology zone.

CONCLUSIONS

Integration of aerial photography and multitemporal, multispectral high spatial resolution satellite imagery within a GIS was used to classify and characterize the status of mangrove forests in Twin Cays. We have developed a number of functional classification themes of mangrove forests based on species, growth status, and deforestation. Results from this new classification will be the basis for future geospatial modeling of the spatial connectivity between nutrient accumulation zones (i.e., algal mats), water-flow changes, and mangrove growth in the island. We have also quantified the changes in mangrove forest cover during the 1986 to 2003 period. Deforestation of mangrove forests has been concentrated mostly on East Island, and seem to continue unabated as recent visits to the island confirm it.

To test and develop new hypotheses of forest change and fragmentation resulting from anthropogenic pressures, the integration of high-spatial multitemporal and multispectral remote sensing/GIS techniques should be applied to the rest of the Mesoamerican Barrier Reef Ecosystem and mainland coastal mangrove ecosystems. From the point of view of mangrove forest conservation and sustainable resource development in the barrier-reef ecosystem, it is clear that new approaches and mechanisms, such landscape and regional monitoring, as well as the creation of buffer zones around protected areas, could be important goals of government and park managers.

ACKNOWLEDGMENTS

Land Cover Classes

- Algal Mats
- Avicennia Basin
- Avicennia Dwarf
- Avicennia Orchard
- Avicennia Scrub
- Avicennia Woodland
- Beach
- Clearcut
- Coastal Scrub
- Dieback
- Eroded Shoreline

- Experimental Trees
- Fringe
- Hydrology Zone Trees
- Lone Avicennia
- Lone Laguncularia
- Lone Rhizophora

- Mixed Woodland
- Moribund Rhizophora
- Open Pond Dwarf
- Planted Coconuts
- Pond
- Regenerating Avicennia Forest

- Relic Fringe
- Rhizophora Dwarf
- Rhizophora Floc Zone
- Rhizophora Woodland
- Rhizophora/Avicennia Dwarf
- Saplings
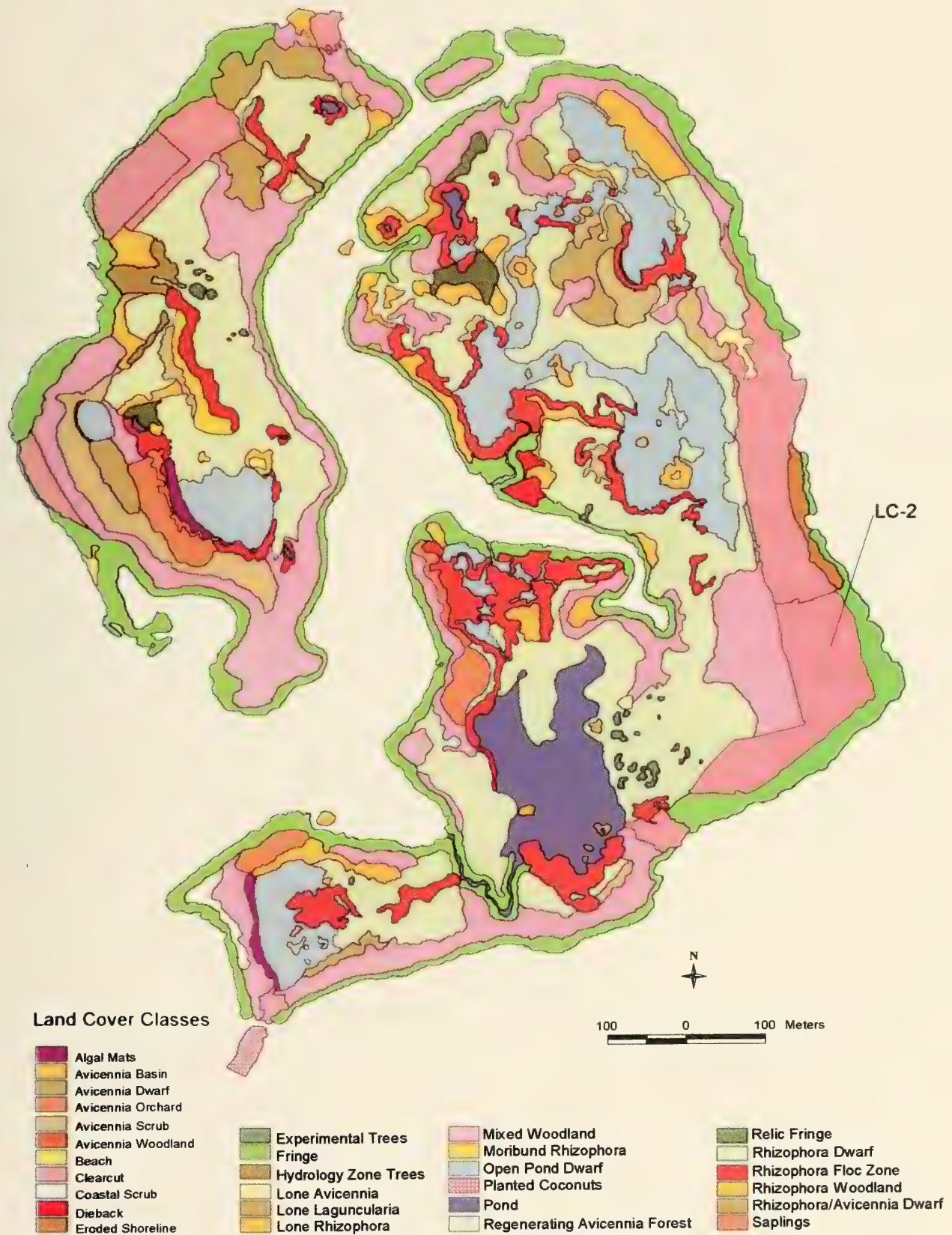
Figure 2. Classification of mangrove vegetation and land cover in Twin Cays, Belize.

16

TWIN CAYS, BELIZE

East Island

West Island

100   0   100 Meters

Land Disturbance in 1986
Deforested
1986 Boundary
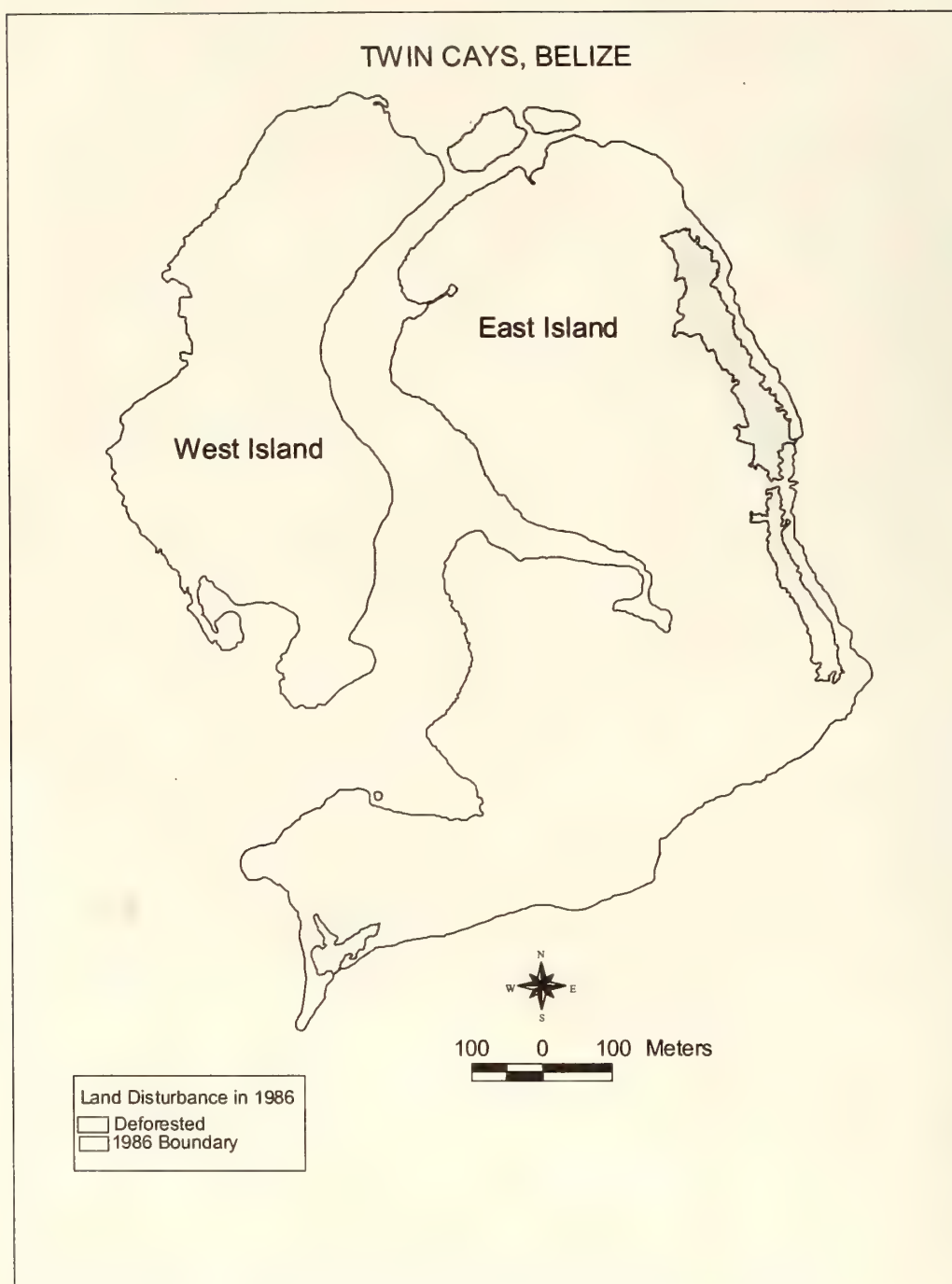
Figure 3. Spatial extent of land clearing in the east section of Twin Cays as quantified from a black-and-white photograph taken in 1986.
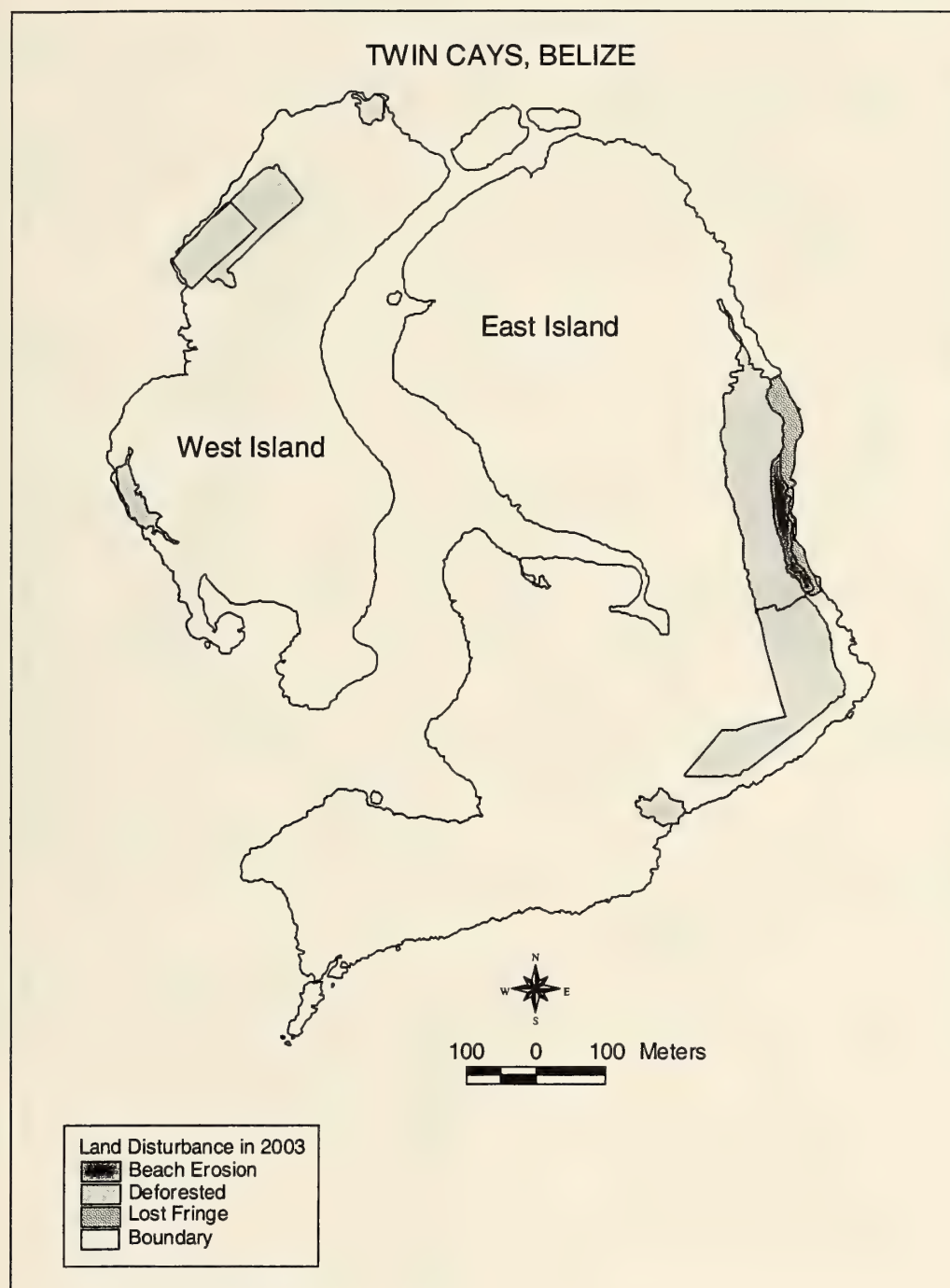
Figure 4. Spatial extent of land clearing, fringe loss, and beach erosion in Twin Cays as quantified from aerial photography (March 2003) and IKONOS satellite imagery (December 2003).

18

Figure 5. Spatial distribution of land-cover change at Twin Cays from 1986 to 2003. This analysis only considered the 'from-to' information from land cover classes of undisturbed and deforested mangrove forests from 1986 to undisturbed, deforested, beach erosion, and lost fringe classes in 2003.

REFERENCES

Anderson, J., E. Hardy, J. Roach, and R. Witmer
  1976. *A land use classification system for use with remote-sensor data.* U.S.
    Department of Interior, U. S. Geological Survey Professional Paper 964,
    Washington, DC.
August, P. V., L. Iverson, and J. Nugranad
  2002. Human conversion of terrestrial habitats. In *Applying Landscape
    Ecology in Biological Conservation*, edited by K. Gutzwiller, 198-224,
    Springer-Verlag, NY.
Blasco, F.
  1988a. The international vegetation map (Toulouse, France). In. *Vegetation Mapping,*
    edited by A. W. Kuchler and I. S. Zonnefeld, 443-460, Junk Publishers,
    Amsterdam.
Blasco, F.
  1988b. *Estudio Sobre los Manglares y la Vegetacion Tropical Utilizando
    Datos Proporcionados por Satellites.* Institute de la Carte Internationale
    de la Vegetation. University Paul Sabatier, Toulouse.
Chapin, F. S. III, E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L.
Reynolds, D. U. Hopper, S. Lavorel, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Diaz.
  2000. Consequences of changing biodiversity. *Nature* 405:234-242.
Chapman, V. J.
  1969. Lagoons and mangrove vegetation. In *Lagunas Costeras, un Simposio*, edited
    by A. Ayala-Castanares and F. B. Phleger, 504-514, UNAM-UNESCO, No. 28-
    30, Mexico.
Chauvaud, S., C. Bouchon, and R. Maniere
  2001. Cartographie des biocenoses marines de Guadeloupe a partir de donnees SPOT
    (recifs coralline, phanerogames marines, mangroves). *Oceanologica Acta* 24:
    S3-S16.
Cowardin, L., V. Carter, F. Golet, and E. LaRoe.
  1979. *Classification of Wetlands and Deepwater Habitats of the United States.* U.S.
    Department of Interior, U.S. Fish and Wildlife Service, Report No. FWS/OBS-
    79/31, Washington, DC.
Dial, G., H. Bowen, F. Gerlach, J. Grodecki, and R. Oleszczuk
  2002. IKONOS satellite imagery, and products. *Remote Sensing of the Environment*
    88:23-36.Douglas, I.
  1999. Hydrological investigations of forest disturbance and land cover impacts in
    South–East Asia: a review, *Philosophical Transactions of the Royal Society of
    London, Series B,* 354:1725-1738.
ERDAS.
  2003. *Earth Resources Data Analysis System.* Field Guide, ERDAS Inc., Atlanta, GA.
Feller, I. C.
  1995. Effects of nutrient enrichment on growth and herbivory in dwarf red mangrove
    (*Rhizophora mangle*). *Ecological Monographs* 65:477-505.
Feller, I. C.
  1996. Effects of nutrient enrichment on leaf anatomy of dwarf *Rhizophora mangle* L.

20

(Red Mangrove). Biotropica 28:13-22.

Feller, I. C., and W. N. Mathis
  1997. Primary herbivory by wood-boring insects along an architectural gradient of *Rhizophora mangle* L. *Biotropica* 29:440-451.

Feller, I. C., K. L. McKee, D. F. Whigham, and J. P. O'Neill
  2003a. Nitrogen vs. phoshphorous limitation across an ecotonal gradient in a mangrove forest. *Biogeochemistry* 62:145-175.

Feller, I. C., D. F. Whigham, K. L. McKee, and C. E. Lovelock
  2003b. Nitrogen limitation of growth and nutrient dynamics in a disturbed mangrove forest, Indian River Lagoon, Florida. *Oecologia* 134:405-414.

Feller, I. C., C. E. Lovelock, K. L. McKee, M. C. Ball, and J. Ellis
  2003c. Biocomplexity in mangrove forests: Indo-West pacific vs. Atlantic-East comparison of the effects on nutrient availability on growth and herbivory. *ERF 2003 Conference Abstracts,* September 14-19, 2003, Seattle, WA.

Forman, R. T. T., and M. Godron
  1986. *Landscape Ecology,* John Wiley, NY.

Forman, R. T. T.
  1995. *Land Mosaics: The Ecology of Landscapes and Regions*. Cambridge University Press, Cambridge.

Gill, A. M. and P. B. Tomlinson
  1971. Studies on the growth of red mangrove (*Rhizophora mangle*). Part 3. Phenology of the shoot. *Biotropica* 3:109-124.

Herben, T., H. J. During and R. Law
  2000. Spatio-temporal patterns in grassland communities. In *The Geometry of Ecological Interactions: Simplifying Spatial Complexity*, edited by U. Dieckman, R. Law, and J. A. J. Metz, 48-64. Cambridge University Press.

Ibrahim S., and I. Hashim
  1990. Classification of mangrove forest by using 1:40 000 scale aerial photographs. *Forest Ecology and Management* 33:583-592.

Jensen, J. R.
  1983. Urban-suburban land use analysis. In *Manual of Remote Sensing,* edited by R. Colwell, 1571-1666. American Society of Photogrammetry.

Jensen, J. R.
  1996. *Introductory Digital Image Processing: A Remote Sensing Perspective*. Prentice-Hall, Inc.

Lyon, G. J.
  2001. *Wetland Landscape Characterization: GIS, Remote Sensing, and Image Analysis*. Ann Arbor Press, MI.

Macintyre I. G., M. M. Littler, and D. S. Littler
  1995. Holocene history of Tobacco Range, Belize, Central America. *Atoll Research Bulletin* 430:1-18.

McKee, K. L., I. C. Feller, M. Popp, and W. Wanek
  2002. Mangrove isotopic fractionation (^{15}N and ^{13}C) across a nitrogen versus phosphorous limitation gradient. *Ecology* 83:1065-1075.

Penner, J. E.
  1993. Atmospheric chemistry and air quality. In *Changes in Land Use and Land*

Cover: A Global Perspective, edited by W. B. Meyer and B. L.Turner II, 175-209. Cambridge University Press, Cambridge.

Ramirez-Garcia, P., J. Lopez-Blanco, and D. Ocana
    1997. Mangrove vegetation assessment in the Santiago River Mouth, Mexico, by means of supervised classification using Landsat TM imagery. *Forest Ecology and Management* 105:217-229.

Running, S. W., G. J. Collatz, J. Washburne, S. Sorooshian, T. Dunne, R. E. Dickinson, W. J. Shuttleworth, C. J. Vorosmarty, and E. F. Wood
    1999. Land ecosystems and hydrology. In *EOS Science Plan: The State of Science in the EOS Program,* edited by M. D. King, 197-257). Office of Earth Science, NASA Headquarters, Washington DC.

Rützler, K., and I. C. Feller
    1988. Mangrove swamp communities. *Oceanus* 30:16-24.

Rützler, K, and I. C. Feller
    1996. Caribbean mangrove swamps. *Scientific American* 274:94-99.

Sanchez-Azofeifa, G. A., G. C. Daily, A. S. P. Pfaff, and C. Busch
    2003. Integrity and isolation of Costa Rica's national parks and biological reserves: examining the dynamics of land-cover change. *Biological Conservation* 109:123-135.

Singh, A.
    1986. Change detection in the tropical rain-forest environment of Northeastern India using Landsat. In *Remote Sensing and Tropical Land Management,* edited by M. J. Eden and J. T. Parry, 237-254. John Wiley & Sons, London.

Singh, A,
    1989. Digital change detection techniques using remotely-sensed data. *International Journal of Remote Sensing* 10: 989-1003.

Skole, D., and C. J. Tucker
    1993. Tropical deforestation and habitat fragmentation in the Amazon: Satellite data from 1978 to 1988. *Science* 260:1905-1910.

Snedaker, S.
    1993. Impact on Mangroves. In *Climatic Change in the Intra-Americas Sea: Implications of Future Climate on the Ecosystems and Socioeconomic Structure in the Marine and Coastal Regions of the Caribbean Sea, Gulf of Mexico, Bahamas, and the Northeast Coast of South America,* edited by G. A. Maul, 282-305. Chapman and Hall, Inc, NY.

USGS
    1991. *Standards for Digital Line Graphs for Land Use and Land Cover Technical Instructions.* Referral STO-1-2. US Government Printing Office Washington, DC.

Vicente, V. P., N. C. Singh, and A. V. Botello
    1993. Ecological implications of potential climate change and sea-level rise. In *Climatic Change in the Intra-Americas Sea: Implications of Future Climate on the Ecosystems and Socioeconomic Structure in the Marine and Coastal Regions of the Caribbean Sea, Gulf of Mexico, Bahamas, and the Northeast Coast of South America,* edited by G. A. Maul, 262-281. Chapman and Hall, Inc., NY.

22

Wicox, B. A. and D. D. Murphy
    1985. Conservation strategy: the effects of fragmentation on extinction. *American
        Naturalist* 125: 879-887.
Woodroffe, C. D.
    1995. Mangrove vegetation on Tobacco Range and nearby mangrove ranges, central
        Belize barrier reef. *Atoll Research Bulletin* 427:1-35.
Wright, R., D. Urish, and I. Runge
    1991. The Hydrogeology of a Caribbean Mangrove Island. *Proceedings of the
        Coastlines of the Caribbean Coastal Zone* 1991 Conference, ASCE, Long
        Beach, CA.
Vitousek, P. M.
    1994. Beyond global warming: ecology and global change. *Ecology* 75:1861-1876.
Zanoni, V. M., and S. N. Goward
    2004. A new direction in Earth observations from space: IKONOS. *Remote Sensing of
        Environment* 88:1-2.

THE DINOFLAGELLATES OF TWIN CAYS, BELIZE: BIODIVERSITY,
DISTRIBUTION, AND VULNERABILITY

BY

MARIA A. FAUST

Twin Cays, Belize

2002

North Point **BIG DIPPER** **LITTLE DIPPER**

3 houses built '95-'96

North Bay

Cassiopea Cove

Batfish Point *Cuda Cut*

dredged and filled '95
palm trees planted

Candy's Pond *Aanderaa Flats*

Turtle Pond

WEST ISLAND

Dock

EAST ISLAND

West Bay

Gabriel cut in '80s
recut '90
house built

East Pond

Main Channel

Hummingbird Pond

Sink Hole *Gator Creek*

Sink Hole Flats

Zabané cut, dredged,
& filled '95
cut again, burned,
& filled

Lair Channel

house built '98

Grouper Gardens *The Lair*

East Point

Sponge Haven *Twin Bays*

Hidden Lake

Johnny Cake's house
(former CARICOMP site)

Boston Bay

Hidden Creek

Boa Flats

Crescent Bay

tip of land cut off during Mitch '98

Ranger Station

South Point

0   100
m

☐ Clearcut areas
☐ Dwarf red mangrove
☐ Dense mangrove growth
☐ Dredged & filled areas
☐ Red mangrove fringe

Figure 1. Location Map of Twin Cays showing collection sites: Main Channel, The Lair , Boston Bay and Hidden Lake, Belize.

THE DINOFLAGELLATES OF TWIN CAYS, BELIZE: BIODIVERSITY, DISTRIBUTION, AND VULNERABILITY

BY

MARIA A. FAUST [1]

ABSTRACT

Floating detritus, a unique microcosm, acts as a reservoir of diverse microalgae and meiofauna in mangrove areas found in Twin Cays, Belize. The Lair, Boston Bay, Hidden Creek and Main Channel, four locations within Twin Cays, were used as the study sites. Large suspended detrital aggregates are specialized environments where benthic photosynthetic and heterotrophic organisms thrive as suspended free-floating cells in the water column. On the water surface, patches of detritus, a combination of benthic organisms, dinoflagellates, diatoms, cyanobacteria and dinoflagellate cysts, are enclosed in a matrix of fibers. Heterotrophic organisms are also numerous in floating detritus. Phagotrophic fauna, along with nematodes, ciliates, copepods and crustacean larvae, rely on small algal forms in detritus as their food source. Vertical distribution and species composition of microalgae and associated meiofauna in rising and sinking detritus aggregates are reported using water-depth and time-series studies. The biodiversity of dinoflagellates included a total of 38 species, 15 potentially harmful species, and eight neritic species. Populations of benthic dinoflagellates in floating detritus were measured against total cell counts and found to represent 28-43 % in the Lair and 18-68 % in Boston Bay. The highest concentration of dinoflagellate species were identified as being: *Bysmatrum subsalsum*, *Prorocentrum caribbeanum*, *Prorocentrum elegans* and *Prorocentrum mexicanum*. All other dinoflagellates were one to two orders of magnitude lower in cell numbers. Dominant meiofauna organisms were nematodes and ciliates in detritus. Illegal dumping of domestic waste in the Lair caused dinoflagellates to disappear from floating detritus and their recovery is briefly described.

INTRODUCTION

Shallow subtropical warm waters serve as habitats for assemblages of microalgae, and zooplankton (Frenchel, 1988). Mangrove detritus is relatively high in organic matter (Leichfried, 1988) allowing bacteria (Alongi, 1994), microalgae and meiofauna to thrive within the nutrient-rich environment. In addition, mangrove detritus serves as the food source for fish and shell fish (Robertson, 1987; Boto et al., 1989). However, perceiving microscopic aspects of this environment is just becoming better known with regard to biodiversity and distribution of microalgae and meiofauna assemblages.

---

[1] Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington , D.C., 20560

In shallow mangrove embayments at Twin Cays, Belize, physical relationships exist between pelagic and benthic environments. On the sediment surface, amorphous aggregates and partially decomposed plant matter accumulates. Floating mangrove detritus represents a benthic habitat that is suspended in the water column as it moves vertically upward responding to oxygen gas generated by the attached microalgae. Patches of detritus increase on the water surface as the day progresses and sink out of sight in the late afternoon (Faust, 1996). Mangrove detritus in protected marine habitats, located at depths of 2 to 4 m, is a rising-sinking platform of various microorganisms in the microscopic food web. Benthic microalgal populations are able to maintain high biodiversity in floating detritus, and are the daily food source for the heterotrophic meiofauna organisms.

I investigated the associations of microalgae and meiofauna in floating detritus at the mangrove island, Twin Cays, to show the changing patterns of these organisms in the microscopic marine food web in long-term studies. In addition, I examined temporal and spatial patterns of benthic microorganisms to illustrate their proliferation and functional role in the tropical mangrove detritus.

METHODS

Study Area

Floating detritus samples were collected at Twin Cays, Belize (16°48' 88°05'W), an intertidal mangrove island (Fig. 1), a section of the barrier reef on the western Caribbean extending 220 km from the Mexican border north to the Gulf of Honduras (Ruetzler and Feller, 1988). Twin Cays has distinct habitats such as lagoons, channels, lakes, and mud flats. Floating detritus samples were collected in the Lair, a 2-3 m-deep enclosed embayment containing high amounts of organic matter originating from the red mangrove, *Rhizophora mangle* Linnaeus and anaerobic sediments. In contrast, Boston Bay and Hidden Lake are 0.5 to 1.5 m deep and Main Channel 3-4 m deep with carbonate silt, mud, sand and meadows of turtle grass, *Thalassia testudinium* Bank ex Konin. The water is high in organic detritus as result of plant and animal decay associated with peat and siliceous skeletons derived from diatom, calcareous algae and sponges.

Sampling

Detritus samples were collected in the center of the Lair with 250 ml plastic bottles opened and closed at a given depth and positioned on a 4 m-PVC pipe four times a day: 06:00, 11:00, 15:00 and 18:00. Samples were collected at three different stations approximately 5 m apart and three depths in the water column (below surface, mid-water and bottom) in May, 1991-1995. Time-series samples from the three stations were pooled for each depth and composite samples were used to minimize biological patchiness within detritus and to collect a manageable number of samples for cell counts and organism identification. A detritus sample was then concentrated to 40 ml and fixed with glutaraldehyde to a final concentration of 2% for enumeration of microorganisms (Faust, 1990).

Physical Parameters

Temperature, salinity and irradiance were measured in the water column at three depths with the following instruments: a Yellow Spring Instrument (YSI) 33 S-C-T Meter; a YSI oxygen analyzer Model 57; a YSI 5739 oxygen probe; and a YSI5795A portable, batter-operated submersible stirrer. Irradiance was estimated by the integrating quantum scalar irradiance meter, Biospherical Instruments # QSI-140 meter. Light intensity varied between 150 and 200 $\mu E.m^{-2}.s^{-1}$. During the study the water temperature ranged from 27.1 to 30.8°C, salinity from 22.2 to 30 psu, and dissolved oxygen from 2.2 to 7.5 ppm. Rainfall was 0.2 to 20 mm/day and wind speed ranged from 7.8 to 9.8 $m.s^{-1}$. Nutrient concentrations in the Lair water column were: urea (4 to 6 $nmole.L^{-1}$); ammonium (1.to 1.5 $\mu mole.L^{-1}$); nitrate and nitrite (0.3 to 1.0 $\mu mole.L^{-1}$); and dissolved organic phosphorus (0.15 to 0.22 $\mu mole.L^{-1}$) (Ambler, 1991). Floating detritus had a total nitrogen (TN) content of 18.5 $mg.g^{-1}$ and total particulate organic carbon (POM) content of 34.3 $mg.g^{-1}$ (Leichfried, 1988).

Enumeration and Identification of Organisms

To enumerate microalgae the following procedure was used: 1ml fixed detritus sample was sonicated for 15-20 s, diluted to 10 ml volume with filtered seawater, centrifuged with an International Clinical Centrifuge at top speed for 10 min, supernatant discarded and sample volume adjusted to 2 ml. Cell concentrations were estimated at 100X magnification in a Palmer-Maloney cell chamber (Stein, 1973) and examined under differential interference contrast illumination with Carl Zeiss Axiophot microscope. Abundance of microalgae was enumerated and calculated for dinoflagellates, diatoms, cyanobacteria and dinoflagellate cysts. Relative abundance of microorganisms was determined as the proportion of organisms present in a total of 500 cells. For plate pattern identification, cells were stained with Calcofluor White (Fritz and Triemer, 1985) and observed under the same microscope equipped with an ultraviolet mercury lamp and a Zeiss 01 filter set. The proportion of diatoms and cyanobacteria appeared underrepresented in the data because both form chains or colonies making exact enumeration of populations difficult. Sonication for 15-20 s did not affect the morphology of fixed-cell populations. To enumerate meiofauna, the following procedure was used: the day before counting, 0.2 ml of 1% rose Bengal solution was added to a 20 ml fixed detritus sample from each station-to-strain organism (Higgins and Thiel, 1988). Samples were sonicated (3-5 min) just prior to counting to loosen zooplankton from attached detritus particles. Samples were counted in a 10 x 10 cm square plastic plate. Concentrations of organisms were estimated at 40x magnification using a Carl Zeiss dissecting microscope. Abundance of heterotrophs was calculated for ciliates, copepods, Crustacea, and crustacean larvae. Meiofaunal assemblages are considered underrepresented due to relatively small sample sizes.

Kofoidian nomenclature was used for identifying dinoflagellate species (Kofoid, 1909). Samples of this investigation are deposited in the Dinoflagellate Collection of the U.S. National Herbarium, Department of Botany, NMNH, Smithsonian Institution, Washington, D.C. 20560, United States.

4

RESULTS

Floating Detritus

In the shallow waters of the Lair, Hidden Lake and Boston Bay, patches of detritus float upward to the water surface on sunny days. Detritus is composed of a loosely composed aggregate of associated organisms, organic fibers, mucus, fecal pellets, silt, and brown humus-like particulates. The abundance of floating detritus increases in the water column with sunrise, peaking in mid-afternoon and declining by sunset. Slight variations in light levels and temperature affect the metabolic activity of microbial assemblages within mangrove detritus fibers. Near the sediment surface of the water column, light level is low and water temperature is cool. From this location detritus floats vertically to the water surface via oxygen bubbles (Lewis and Gattie, 1990) generated by the photosynthesis of diverse, attached microalgae. Once the detritus reaches the water surface, cells are exposed to warmer temperatures and higher light levels which induce rapid growth of organisms. Large patches of forming detritus become visible to the human eye on the water surface by early afternoon. These patches can be dispersed by wind or heavy rain which transports the organisms to other habitats.

Physical Parameters

Table 1. Daily mean water temperature, salinity and dissolved oxygen levels for a six day time-series in the Lair habitat measured on May, 1995. Each value is the daily mean of 12 measurements taken at three depths

Parameters	Sampling days					
	1	2	3	4	5	6
Temperature (°C)	29.45	29.45	29.65	29.84	29.52	29.27
Salinity (psu)	35.39	33.69	31.73	28.98	30.04	28.30
Oxygen (ppm)	4.99	4.51	5.45	4.11	5.64	6.18

Temperatures ranged at the water surface from 26.6 to 30.3°C; at mid-water from 28.0 to 30.3°C; and the bottom from 28 to 30.8°C. Temperatures were the lowest at 06:00 at sunrise and the highest at 14:00, declining by 18:00 before sunset. Salinity values ranged at the surface from 22.2 to 35.0 psu, at midwater from 26.7 to 35.8 psu and at bottom from 25.8 to 35.8 psu. Salinity values remained relatively similar from 06:00 to 18:00 but varied daily. Dissolved oxygen concentrations varied within the vertical water column depth from day-to-day. Dissolved oxygen concentrations were the lowest at sunrise 3.5-4.5 O_2 ppm, at 06:00 at the sediment surface and the highest, 6.4 -7.3 O_2 ppm, at 17:00 at 1.5 m depth within the water column.

Association of Microscopic Organisms in Detritus

Detritus provides a protective environment which acts as a nursery for diverse species of toxic and nontoxic dinoflagellates and meiofauna. The following light micrographs illustrate the life-cycle stages of dinoflagellates in detritus (Figs 2-8).
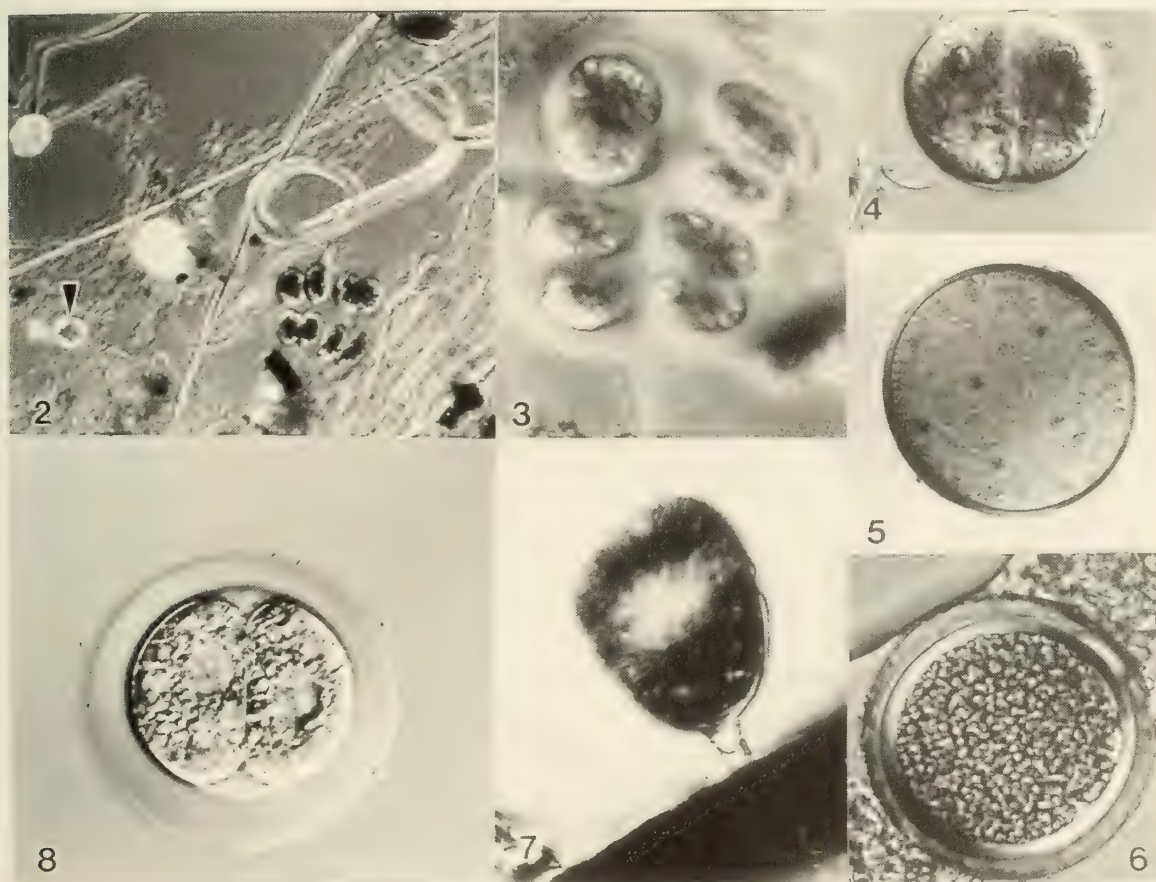
Figures 2-8. Life cycle stages and related associations among benthic dinoflagellates present in floating detritus.

Floating detritus showing loosely bound fibrous aggregates and life-cycle stages viewed at low (100x) magnification (Fig. 2). Photographs were taken at 1000x magnification. Cell pairs of *Prorocentrum* ssp. is embedded in mucilage (Fig. 3). Daughter cells of *Amphidinium* sp. is in a division cyst (Fig. 4). Temporary cyst of *Prorocentrum lima* (Fig. 5). Round, triple-layered dormant cyst of *Prorocentrum foraminosum* has unique granulated cytoplasm (Fig. 6). Prominent stalk of a dinoflagellate sp. attached to plant detritus (Fig. 7). Dividing cell pair of *Prorocentrum* sp. embedded in a ring of mucilage (Fig. 8).

6
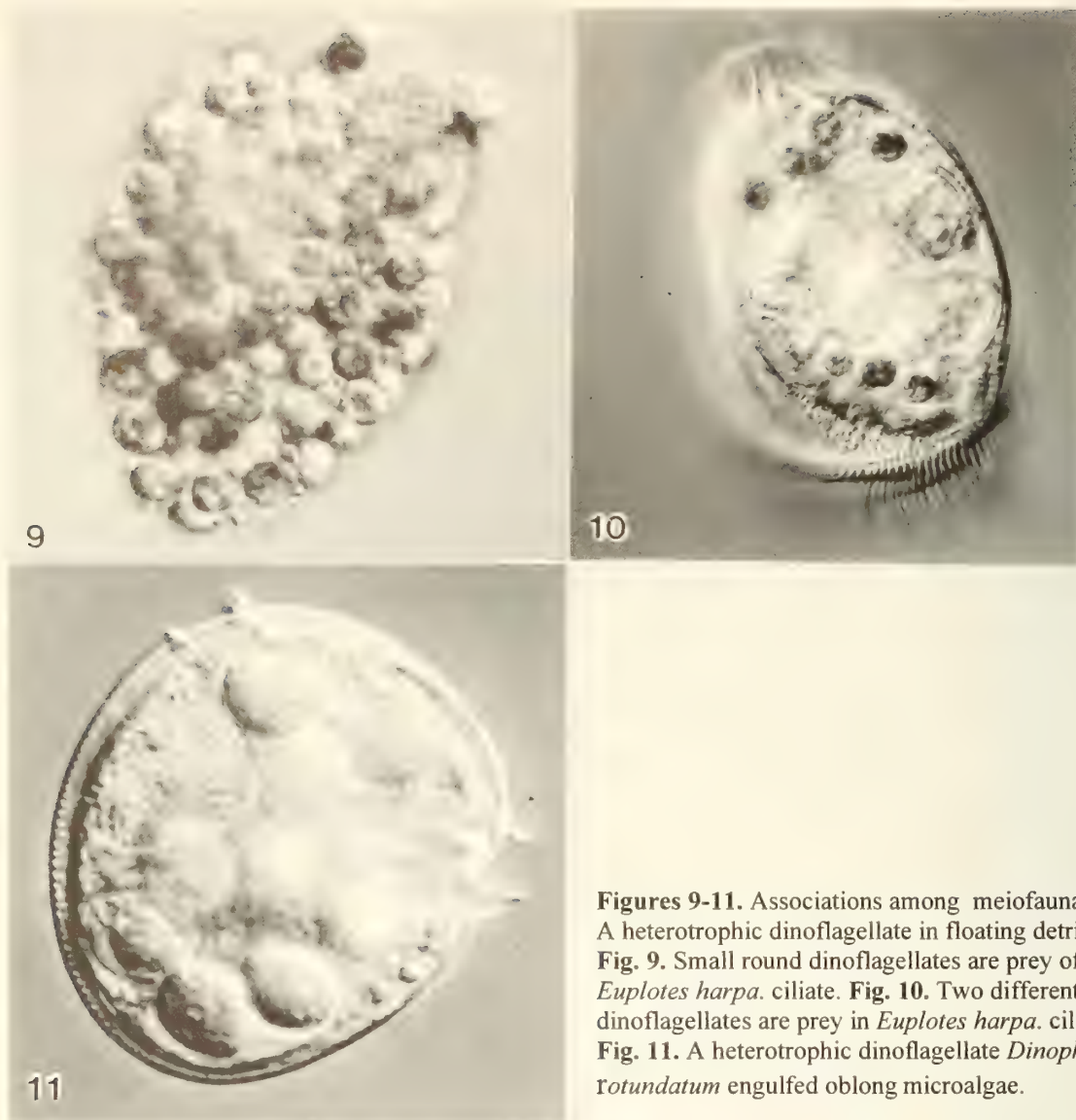
Figures 9-11. Associations among meiofauna and A heterotrophic dinoflagellate in floating detritus. **Fig. 9.** Small round dinoflagellates are prey of an *Euplotes harpa.* ciliate. **Fig. 10.** Two different sized dinoflagellates are prey in *Euplotes harpa.* ciliate. **Fig. 11.** A heterotrophic dinoflagellate *Dinophysis rotundatum* engulfed oblong microalgae.

Examples of dinoflagellates as prey of ciliates and heterotrophic dinoflagellates in floating detritus are in the following light micrographs illustrated at 400x magnification: *Euplotes harpa.* exhibits captured small round dinoflagellates (Fig. 9). A second *Euplotes harpa* shows engulfed large and small dinoflagellate as prey (Fig. 10). *D. rotundatum* heterotrophic dinoflagellate, consumed small, oblong microalgal preys (Fig. 11).

Time-series Study: Microalgae

Floating detritus samples were collected from three levels within the water from the Lair and Boston Bay on May 12, 1991 (Fig. 12). The relative abundance of microorganisms varied with time and collection site. At the Lair, 28 % to 43 % of the floating microorganisms were dinoflagellates whereby in Boston Bay the quantities of dinoflagellates recorded was 18 % to 60 % of the total cell numbers, reaching the highest value at mid-afternoon, 15:00 h. Diatoms were also present in high proportional numbers;
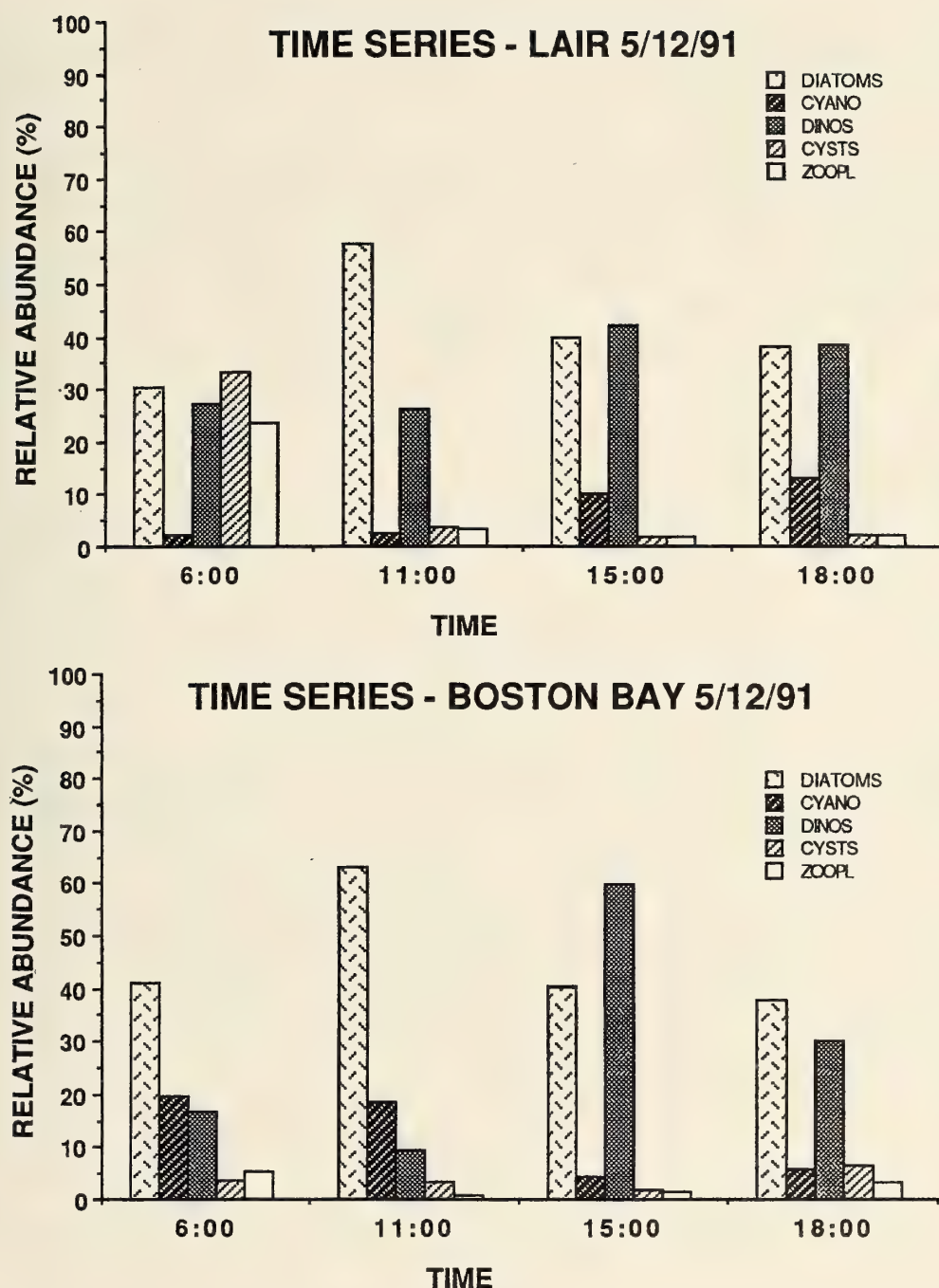
Figure 12. Relative abundance of diatoms, cyanobacteria, dinoflagellates and cysts of dinoflagellates and zooplankton in floating detritus in a time series experiment.

30 % to 68 % at the Lair and 42 % to 65 % in Boston Bay where they the most abundant. Recording was made in the late morning, 11:00 h. Cyanobacteria represented less than 20 % in the samples. Populations increased during time series studies at the Lair whereas decreased in Boston Bay. Benthic cysts represented approximately 35 % of the detritus collected at the Lair at 6:00 h and their numbers rapidly declined from 11:00 h to 18:00 h. In contrast, cysts remained low at Boston Bay from 6:00 h to 18:00 h. Zooplankton

numbers were the lowest at both sampling sites. Dinoflagellates and diatoms were the dominant component in the mangrove detritus, whereas only minor populations of zooplankton were counted.

Table 2. Dinoflagellate species distribution listed as cell numbers present in 500 total cells counted per detritus sample collected in the Lair and Boston Bay on May 12, 1991.

Dinoflagellate species	Samples Taken (Time and Stations)							
	6h		11h		15h		18h	
	L	BB	L	BB	L	BB	L	BB
Amphidinium carterae	0	0	3	4	11	169	29	2
A. operculatum	0	0	11	0	46	15	0	0
Ceratium furca	8	17	6	1	21	2	0	64
Gambierdiscus toxicus*	13	5	18	0	10	9	7	2
Gonyaulax grindleyi	0	57	28	19	50	215	67	61
Ostreopsis lenticularis*	0	0	0	1	3	2	0	3
Bysmatrum subsalsum	41	1	9	3	0	0	14	5
Prorocentrum lima*	0	3	0	0	0	1	0	0
P. mexicanum*	5	3	26	5	12	12	5	2
P. concavum*	0	0	3	0	0	0	0	0
P. emarginatum	2	0	5	1	0	2	0	0
P. ruetzlerianum	0	0	3	0	2	1	4	0
P. norrisianum	66	9	11	1	3	2	18	6

Abbreviations: L = The Lair; BB = Boston Bay; * toxic species

Detritus collected at the Lair and Boston Bay exhibited diverse dinoflagellate species (Table 2). Cell numbers differed at the collection sites and were found to be less in the early morning at 6:00 h. and highest at 15:00 h. These findings directly coincided with the abundance levels of less floating detritus with the least observed at 6:00 h. and the most seen at 15:00 h. Species of *Amphidinium carterae, A operculatum,* Claparède et Lachmann, *Gonyaulax grindleyi* Reinecke, *Prorocentrum mexicanum* Tafall, and *P. norrisianum* Faust were the most numerous in the samples. Several toxins-producing species were also present in low numbers: *G. toxicus* Adachi et Fukuyo, *O. lenticularis* Fukuyo, *P. lima* (Ehrenberg) Dodge, *P. mexicanum* and *P. concavum* Fukuyo. One planktonic species, *Ceratium furca* (Ehrenberg) Claparède et Lachmann, was recognized.

Cell populations of the microalgae in suspended detritus varied daily with time and depth as observed at the Lair study site. Vertical distribution of dinoflagellates, diatoms, cyanobacteria and cysts was compared as a relative percentage of total cells. Three depths (below surface, mid-water, bottom) were examined over a six-day time period in May 1994. In Figure 13, vertical distribution of dinoflagellates, diatoms, cyanobacteria, and dinoflagellate cysts is compared via a relative percentage of total number of cells. The resulting distribution is the following: dinoflagellates represent 50-90 %, diatoms 5-15 %, cyanobacteria 3-25 %, and dinoflagellate cysts 1-7 % of the total cells in floating detritus.
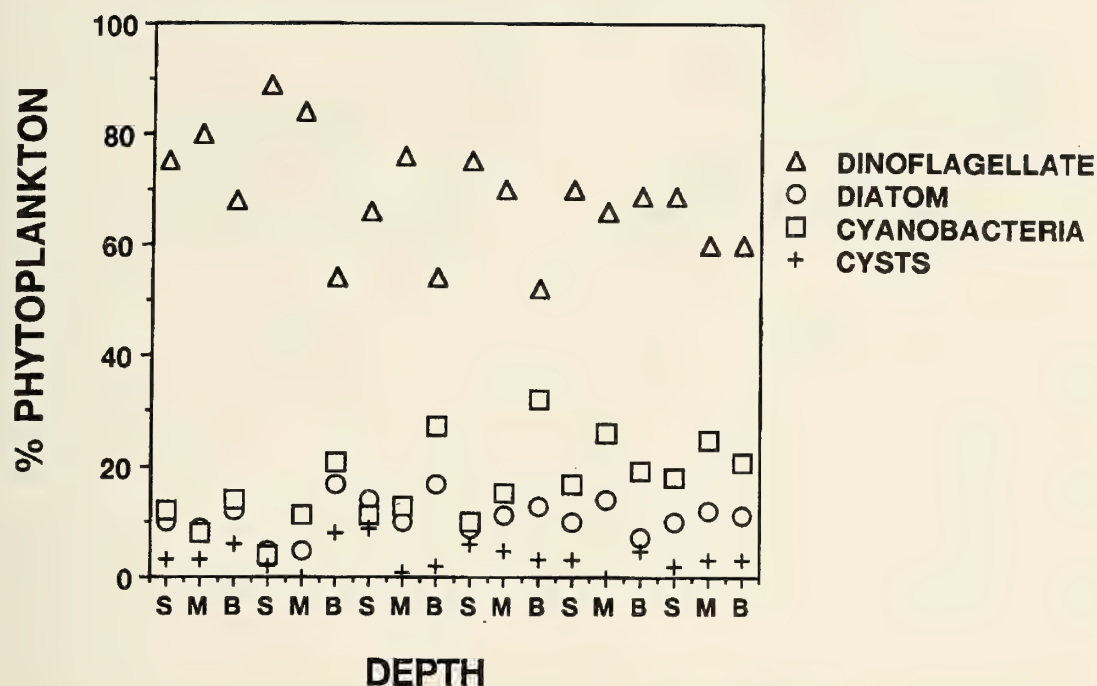
Figure 13. Vertical distribution of dinoflagellates, diatom, cyanobacteria, and cysts of dinoflagellates expressed as relative percentage of total cells in detritus of six-days time series experiment. S, surface; M, midwater; B, bottom detritus.

Vertical distribution and patterns of microalgae in floating detritus for day three are illustrated as one example in (Figure. 14 A). Cell numbers were the highest in surface and bottom of samples and lowest in mid-water with their numbers increasing with time. In bottom detritus samples cysts with a red body were present (Fig. 2), whereas in mid-water and surface detritus samples division cyst enclosed in hyaline membrane (Figs 4-5) were observed. During their journey in rising detritus, cysts became metabolically active and divided (Figs 2-4, 8). The freshly divided microalgae provide an abundant food source for meiofauna that follow.

Composition of meiofauna in floating detritus is diverse. Dominant taxa include nematodes, ciliates, copepods and crustacean-larvae (Fig. 14 B). Meiofauna cell densities in detritus varied with depth and time. Most numerous taxa in surface and bottom detritus were nematodes, crustacean-larvae, and ciliates and copepods were more abundant in mid-waters. Usually meiofauna assemblages in mid-water detritus samples were few.

10
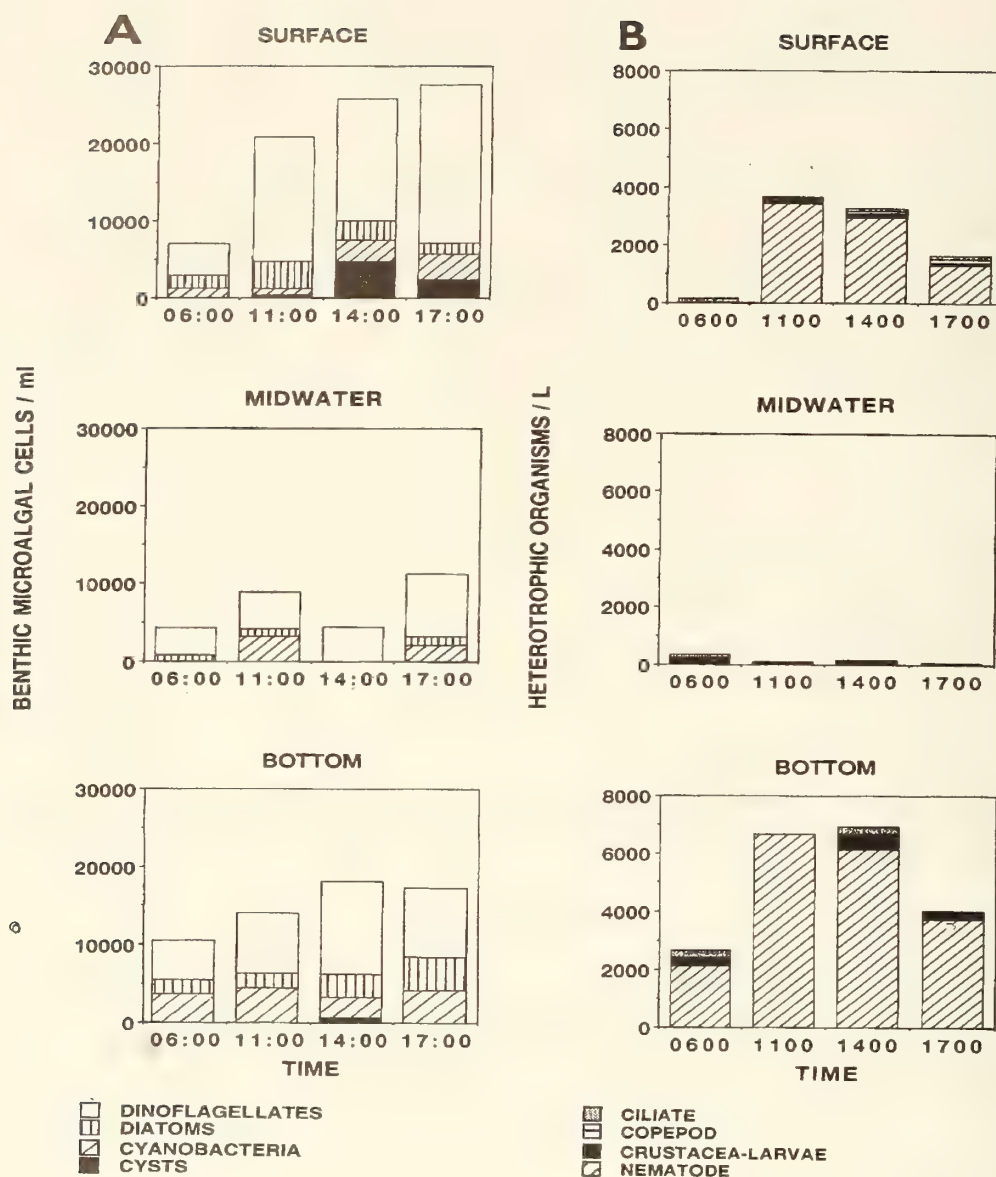
Figure 14. Vertical distribution of microalgae (A) and meiofauna (B) assemblages in floating detritus samples collected at surface, midwater and bottom floating detritus samples from sunrise to sunset in time series

Biodiversity of Dinoflagellates

The biodiversity of dinoflagellates in floating detritus at the Lair, Boston Bay, Hidden Lake, and Main Channel include 38 species, 15 toxins producing species (*) and 8 neritic species (Table 3).

Table 3. Biodiversity of dinoflagellate species in detritus from the Lair (L), Boston Bay (BB), Hidden Lake (HL) and Main Channel (MC), Twin Cays, * toxic species, and species in detritus (D), sediment (S) and plankton (P) are presented.

Dinoflagellate species	L	BB	HL	MC	Ecology
Amphidinium carterae*	+	+	+	-	DS
A. operculatum	-	-	+	-	D
Bysmatrum capony	+	-	-	-	D
B. subsalsum	+	+	+	+	DSP
Ceratium furca	+	-	-	+	P
C. hircus	+	-	-	+	P
C. lineatum	+	-	-	+	P
Cochlodinium polykrikoides*	+	-	-	+	P
Coolia monotis*	+	+	+	+	DSP
Dinophysis caudata*	-	-	-	+	DP
D. mitra	-	-	-	+	DP
D. rotundatum*	-	-	-	+	DP
Gambierdiscus toxicus*	+	-	+	+	DSP
Gonyaulax grindleyi*	+	+	-	+	DSP
G. polyedra	+	-	-	+	PD
G. polygramma	+	-	-	+	PD
G. spinifera	-	-	-	+	PD
Gymnodinium sanguineum	+	+	+	+	P
G. estuariale	+	+	+	+	P
Ostreopsis lenticularis*	+	+	-	+	DPS
O. ovata*	+	+	-	+	DPS
Plagodinium belizeanum	+	-	-	+	DPS
Prorocentrum belizeanum*	+	+	+	+	DPS
P. caribbeanum	+	+	-	-	DS
P. concavum*	+	+	+	+	DS
P. elegans	+	+	-	-	PD
P. emarginatum	+	+	+	-	DS
P. formosum	+	+	+	-	P
P. foraminosum	+	-	+	-	DS
P. hoffmannianum*	+	+	+	+	DSP
P. lima*	+	+	+	+	DS
P. maculosum*	+	+	+	-	DS
P. mexicanum*	+	+	+	+	DSP
P. norrisianum	+	+	+	-	DP
P. ruetzlerianum	+	+	+	-	DSP
Pyrodinium bahamense	-	-	-	+	P
Scrippsiella trochoidea-	+	+	+	+	PD
Synophysis microcephalus	+	-	+	-	SD

12

Most numerous species were benthic dinoflagellates associated with detritus and sand, and eight species considered planktonic/neritic (Table 4). Detritus samples with high species counts were collected in the Lair and Main Channel. Collections of detritus samples were relatively few from Boston Bay and Hidden Lake which may limit the assessment of the biodiversity of dinoflagellates.

Table 4. Concentrations of benthic and neritic dinoflagellate species within floating detritus in the Lair collected at two different dates, May 1991 and 2003, are compared.

Dinoflagellate species	Concentrations (cells. 10^{-3}. L^{-1})	
	1991	2003
Benthic		
Amphidinium carterae	3.01	0.15
Bysmatrum subsalsum	18.45	0.25
Coolia monotis *	4.24	
Dinophysis caudata *	0.31	
D. rotundatum *	0.28	
Gambierdiscus toxicus *	0.25	
Gonyaulax grindleyi *	2.72	0.06
Ostreopsis lenticularis *	0.72	
O. ovata *	0.37	
Plagodinium belizeanum	4.75	0.20
Prorocentrum belizeanum *	2.26	0.32
P. caribbeanum	10.52	
P. concavum *	0.06	
P. elegans	80.23	0.88
P. emarginatum	1.56	0.08
P. formosum	1.69	
P. foraminosum	1.52	
P. hoffmannianum *	1.12	
P. lima *	0.81	
P. maculosum *	0.75	
P. mexicanum *	27.84	0.16
Synophysis microcephalus	0.07	
Neritic		
Ceratium furca	1.83	
Gonyaulax grindleyi	2.72	
Cochlodinium polykrikoides	3.14	
G. polyedra	3.11	
Gymnodinium sanguineum	2.32	
G. estuariale	0.42	
Protoperidinium quinquecorne	0.32	
Scrippsiella trochoidea	2.87	

Dinoflagellate concentrations in floating detritus from the Lair illustrate the distribution of species collected in early afternoon in May 1994 (Table 4). Four dinoflagellates reached the highest populations: *Bysmatrum subsalsum* (Ostenfeld) Faust et Steidinger 1998, *Prorocentrum caribbeanum* Faust 1993, *P. elegans* Faust 1993, and *P. mexicanum* Tafall 1942. Thirteen toxic species were also present in the samples which are marked with the * symbol. The majority of other species were one-two orders of magnitudes lower in cell numbers. *Dinophysis caudata* Saville-Kent 1881 and *D. rotundatum* (Claparède and Lachmann) Kofoid and Michener 1911 are heterotrophic void of chloroplasts whereas all other species are photosynthetic.

Concentrations of dinoflagellates in detritus varied with species (Table 4). The highest concentration of dinoflagellate species were identified as being: *Bysmatrum subsalsum*, *Prorocentrum caribbeanum*, *P. elegans,* and *P. mexicanum.* All other dinoflagellates were one-to-two orders of magnitude lower in cell numbers. Dinoflagellate assemblages in floating detritus at the Lair totaled 22 benthic species, 13 toxin-producing species, and eight neritic species. Among the dinoflagellates, nine were new species described from Twin Cays and also reported for the first time from a coral reef-mangrove habitat: *Plagodinium belizeanum* Faust et Balech 1993, *Prorocentrum hoffmannianum* Faust 1990, *P. maculosum* Faust 1993, *P. formosum* Faust 1993, *P. ruetzlerianum* Faust 1990, *P. belizeanum Faust* 1990, *P. foraminosum* Faust 1993, *P. elegans*, and *P. caribbeanum. Sinophysis microcephalus* Nie et Wang 1944.

Illegal dumping of domestic waste occurred in the Lair beginning in 1995. The next four years, 1997 to 2000, dinoflagellates disappeared from floating detritus. The first sign of recovery of dinoflagellate was noted in 2001; since then eight species were identified in floating detritus in 2003. Note the low cell numbers as shown in Table 4.

DISCUSSION

Floating Detritus: a Specialized Environment

In Twin Cays, floating detritus is a unique microcosm in protected mangrove habitats. It consists of organic fibers, decomposed organic matter, and various taxa of photosynthetic microalgae and heterotrophic meiofauna (Faust, 1995). Patches of detritus originate at the sediment-water interface and appear floating on the water surface visible to the human eye. Detritus dislodges from sediment where dissolved oxygen and light levels are low and organic matter high (Amble, 1991). Benthic microorganisms enclosed in aggregates move upward, and re-enter the water column daily. As the sun rises, microalgal photosynthesis increases generating oxygen gas bubbles clearly visible as patches of detritus float upward to the water surface propelled by the buoyant force. Microalgal assemblages proliferate in rising detritus aggregates where they receive optimum light to photosynthesize, grow and sexually reproduce (Faust, 1993). During this journey detrital aggregates exhibit dividing microalgae that are consumed by the meiofauna (Faust and Gulledge, 1996). Once the patches reach the water surface, cells are exposed to warmer temperatures and higher levels of light induce excystment of dinoflagellates and rapid bacterial growth (Herndl, 1991). These adhering organisms in the detritus can be reintroduced into the water column or detritus patches can be broken

14

up and dispersed by high winds and heavy rains. The observation described in Twin Cays is less complementary to the formation and sinking aggregates of shallow coastal waters (Sherr et al., 1986) or of deep ocean (Alldredge, 1989).

Dinoflagellate-adhering surface relationships may involve physical and chemical interactions as well as structural and surface interactions (Bomber et al., 1985). Bomber et al., (1985) referred to the attachment of dinoflagellates to microalgal surfaces. In the detritus-dinoflagellate interaction the detritus probably supplies nutrients and provides a stabilizing structure for colonization. Because detritus patches are easily broken up by the water movement, cells are nourished and benthic organisms become free floating. In this situation, detritus patches serve as a nursery for dinoflagellate populations as well as other associated microorganisms. When such dinoflagellate populations are producers of toxins, floating detritus in mangrove embayments, near-shore areas and coral reefs may be extremely important to the balance of this ecosystem.

Twin Cays mangrove ecosystem is a unique floating detritus habitat and a masterpiece of nature (Along, 1998; Newel, 1984). In the protected quiescent physical habitats found at the Lair, Boston Bay and Hidden Lake, low-wave energy enables mangrove leaves to decompose and facilitate development of soft sediments (Faust, 1996). Floating detritus is processed and recycled rapidly through the aquatic food chain and controlled by hydrodynamic forces, temperature, light and nutrient availability. Assemblages of microalgae including dinoflagellates proliferate in rising detritus aggregates. On the sediment surface, unfavorable environmental conditions hinder microalgal proliferation and their number is further reduced by the grazing activities of nematodes, ciliates and other consumer organisms (Fig. 14B). These microbial communities are highly dynamic undergoing rapid population changes that are measured in hours (Fig. 14A). At the bottom, detritus predation of nematodes may regulate population size of dinoflagellates similarly to phagotrophic protozoa (Sherr et al., 1986). In surface detritus where predator populations were lower, dinoflagellates developed into densities two-three orders of magnitude higher than found in bottom detritus. As part of the microbial community, microalgae produced new cells and dissolved oxygen for heterotrophs residing in the mangrove food webs.

Benthic food chains in mangroves obtain their sustenance not only from detritus but also from a variety of living nutritious microalgae (Alongi, 1998). Floating detritus acts as a vehicle that transports benthic microbial assemblages daily into the suspended pelagic tropical waters where they become actively dividing populations and, as a consequence, a continuous shift exists between actively growing and inactive cell populations. These phototrophs, such as cyanobacteria, dinoflagellates and diatoms, are a quality food source of meiofauna (Porter et al., 1985, Sherr et al., 1986), microfaunal assemblages (Frenche, 1988), and large particle feeders including crustaceans and juvenile fish (Stoner and Zimmerman, 1988). Suspended floating detritus provide physical surfaces where microorganisms can attach and grow rapidly in an environment highly enriched and protected from predators. In detritus the proliferation of benthic microbial communities occur, a nutrient-enriched environment, resulting in diverse microalgal and meiofaunal populations and dinoflagellate life-cycle events (Faust, 1993, 1991).

Microbial Associations and Biodiversity

Microorganisms in floating detritus at Twin Cays represent assemblages that are associated in benthic planktonic habitats (Tables 3 and 4). Dinoflagellates are the most dominant microalgae, with nematodes and ciliates as the most common consumers. Benthic assemblages of microalgae in a time series are illustrated in Figure 14. The biodiversity of dinoflagellates at four collection sites included a total of 38 species, 15 toxins-producing species (designated by * symbol), and eight neritic species. Dinoflagellate populations were most numerous in floating detritus. Smetacek (1985) proposed that aggregation is an adaptation-promoting sedimentation of diatoms out of warm, nutrient-depleted water into cold water resulting in the formation of resting spores. The result of this study illustrates a similar occurrence where benthic dinoflagellate assemblages of resting cysts reenter the water column and form new surface dinoflagellate populations beginning at sunrise via floating detritus. Floating detritus containing dinoflagellates in late afternoon sink down from warm surface water into colder nutrient-enriched bottom waters to produce resting stages with sunset. This floating platform of mangrove detritus creates microhabitats for a variety of benthic organisms within the water column. This aggregated platform has its own diverse microbial assemblages, nutrient gradients on solid-liquid interfaces as illustrated in ecological analogies of the aggregated micro-environments (Alldredge and Youngbluth, 1985; Sherr et al., 1986; Aldredge and Silver, 1988; Stoner and Zimmerman, 1988; Caron, 1991; Alongi, 1994, 1992).

Functional Roles

Floating mangrove detritus at Twin Cays has microbial assemblages that include photosynthetic and heterotrophic organisms that form the base of benthic food webs. Large suspended aggregates are specialized environments rich in communities of bacteria, cyanobacteria and photosynthetic eukaryotes constituting patches of highly elevated abundances of prey for many species of protozoa (Porter et al., 1985), ciliates (Sherr et al., 1986) and flagellates (Caron, 1991). In mangrove detritus, phytoplankton exudates in the form of dissolved nutrients (organic carbon and nitrogen) that may provide nutrients for heterotrophs as described for marine snow (Alldredge, 1979) and mangrove detritus (Boto et al., 1989). Mangrove detritus creates a specialized environment where mixed species of organisms exist. Benthic microorganisms may grow at different rates, in part depending on whether they are free-living motile species in the water or aggregated in biofilms (Lewis and Gattie, 1990).

Nematodes were by far the most abundant heterotroph in the floating detritus samples. They were most abundant at 11:00 in bottom detritus (Fig. 14B) when algal densities were relatively low. Nematodes were less abundant in surface detritus where algal densities were more prominent (Fig. 14A). This is similar to the findings of Alongi and Christoffers, (1992) who reported comparable numbers of nematodes in near-bottom mangrove sediments of the Great Barrier Reef, Australia. In this study, 15-25 engulfed dinoflagellates were often observed in a nematode indicating that they are important consumers of benthic dinoflagellates in Twin Cays mangroves. The slender, flexible body structure of a nematode allows it to move easily between detrital fiber-webs in search of food. They may be the major predators of benthic dinoflagellates. Nematode densities

16

also varied significantly with depth in Australian sediments at Queensland (Alongi and Christoffers, 1992).

Dinoflagellate-ciliate prey associations are complex. Ciliate species exhibited size-specific dinoflagellate-prey preferences (Figs 9-10). Certain ciliates capture only small dinoflagellates, but other ciliates have a more diverse food preference (Faust and Gulledge, 1996). Ciliates also have some advantage in floating detritus; they are small and readily navigate within detritus fibers and often consume small centric diatoms readily available. In floating detritus the movements of calanid copepods are restricted due to their larger size and feed primarily on attached bacteria (Herndl, 1991). Heterotrophic dinoflagellates were the major consumers of small dinoflagellates in floating detritus (Fig. 11).

The function of floating mangrove aggregates appears to be somewhat similar to marine snow (Alldredge and Silver, 1988; Alldredge, 1989; Jackso, 1993) as observed in Twin Cay mangrove habitats. It is difficult, however, to make exact comparison between the ecology and dynamics of these two marine aggregates. One major difference is that in the shallow water column, mangrove aggregates rise and sink daily and have a short residence time in water measured in hours. 'Marine snow' aggregates in deep oceans have a long residence time and move downward in the water and sink to the sea floor in a matter of months (Alldredge, 1989).

This study suggests that floating mangrove detritus is a location where food sources are generated for meiofauna. Regeneration of algae and the predation cycle repeated daily in the water column regulate benthic assemblages of organisms. Detrital aggregates are sites of active algal and bacterial production (Alongi, 1994; Gotschalk and Alldredge, 1989) that serve as the food source for a variety of heterotrophic organisms (Sherr et al., 1986; Alongi, 1990). The main function of floating mangrove detritus is as a highly effective, self-sufficient microhabitat for a variety of benthic taxa, somewhat similar in function to marine snow for pelagic microorganisms.

Vulnerability of Dinoflagellates

Illegal dumping of domestic waste in black plastic bags in shallow waters of the Lair, Twin Cays was discovered during field studies. Waste disposal took place beginning in 1995 and continued for three years. Released pollutants during the decomposition process resulted in dramatic loss of phytoplankton populations diverse in these waters. First sign of population recovery occurred in May 2001 when benthic dinoflagellates were again seen attached to floating detritus. However, species composition and cell density were significantly reduced after to dumping of domestic waste (Table 4).

While dinoflagellates have little economic value, indirectly they may signal change in the health of an undisturbed aquatic ecosystem as illustrated in Twin Cays. Mangrove swamps indeed constitutes an extremely delicate natural ecosystem. Three years of waste disposal in Twin Cays significantly altered dinoflagellate assemblages, greatly changing the microscopic food web of this habitat. Continuation of waste disposal would probably have caused irreversible harm. The effect of household waste ultimately could have resulted in a loss of habitat for microscopic food web organisms and, in turn, the loss of a food source for marine fish and shell fish. Our knowledge is limited in

understanding the effect of household waste on the microscopic biodiversity of shallow, slow-flowing mangrove environments.

CONCLUSIONS

Long-term studies at the Twin Cays mangrove forest provided the opportunity to examine not only spatial differences in assemblages of microalgae and meiofauna in floating detritus but also to document temporal changes in abundance, biodiversity and distribution in the microscopic food web. Floating detritus aggregates are specialized protective environments and significantly affect the productivity of mangroves, where benthic organisms sweep into the water column as fre- floating photosynthetic and heterotrophic organisms. On the water surface microalgae proliferate in light and provide the food source for the meiofauna grazers and in turn, maintain nursery for fish and shellfish. Detritus is a nutrient-enriched microcosm for toxic dinoflagellates and a possible site for ciguatera outbreaks. When dinoflagellate populations are ciguatoxigenic, the existence of floating detritus originating in mangrove waters may take on extreme importance to near-shore areas and coral reefs. Illegal dumping of domestic waste in the Lair for four years caused the disappearance of dinoflagellates. Our knowledge is limited in understanding the effects of household waste on microscopic aquatic organisms. This report is the first to provide empirical data on this topic.

ACKNOWLEDGEMENTS

18

REFERENCES

Alongi, D. M.
   1990. Abundance of bentic microfauna in relation to outwelling of mangrove
         detritus in a tropical coastal region. *Marine Ecology Progress Series* 63:53-63.
Alongi, D. M.
   1994. The role of bacteria in nutrient cycling in tropical mangrove and other coastal
         benthic ecosystems. *Hydrobiologia* 285:19-32.
Alongi, D. M.
   1998. *Coastal Ecosystems Processes*. Marine Science Series, CRC Press LLC Boca
         Raton, Florida, 1-419 pp.
Alongi, D. M., and P. Christoffersen
   1992. Benthic infauna and organism-sediment relations in a shallow, tropical coastal
         area: influence of outwelled mangrove detritus and physical disturbance.
         Marine Biology Progress Series 81:229-245.
Alldredge, A. L.
   1979. The chemical composition of macroscopic organic aggregates in two neritic
         seas. *Limnology and Oceanography* 24:855-866.
Alldredge, A. L.
   1989. The significance of suspended detrital aggregates of marine snow as micro
         habitats in the pelagic zone of the ocean. In: Hattori, H., Ishida, Y., Maruyama,
         Y. Morita, R. Y, and A. Uchida, eds., *Recent Advances in Microbial Ecology.*
         Japan Scientific Society Press, Tokyo, pp. 108-112.
Alldredge, A. L., and M. J. Youngbluth
   1985. The significance of macroscopic aggregates (marine snow) at sites of
         heterotrophic bacterial production in the mesopelagic zone of the subtropical
         Atlantic. *Deep-Sea Research,* 32:1445-1456.
Alldredge, A. L., and M. V. Silver
   1988. Characteristics, dynamics and significance of marine snow. *Progress in*
         Oceanography 20:41-82.
Ambler, J. W.
   1991. Population dynamics of *Dioithona oculata* neared mangrove roots. *Progress*
         Report, Caribbean Coral Reef Ecosystem Program, Smithsonian Institution,
         12 pp.
Bomber, J. W., D. R. Norri, and L. E. Mitchell
   1985. Benthic dinoflagellates associated with ciguatera from Florida Keys. II.
         Temporal, spatial and substrate heterogeneity of *Prorocentrum lima.* In:
         Anderson, D. M., A.W. White and D. G. Baden, eds., *Toxic Dinoflagellates.*
         Elsevier Publisher, pp. 39-44.
Boto, K. G., D. M. Alongi, and A. L. J. Nott
   1989. Dissolved organic carbon-bacterial interactions at sediment-water interface in
         a tropical mangrove system. *Marine Ecology Progress Series* 51:243-251.
Caron, D. A.
   1991. Heteroflagellates associated with sedimenting detritus. In: Patterson, D. J. and
         J. Larsen. *The Biology of Free-living Flagellate, Carnedon Press, Oxford, UK*
         pp. 77-92.

Faust, M. A.
  1990. Morphologic details of six benthic species of *Prorocentrum* (Pyrrophyta) from a mangrove island, Twin Cays, Belize, including two new species. *Journal of Phycology* 26:548-558.
Faust, M. A.
  1991. Observation on the morphology and sexual reproduction of *Coolia monotis* (Dinophyceae). *Journal of Phycology,* 28:94-104.
Faust, M. A.
  1993. Sexuality in a toxic dinoflagellate *Prorocentrum lima*. In: Smayda, T. J, and Y. Shimizu, eds., *Toxic Marine Phytoplankton. Elsevier Scientific Publisher, Amsterdam,* pp. 121-126.
Faust, M. A.
  1995. Benthic, toxic dinoflagellates: an overview. In: *Harmful Marine Algal Blooms*, Lassus, P. et al. eds. Lavoisier Science Publisher, Paris, pp. 847-854.
Faust, M. A.
  1996. Dinoflagellates in a mangrove ecosystem, Twin Cays, Belize. Nova Hedwigia 112:445-58.
Faust, M. A., and R. A. Gulledge
  1997. Associations of microalgae and meiofauna in floating detritus at a mangrove island, Twin Cays, Belize. *J. Experimental Marine Biology and Ecology* 197:159-175.
Frenchel, T.
  1988. Marine Plankton Food Chain. *Annual Reviews Ecology System.* 18:19-38.
Fritz, L., and R. E. Triemer
  1985. A rapid simple technique utilizing Calcofluor White M2R for the visualization of dinoflagellate thecal plates. *Journal of Phycology,* 21:662-664.
Gotschalk, C. C., and A. L. Alldredge
  1989. Enhanced primary production and nutrient regeneration within aggregated marine diatoms. *Marine Biology,* 103:119-129.
Herndl, G. J.
  1988. Ecology of amorphous aggregations (marine snow) in the northern Adriatic Sea. 2. Microbial density and activity in marine snow and its implication to overall pelagic processes. *Marine Ecology Progress Series,* 48:265-275.
Herndl, G. J.
  1991. Microbial biomass dynamics along a tropical gradient at the Atlantic Barrier Reef off Belize (Central America). *Marine Ecology,* 12:41-51.
Higgins, P. R., and H. Thiel
  1988. *Introduction to the Study of Meiofauna .*Smithsonian Institution Press, Washington, DC, p. 488.
Jackson, G. A.
  1992. Flux feeding as a mechanism for zooplankton grazing and its implications for verticulate particulate flux. *Limnology and Oceanography* 38:1328-31.
Leichfried, M.
  1988. Distribution and quality of POM in mangrove sediments. In: Ruetzler, K., ed., *Mangrove Ecosystem: Twin Cays, Belize*, edited by (workshop held at Solomons, Maryland, 16-18 November, 1988).

Lewis, D. L., and D. K. Gattie
   1990.   Effects of cellular aggregations on the ecology of microorganisms. *American Microbiology News* 56:263-268.
Kofoid, C. A.
   1909.   On *Peridinium steinii* Joergensen, with a note on the morphology on the nomenclature of the skeleton of the Peridinidae. *Archiv fur Protistenkunde* 16:25-47.
Newell, R. C.
   1984.   The biological role of detritus in the marine environment. In: Fasham, M. J. R. ed., *Flows of Energy and Materials in Marine-ecosystems.* Plenum Press, New York, NY, pp. 317-343.
Porter, K. G., E. B. Sherr, B. F. Sherr, M. Pac, and R. W. Sanders
   1985.   Protozoa in Planktonic food webs. *Journal of Protozoology* 32:409-415.
Robertson, A. I.
   1986.   The determination of trophic relationships in mangrove-dominated systems. In: *Mangrove Ecosystems of Asia and the Pacific: Status, Exploitation and Management.* AIMS and Australian Committee for Mangrove Research, Townsville, Australia, pp. 292-304.
Ruetzler, K., and C. Feller
   1988.   Mangrove swamp communities. *Oceanus* 30:16-24.
Sherr, E. B., B. F. Sherr, and G. A. Paffenhoffer
   1986.   Phagotrophic protozoa as food for metazoans: a 'missing' trophic link in marine pelagic food webs. *Marine Microbial Food Webs* 1:61-80.
Smetacek, V. S.
   1985.   Role of sinking in diatom life history cycles: Ecological, evolutionary and geological significance. *Marine Biology,* 84:239-251.
Stein, J. R.
   1973.   *Handbook of Phycological Methods. Culture, Methods and Growth.* Cambridge University Press, Cambridge, p. 448.
Stoner, A. W., and R. J. Zimmerman
   1985.   Food pathway associated with penaeid shrimps in a mangrove-fringed estuary. *Fisheries Bulletin* 86:543-551.

ATOLL RESEARCH BULLETIN

NO. 515

EXTRAORDINARY MOUND-BUILDING FORMS OF *AVRAINVILLEA*
(BRYOPSIDALES, CHLOROPHYTA): THEIR EXPERIMENTAL TAXONOMY,
COMPARATIVE FUNCTIONAL MORPHOLOGY AND ECOLOGICAL
STRATEGIES

BY

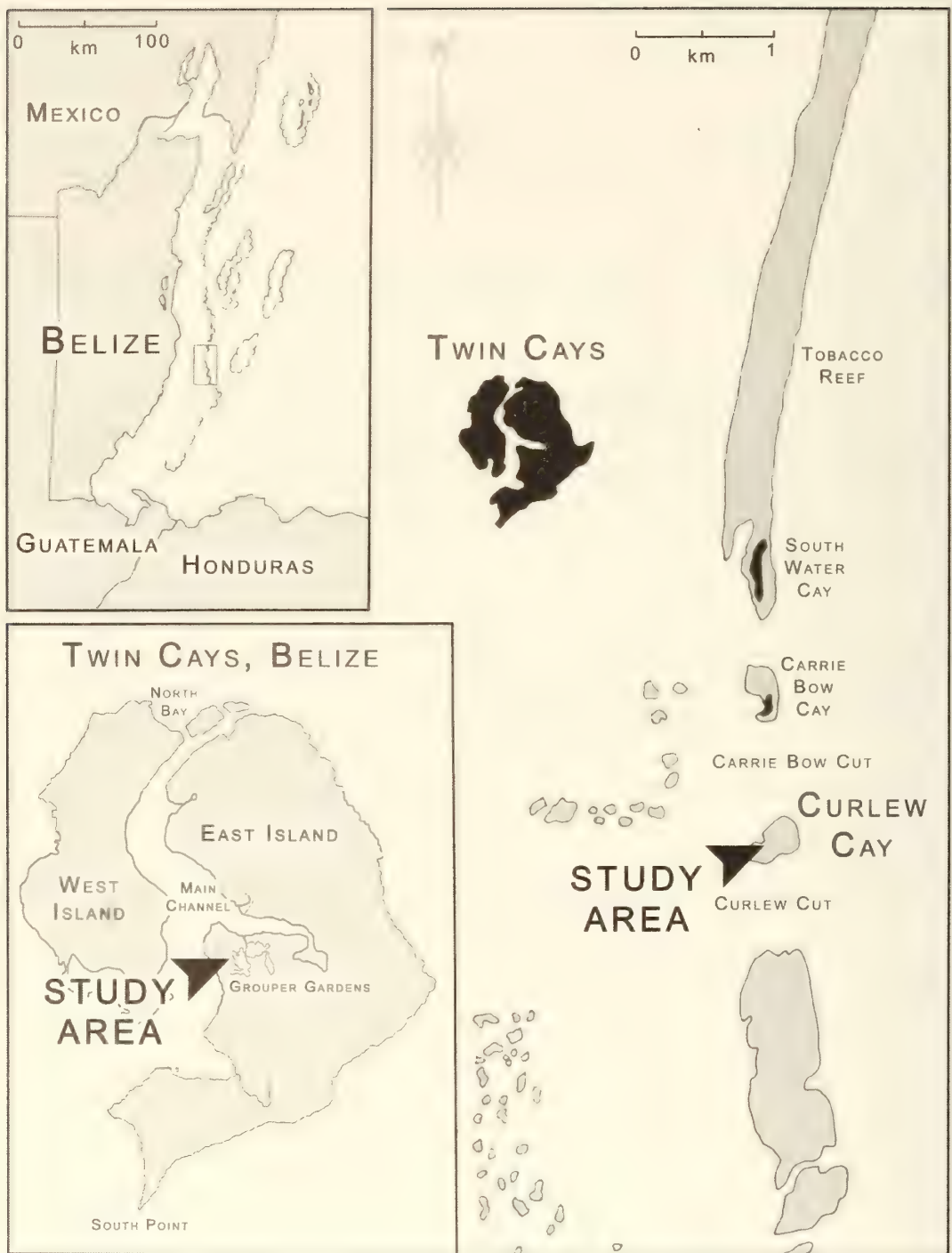MARK M. LITTLER, DIANE S. LITTLER, AND BARRETT L. BROOKS

Figure 1. The Central Province of the Belize Barrier Reef showing the study sites on Twin Cays and Curlew Cay.

EXTRAORDINARY MOUND-BUILDING FORMS OF *AVRAINVILLEA* (BRYOPSIDALES, CHLOROPHYTA): THEIR EXPERIMENTAL TAXONOMY, COMPARATIVE FUNCTIONAL MORPHOLOGY AND ECOLOGICAL STRATEGIES

BY

MARK M. LITTLER, DIANE S. LITTLER, AND BARRETT L. BROOKS

ABSTRACT

The discovery of astounding mound-building forms of *Avrainvillea* (to 30 m diam.) catalyzed this study. These colonial (possibly clonal) mounds dominate the standing stocks and productivity of protected, shallow, eutrophic interiors of Belizean mangrove islands. A common-garden reciprocal-transplant experiment showed that the mound formers (*A. longicaulis* f. *laxa* and *A. asarifolia* f. *olivacea* from Twin Cays), which we initially hypothesized to be undescribed species, readily acquired the morphological features consistent with the taxa characteristic of open-water habitats (*A. longicaulis* f. *longicaulis* and *A. asarifolia* f. *asarifolia* from Curlew Cay), thereby falsifying the hypothesis that the mound formers are distinct species.

In support of the coloniality hypothesis, the Twin Cays f. *laxa* and f. *olivacea* morphs were uniquely adapted to produce flabellar stipes that serve as shallow subterranean rhizomes which spread laterally to overgrow rich organic peat bottoms. The massive columnar rhizoidal holdfasts found in the Curlew Cay f. *longicaulis* and f. *asarifolia* morphs were adaptive for both anchoring and obtaining pore-water nutrients, but proved to be superfluous under placid enriched water-column nutrient conditions and were incapable of surviving the deeper anoxic conditions of the composting peat deposits. The large colonial mangrove morphs (i.e., f. *laxa* and f. *olivacea*) were not physically resistant to even the moderate current levels (3.6±0.5 cm per sec) encountered in the back-reef lagoon habitats of the deeply anchored morphs (i.e., f. *longicaulis* and f. *asarifolia*). However, smaller 2-3 blade clumps, with their stipes deeply buried, survived and grew.

Consistent with the perennation hypothesis, only the experimentally amputated Curlew Cay morphs (both f. *longicaulis* and f. *asarifolia*) showed significantly more proliferations (100 %) than either the amputated Twin Cays morphs (both f. *laxa* and f. *olivacea*) or the uncut Curlew and Twin Cays control plants. The stipes and blades of the open-water morphs (f. *longicaulis* and f. *asarifolia*) serve as expendable assimilators with a major function of building a massive perennating/storage organ, the columnar holdfast, which comprises the bulk of the plant. Physical disturbances (such as storms and herbivory), as well as physiological stresses (such as epiphyte loading), can cause disproportionate losses of the relatively delicate expendable assimilators which are replaced subsequently by perennation from the long-lived subterranean holdfast during more favorable conditions.

Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington DC 20560-0166

INTRODUCTION

Overall, the ecology of siphonaceous green algae (Bryopsidales) is not well known even though members of this group occur abundantly in virtually all tropical open-water reef and lagoon habitats. The discovery of three incredible mound-building colonial forms [=morphs or forma (f.)] of *Avrainvillea*, dominating the standing stocks and productivity of submerged habitats within Belizean mangrove island interior creeks, ponds and lakes, literally demanded this study. These persistent mound-formers are restricted to shallow (<3 m), calm, peat-bottom, high-nutrient waters in the protected interiors of mangrove islands.

Lagoons of the Belize Barrier Reef Central Province, such as those westward of Carrie Bow Cay and Curlew Cay (Fig. 1), are the most extensive of the entire reef tract including diverse and abundant populations of sand-dwelling macroalgae and seagrasses. These back-reef environs comprise a well-developed lagoonal system remote from major human pollutants. Organic detritus rarely accumulates on coral-dominated reefs because of intense herbivory and export processes. However, the characteristic nutrient limitation patterns typically observed in tropical reef systems are not applicable to the detritus-rich mangrove-peat systems of Twin Cays (Lapointe et al., 1987), which are characterized by elevated nutrient availability.

In general, mangrove ecosystems are well-known for their high levels of marine compost (Fell et al., 1980; Newell et al., 1984) that release relatively high concentrations of dissolved inorganic nitrogen and phosphates (Snedaker and Brown, 1981) into the adjacent water column. Considering that nutrient uptake kinetics in macroalgae are highly concentration dependent, mangrove macroalgae have been shown (Lapointe et al., 1987) to be far less nutrient-limited compared to macroalgae on coral reefs, based on seawater and tissue analyses as well as nutrient limitation/bioassays performed at the identical sites investigated here (i.e., Curlew Cay and Twin Cays, Fig. 1). Also, the geology, natural history and biology of these systems are comparatively well-known as a result of over three decades of multidisciplinary investigations (see Rützler and Macintyre 1982, Rützler and Macintyre this volume).

The rhizomatous ("rooted") Bryopsidales are considered to be important stabilizers of both organic and carbonate sediments. It also has been documented (Williams and Fisher, 1985; Littler et al., 1988; Littler and Littler, 1990) that the rhizoidal sand-dwelling forms of the open lagoon play a significant role in cycling nutrients from sediment pore waters. Rhizophytes such as *Avrainvillea*, *Udotea*, *Halimeda*, *Penicillus*, *Rhipocephalus*, *Cladocephalus* and *Caulerpa* (Chlorophyta, Bryopsidales) are among the predominant contributors to macroalgal cover and primary productivity within the vast seagrass meadows throughout the tropical western Atlantic. Such "rooted" plants, by tapping into the nutrient-rich interstitial pore waters (Littler and Littler, 1988), can avoid many of the nutrient-limitation problems experienced by their rock-dwelling counterparts.

While seagrasses and diverse macroalgal phyla are abundant on the outer perimeters of Twin Cays (Fig. 2, Littler et al., 1985), it is the siphonaceous Chlorophyta that dominate the standing stocks and productivity of submerged interior habitats within the mangrove island proper. In particular, the genus *Avrainvillea* is conspicuous among the predominant contributors to biomass and primary productivity within the vast array of creeks, ponds, lakes and borders of Twin Cays. Some members of the siphonaceous green algae characteristic of Twin Cays contain unique and interesting secondary chemical compounds (Sun et al., 1983;

Figure 2. Oblique aerial view of Twin Cays (looking east) showing the many hidden lakes and ponds. The Grouper Gardens study site is labeled on the upper right.

Figure 3. Colonial sea anemones using *Avrainvillea* blades as an attachment substrate.

4

Figure 4. The cryptic crab
Thersandrus compressus (arrow) is
a specialist feeder on *Avrainvillea*
that has a negative impact (Hay et
al., 1990; Littler and Littler, 1999).
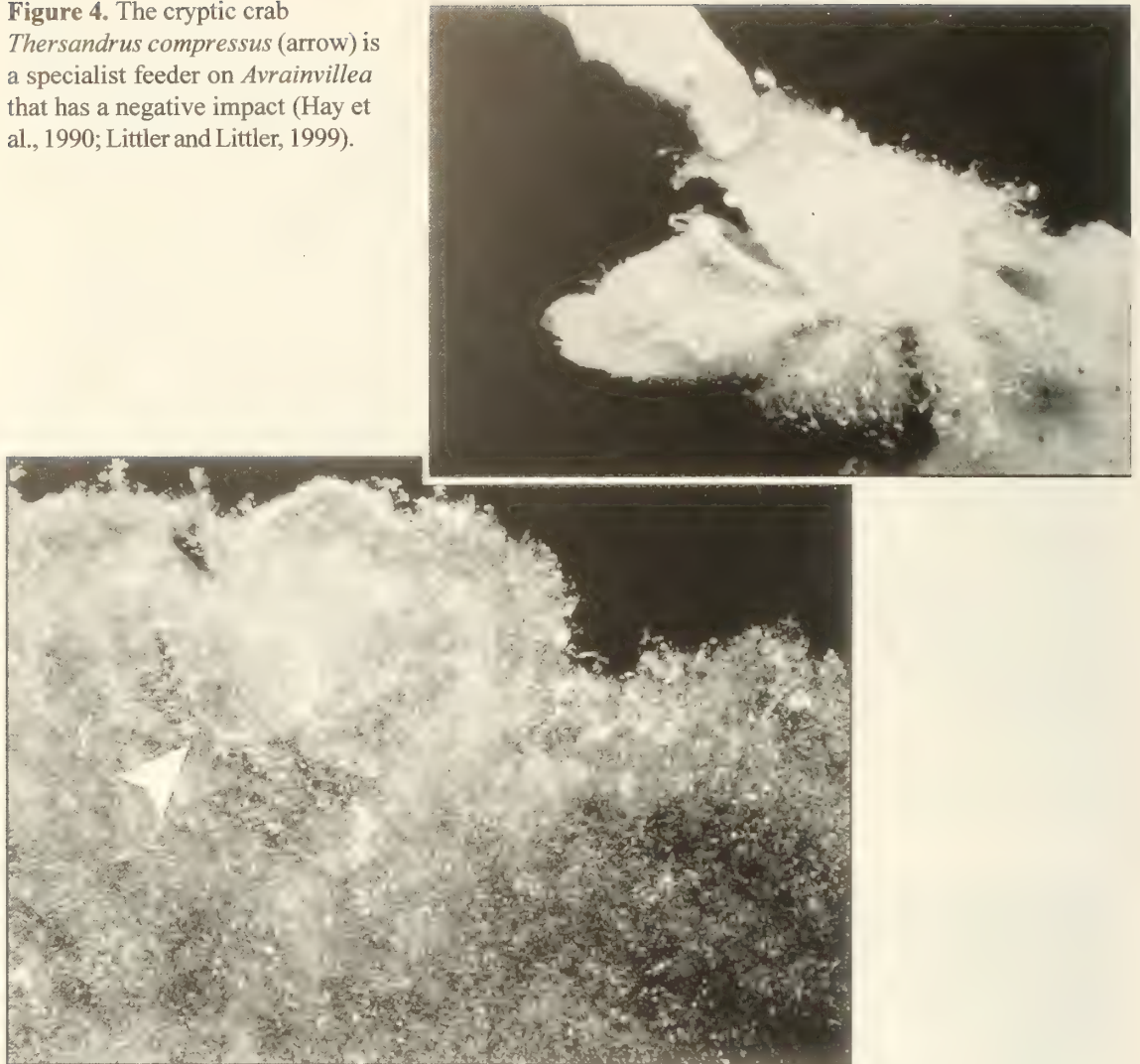
Hay and Fenical, 1988) and highly specialized interactions between the larger forms (e.g.,
Udotea, *Avrainvillea*, *Caulerpa*, *Penicillus*) and such herbivorous invertebrates as crabs and
molluscs have been observed (Hay et al., 1990; Littler and Littler, 1999). *Avrainvillea*
provides microhabitats (Fig. 3), as well as food and shelter (Fig. 4), for many meso- and micro-
invertebrates. These one-sided associations have proven (Hay et al., 1990) to be primarily
beneficial to the invertebrates and detrimental to the algal host.

The mucilage-free spongy textures of *Avrainvillea* would seem to make them
susceptible to epiphytic plant/animal loading. However, we showed in an earlier study (Fig. 5,
Littler and Littler, 1999) that the solitary lagoon morphs are able to rapidly produce new fronds
by cytoplasmic streaming and translocation through their siphons, a process that is not impaired
by cross walls (as is the case of cellular plants). This represents a unique antifouling mechanism
(Littler and Littler, 1999) whereby old assimilators and their inhibitory epiphytes can be shed by
"blade abandonment/proliferation" at relatively low cost to the plant.

A major obstacle to understanding the ecological role of siphonaceous algae at Twin Cays has been the high biodiversity of the taxonomically problematical genera named above. Six distinct species of *Avrainvillea* co-occur in the creeks, ponds and lakes of Twin Cays (Figs. 6, 7). Using treatments prior to the beginning of this investigation (Taylor, 1960; Norris and Bucher, 1982), it would have been possible to discern only a small fraction of the taxa that are actually present. As one example, *Avrainvillea longicaulis* f. *longicaulis* (Fig. 6) and the similar appearing *A. mazei* (Fig. 7) co-occur at Twin Cays as well as throughout lagoonal grass bed habitats and require precise discrimination (see misidentification of *A. mazei*, as *A. longicaulis*, on page 225 of Humann and DeLoach, 2002). In fact, there had been no serious systematic work on the group since the turn-of-the-century (Gepp and Gepp, 1911) with the
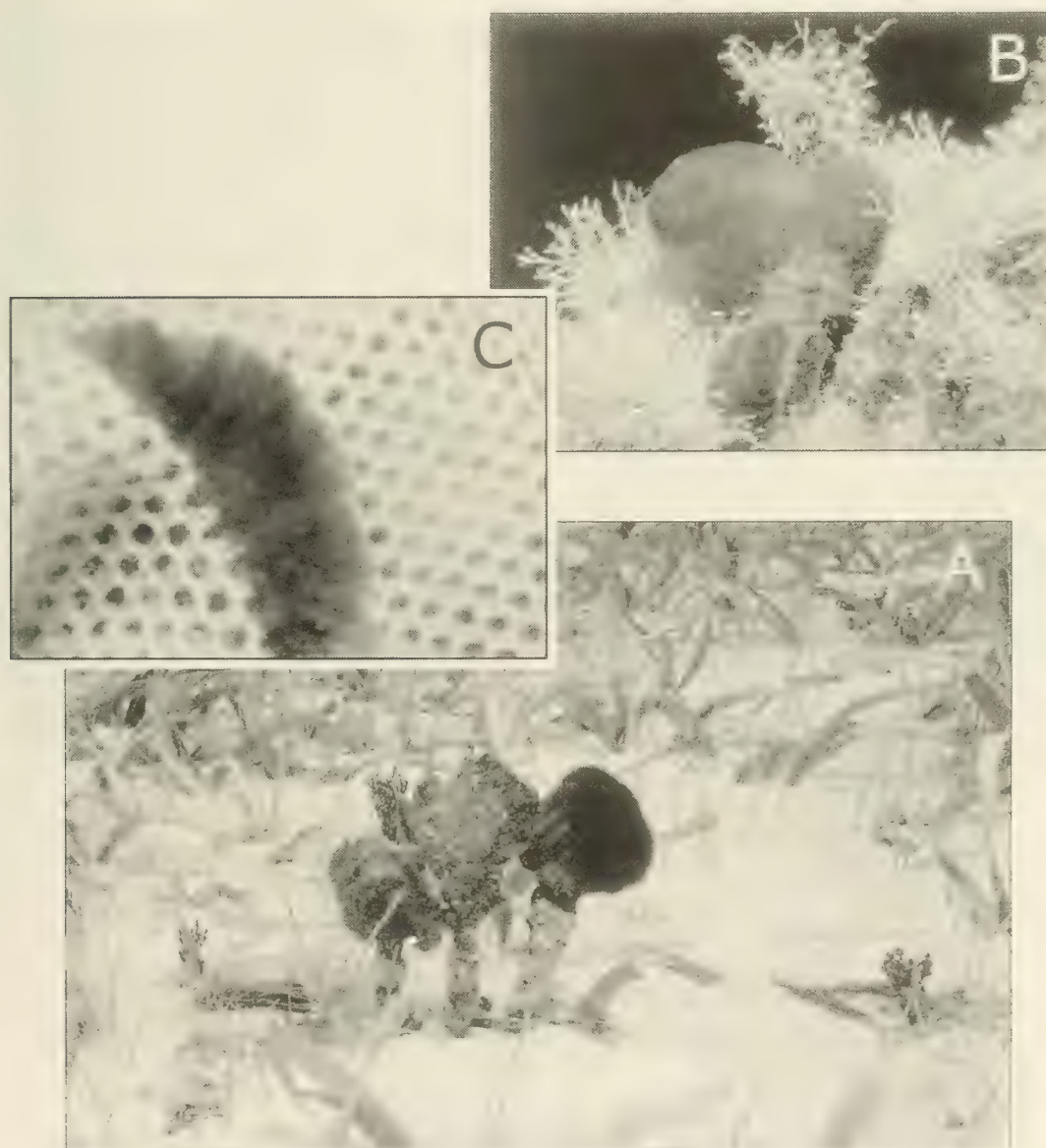
Figure 5. The paddle-like blades of the lagoon forms of *Avrainvillea* (A) can rapidly translocate protoplasm to proliferate new epiphyte-free blades (B-natural epiphytes, C-mesh bag).

6

exceptions of herbarium-based treatments of *Halimeda* (Hillis-Colinvaux, 1980) and Pacific *Avrainvillea* (Olsen-Stojkovich, 1985). The systematic monograph on tropical western Atlantic *Avrainvillea* (Littler and Littler, 1992), as well as the floristic field guide for the nearby Pelican Cays (Littler and Littler, 1997), alleviated the major taxonomic stumbling blocks and enabled this study.

Experimental Organisms

As mentioned, the siphonaceous green algal genus *Avrainvillea* often dominates the standing stocks and productivity of submerged habitats within mangrove island creeks, ponds and lakes as well as occurring abundantly throughout virtually all calm-water reef systems.
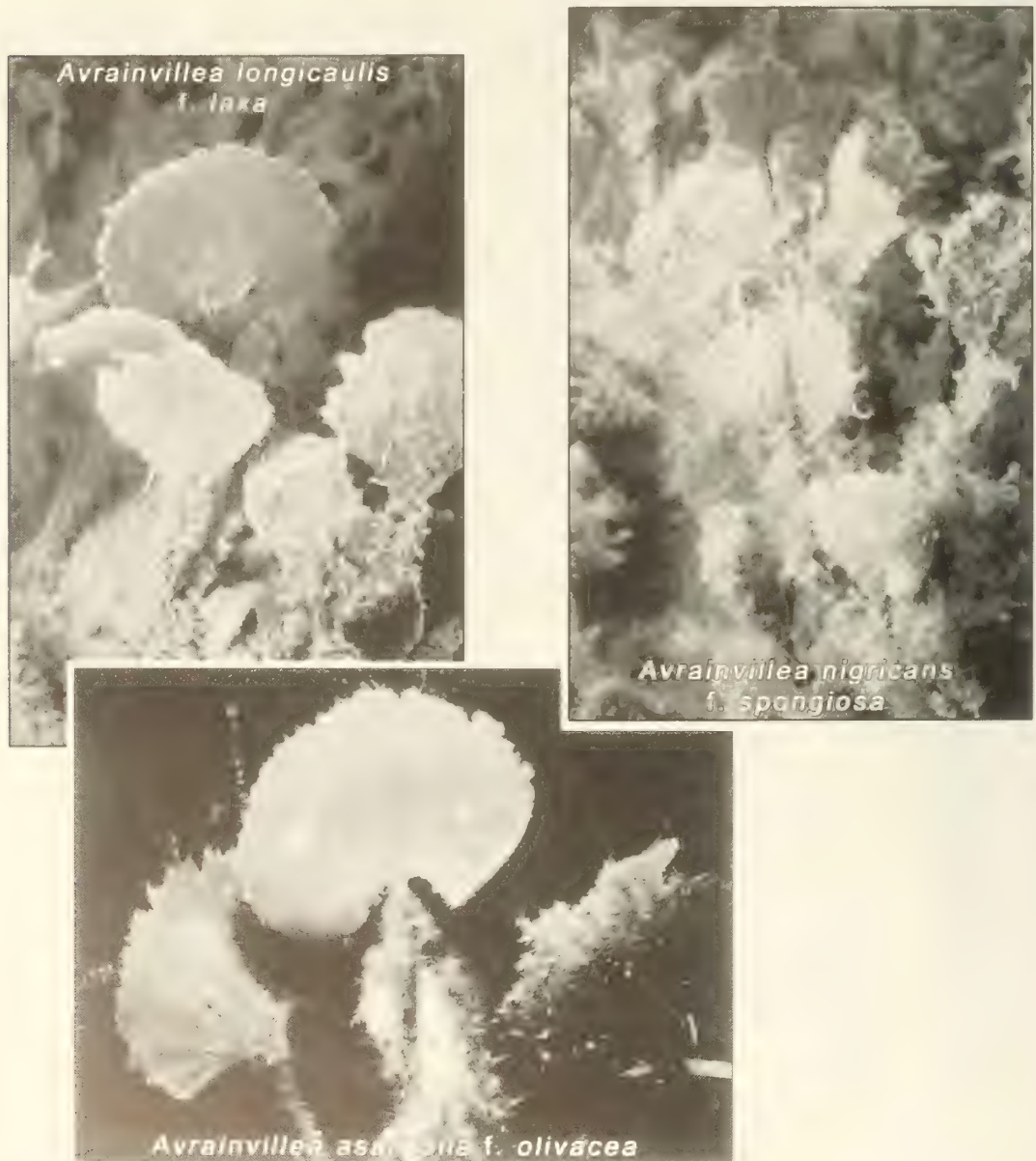
Figure 6. The three species shown here (*Avrainvillea nigricans* f. *spongiosa, A. asarifolia* f. *olivacea* and *A. longicaulis* f. *laxa*) create large mound-like colonies in mangrove lakes and ponds.

Figure 7. The three species shown here occur as individuals at Twin Cays but do not form colonial mounds.

Although sporogenic reproduction has never been reported for Belizean *Avrainvillea*, rare club-shaped release structures produced at the tips of individual blade siphons have been observed elsewhere (Littler and Littler, 1992). Unlike other Bryopsidales, species of *Avrainvillea* are long-lived (see Littler and Littler, 1992) and do not undergo holocarpy [i.e., mass synchronous sporogenesis (Clifton, 1997) followed by death and disintegration of the entire thallus].

The experimental macroalgae *Avrainvillea longicaulis* f. *longicaulis*, *A. longicaulis* f. *laxa* (Fig. 8), *A. asarifolia* f. *asarifolia* and *A. asarifolia* f. *olivacea* (Fig. 9) are particularly abundant but mostly unstudied in the Belize Barrier Reef lagoon and mangrove islands. The paddle-shaped blades (=flabella, caps or assimilators) of *Avrainvillea* number from one to many and are broadly oval (to 24 cm high, to 29 cm wide) with truncated lower margins. They are thick (> 4 mm) and spongy (lacking a mucilaginous coating) with cylindrical or flattened stipes (to 12 cm long, 13 mm diam.). The blades, stipes and holdfasts are composed of dichotomously branched interconnected siphons entirely lacking cross walls. The thalli of *A*.

Figure 8. The two dramatically different morphological forms (morphs) of *Avrainvillea longicaulis* (f. *longicaulis* & f. *laxa*). However, note the anatomical (siphons) similarities.
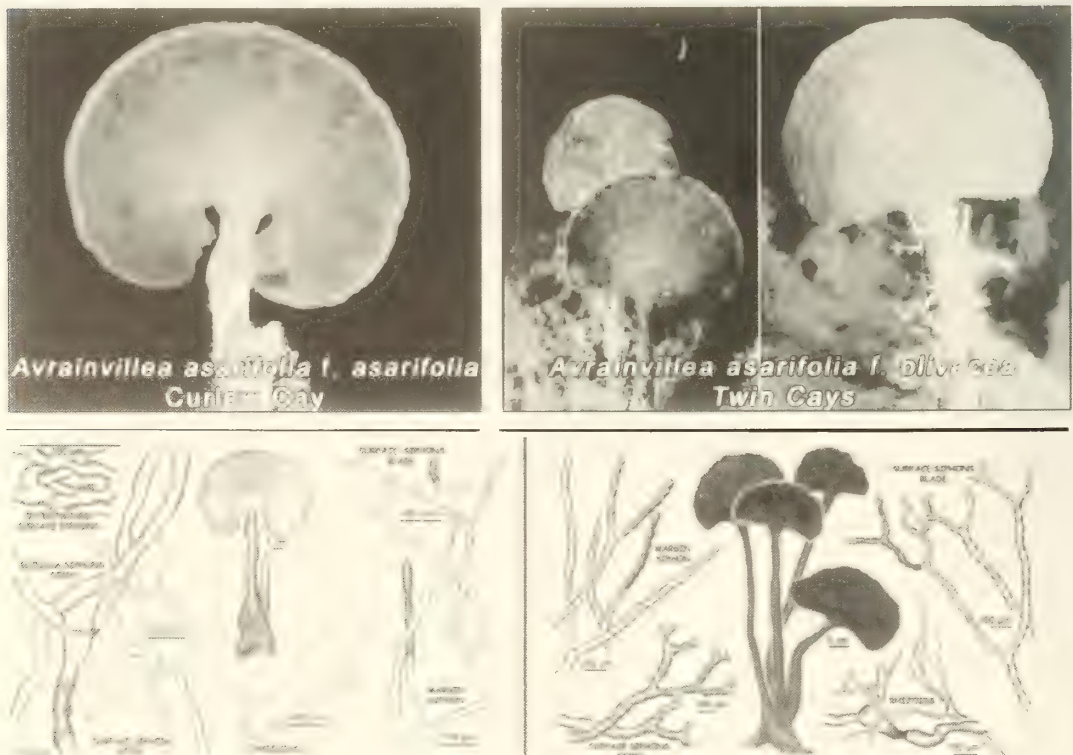
Figure 9. The two dramatically different morphological forms of *Avrainvillea asarifolia* (f. *asarifolia* & f. *olivacea*). However, note the anatomical (siphons) similarities.

longicaulis f. *longicaulis, A. asarifolia* f. *asarifolia* and *A. nigricans* f. *nigricans* (Figs. 8, 9, 10) are typically anchored by a massive, perennating, bulbous, rhizoidal holdfast (Fig. 11) in open sandy or seagrass areas of shallow (to 30 m) pristine waters.

As emphasized above, the discovery of incredible mound-building colonial morphs of *Avrainvillea* [*A. longicaulis* f. *laxa* (Fig. 8), *A. asarifolia* f. *olivacea* (Fig. 9) and *A. nigricans* f. *spongiosa* (Fig. 10)] catalyzed this study. These three colossal mound-formers are restricted to shallow (<3 m), placid, peat-bottom, high-nutrient waters in the protected interiors of mangrove islands.

HYPOTHESES TESTED

Coloniality Hypothesis

To reiterate, *Avrainvillea longicaulis* f. *longicaulis* and *A. asarifolia* f. *asarifolia* (Figs. 8, 9) are solitary in open lagoonal sandy environments with consistent wave action but can form extraordinary decades-old colonial (possibly clonal?) mounds (Fig. 12). The taxa, described (Littler and Littler, 1992) as *A. longicaulis* f. *laxa* (Fig. 8) and *A. asarifolia* f. *olivacea* (Fig. 9), are persistent in peaty, highly eutrophic, placid, interior mangrove habitats. The f. *laxa* and f. *olivacea* morphs hypothetically (i.e.,
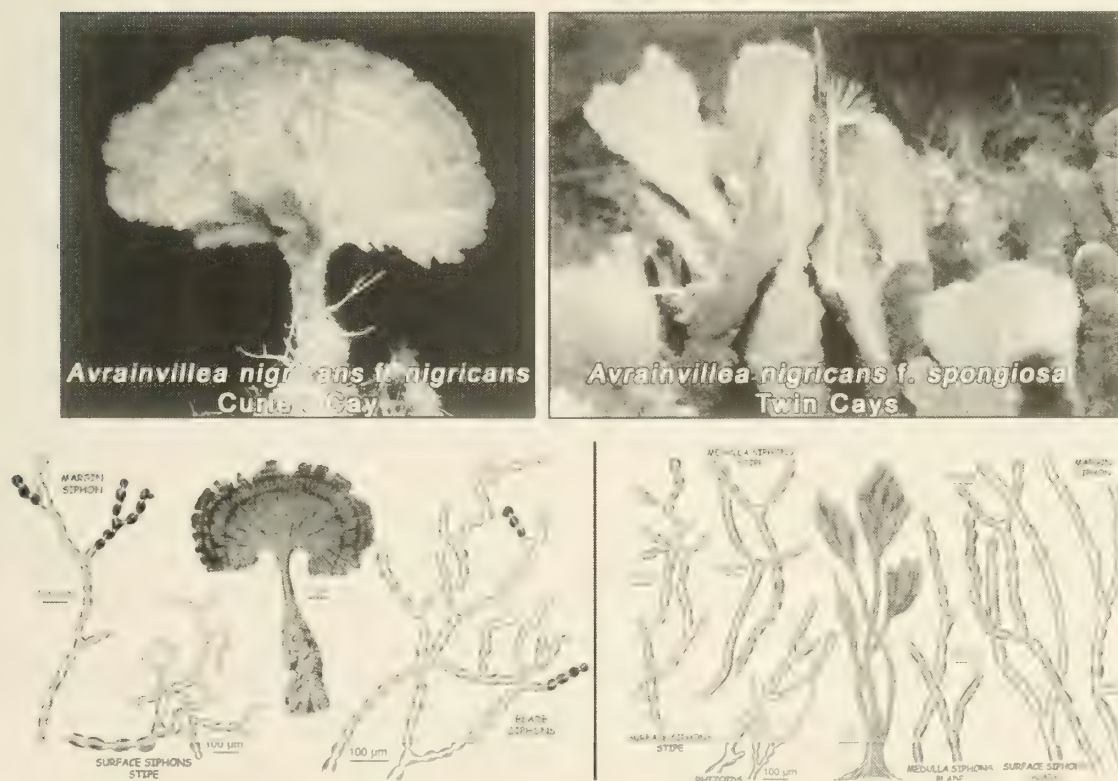
Figure 10. The two dramatically different morphological forms of *Avrainvillea nigricans* (f. *nigricans* & f. *spongiosa*). However, note the anatomical (siphons) similarities.

Figure 11. The massive perrenating, bulbous, rhizoidal holdfast of *Avrainvillea longicaulis* f. *longicaulis* characteristic of open sandy lagoonal areas.

Figure 12. Portion of a colossal colonial mound of *Avrainvillea longicaulis* f. *laxa* supporting diverse epiphytes at Twin Cays.

"coloniality hypothesis") are uniquely adapted to utilizing flabellar stipes as shallow subterranean rhizomes that spread laterally to produce enormous (several meters-thick, to 30 m diameter, Fig. 12) mound-like colonies that overgrow rich organic peat bottoms. Massive columnar rhizoidal holdfasts, such as those found in the f. *longicaulis* (Fig. 11) and f. *asarifolia* (Fig. 9) morphs, hypothetically would be superfluous under placid enriched water-column nutrient conditions as well as incapable of surviving the deeper anoxic conditions of the composting peat deposits. Conversely, open-water wave surge and current drag on the huge colonial morphs (lacking a strong anchoring rhizoidal holdfast that could augment the low nutrient conditions in open lagoonal waters) should result in uprooting, wave-shearing damage and general attrition.

Perennation Hypothesis

In the open-water morphs of *Avrainvillea longicaulis* f. *longicaulis* and *A. asarifolia* f. *asarifolia* (Littler and Littler, 1992), we had observed what appeared to be perennation; where the remains of lost blades were indicated by breakage points and scars with newly forming flabella arising from either the former stipes or columnar holdfasts. We postulated (i.e., "perennation hypothesis") that the stipes and blades of *Avrainvillea longicaulis* f. *longicaulis* and *A. asarifolia* f. *asarifolia* serve as expendable photosynthetic assimilators with a major function of building a massive perennial storage organ, the columnar rhizoidal holdfast. This structure (Fig. 11) can comprise up to 90% of the total thallus (Olsen-Stojkovich, 1985; Littler and Littler, 1999). In other words, physical disturbances (such as storms and herbivory) as well

Figure 13. Conducting primary productivity experiments on *Avrainvillea longicaulis* f. *laxa* at Twin Cays using oxygen electrode methods (Littler and Littler, 1987).
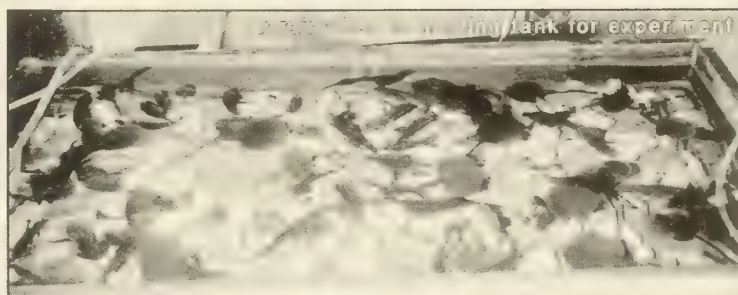
as physiological stresses (such as epiphyte loading) should result in disproportionate losses of the relatively delicate above-ground assimilators, which can be replaced by perennation from the massive subterranean holdfasts (Fig. 11) during more favorable conditions.

METHODS AND MATERIALS

Experimental Taxonomy

The critical initial phase of this research included completion of a systematic and phylogenetic monograph of Caribbean *Avrainvillea* based on intensive and extensive collections throughout Twin Cays and surrounding environments (Littler and Littler, 1992). As emphasized, the discovery of astounding mound-building persistent colonial morphs of *Avrainvillea* motivated this study. However, taxonomic issues still prevailed regarding these mound formers, which were initially thought by us to be distinct species although the internal anatomical data suggested (Littler and Littler, 1992) otherwise. The experimental "common garden" reciprocal transplant approach used here (see below) provided quantitative resolution of these issues.

Coloniality Hypothesis

We used a costs vs. benefits approach to test the coloniality hypothesis (N=10/ treatment). We posited that the two sets of remarkably different morphs (i.e., f. *laxa* vs. f. *longicaulis* and f. *olivacea* vs. f. *asarifolia*) are adaptive for their respective habitats. We attempted to experimentally induce colony formation in the f. *longicaulis* and f. *asarifolia* morphs by burial of the flabellar stipes as well as by conducting reciprocal transplant

12

experiments with appropriate control (C= tagged only) and transplant controls for both morphs. In each habitat (i.e., Curlew Cay and Twin Cays, Grouper Gardens, Figs. 1, 2), the transplant controls (TC) were completely removed by careful digging and gently replanted in holes wedged opened by titanium crowbars in the nearby general area. The experimental transplants (T) were carefully removed, floated into a 100-liter cooler of seawater and transferred to the reciprocal field sites where they were carefully replanted as above.

As described earlier, this "common garden" approach also led to an experimental taxonomic analysis of whether or not the various morphs might represent distinct species. All replicates were tagged by nearby surveyors' flags. After one year of growth, the plants were returned to the laboratory for final photography and morphometric documentation. If we could (1) induce coloniality in the individuals of f. *longicaulis* and f. *asarifolia* (under the presence of high nutrients and benign physical conditions) within calm interior mangrove ponds and (2) show that large colonies of f. *laxa* and f. *olivacea* are susceptible to removal by natural levels of current and wave surge, then the coloniality hypothesis would be deemed to be supported. A further bonus in support of the hypothesis would be (3) any sign of long-term induction of columnar holdfasts in the f. *laxa* and f. *olivacea* transplants moved from mangrove island pools into open water habitats.

Perennation Hypothesis

In addition, we concurrently tested the perennation hypothesis as follows: (1) in experimental lagoon thalli with blades physically amputated, proliferation of new blades should be stimulated and (2) moderate losses of blades should not lead to high levels of mortality relative to control plants not subjected to such mutilation. Twenty separate plants of *Avrainvillea longicaulis* f. *longicaulis* and another 20 of *A. asarifolia* f. *asarifolia* were assessed in the lagoon behind Curlew Cay. Both sets were divided into the following two replicate groups (N=10) by double randomization to provide: controls (CO, to correct for natural changes and possible stochastic events) and cut plants (C, to simulate natural physical damage). All were marked by surveyors' flags.

The same procedure was repeated at Grouper Gardens for *Avrainvillea longicaulis* f. *laxa* and *A. asarifolia* f. *olivacea* (N=10/treatment). After one year, the controls (CO, which had been left intact) and the amputated/cut plants (C, which were trimmed with scissors, leaving the intact holdfast and 2-cm stipe lengths) were assessed for blade numbers and new proliferations. Data analysis employed ANOVA and the Bonferroni Test for significant differences.

Ecological Role

To assess the ecological importance of the *Avrainvillea longicaulis* f. *laxa* mounds, quantitative transect surveys of biotic cover were conducted using the nondestructive photogrammetric techniques developed by Littler and Littler (1985). This entailed video transects at right angles to the substrata that were then scored in stop action on a high-resolution video monitor. Cover was determined by recording the percentages of point intercepts from a randomized array superimposed over the video images. Two randomly selected 0.25 m sections at the edges of two separate mounds were harvested for biomass determinations.

These were cleaned of peat deposits and epiphytes, photographed and weighed wet. A set of subsamples from these were rinsed in freshwater, weighed, dried and reweighed to determine wet-to-dry weight relationships. Organic dry-weight (ODW) was determined by igniting the dried samples in a muffle furnace to constant weight at 500° C.

Primary productivity measurements were made for the dominant *Avrainvillea longicaulis* f. *laxa* using traditional light-dark bottle oxygen electrode techniques (Fig. 13). This, and the transect data, were used to ascertain an average mound's contribution to primary production at Twin Cays. We measured photosynthetic rates of the assimilators during early summer under ambient environmental conditions (30–31° C, 36 ppt salinity, 1500–2100 µmol photons per m² per sec) using the same methods detailed in Littler and Littler (1990). We incubated healthy assimilators containing natural levels of epiphytes and replicate blades with the epiphytes carefully removed by pinching (Fig. 13), as well as incubating the epiphytes separately (N =6 for all treatments), to ascertain the primary productivity contributions of the epiphytes. We chose photosynthesis as an indicator of physiological production since growth is relatively intractable due to the continual translocation processes and the inaccessibility of the stipe/holdfast system. Data analysis employed ANOVA and the Bonferroni Test for significant differences.

RESULTS

Experimental Taxonomy

The mound formers (f. *laxa* and f. *olivacea*), which were initially hypothesized to be putative species (Littler and Littler, 1992), presented unresolved taxonomic questions. The common-garden reciprocal-transplant approach provided definitive resolution of these issues. Following one year of transplantation, all experimental transplants had acquired the morphological features consistent with the taxa characteristic of the new habitats (Fig. 14, 15, 16, 17), thereby falsifying the hypothesis that the mound formers were distinct species.

Coloniality Hypothesis

The large colonial mangrove morphs (i.e., f. *laxa* and f. *olivacea*) were not resistant to even the moderate current levels (3.6±0.5 cm per sec) encountered in the back-reef lagoon habitats of the deeply anchored morphs (i.e., f. *longicaulis* and f. *asarifolia*). When buried to normal depths, the massive natural colonial morphs would pull free and begin to drift downstream (Figs. 18, 21). However, the majority of the 2- to 3-blade clumps (Fig. 15), with their stipes deeply buried in the sandy sediments, were able to survive and grow.

After one year, the surviving 2- to 3-blade clumps transplanted from Twin Cays to Curlew Cay had developed rudimentary stages of the massive holdfast with the adherent sand grains (Fig. 15) characteristic of f. *longicaulis* and f. *asarifolia*. Conversely, all of the surviving morphs with normally massive holdfasts that were transplanted to the mangrove pools at Grouper Gardens showed degeneration of the columnar holdfasts (Fig. 17), with only remnant rhizoids containing clumped sand grains present at the end of one year. Concurrently, they had developed new assimilators/stipes characteristic of the colonial morphs (f. *laxa* and f. *olivacea*). Both sets of surviving controls and transplant controls were 100% uniform in retaining morphs consistent with their original habitats.

14
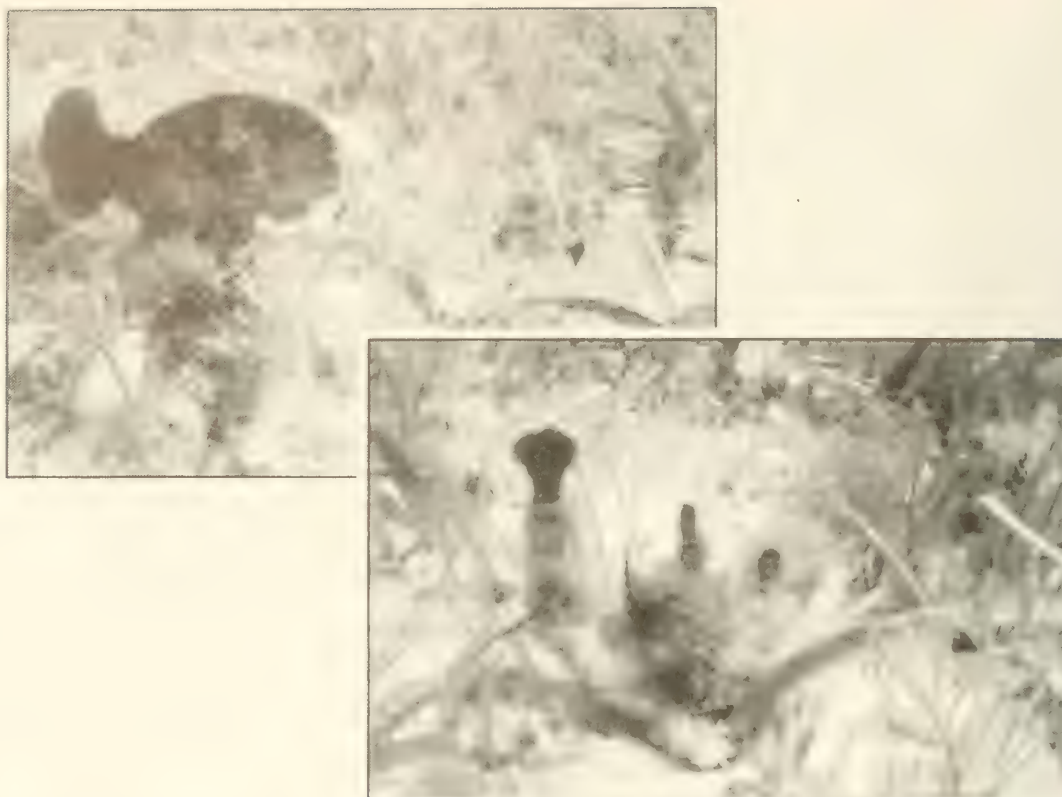
Figure 14. Examples of *Avrainvillea longicaulis* f. *laxa* transplanted from twin Cays to Curlew Cay after 12 months. Blades now are consistent with the f. *longicaulis* morph.

Figure 15. Examples of *Avrainvillea longicaulis* f. *laxa* transplanted from twin Cays to Curlew Cay and harvested after 12 months. Holdfasts now are consistent with the f. *longicaulis* morph.

15
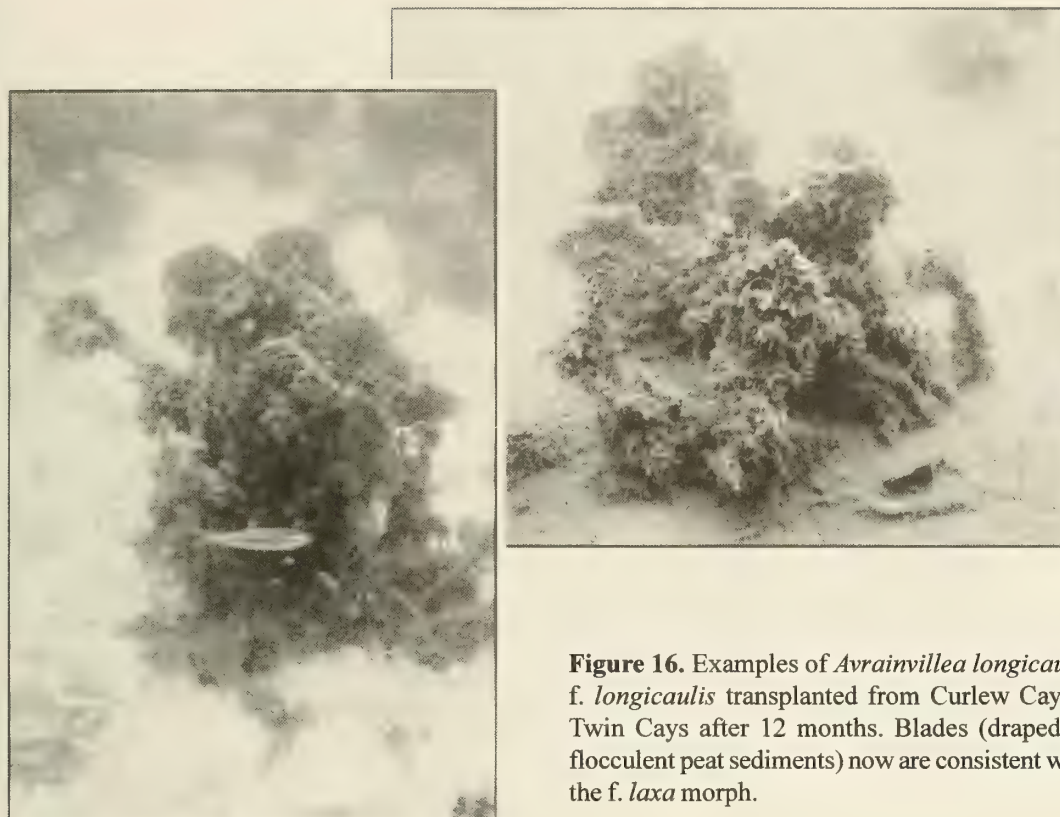
Figure 16. Examples of *Avrainvillea longicaulis* f. *longicaulis* transplanted from Curlew Cay to Twin Cays after 12 months. Blades (draped in flocculent peat sediments) now are consistent with the f. *laxa* morph.
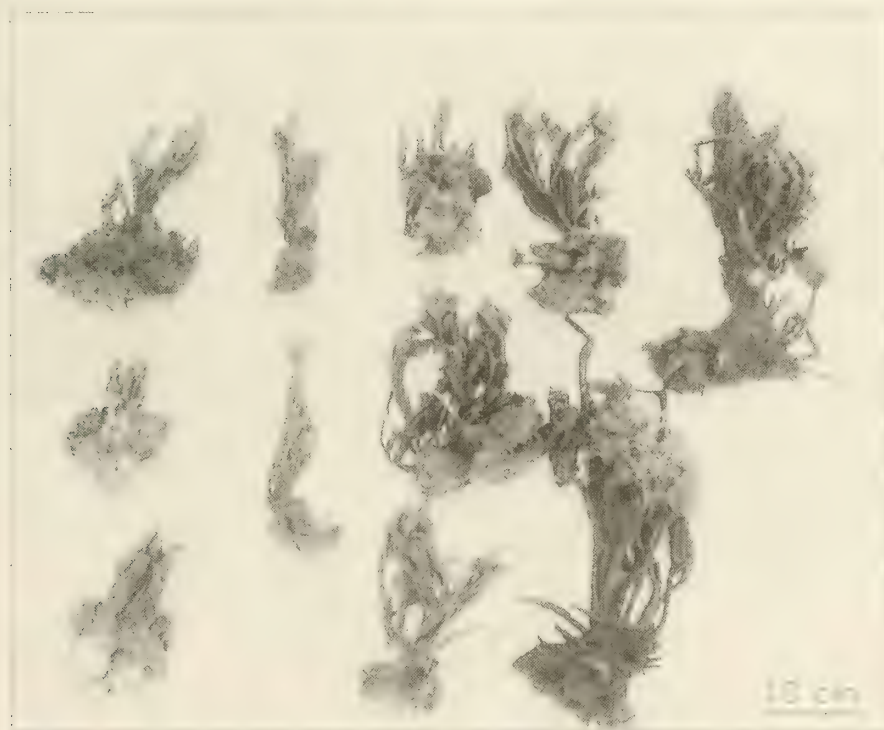
Figure 17. Examples of *Avrainvillea longicaulis* f. *longicaulis* transplanted from Curlew Cay to Twin Cays and harvested after 12 months. Pseudo-rhizomatous holdfasts and stipes now are consistent with the f. *laxa* morph.

16

We also discovered unexpected evidence in further support of the coloniality hypothesis in the case of *Avrainvillea longicaulis* f. *laxa*. We found that the colonial morphology is uniquely reinforced by the intermingling of blade and stipe siphons at areas of contact (Figs. 19, 25). Contact frequently occurs for prolonged periods in such calm habitats, leading to abundant anastomosing points of fusion/adhesion.
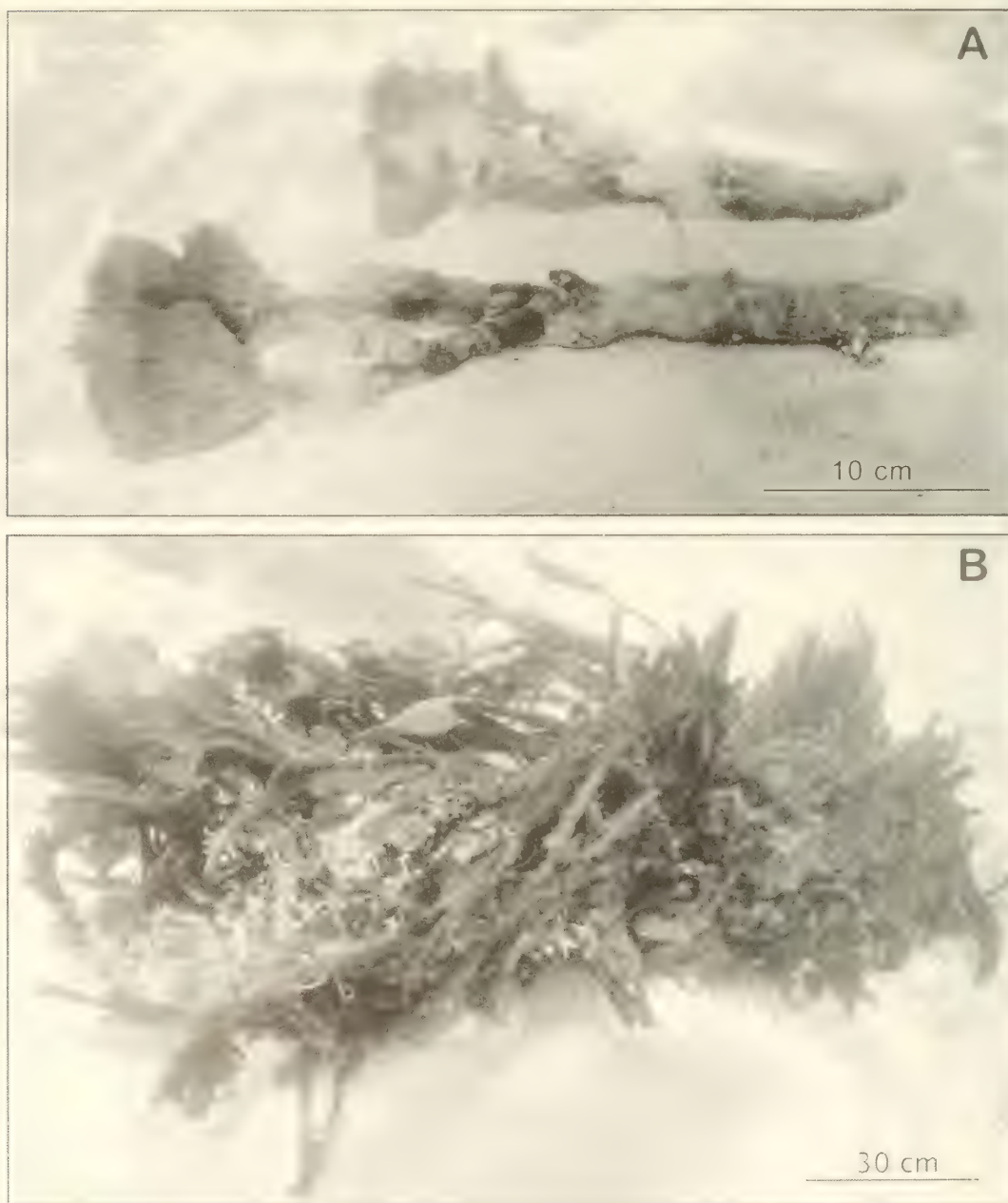
Figure 18. A – Two individuals of *Avrainvillea asarifolia* f. *asarifolia* from Curlew Cay. B – Colony of f. *olivacea* from Twin Cays. When both forms were transplanted to the back-reef sandy habitat, the f. *olivacea* colony was uprooted by current within hours, whereas the f. *asarifolia* thalli remained indefinitely.

Figure 19. Typical inter-thallus fusions characteristic of the colonial mound-forming species.

Perennation Hypothesis

In support of the hypothesis (Fig. 20), only the experimentally amputated Curlew Cay morphs (both f. *longicaulis* and f. *asarifolia*) showed significantly more proliferations (100 %) than either the experimentally amputated Twin Cays morphs (both f. *laxa* and f. *olivacea*) or the uncut Curlew or Twin Cays control plants. In particular, the amputated Curlew Cay *Avrainvillea longicaulis* f. *longicaulis* showed 100 % new proliferations, a significant fivefold increase relative to the Twin Cays f. *laxa* (20 %). The uncut controls from Curlew Cay f. *longicaulis* showed significantly fewer (70 %) new proliferations, whereas the Twin Cays experimental f. *laxa* plants also had significantly fewer (50 %) new proliferations.

In the case of *Avrainvillea asarifolia* f. *asarifolia* from Curlew Cay (Fig. 20), the experimentally amputated plants also had 100 % new proliferations paralleling the results for *A. longicaulis* f. *longicaulis*. The experimentally amputated f. *olivacea* also showed significantly fewer proliferations comparable to those for f. *laxa* (only 20 % new proliferations, significantly less at P < 0.05). The uncut control morphs of *A. asarifolia* from both Curlew Cay (f. *asarifolia*) and Twin Cays (f. *olivacea*) produced comparably low results as well, with significantly fewer (40 %) new proliferations (Fig. 20).

18

Ecological Role

One of the smaller *Avrainvillea longicaulis* f. *laxa* colonies measuring 0.6 X 1.1 m in diam. (Fig. 21) and hand-cleaned of debris and epiphytes (mostly unusual forms of *Laurencia intricata*, *Cladophoropsis membranacea* and *Polysiphonia flaccidissima*, Fig. 22) weighed 19 kg. Similar weights were recorded for comparable colonies of *A. asarifolia* f. *olivacea* (Fig. 18). Given that transect studies documented that both *A. longicaulis* f. *laxa* and *A. asarifolia* f. *olivacea* form colonies in excess of 30 m diam. (Fig. 12), their contribution to biomass in Twin Cays ponds is enormous.

Epiphyte-free *Avrainvillea longicaulis* f. *laxa* blades showed a net photosynthetic rate of about two-and-a-half mg C fixed per gram of organic dry mass (ODM) per h, with a dark respiration rate of about half a mg C consumed per g ODM per h (Fig. 23). Twenty newly formed blades (mean area = 50 cm^2) contained an average of 12 mg ODM per cm^2 (28 % C), which converts to about four mg C per cm^2 of proliferating blade. Given the net photosynthetic production determined above (with the normal inhibitory effects of natural levels of epiphytes) and assuming that this rate could be sustained throughout a 10 h-day, with dark respiration at half a mg C consumed per g ODM per h for 14 h (not including respiration of the pseudo-
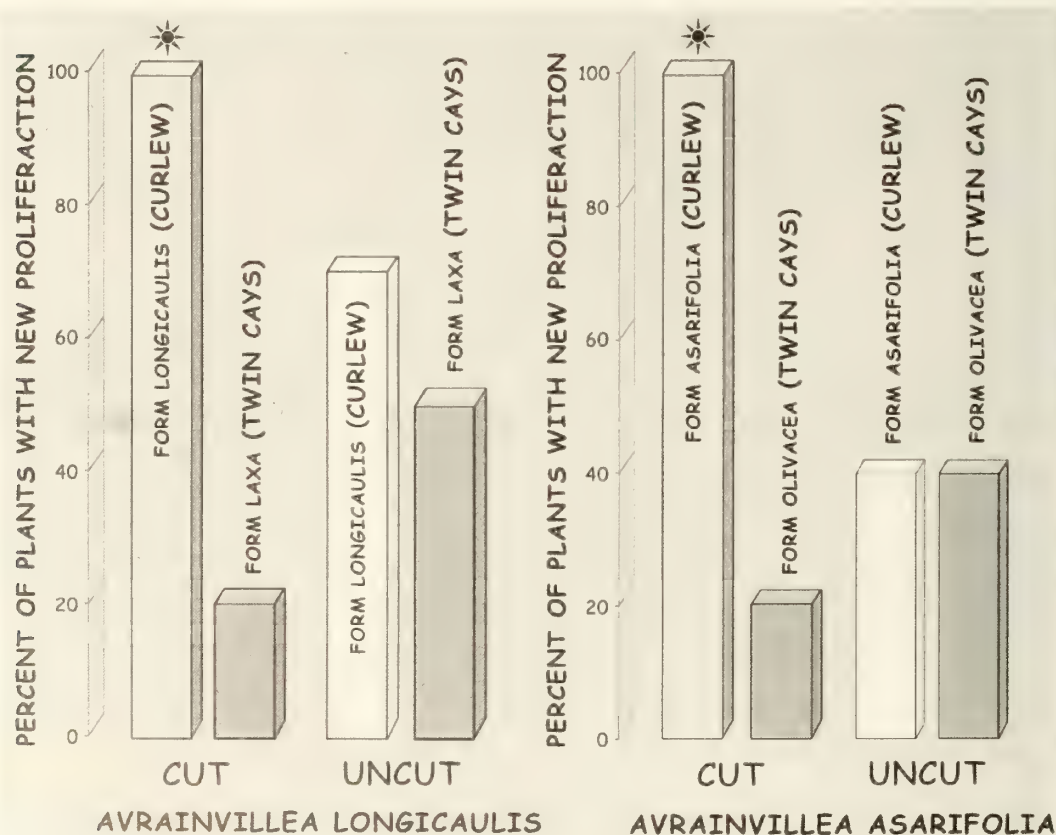
Figure 20. The percent of plants with new proliferations following mutilation (blade decapitation by cutting) after 12 mos. The massive holdfast morphs f. *longicaulis* and f. *asarifolia* from Curlew Cay showed significantly greater proliferation following cutting than the uncut treatments or the uncut and cut colonial mangrove morphs, f. *laxa* and f. *olivacea*. (* indicates significant differences at P<0.05)

Figure 21. This small colony of *Avrainvillea longicaulis* f. *laxa* from Twin Cays weighed 19 kilograms (spun wet weight).

Figure 22. Twenty meter diameter colony of *Avrainvillea asarifolia* f. *olivacea* at Twin Cays showing the extensive coverage of epiphytes (predominantly unusual forms of *Laurencia intricata, Cladophoropsis membranacea* and *Polysiphonia flaccidissima*).

rhizomatous mass), we arrived at a net rate of about four mg C per g ODM per day. The wet samples yielded an average dry weight (DW) of about 7% of the WW. When ignited to constant ash weight at 500° C, the organic dry mass averaged 79 % of the DW. Mature assimilators produced about two mg C per plant (single stipe with blade) per day. Based on these calculations, the productivity of an average single mound of *Avrainvillea* at Twin Cays would conservatively yield an astounding 4 kg of carbon fixed per day. The productivity of a square meter of an average mound calculates at 6.2 g of C fixed per day.

DISCUSSION

Taxonomy

Experimental field approaches to macroalgal taxonomic questions are seldom utilized even though the rapid growth of most seaweeds makes them amenable to manipulative techniques. The "common garden" reciprocal transplant experiment provided definitive resolution of the hypothesis that the mound formers (f. *laxa* and f. *olivacea*) were discrete species. Following one year of transplantation, all experimental transplants had acquired morphological features that were consistent with the morphs characteristic of their new habitats (Fig. 24). This result, and the internal anatomical data (Littler and Littler, 1992), supports the hypothesis that the mound forms are not distinct from the solitary forms (f. *longicaulis* and f. *asarifolia*) and, therefore, falsifies the hypothesis that mound-forming colonial taxa are separate species.
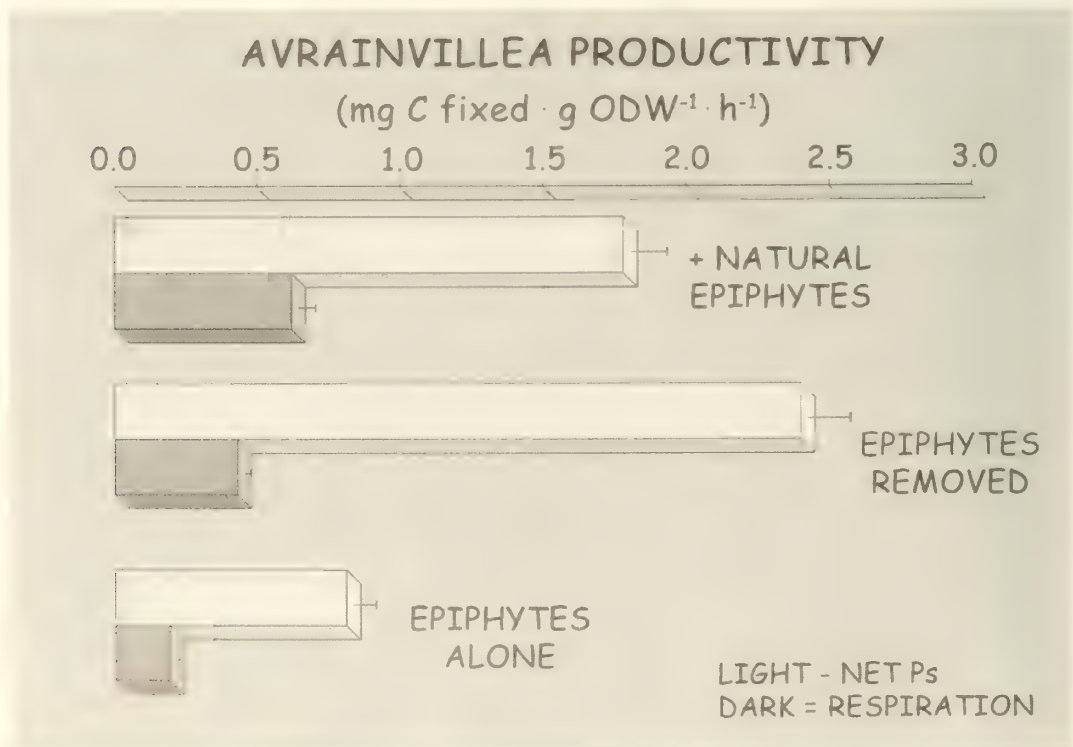
Figure 23. The net primary productivity (light histograms) and respiration (dark) of *Avrainvillea longicaulis* f. *laxa* blades with natural epiphytes, epiphytes removed and epiphytes alone.

Avrainvillea transplanted from Twin Cays ponds to Curlew Cay (Fig. 15).

5 cm

Avrainvillea transplanted from Curlew Cay to Twin Cays Ponds (Fig. 17)

10 cm
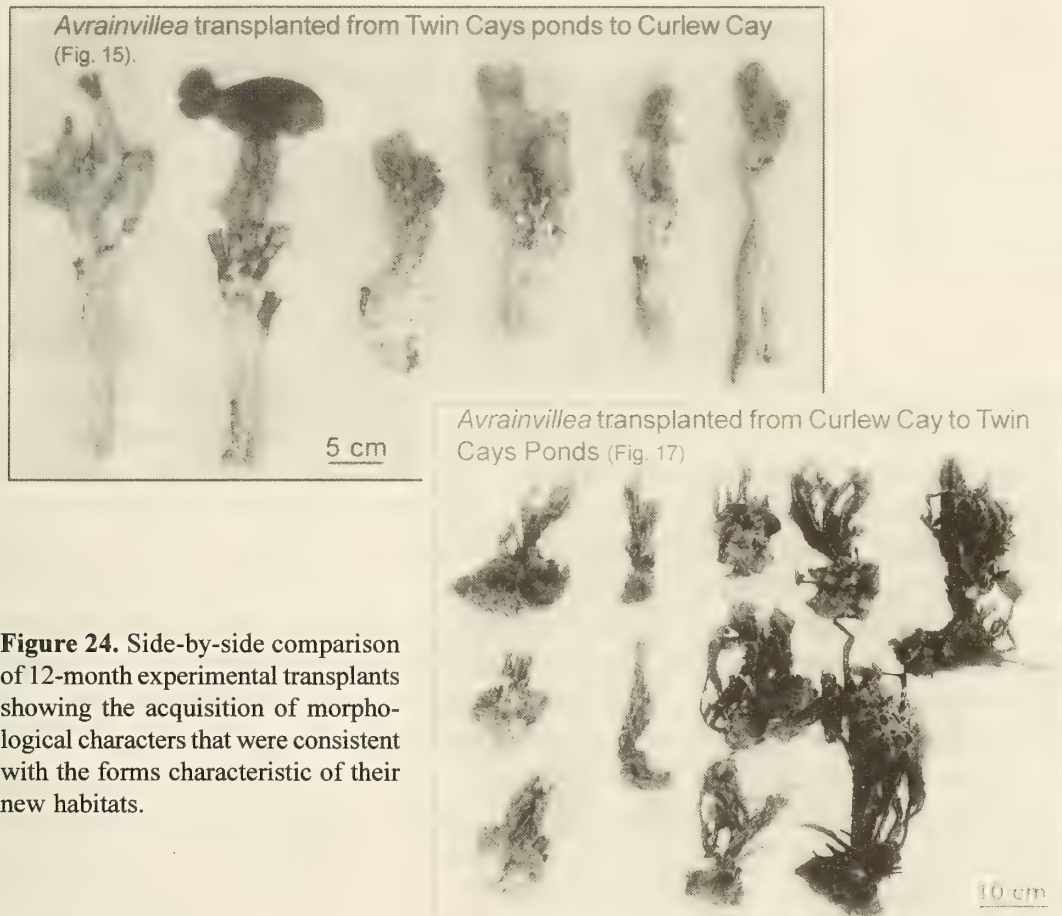
Figure 24. Side-by-side comparison of 12-month experimental transplants showing the acquisition of morphological characters that were consistent with the forms characteristic of their new habitats.
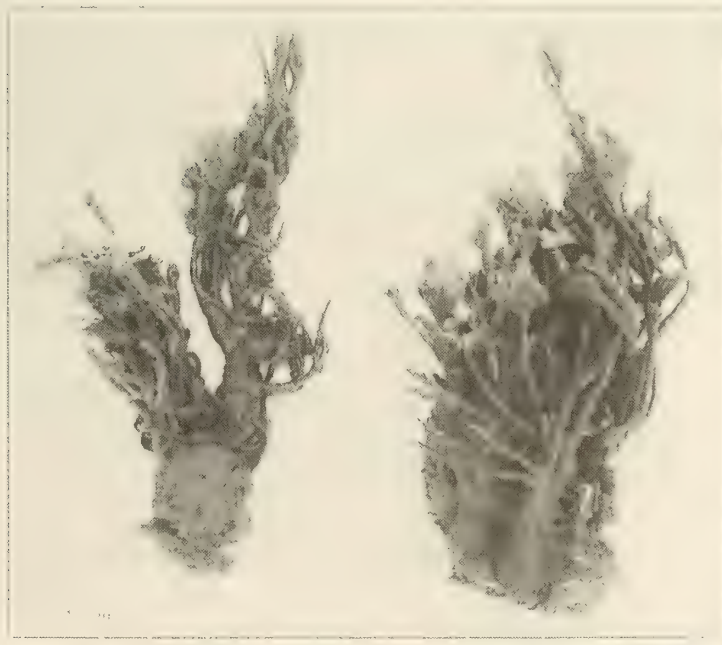
Figure 25. *Avrainvillea longicaulis* f. *laxa* showing tangled jumble of stipes and blades forming extensive mounds in Twin Cays ponds. Note the tangled fused pseudo-rhizomatous stipe structure adaptive for the flocculent anoxic peat substrate.

Coloniality

This study suggests that the ecological attributes of mangrove interior ponds, lakes and creek areas select for the colonial morphs of *Avrainvillea*, not only by providing refuge habitats from the intense fish-and-sea-urchin herbivory (Taylor et al., 1986) that is associated with open-water systems (e.g., Littler et al., 1983; Lewis, 1986) but also by ameliorating the nutrient stresses that frequently occur in such reef- and lagoon-ecosystems. This enables the more delicate colonial morphology to prevail, spreading by means of the unique pseudo-rhizomatous stipe structure (Fig. 25) to cover the otherwise unavailable flocculent anoxic peat substrate.

It is interesting to note that the mound formers, while capable of overtopping other psammophytic (sediment dwelling) organisms, tend to bear prodigious quantities of epiphytes such as *Laurencia intricata*, *Cladophoropsis membranacea* and *Polysiphonia flaccidissima* (Figs. 12, 22), which, given sufficient light, would add about 30% to overall colony productivity in this shallow light- and nutrient-rich environment (Fig. 23). An earlier study at Twin Cays (Littler and Littler, 1985) recorded 17.2 and 13.4 grams of carbon fixed per square meter per day at outer fringe, dense seagrass/algal, bay- and channel-sites, respectively. These values rank among the higher productivity rates recorded and were two-to-three times the production rates of the mangrove pond *Avrainvillea* colonies calculated in the present study. In contrast to the epiphytized colonial morphs, the deeper occurring lagoon morphs have been shown (Littler and Littler, 1999) to actively expel their more harmful epiphyte loads by translocation followed by senescence and shedding (i.e., blade proliferation/ abandonment, Fig. 5).

The theoretical costs vs. benefits of coloniality in terrestrial plants and marine animals (e.g., see review by Jackson, 1977) have received substantial attention. However, consideration of this phenomenon for marine plants previously had been limited to the advantages/disadvantages of the algal-turf morphology (Hay, 1981). In comparison to the extraordinary mound-forming species of *Avrainvillea*, it should be noted that two other genera of Bryopsidales also form knoll-like colonies. *Caulerpa* species, particularly the various forms of *C. racemosa*, can overgrow reef habitats to create small (tens-of-centimeters high), but often extensively spreading, humps. *Halimeda* is unique for the massive (tens-of-meters high) fossil bioherms recorded (Drew 1997) from the Great Barrier Reef lagoon. Although time and resources did not allow us to do comparative functional morphology studies on the two morphs of *A. nigricans* (i.e., f. *nigricans* and f. *spongiosa*), we predict that the findings would have closely paralleled those for the morphs of *A. longicaulis* and *A. asarifolia*.

Perennation

We also showed that the stipes and blades of the open-water morphs (*Avrainvillea longicaulis* f. *longicaulis* and *A. asarifolia* f. *asarifolia*) indeed serve as expendable assimilators, with a major function of building a massive perennating/storage organ, the columnar holdfast (Fig. 11), which comprises the bulk of the thallus biomass (Olsen-Stojkovich, 1985). Among all the other Bryopsidales, *Avrainvillea* is uniquely long-lived (see Littler and Littler, 1992) and does not undergo holocarpic reproduction (Clifton, 1997) leading to death. Physical disturbances (such as storms and herbivory), as well as physiological stresses (such as epiphyte loading), result in disproportionate losses of the relatively delicate expendable assimilators, which can be readily replaced by perennation from the long-lived subterranean holdfast during

more favorable conditions. Selection for this strategy is amply represented in terrestrial environments as shown by the multitude of vascular plants that crown sprout after physical forces such as severe storms, fires, freezes or overgrazing have destroyed the above-ground canopies. However, the only relevant marine example (Heck and Valentine, 1995) is the seagrass *Thalassia testudinum* which is able to compensate for short-term grazing losses on emergent shoots by mobilizing stored carbohydrates from the rhizomes (see Tomasko and Dawes, 1989).

Ecological Role

The advantage of the deeply rooted morphs of *Avrainvillea* in open-water sedimentary seagrass environments, such as Curlew Cay where the water column nutrients are consistently low, lies in the fact that these plants can avoid physical catastrophic losses while tapping into the much higher concentrations of interstitial pore-water nutrients (e.g., >200 μmol N, Williams and Fisher, 1985). These findings add a further dimension to observations of nutrient-limited productivity of benthic algae on tropical reefs (Kinsey and Domm, 1974; Kinsey and Davies, 1979; Smith et al., 1979; Hatcher and Larkum, 1983; Lapointe et al., 1987). Conversely, we have shown that colonial adaptations of *Avrainvillea* that take advantage of high nutrient, but anoxic, environments, such as commonly found in mangrove interior creeks, lakes and ponds, result in some of the most prolific communities known. Documentation of such ecosystem level differences in nutritional state and productivity, relative to the functional morphology of the dominant primary producers, is critically needed in the construction of successful models of benthic productivity for tropical marine systems.

ACKNOWLEDGMENTS

REFERENCES

Clifton, K.E.
  1997. Mass spawning by green algae on coral reefs. *Science* 275:1116–1118.
Drew, E.A.
  1983. *Halimeda* biomass, growth rates and sediment generation on reefs in the central Great Barrier Reef province. *Coral Reefs* 2:101–110.
Fell, J.W., I.M. Master, and S.Y. Newell
  1980. Laboratory model of the potential role of fungi in the decomposition of red mangrove (*Rhizophora mangle*) leaf litter. Pages 359–372 in K. Tenore, and B. Coull (eds). *Marine benthic dynamics. Eleventh Belle W. Baruch symposium in marine science, 1979, at Georgetown, SC, U.S.A.* University of South Carolina Press, Columbia.
Hatcher, B.G., and A.W.D. Larkum
  1983. An experimental analysis of factors controlling the standing crop of the epilithic algal community on a coral reef. *Journal of Experimental Marine Biology and Ecology* 69:61–84.
Hay, M.E.
  1981. The functional morphology of turf-forming seaweeds: persistence in stressful marine habitats. *Ecology* 62:739–750.
Hay, M.E., J.E. Duffy, V.J. Paul, P.E. Renaud, and W. Fenical
  1990. Specialist herbivores reduce their susceptibility to predation by feeding on the chemically defended seaweed *Avrainvillea longicaulis. Limnology and Oceanography* 35:1734–1743.
Hay, M.E., and W. Fenical
  1988. Marine plant-herbivore interactions: the ecology of chemical defense. *Annual Review of Ecology and Systematics* 19:111–145.
Heck, K.L. Jr., and J.F. Valentine
  1995. Sea urchin herbivory: evidence for long-lasting effects in subtropical seagrass meadows. *Ecology* 53:125–134.
Hillis-Colinvaux, L.
  1980. Ecology and taxonomy of *Halimeda*: primary producer of coral reefs. *Advances in Marine Biology* 17:vii + viii + 1–327.
Humann, P., and N. Deloach
  2002. *Reef coral identification: Florida Caribbean Bahamas, including marine plants.*, 2nd ed. New World Publications, Jacksonville, FL. 276 pp.
Jackson, J.B.C.
  1977. Competition on marine hard substrata: the adaptive significance of solitary and colonial strategies. *American Naturalist* 111:743–767.
Kinsey, D.W., and A. Domm
  1974. Effects of fertilization on a coral reef environment - primary production studies. *Proceedings of the Second International Seaweed Symposium, Brisbane* 1:49–66.

Kinsey, D.W., and P.J. Davies
1979. Effects of elevated nitrogen and phosphorus on coral reef growth. *Limnology and Oceanography* 24:935–940.

Lapointe, B.E., M.M. Littler, and D.S. Littler
1987. A comparison of nutrient-limited productivity and physiological state in macroalgae from a Caribbean barrier reef and mangrove ecosystem. *Aquatic Botany* 28:243–255.

Lewis, S.M.
1986. The role of herbivorous fishes in the organization of a Caribbean reef community. *Ecological Monographs* 56:183–200.

Littler, D.S., and M.M. Littler
1992. Systematics of *Avrainvillea* (Bryopsidales, Chlorophyta) in the tropical western Atlantic. *Phycologia* 31:375–418.
1997. An illustrated marine flora of the Pelican Cays, Belize. *Bulletin of the Biological Society of Washington* 9:1–149.

Littler, M.M., and D.S. Littler
1985. Nondestructive sampling. Pages 161–175 in M. M. Littler, and D. S. Littler (eds). *Handbook of phycological methods. Ecological field methods: macroalgae.* Cambridge University Press, Cambridge.
1990. Productivity and nutrient relationships in psammophytic versus epilithic forms of Bryopsidales (Chlorophyta): comparisons based on a short-term physiological assay. *Hydrobiologia* 204/205:49–55.
1999. Blade abandonment/proliferation: a novel mechanism for rapid epiphyte control in marine macrophytes. *Ecology* 80:1736–1746.

Littler, M.M., D.S. Littler, and B.E. Lapointe
1988. A comparison of nutrient- and light-limited photosynthesis in psammophytic versus epilithic forms of *Halimeda* (Caulerpales, Halimedaceae) from the Bahamas. *Coral Reefs* 6:219–225.

Littler, M.M., P.R. Taylor, and D.S. Littler
1983. Algal resistance to herbivory on a Caribbean barrier reef. *Coral Reefs* 2:111–118.

Newell, S.Y., J.W. Fell, A. Statzell-Tallman, C. Miller, and R. Cefalu
1984. Carbon and nitrogen dynamics in decomposing leaves of three coastal marine vascular plants of the subtropics. *Aquatic Botany* 19:183–192.

Olsen-Stojkovich, J.L.
1985. A systematic study of the genus *Avrainvillea* Decaisne (Chlorophyta, Udoteaceae). *Nova Hedwigia* 41:1–68.

Sun, H.H., V.J. Paul, and W. Fenical
1983. Avrainvilleol: a brominated diphenylmethane derivative with feeding deterrent properties from the tropical green alga *Avrainvillea longicaulis. Phytochemistry* 22:743–745.

Taylor, P.R., M.M. Littler, and D.S. Littler
1986. Escapes from herbivory in relation to the structure of mangrove island macroalgal communities. *Oecologia* 69:481–490.

26

Taylor, W.R.

    1960. *Marine algae of the eastern tropical and subtropical coasts of the Americas.* University of Michigan Press, Ann Arbor. 870 pp.

Tomasko, D.A., and C.J. Dawes

    1989. Effects of partial defoliation on remaining intact leaves in the seagrass *Thalassia testudinum* Banks ex Konig. *Botanica Marina* 32:235–240.

Williams, S.L., and T.R. Fisher

    1985. Kinetics of nitrogen-15 labelled ammonium uptake by *Caulerpa cupressoides* (Chlorophyta). *Journal of Phycology* 21:287–296.

SESSILE CILIATES WITH BACTERIAL ECTOSYMBIONTS FROM
TWIN CAYS, BELIZE

BY

JÖRG OTT AND MONIKA BRIGHT

Figure 1. SEM photomicrograph of *Zoothamnium niveum* showing microzooids covered by symbiotic bacteria.

SESSILE CILIATES WITH BACTERIAL ECTOSYMBIONTS FROM TWIN CAYS, BELIZE

BY

JÖRG OTT AND MONIKA BRIGHT

INTRODUCTION

Two species of sessile peritrich ciliates with bacterial ectosymbionts are common in the mangrove islands of the Belize Barrier Reef. One is the large colonial *Zoothamnium niveum* (Hemprich & Ehrenberg, 1831) Ehrenberg, 1838 (redescribed in Bauer-Nebelsick et al. 1996a). The second species is an as yet undescribed solitary vorticellid, provisionally called *Vorticella* sp. below. Both species occur together on a variety of sulfidic habitats. They are conspicuous because of their white color in incident light which is due to chemoautotrophic sulfur oxidizing bacteria which almost completely cover their surface. The bacteria need both sulfide and oxygen for autotrophic carbon fixation and growth. These compounds, however, coexist in nature only in narrow, changing and often unpredictable micro zones. The association with the protozoans insures a constant supply of these compounds as will be described below.

SPECIES DESCRIPTION

Zoothamnium niveum

  Zoothamnium niveum belongs to a large genus of peritrich ciliates which includes marine and freshwater species many of which are epizootic or epiphytic. With the exception of *Z. pelagicum* all species are attached to a substrate. The colonies of *Z. niveum* are feather-shaped, consisting of a central stalk which may be up to 15 mm long from which side branches originate in an alternating arrangement (Fig. 1 and Fig. 2). The branches bear up to 20 microzooids, which are the feeding zooids and are equipped with the complete ciliary apparatus of peritrichs and have a well developed cytopharynx. The tip of the stalk and of those branches which are still growing is occupied by a club-shaped terminal zooid lacking cilia and cytopharynx. At the basis of the branches large globular macrozooids devoid of a cytopharynx are developed, which eventually detach and act as dispersal units (Bauer-Nebelsick et al. 1996a). Older and larger colonies often show one or more secondary feather-shaped fans which apparently originate on the stalk. Such colonies may consist of more than 3000 microzooids and bear up to 80 macrozooids.

Institute of Ecology and Conservation Biology, University of Vienna, Austria

Figure 2. *Zoothamnium niveum.* A) Tip of colony with dividing terminal zooid B) Middle part of colony showing side branches with microzooids and macro-zooids C) Contracted large colony with secondary stalk branching off to the left. Light micrograph.

The stalk is attached to the substrate with a disk and its basal portion is not contractile. The remaining part of the stalk and the branches contain a contractile fiber (spasmoneme) which contracts spontaneously or upon disturbances with extremely high speed (0.5 m.s^{-1}, Vopel et al. 2002). Except for the non-contractile part of the stalk and the peristomal disc of the microzooids all surfaces are densely covered by a single layer of rod-shaped bacteria 1.4μm x 0.4μm, which increase in size and change to a more coccoid shape (1.9 x 1μm) towards the distal part of the microzooids (Fig. 1). At the edge of the peristomal disc they appear to form more than one layer and not all bacteria seem to be in contact with the host. The bacteria appear pure white in incident light, which is the reason for the species name of the host, and black in transmitted light (Bauer-Nebelsick et al. 1996b).

According to the 16S rRNA gene the bacteria belong to the γ-proteobacteria (Molnar et al. 2000). Their ultrastructure is similar to free-living thiobacilli, their oxygen uptake decreases upon prolonged exposure to oxygen and is stimulated by incubation in sulfide-containing water. This suggests a sulfur oxidizing chemolithoautotrophic nature of the bacteria, which is further corroborated by the presence of RuBisCO (H. Felbeck, pers. comm.) and the uptake of radiolabelled bicarbonate (Rinke 2002).

Zoothamnium niveum occurs regularly on subtidal sulfidic mangrove peat exposed along the walls of tidal channels and on a variety of natural or artificial substrates in the vicinity of sulfide sources (Ott 1996, Ott et al. 2004). It has been observed within dense macroalgal mats (*Avrainvillea* spp, *Halimeda* spp), sea grass leaves and rhizomes on sulfidic mud. Large numbers of colonies have been observed on garbage bags and on rotting vegetables dumped in mangrove ponds (own unpubl. observations).

In the tidal channels groups of up to about 100 colonies are found around disturbed patches on the surface of the peat, where the microbial surface biofilm has been interrupted, e.g. where a rootlet has rotted and fallen out. At such spots, which are commonly just a few mm in diameter, sulfide diffuses into the ambient, oxygen containing water at a higher rate than through the undisturbed peat surface, where a

3

variety of microbes consume most of it before it enters the boundary layer (Ott et al. 1998). A wave induced mechanism sucks sulfidic water from holes in the peat (Vopel et al., in press), creating a favorable environment for the symbiotic ciliates. The ciliary beat of the microzooids efficiently mixes the ambient oxygen-containing water with the sulfide diffusing from the peat (Vopel et al. 2001). Occasionally the zooids rapidly contract and completely immerse in the sulfidic boundary layer, which is about 3mm thick. During the subsequent slow expansion they drag sulfidic water into the oxic ambient water (Ott et al. 1998, Vopel et al. 2001, 2002).

The life cycle starts with the settlement of a swarmer (macrozooid) which within a few hours grows a 0.5 mm long stalk while transforming into a terminal zooid. This starts to divide, producing 2-3 groups of 1-3 microzooids before the first branch is formed. Growth is rapid and the colonies reach their maximum size within 4 days. From then on the basal parts begin to deteriorate. First the non-contractile part is overgrown by filamentous sulfur bacteria and a variety of other bacteria and diatoms. This epigrowth extends to those parts of stalk and proximal branches that in time lose the microzooids and the original bacterial coat. Mean life span of a colony is 7 days (M. Bright, J. Ott, unpubl. observations).

Zoothamnium niveum occupies ephemeral habitats. The disturbed patches on the mangrove peat deliver enough sulfide for approximately 20 days, before they are overgrown by a microbial mat which scavenges the entire sulfide (Ott et al. 1998). Loose debris accumulations are subject to occasional strong wave action, which redistributes the particles and aerates the pore water. The swarmers therefore play a decisive role in finding suitable environmental patches where they can grow into mature colonies, produce swarmers again and thus maintaining the ciliate population in such a changing environment. Swarmers precisely find sulfide sources which may be only a few mm across. Swimming at a speed of about 5 mm.s^{-1} which may be maintained for at least 24 hours they may cover a distance of 400 m before settling. The so far measured maximum distance from the nearest source of a swarmer settled on an artificial sulfide producing systems (ASPROS), however, is <5m. During dispersal they seem to stay close to the bottom surface, since ASPROS are readily colonized by Z. niveum when exposed flush with the bottom surface, but never when placed at a distance >3cm from it. Macrophyte debris accumulations may act as stepping stones between suitable habitats and even may allow rafting (M. Bright, A. Nussbaumer, J. Ott, unpubl. observations).

Zoothamnium niveum was originally described from the Red Sea (Hemprich & Ehrenberg, 1831). In the original description there is no mention of the bacteria, but the white color was noted. The material for the redescription was collected at Twin Cays. Since then it has been found at Blue Ground Range, Tobacco Range and the Pelican Cays. Other reports of this conspicuous species come from the Florida Keys, the Canary Islands and the Eastern (Greece) and Western Mediterranean (Corsica, Elba, Giglio) where it grows on decaying macrophyte debris or hard substrates in close contact with debris accumulations (M. Bright, P. Wirtz, G. Scattolin, J. Pillen, pers. comm. and own unpubl. observations).

4

Vorticella sp.

There is much less data on the second symbiotic peritrich ciliate species and most of the data below are from own unpublished observations. *Vorticella* sp. is solitary like all members of the genus. The zooids have a 350-500µm long stalk which is attached to the substrate with a basal disk. The zooids appear conical when retracted. When fully extended, however, they are disc- or even mushroom-shaped. The aboral side and the fully contractile stalk are densely covered by bacteria which appear similar to those in *Zoothamnium niveum* (Fig. 3). Likewise they appear white in incident light. There are no data as yet on the fine structure, enzyme repertoire and physiology, nor are gene sequences available. The white color which is indicative of sulfur storage and the habitat preference of the symbiosis, however, suggest a chemoautotrophic sulfur-oxidizing nature for the bacteria.
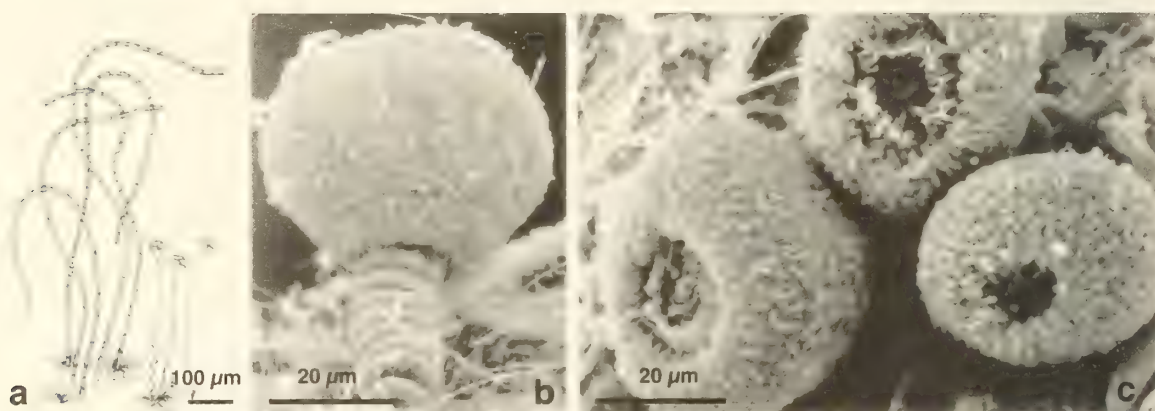
Figure 3. *Vorticella* sp. A) Sketch of microbial community with large filamentous sulfur bacteria (*Beggiatoa* sp.) (adapted from Vopel et al. 2001) B) Contracted individual covered by symbiotic bacteria. SEM C) Three individuals seen from the oral side. SEM

Vorticella sp. co-occurs regularly with *Zoothamnium niveum* in the vicinity of disturbed peat patches. It seems to be more abundant on older patches where most *Z. niveum* colonies are mature to senescent forming dense lawns of zooids. Due to their small size the zooids are constantly within the sulfidic boundary layer and rely heavily on the current created by beating their cilia (Vopel et al. 2001, 2002) (Fig. 4)

The zooids divide, forming a stalk less dispersal stage which detaches and swims away. It apparently settles not far from the parent zooid, thus rapidly producing dense lawns of individuals. There are no data yet on the life span of the swarmer stages. The dispersal capacity seems to be much less than that of the swarmers of *Z. niveum*, which is indicated by the rather late arrival at sulfide sources. In addition, *Vorticella* sp. was only rarely observed on ASPROS used to attract swarmers both in the laboratory and the field, although once settled on an ASPROS it grows well and reproduces rapidly.

The symbiotic *Vorticella* sp. has so far been observed at all locations of the Belize Barrier Reef system where *Z. niveum* has been found. A similar symbiotic species co-occurs with *Z. niveum* on sea grass debris in Corsica, Western Mediterranean.
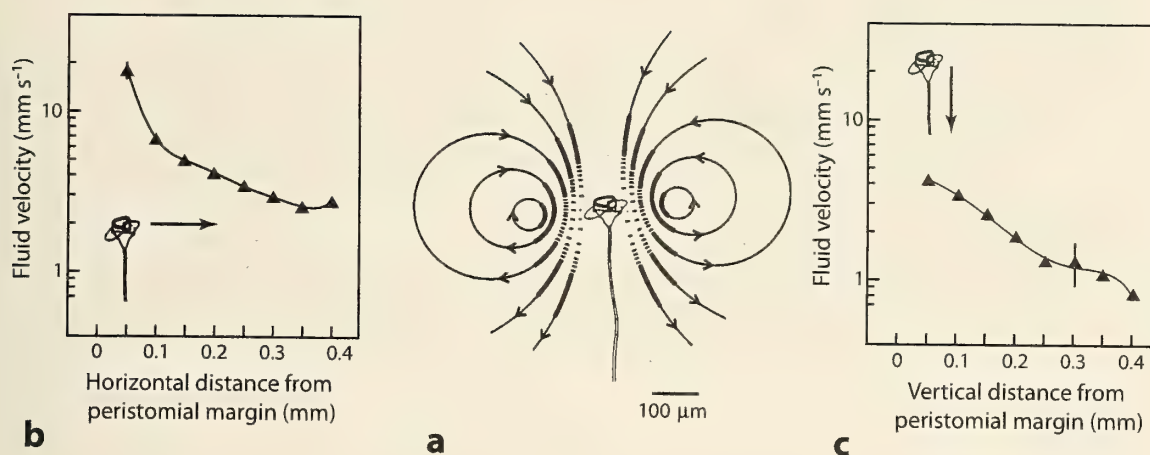
Figure 4. *Vorticella* sp. a) velocity field around an individual of Vorticella sp. b) horizontal velocity profile c) vertical velocity profile (after Vopel et al. 2001, 2002)

Symbiotic partnership

Benefits of the symbiotic partnership are a constant and rich food supply for the ciliates, which apparently feed largely on the detached symbiotic bacteria and can maintain high growth rates. *Z. niveum* grows several times faster than its non-symbiotic relatives and attains the largest colony size within the genus. The bacteria in turn are supplied with sulfide and oxygen at a predictable and high rate and the motile swarmer stages carry them to new microhabitats when the old ones deteriorate. The bacteria in *Zoothamnium niveum* show a complex cell cycle as adaptation to the symbiotic life (Bright 2002). Both species are extremely patchy distributed wherever they occur. This seems to be correlated with the patchy distribution of sulfide sources. Non-symbiotic *Zoothamnium* and *Vorticella* species occur in low densities in the same habitats but away from the sulfide point sources. The non-symbiotic *Zoothamnium* colonies are most probably *Z. alternans*, which is closely related to *Z. niveum*. Both species have been cultivated in the laboratory for a few generations using ASPROS. Both have, however, resisted long term maintenance and cultivation. Maintaining suitable conditions for the bacteria seems to be critical, since both species first loose the symbionts and are able to survive aposymbiotically for a while. They may even produce dispersal stages and an aposymbiotic generation, which however is incapable of further reproduction.
Both ciliates exploit the sharpest sulfide/oxygen gradient of all known sulfide symbioses (Polz et al. 2000). Despite their small size they effectively manipulate the microenvironment within their habitat in order to thrive in an otherwise hostile environment.

6

REFERENCES

Bauer-Nebelsick, M., Bardele, C.F. & Ott, J.A.
  1996a. Redescription of *Zoothamnium niveum* (Hemprich & Ehrenberg, 1831) Ehrenberg, 1838 (Oligohymenophora, Peritrichida), a ciliate with ectosymbiotic, chemoautotrophic bacteria. *European Journal of Protistology.* 32:18-30.

Bauer-Nebelsick, M., Bardele, C.F. & Ott, J.A.
  1996b. Electron microscopic studies on *Zoothamnium niveum* (Hemprich & Ehrenberg, 1831) Ehrenberg 1838 (Oligohymenophora, Peritrichida), a ciliate with ectosymbiotic, chemoautotrophic Bacteria. *European Journal of Protistology.* 32:202-215.

Bright, M.
  2002. Life strategies of thiotrophic ectosymbioses. In *The Vienna School of Marine Biology: A Tribute to Jörg Ott*, M. Bright et al. (Eds.). Wien: Facultas Universitätsverlag, 19-32.

Molnar, D.A., Nikolausz, M., Bright, M., Márialigeti, K., Vanura, K., Buchholz, T.G. & Ott, J.A.
  2000. Phylogenetic characterization of *Zoothamnium niveum's* (Ciliophora, Peritrichida) thiotrophic ectosymbiotic bacterial community based on comparative 16S rRNA sequence analysis. In: *Programs, Abstracts and Papers of the Third International Congress on Symbiosis*. H.C. Weber et al. (Eds.). Philipps University of Marburg, Germany, 153.

Ott, J.A.
  1996. Sulphide ectosymbioses in shallow marine habitats. In *Deep-sea and extreme shallow-water habitats: affinities and adaptations,* F. Uiblein et al. (Eds.). Vienna: Austrian Academy of Sciences Press, Biosystematics and Ecology Series. 11:369-382.

Ott, J.A., Bright, M. & Bulgheresi, S.
  2004. Marine Microbial Thiotrophic Ectosymbioses. *Oceanography and Marine Biology: An Annual Review.* 42:95-118.

Ott, J.A., Bright, M. & Schiemer, F.
  1998. The ecology of a novel symbiosis between a marine peritrich ciliate and chemoautotrophic bacteria. *Pubblicazioni della Stazione Zoologica di Napoli*: *Marine Ecology.* 19:299-243.

Polz, M. F., Ott, J.A., Bright M. & Cavanaugh, C.M.
  2000. When bacteria hitch a ride Associations between sulfur-oxidizing bacteria and eukaryotes represent spectacular adaptations to environmental gradients *ASM News.* 66:531-532.

Rinke, Ch.
  2002. Carbon fixation, incorporation, and transfer in the chemoautotrophic *Zoothamnium niveum* symbiosis with 14C bicarbonate autoradiography. Diploma Thesis, University of Vienna.

Vopel, K., Pöhn, M., Sorgo, A. & Ott, J.
    2001. Ciliate-generated advective seawater transport supplies chemoautotrophic ectosymbionts. *Marine Ecology Progress Series.* 210:93-99.

Vopel, K., Reick, C.H., Arlt, G., Pöhn, M. & Ott, J.A.
    2002. Flow microenvironment of two marine peritrich ciliates with ectobiotic chemoautotrophic bacteria. *Aquatic Microbial Ecology.* 29:19-28.

Vopel, K., Roy H., Huettel M., Jorgensen B.B.
    (In press). Transport of water and solutes around the symbiont bearing sessile ciliate *Zoothamnium* niveum. *Limnology and Oceanography.*

SEASONAL VARIATION IN EPIPHYTIC FORAMINIFERAL BIOTAS FROM *THALASSIA* SEAGRASS HABITATS, TWIN CAYS, BELIZE

BY

SUSAN L. RICHARDSON

Figure 1. Map of Twin Cays showing location of sites sampled in June 2001 and February 2002. **A.** Boston Bay, **B.** Sponge Haven, **E.** Main Channel near Dock, **J.** 'Cuda Cut.

SEASONAL VARIATION IN EPIPHYTIC FORAMINIFERAL BIOTAS FROM *THALASSIA* SEAGRASS HABITATS, TWIN CAYS, BELIZE

BY

SUSAN L. RICHARDSON

ABSTRACT

The epiphytic foraminiferal biotas living on the seagrass *Thalassia testudinum* were surveyed at four sites in the Twin Cays, Belize (Boston Bay, Sponge Haven, the Main Channel near the dock, and 'Cuda Cut) during June 2001 and February 2002 in order to investigate seasonal variation in diversity, distribution, and abundance. Twelve blades were surveyed from each site and all live epiphytic foraminiferans were identified to species (S=41) and counted (N=15,455). The sites surveyed showed distinct seasonal differences in mean density of individuals per unit blade area (N/cm^2), species richness (S), and evenness (E), between June 2001 (the warmer, wet season) and February 2002 (the cooler, dry season). The mean density of individuals per unit blade area was higher at all sites in June 2001, compared with February 2002. Although mean species richness per blade did not show a significant seasonal signal, the total species richness recorded at each site was higher during June 2001, relative to February 2002, for all sites except Boston Bay. Likewise, values of evenness were higher in June 2001, relative to February 2002, except for the Boston Bay site. Both abiotic and biotic factors are considered to influence the seasonal differences in the population densities and species composition of the epiphytic foraminiferans living on *T. testudinum* in the vicinity of the Twin Cays mangrove island. It is suggested that seasonal differences at this locality may result from an influx of nutrients from the adjacent mangrove fringe during the wet season, an incursion of open-water species into the mangrove habitats during the dry season, and the thermal tolerances of individual species. The high dominance of the encrusting, milioline species *Rhizonubecula* sp. observed at all sites in the Main Channel in June 2001, and again at the Dock site in February 2002, highlights the potential utility of this species as a bioindicator of increased nutrients in mangrove habitats.

INTRODUCTION

Foraminifera is a monophyletic clade of single-celled eukaryotes that has recently been found to be most closely related to chlorarachniophytes, cercomonads, euglyphiid amoebans, and other cercozoans (Archibald et al., 2003; Keeling, 2000; Pawlowski et al., 2003). The clade has a long geological history, reaching back to at least the Early Cambrian (Culver, 1991; Lipps and Rozanov, 1996; McIlroy et al., 2001), and the group

Smithsonian Marine Station at Fort Pierce, 701 Seaway Drive, Fort Pierce, FL 34949 U.S.A.;
richardson@sms.si.edu

is considered by many to have one of the best fossil records of any organism (Culver and Buzas, 1998). Fossil foraminiferans are widely used in biostratigraphic, paleoecological, paleoenvironmental, and evolutionary studies (Culver and Buzas, 2000). Extant species are ubiquitous in the marine realm and occupy a diversity of habitats in near-shore tropical to subtropical waters. Foraminiferans can be found dwelling within the sediments of mangrove swamps and seagrass meadows as well as living attached to seagrass blades, macroalgae, coral rubble, and other firm substrates (Sen Gupta, 1999).

Traditionally, ecological studies of extant foraminiferans have focused primarily on the sediment-dwelling species and there exists an extensive literature documenting the diversity, distribution and abundance of living foraminiferal communities from an array of sediment types in different marine environments (Culver, 1990; Murray, 1973; 1991a, b). More recently, a number of studies have examined the ecology of foraminiferans living attached to phytal substrates, such as seagrasses and macroalgae, in nearshore marine ecosystems (Fujita and Hallock, 1999; Langer, 1993; Richardson 2000, Semeniuk, 2000, 2001). These studies indicate that the standing stock of epiphytic foraminiferal populations may be as high as those of the sediment-dwelling biota, suggesting that a considerable component of the overall foraminiferal biodiversity has been overlooked by surveys employing traditional sampling methods. In his study of the distribution of foraminiferans in the sediments of the Belize Shelf, Wantland (1975: 358) observed that the highest species diversity occurred in regions colonized by moderate-to-dense stands of turtle grass (*Thalassia testudinum*) and speculated that "many, if not most, of the benthonic foraminifera inhabiting shallow back-reef environments live attached to plants and other floral and faunal elements above the sediment surface."

Thalassia testudinum is the dominant seagrass in the Caribbean and its broad, strap-like leaves provide a substantial amount of surface area (LAI = 4-12 m^2 blade surface area/m^2 seafloor) for the settlement of epiphytic organisms (Philips and Meñez, 1988; Zieman, 1975). The species richness of epiphytic foraminiferans from *Thalassia* habitats in the central province of the Belizean Barrier Reef Complex (i.e., Carrie Bow Cay, Twin Cays, Man O'War Cay, and the Pelican Cays) totals 49 species to date (Richardson, 2000; S. Richardson, unpublished data). These foraminiferal species compete for space and resources with numerous multicellular organisms, such as encrusting calcareous algae, filamentous microalgae, hydroids, bryozoans, copepods, spirorbids, ascidians, sponges, barnacles, and oysters (Richardson 2000; S. Richardson, unpublished observations).

Estimates of the biomass of *T. testudinum* from this region are among the highest in the Caribbean, with above-ground biomass ranging from 883±254 g m^{-2} dry weight (August) to 731±211 g m^{-2} dry weight (December) (Koltes et al., 1998; Zieman and Zieman, 1989). Areal productivity shows minimal seasonal variation, with slightly higher values of productivity recorded during August (2.86±0.96 g dry weight m^{-2} day^{-1}) relative to December (2.30±0.88 g dry weight m^{-2} day^{-1}), and the turnover rate for *T. testudinum* leaves averages about 2.4% per plant per day (Koltes et al., 1998).

Since *T. testudinum* provides a relatively stable and predictable substratum for the settlement, growth and reproduction of epiphytic organisms throughout the year, one might ask whether or not the epiphytes colonizing the leaf blades also exhibit minimal seasonality? The objective of the present study was, therefore, to investigate the seasonal

changes in abundance, diversity, and species composition of the epiphytic foraminiferal biotas living on *T. testudinum* growing in the vicinity of the Twin Cays, Belize.

MATERIAL AND METHODS

Description of Study Sites

Seagrass samples for this study were collected from the vicinity of Twin Cays, Belize, a 91.5 ha-mangrove island that is located within the central province of the Belizean Barrier-Reef Complex, approximately 21 km SE of Dangriga and 2 km west of the reef crest (Koltes et al., 1998). Sampling sites were located along a north-south transect, in the Main Channel between East and West Islands as well as in the adjacent embayment of Boston Bay (Fig. 1). Blades of *T. testudinum* with their associated epiphytes were collected during field excursions in the summer (wet season) of 2001 (22 June-03 July) and again in the winter (dry season) of 2002 (31 January-11 February).

Site A was located in Boston Bay in approximately 1.5-m water depth in a monospecific stand of *T. testudinum*. The overlying water column at this site was reddish-brown in color due to the high tannin content and the sediment consisted of a thick accumulation of mangrove-derived, organic flocculent. Numerous individuals of the "upside-down" jellyfish, *Cassiopeia xamachana*, were present in sand patches interspersed throughout the seagrass stands. Site B was located in approximately 2-m water depth in a monospecific stand of *T. testudinum* at Sponge Haven. The overlying water was cloudy, presumably due to suspended sediment load in the channel, and the sediment surface consisted of a thick accumulation of mangrove-derived, organic flocculent (Calem and Pierce, 1993). Site E was located southeast of Turtle Cove, near the dock, in 1.5-m water depth in a dense, monospecific stand of *T. testudinum* growing in the middle of the channel (Fig. 1). The overlying water column was cloudy with suspended sediment, and the bottom sediments were comprised of a fine silty, calcareous mud (Calem and Pierce, 1993). Site J was located at 'Cuda Cut in 1.5-m water depth in a monospecific stand of *T. testudinum*. The overlying water column at this site was cloudy with suspended sediment and the bottom sediments were comprised of a silty, calcareous mud (Calem and Pierce, 1993).

Field and Laboratory Protocols

Twelve shoots of *T. testudinum* were collected haphazardly (*sensu* Hayek and Buzas, 1997) at each site from within an area of approximately nine m² of sea floor. Seagrass samples were stored in Ziploc bags immersed in seawater and kept in a cooler until return from the field. Collections from each sampling site were maintained temporarily in separate tanks with running seawater in the wet lab on Carrie Bow Cay until the leaf blades could be examined.

The longest blade from each shoot was selected and examined for live foraminiferans using a binocular, dissecting microscope (Leica M5). Living individuals were recognized by their cytoplasmic coloration and/or the presence of pseudopodial arrays and feeding cysts. Notes on the abundance, reproductive state of each specimen,

4

and the occurrence and distribution of other epiphytic organisms were also recorded. Several voucher specimens of each foraminiferal species identified were removed from the blades and stored on microslides for comparison with the type Foraminifera collections housed in the National Museum of Natural History, Smithsonian Institution, Washington, DC.

Specimens illustrated by scanning electron microscopy (SEM) were cleaned with distilled water, air-dried, mounted on stubs, coated with a 400-Å layer of gold in a Technics Hummer II sputter coater, and examined in a JEOL JSM-6400V model SEM (Smithsonian Marine Station at Fort Pierce, FL).

RESULTS

Forty-one species of epiphytic foraminiferans were identified among 15,455 individuals identified from a total of 96 seagrass blades (Appendix I). The total leaf-surface area surveyed was 6568.58 cm^2 (0.66 m^2)--3267.66 cm^2 blade surface was surveyed in June 2001 and 3300.92 cm^2 was surveyed in February 2002. The mean total blade area surveyed per site was slightly lower in June 2001 (816 cm^2, σ=213.04, σ^2=45,386.95) than in February 2002 (825.23 cm^2, σ=107.21, σ^2=11,493.50); however, the mean blade surface area surveyed at each sampling site was observed to be approximately the same in both seasons (Fig. 2, Tables 1, 2).
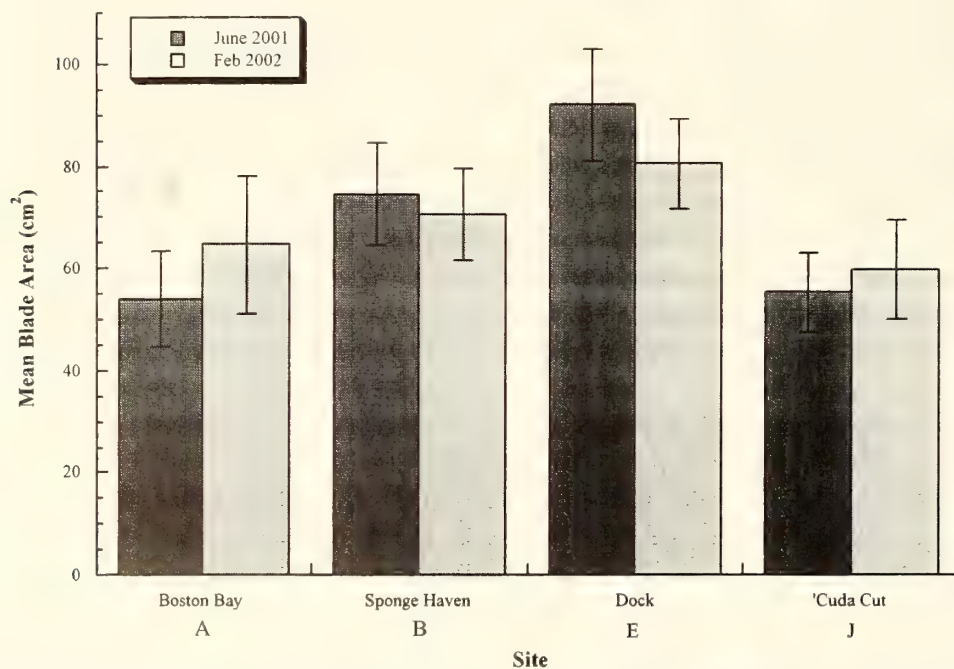
Figure 2. Mean seagrass blade surface area surveyed at each sampling site, Twin Cays, Belize (June 2001 and February 2002). 95% confidence limits were calculated following Hayek and Buzas (1997).

Table 1. Summary data table for sites sampled within Twin Cays, Belize, in June 2001.

	Boston Bay	Sponge Haven	Dock	'Cuda Cut
Field ID #	BZE01A	BZE01B	BZE01E	BZE01J
Collecting date	22 June 2001	24 June 2001	28 June 2001	03 July 2001
Total species richness (S)	19	25	27	31
Mean species richness	10.08 (σ=2.23, σ^2=4.99)	15.67 (σ=2.02, σ^2=4.06)	13.17 (σ=5.18, σ^2=26.88)	14.17 (σ=3.69, σ^2=13.61)
Shannon's H	1.6557 (σ=0.0325, σ^2=0.0011)	2.1965 (σ=0.0207, σ^2=0.0406)	1.4699 (σ=0.0204, σ^2=0.0399)	1.9324 (σ=0.0204, σ^2=0.0399)
Evenness (E)	0.2756	0.6824	0.4460	0.5627
Total blades surveyed	12	12	12	12
Total individuals (N)	1332	2617	4302	1862
Mean density (N/blade)	111.00 (σ=48.77, σ^2=2378.18)	218.08 (σ=74.95, σ^2=5616.99)	358.50 (σ=208.57, σ^2=43,500.82)	155.17 (σ=96.10, σ^2=9234.33)
Total blade area (cm^2)	646.26	854.54	1103.40	663.46
Mean blade area (cm^2)	99.42 (σ=164.90, σ^2=27192.14)	71.21 (σ=15.46, σ^2=238.96)	91.95 (σ=17.14, σ^2=293.84)	55.29 (σ=12.05, σ^2=145.26)
Mean density per unit blade area (N/cm^2)	2.26 (σ=1.20, σ^2=1.45)	3.10 (σ=0.96, σ^2=0.92)	4.27 (σ=2.26, σ^2=5.09)	2.92 (σ=1.39, σ^2=1.92)

The site with the lowest total blade surface area surveyed was the Boston Bay site in June 2001 (646.26 cm^2) and the site with the highest total blade surface area was the Dock site in June 2001 (1103.40 cm^2) (Tables 1, 2). A higher number of individuals was counted in June 2001 (10,113 individuals), relative to February 2002 (5,342 individuals), with the highest populations observed at the Dock site in June 2001 (4,320 individuals) (Fig. 3). The lowest population numbers were recorded at the Boston Bay site in February 2002 (626 individuals). Mean densities per blade (N/blade) were higher at all sites in June 2001 relative to February 2002 (Tables 1, 2). Higher mean densities per unit-blade area (mean N/ cm^2) were recorded during June 2001, compared to February 2002, at all sites

except for 'Cuda Cut which had approximately the same density per unit blade area (cm^2) during both seasons (Fig. 4). The highest mean densities were recorded at the Dock site

Table 2. Summary data table for sites sampled within Twin Cays, Belize, in February 2002.

	Boston Bay	Sponge Haven	Dock	'Cuda Cut
Field ID #	BZE02A	BZE02B	BZE02E	BZE02J
Collecting date	31 Jan. 2002	06 Feb. 2002	11 Feb. 2002	11 Feb. 2002
Total species richness (S)	21	24	19	23
Mean species richness	10.08 (σ=3.00, σ^2=8.99)	10.83 (σ=3.79, σ^2=14.33)	9.58 (σ=2.07, σ^2=4.27)	9.00 (σ=2.09, σ^2=4.36)
Shannon's H	2.1989 (σ=0.0389, σ^2=0.0015)	2.3493 (σ=0.0349, σ^2=0.0012)	1.1521 (σ=0.0280, σ^2=0.0008)	1.8030 (σ=0.0283, σ^2=0.0008)
Evenness (E)	0.4293	0.4366	0.1666	0.2638
Total blades surveyed	12	12	12	12
Total individuals (N)	626	786	2220	1710
Mean density (N/blade)	52.17 (σ=31.68, σ^2=1003.79)	65.50 (σ=49.33, σ^2=2433.91)	185.00 (σ=78.64, σ^2=6184.91)	142.50 (σ=62.51, σ^2=3907.73)
Total blade Area (cm^2)	775.62	845.76	964.62	714.92
Mean blade area (cm^2)	64.64 (σ=21.01, σ^2=441.31)	70.48 (σ=13.79, σ^2=190.23)	80.39 (σ=13.55, σ^2=183.67)	59.58 (σ=15.25, σ^2=232.63)
Mean density per unit blade area (N/cm^2)	0.84 (σ=0.43, σ^2=0.18)	0.91 (σ=0.65, σ^2=0.42)	2.35 (σ=0.1.07, σ^2=1.15)	2.50 (σ=1.18, σ^2=1.40)

in June 2001 (4.27 individuals/cm^2) while the lowest densities were recorded at the Boston Bay and Sponge Haven sites in February 2002 (0.84 and 0.91 individuals/cm^2, respectively). Table 3. Relative abundance of epiphytic species at sites sampled within Twin Cays, Belize, in June 2001. 95% confidence intervals were calculated from

standard error for species proportions for cluster sampling (Hayek and Buzas 1997).
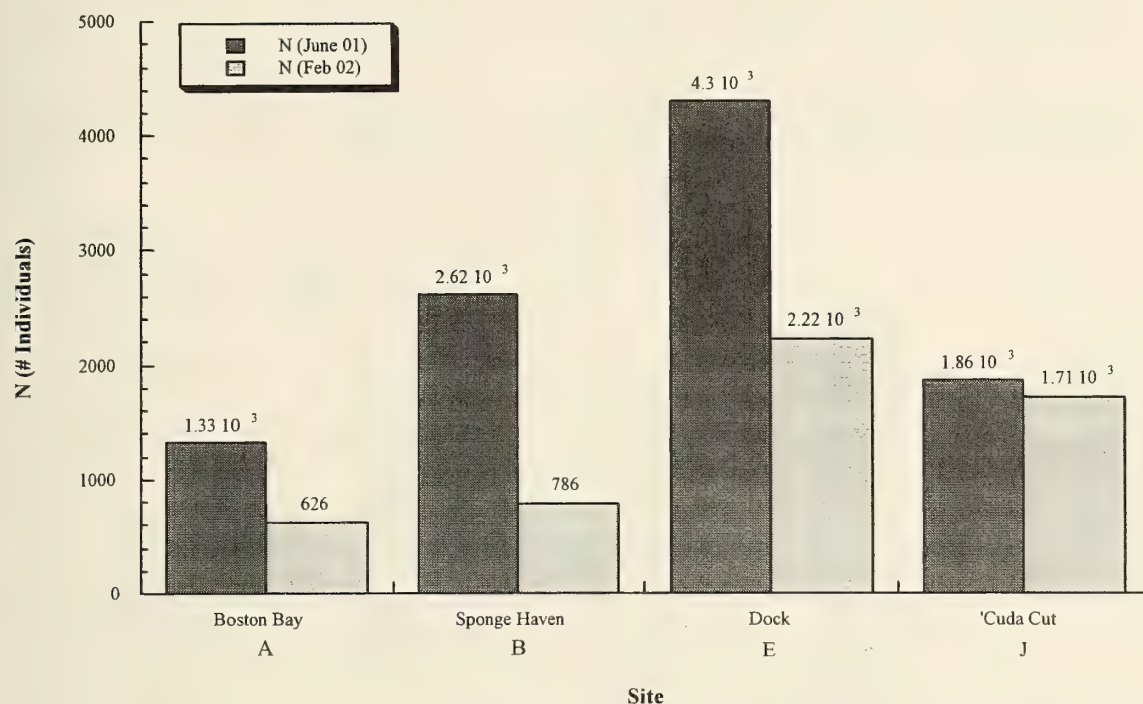
Figure 3. Total number of foraminiferal individuals counted at each site, Twin Cays, Belize (June 2001 and February 2002).
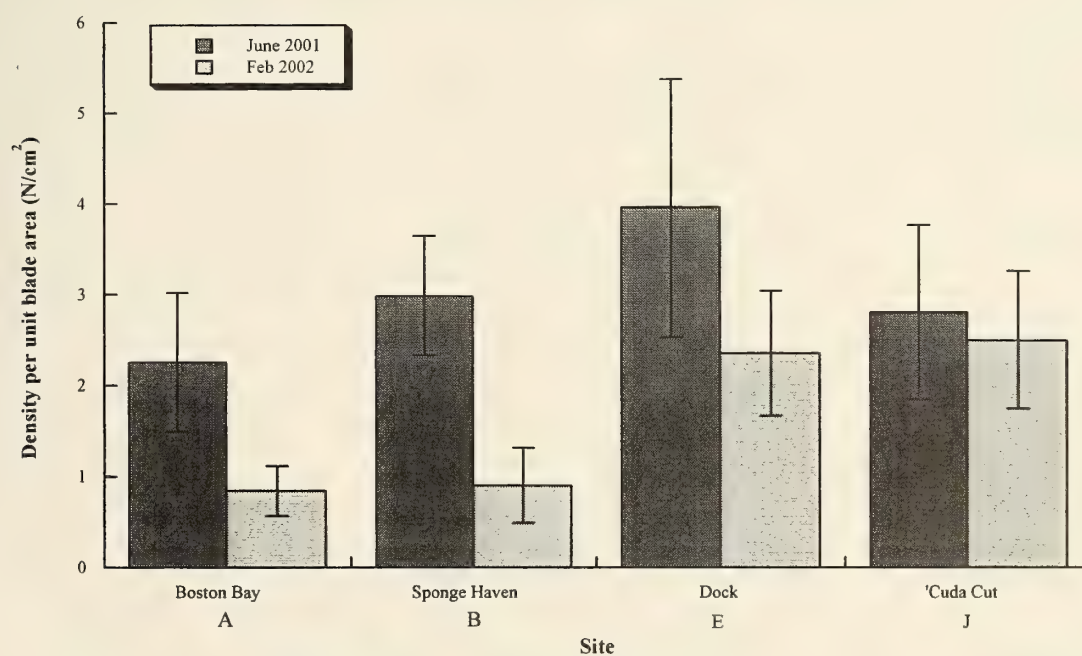
Figure 4. Density of epiphytic foraminiferans per unit blade area (N/cm^2) on *Thalassia testudinum* at each site, Twin Cays, Belize (June 2001 and February 2002). 95% confidence limits were calculated following Hayek and Buzas (1997).

Diversity indices calculated for each site include: S (species richness), H (Shannon's H), and E (evenness) (Hayek and Buzas, 1997) (Tables 1, 2). Except for Boston Bay, the total species richness recorded at each site was higher in June 2001 than in February 2002 (Fig. 5). The highest values of S were recorded at the 'Cuda Cut site in

Table 3. Relative abundance of epiphytic species at sites sampled within Twin Cays, Belize, in June 2001. 95% confidence intervals were calculated from standard error for species proportions for cluster sampling (Hayek and Buzas, 1997).

Species	Boston Bay	Sponge Haven	Dock	'Cuda Cut
A. cf. parkinsoniana	0.00	0.00	0.00	0.16±0.82%
A. gibbosa	0.00	0.00	0.00	0.00
A. cf. lucasi	0.08±2.01%	0.61±0.85%	0.26±0.77%	0.16±1.46%
A. antillarum	0.00	0.00	0.07±1.16%	0.00
A. mucronata	0.15±1.89%	0.23±1.08%	0.05±1.27%	0.27±0.97%
B. rhomboidalis	0.00	0.04±1.99%	0.02±1.83%	0.00
C. planorbis	0.83±0.95%	0.65±0.59%	0.02±2.05%	0.38±0.55%
Cor. antillarum	0.53±0.53%	2.41±0.23%	0.40±0.71%	0.43±0.82%
Cos. antillarum	0.23±1.02%	0.42±0.70%	0.00	0.11±1.31%
C. cushmani	0.38+1.11%	0.11±1.43%	0.05±1.43%	0.05±2.05%
Crithionina sp.	0.00	0.00	0.09±0.96%	0.05±1.95%
C. squammosa	0.15±1.92%	0.00	0.00	0.00
C. tobagoensis	2.85±0.40%	5.31±0.22%	0.46±0.34%	0.64±0.43%
C. poeyanum	0.00	0.04±1.96%	0.00	0.00
F. labiosa	3.83±0.41%	1.41±0.59%	0.44±0.44%	1.34±0.48%
H. palabunda	1.95±0.49%	0.53±0.56%	1.79±0.28%	3.11±0.51%
H. cf. cribrostoma	0.30±0.70%	0.38±1.15%	9.34±0.69%	0.59±0.56%
I. cf. diaphana	39.26±0.20%	12.99±0.29%	17.76±0.17%	23.85±0.24%
L. cf. karreri	0.45±0.68%	3.02±0.36%	1.44±0.43%	0.70±0.67%
M. fusca	0.00	0.00	0.00	0.00
N. cf. terquemi	0.15±1.92%	0.80±1.39%	0.14±0.79%	0.27±0.94%
P. corrugata	0.00	0.04±1.85%	0.00	0.00
P. acervalis	7.21±0.31%	5.96±0.30%	3.51±0.28%	12.89±0.30%
P. mediterranensis	0.00	0.00	0.16±0.60%	0.16±0.0.96%
P. cf. squamiformis	0.00	0.04±1.96%	0.02±1.83%	0.00
P. occidentalis	0.00	0.00	0.00	0.11±1.19%
P. goesi	2.03±0.43%	5.20±0.39%	0.21±0.58%	1.77±0.57%
Quinqueloculina sp.	0.00	0.31±0.67%	0.00	0.43±0.96%
Q. bicostata	0.00	0.00	0.00	0.00
R. adherens	0.15±1.86%	10.51±0.39%	0.19±0.74%	0.21±1.29%
Rhizonubecula sp.	5.63±0.44%	29.61±0.30%	57.37±0.11%	33.24±0.26%
R. candeiana	0.00	0.96±0.45%	0.93±0.97%	2.15±0.52%
Rosalina sp.	0.00	0.00	0.00	0.00
R. auberii	0.00	0.00	0.00	0.21±1.99%
Sagenina sp.	0.00	0.00	0.00	0.05±2.07%

Table 3 (cont'd.)

S. atlantica	0.00	0.38±0.81%	0.05±1.07%	0.21±0.78%
S. dominicensis	33.86±0.20%	18.04±0.32%	4.88±0.49%	15.41±0.52%
T. agglutinans	0.00	0.00	0.02±1.96%	0.05±1.99%
Tretomphalus sp.	0.00	0.00	0.21±0.73%	0.27±1.12%
T. bicarinata	0.00	0.00	0.00	0.11±1.22%
T. cf. *trigonula*	0.00	0.00	0.12±1.02%	0.59±0.75%
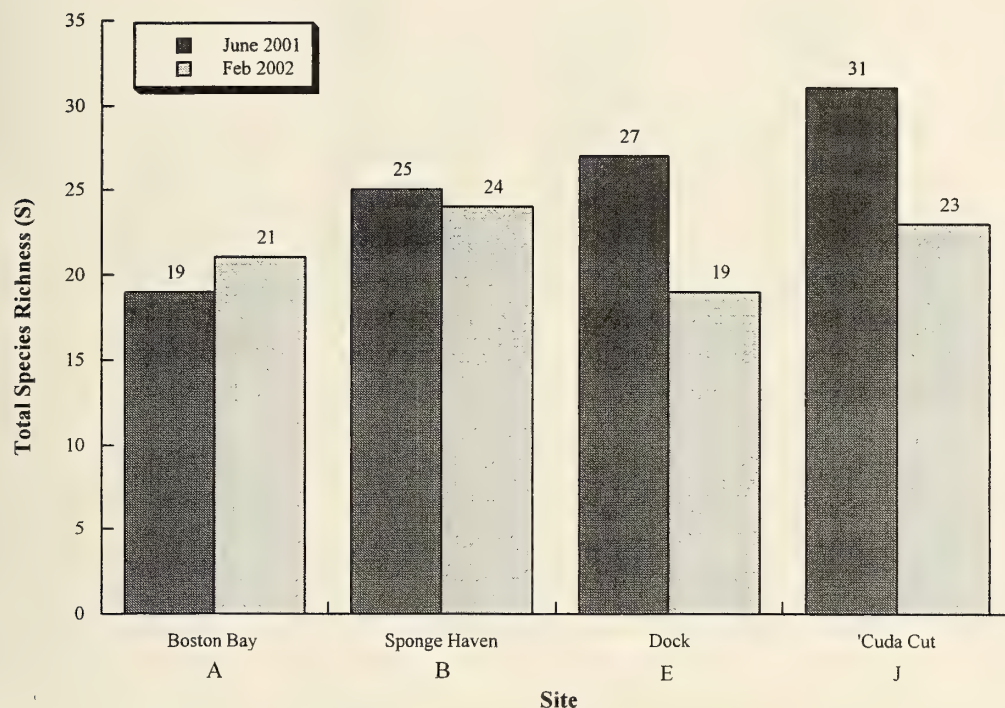
Figure 5. Total species richness of epiphytic foraminiferans identified at each site, Twin Cays, Belize (June 2001 and February 2002).

Table 4. Relative abundance of epiphytic species at sites sampled within Twin Cays, Belize, in February 2002. 95% confidence intervals were calculated from standard error for species proportions for cluster sampling (Hayek and Buzas, 1997).

SPECIES	Boston Bay	Sponge Haven	Dock	'Cuda Cut
A. cf. *parkinsoniana*	0.00	0.00	0.00	0.00
A. gibbosa	0.00	0.13±1.91%	0.00	0.00
A. cf. *lucasi*	0.00	0.00	0.00	0.00
A. antillarum	0.00	0.00	0.00	0.00
A. mucronata	0.00	0.25±1.86%	0.36±0.93%	0.18±1.46%
B. rhomboidalis	0.00	0.00	0.00	0.00
C. planorbis	0.80±1.33%	1.40±0.37%	0.14±1.04%	0.12±1.98%
Cor. antillarum	17.41±0.36%	5.34±0.96%	0.36±1.08%	0.88±1.05%
Cos. antillarum	0.00	0.00	0.00	1.17±1.14%

Table 4 (cont'd)

C. cushmani	8.95±0.49%	12.72±0.43%	0.00	0.00
Crithionina sp.	0.96±1.07%	0.64±1.67%	0.00	0.00
C. squammosa	0.00	0.38±1.34%	0.09±1.21%	0.12±1.42%
C. tobagoensis	0.96±0.75%	2.16±1.06%	0.00	0.06±1.98%
C. poeyanum	0.00	0.00	0.00	0.00
F. labiosa	7.67±0.32%	5.47±0.83%	0.86±0.44%	0.12±1.98%
H. palabunda	1.44±0.76%	0.89±0.84%	0.99±0.44%	6.37±0.86%
H. cf. *cribrostoma*	0.16±2.08%	0.00	0.05±1.93%	0.00
I. cf. *diaphana*	24.76±0.35%	16.67±0.32%	10.68±0.17%	18.36±0.37%
L. cf. *karreri*	0.00	0.25±1.44%	0.14±1.00%	0.00
M. fusca	0.00	0.13±2.05%	0.00	0.00
N. cf. *terquemi*	0.00	0.76±0.62%	0.18±1.03%	0.18±1.07%
P. corrugata	0.00	0.00	0.00	0.06±1.91%
P. acervalis	16.77±0.38%	19.72±0.32%	12.79±0.14%	28.07±0.32%
P. mediterranensis	0.00	0.00	0.00	0.12±1.32%
P. cf. *squamiformis*	0.80±0.89%	1.15±0.74%	0.36±0.77%	0.64±0.50%
P. occidentalis	0.00	0.00	0.00	0.00
P. goesi	1.12±0.79%	0.76±0.91%	1.13±0.74%	0.18±1.50%
Quinqueloculina sp.	0.16±1.92%	0.25±1.94%	0.00	0.06±1.98%
Q. bicostata	0.16±1.92%	0.00	0.00	0.00
R. adherens	3.04±0.46%	2.29±0.46%	0.05±1.92%	0.06±1.94%
Rhizonubecula sp.	1.92±0.70%	14.63±0.82%	68.24±0.04%	26.55±0.25%
R. candeiana	0.16±2.08%	0.51±0.92%	1.26±0.59%	3.51±0.34%
Rosalina sp.	0.48±0.95%	0.00	0.00	0.00
R. auberii	0.00	0.00	0.00	0.06±2.01%
Sagenina sp.	0.00	0.00	0.00	0.00
S. atlantica	0.00	0.00	0.00	0.06±2.01%
S. dominicensis	10.70±0.60%	11.83±0.30%	2.21±0.51%	12.98±1.02%
T. agglutinans	0.00	0.00	0.00	0.00
Tretomphalus sp.	0.16±1.92%	0.89±0.55%	0.19±1.37%	0.12±1.32%
T. bicarinata	0.00	0.00	0.00	0.00
T. cf. *trigonula*	1.44±0.45%	0.76±1.05%	0.05±1.90%	0.00

June 2001 (S=31); the lowest values of S were recorded at Boston Bay (S=19) in June 2001 and the Dock (S=19) in February 2002. Mean species richness per blade varied from 9.02 at 'Cuda Cut in February 2002, to 15.67 at Sponge Haven in June 2001 (Tables 1, 2). Although the means species richness was higher at all sites located in the Main Channel in June 2001 than February 2002 the width of the confidence intervals calculated from these values indicates that these differences are not significant (Fig. 6). There was not a clear cut temporal difference in values of Shannon's H calculated for each site (Tables 1, 2). Values of H were higher for the Dock and 'Cuda Cut samples collected in June 2001 relative to those collected in February 2002, and lower for the Boston Bay and Sponge Haven samples collected in June 2001 relative to those collected in February 2002 (Fig. 7). The lowest value of Shannon's H (H=1.4699) was observed at the Dock
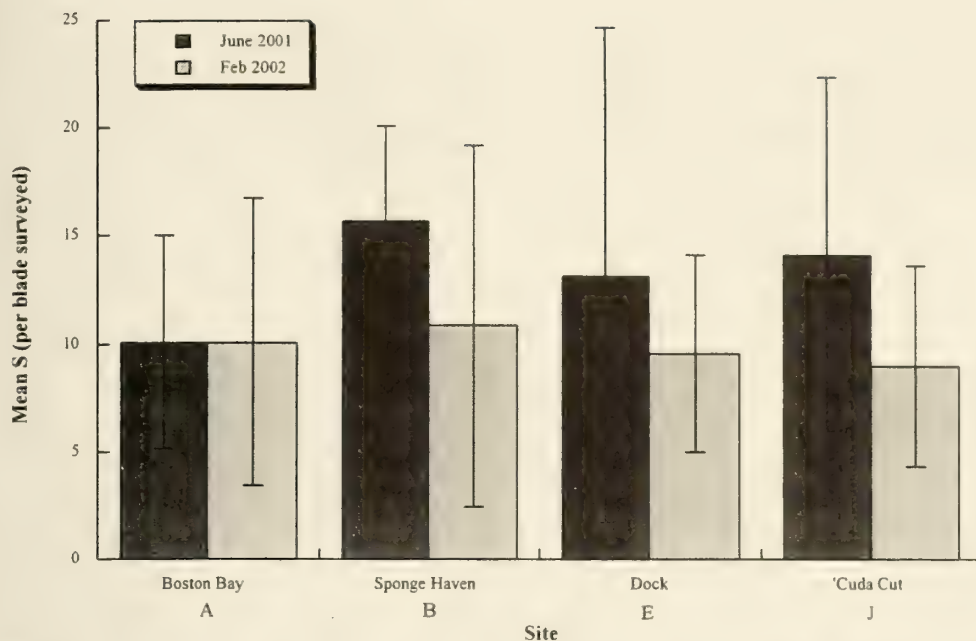
Figure 6. Mean species richness per blade surveyed at each site, Twin Cays, Belize (June 2001 and February 2002). 95% confidence limits were calculated following Hayek and Buzas (1997).

site in June 2001; the highest value of Shannon's H (H=2.3493) was calculated for the sample collected from Sponge Haven in February 2002. Evenness was highest at the Sponge Haven site in June 2001 (E=0.6824) and lowest at the Dock site in February 2002 (E=0.1666), reflecting the high dominance of the species *Rhizonubecula* sp. (68.24%) at the latter site (Fig. 8, Tables 3, 4).

The proportions of all species recorded during the course of this study are presented in Table 3 (June 2001) and Table 4 (February 2002). Ninety-five percent confidence intervals were calculated from the standard error for species proportions for cluster sampling as outlined in Hayek and Buzas (1997:189). In most instances, when the proportion of a species is less than 1%, the width of the confidence limits for these rare species is approximately the same value (or wider) than the proportion. Figures 9 (June 2001) and 10 (February 2002) graphically depict the relative abundances at each site of those species for which the overall proportion (relative abundance) falls between the calculated 95% confidence limits. Likewise, Tables 5 and 6 list the rank abundance for these same species. Of the 41 total species identified in this study, only one species, *Iridia* cf. *I diaphana*, a single-chambered agglutinated foraminiferan, was common in the samples surveyed at all sites during both seasons (Tables 3-6). In June 2001, *Rhizonubecula* sp., an encrusting milioline foraminiferan, was the dominant species at all collecting sites located within the Main Channel (Sponge Haven, Dock, and 'Cuda Cut) but ranked only forth at the Boston Bay site (Tables 3, 5). *Sorites dominicensis*, a milioline species that possesses dinoflagellate endosymbionts, was found to be the second most common species at the Boston Bay, Sponge Haven, and 'Cuda Cut sites and the third most common species at the Dock site in June 2001. The patterns of relative

12
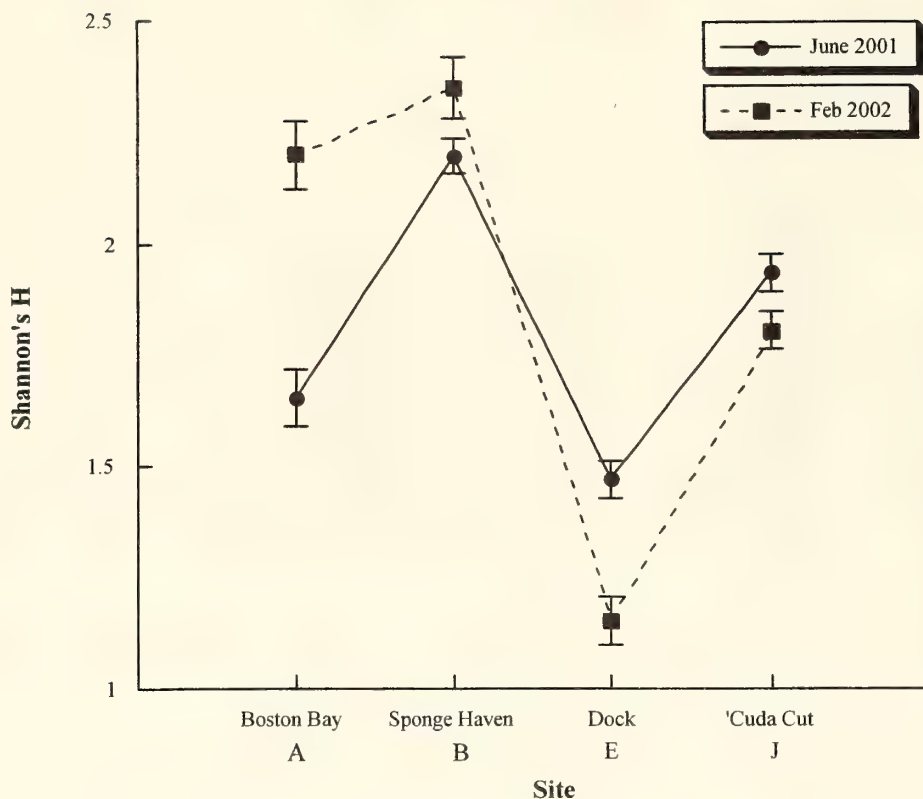
Figure 7. Values of H (Shannon Information Function) calculated for each site, Twin Cays, Belize (June 2001 and February 2002). H = - $\sum$ p$_i$ln(p$_i$) (Hayek and Buzas 1997). Sponge Haven (B=0.2239) and the sites that were the most dissimilar were again Boston Bay and the Dock (B=0.5601).

abundance were more varied at each site in February 2002. In February 2002, *Iridia* cf. *I. diaphana* was again the most common species in Boston Bay, the second most common species at Sponge Haven, and the third most common species at both the Dock and 'Cuda Cut sites (Fig. 3, Tables 4, 6). *Rhizonubecula* sp. was observed to be the dominant species at the Dock site only, falling to the second most common species at 'Cuda Cut, the third most common species at Sponge Haven, and the eighth most common species in Boston Bay. *Planorbulina acervalis,* a multichambered, calcareous hyaline species, was observed to be the most common species at the Sponge Haven and 'Cuda Cut sites, the second most common species at the Dock site, and the third most common species in Boston Bay. *Cornuspiramia antillarum* was the second most common species at the Boston Bay site in February 2002 but ranked only seventh at Sponge Haven and was present, in proportions below 1% at both the Dock and 'Cuda Cut sites (Table 4).

The Bray-Curtis measure of dissimilarity (B) was calculated for each pair of sites in June 2001 (Table 7) and February 2002 (Table 8). The mean dissimiliarity calculated for all sites was slightly lower in June 2001 (B$_{av}$=0.3052) than in February 2002 (B$_{av}$=0.3852). In June 2001, the sites with the lowest values of dissimilarity (or highest
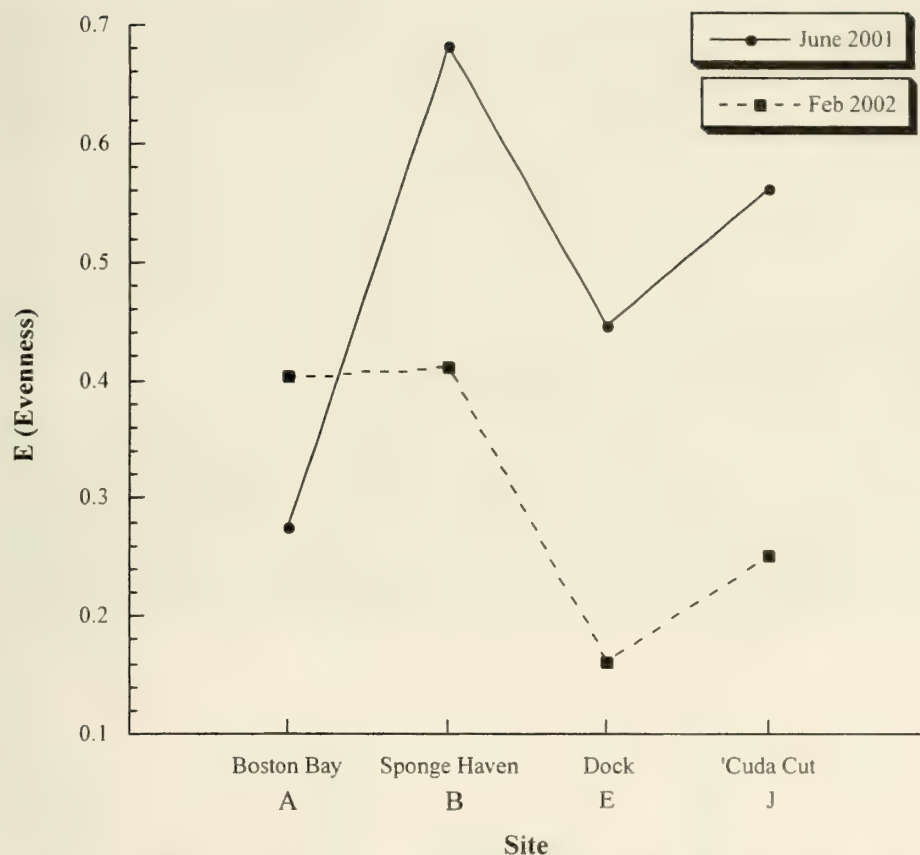
Figure 8. Values of E (Buzas-Gibson Evenness) calculated for each site, Twin Cays, Belize, during June 2001 and February 2002.

similarity) were Boston Bay and 'Cuda Cut (B=0.1659) and Sponge Haven and 'Cuda Cut (B=0.1686); the sites that were the most dissimilar were Boston Bay and the Dock (B=0.5272). In February 2002, the sites that were the most similar were Boston Bay and Sponge Haven (B=0.2239) and the sites that were the most dissimilar were again Boston Bay and the Dock (B-0.5601).

14
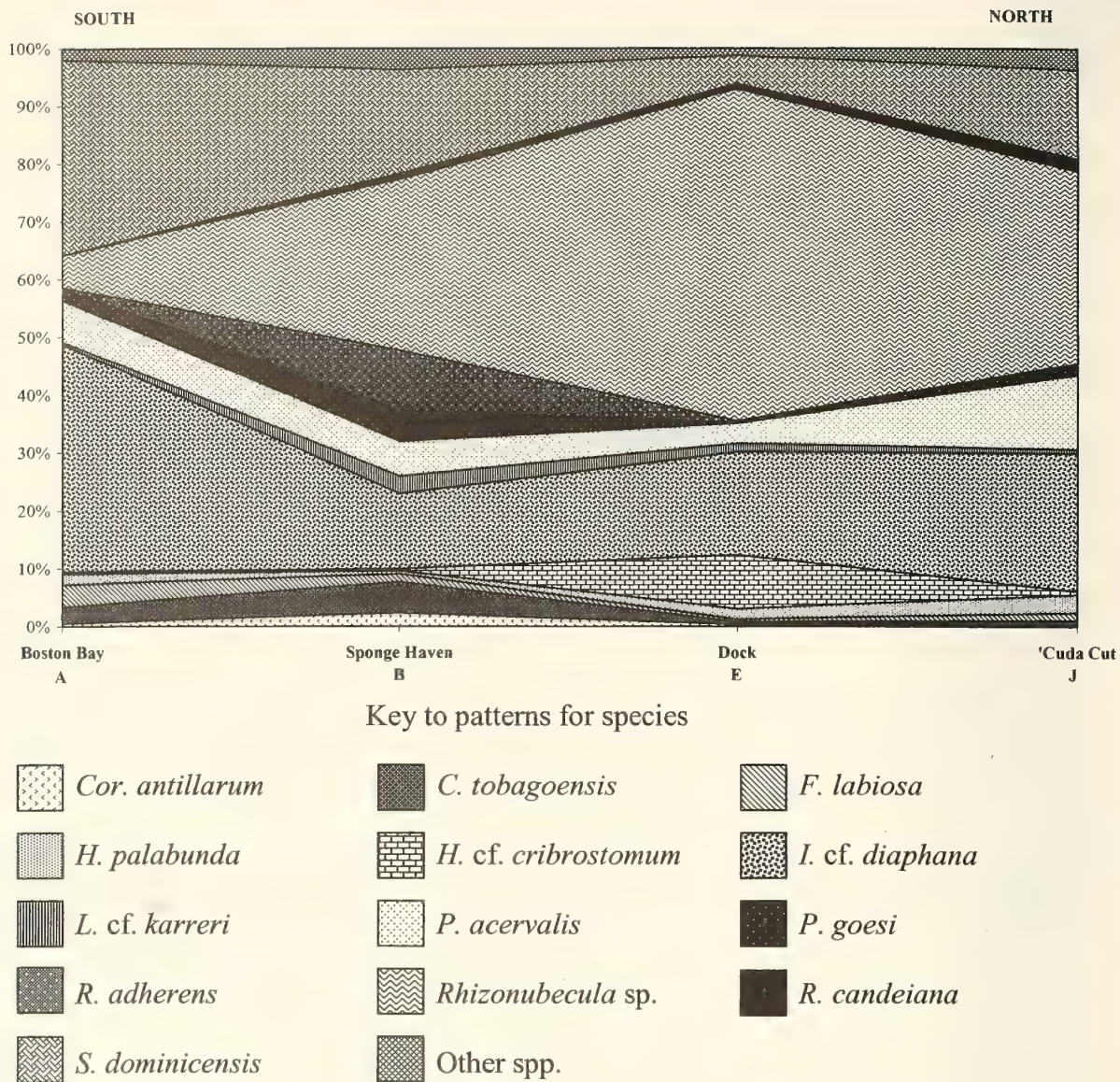
Figure 9. Relative abundance of the 13 most common epiphytic foraminiferal species at sites sampled within Twin Cays, Belize, in June 2001. Species arranged in alphabetical order from bottom to top of diagram. See key for species identification.
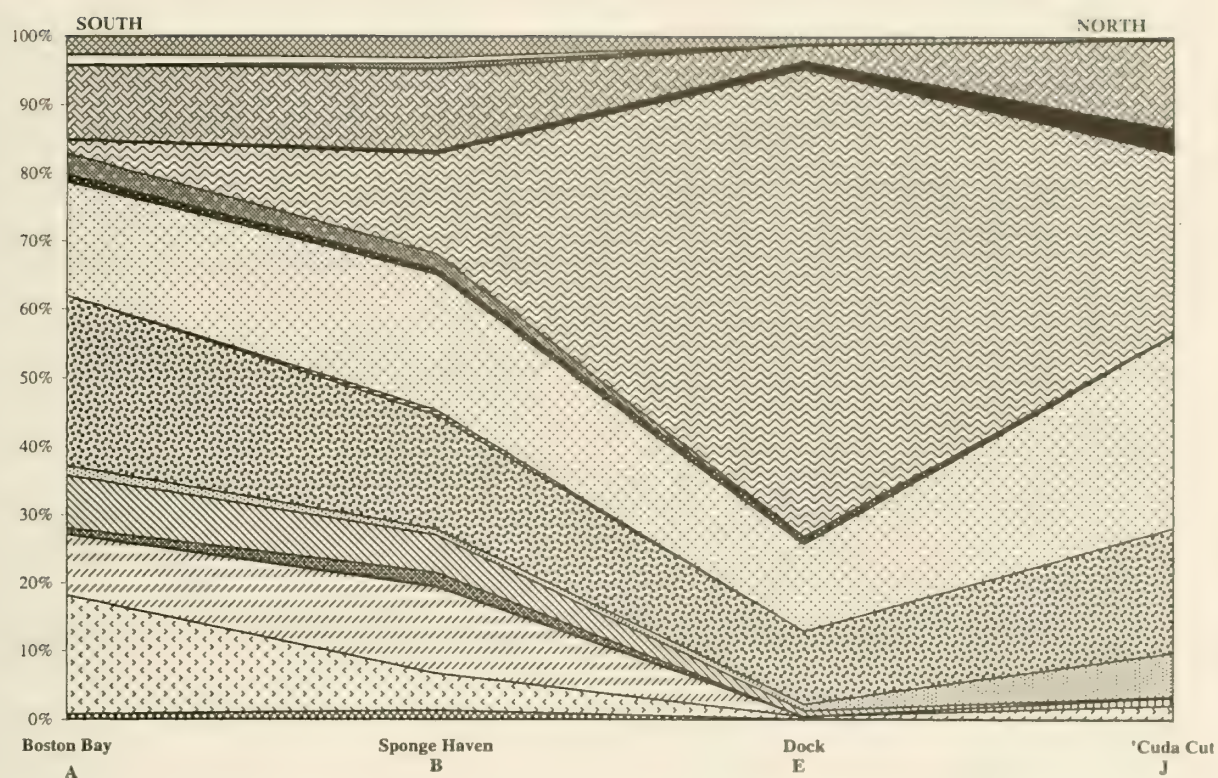
Key to patterns for species depicted in Figure 10.

C. planorbis   *Cor. antillarum*   *Cos. Antillarum*

C. cushmani   *C. tobagoensis*   *F. labiosa*

H. palabunda   *I.* cf. *diaphana*   *N.* cf. *terquemi*

P. acervalis   *P. goesi*   *R. adherens*

Rhizonubecula sp.   *R. candeiana*   *S. dominicensis*

Tretomphalus sp.   *T.* cf. *trigonula*   Other spp.

Figure 10. Relative abundance of the 17 most common epiphytic foraminiferal species at sites sampled within Twin Cays, Belize, in February 2002. Species arranged in alphabetical order from bottom to top of diagram. See key for species identification.

16

Table 5. Rank abundance of the most common species identified at each collecting site in June 2001.

| Boston Bay	Sponge Haven	Dock	'Cuda Cut
1. *I.* cf. *diaphana*	1. *Rhizonubecula* sp.	1. *Rhizonubecula* sp.	1. *Rhizonubecula* sp.
2. *S. dominicensis*	2. *S. dominicensis*	2. *I.* cf. *diaphana*	2. *I.* cf. *diaphana*
3. *P. acervalis*	3. *I.* cf. *diaphana*	3. *H. cribrostoma*	3. *S. dominicensis*
4. *Rhizonubecula* sp.	4. *R. adherens*	4. *S. dominicensis*	4. *P. acervalis*
5. *F. labiosa*	5. *P. acervalis*	5. *P. acervalis*	5. *H. palabunda*
6. *C. tobagoensis*	6. *C. tobagoensis*	6. *H. palabunda*	6. *R. candeiana*
7. *P. goesi*	7. *P. goesi*	7. *L.* cf. *karreri*	7. *P. goesi*
8. *H. palabunda*	8. *L.* cf. *karreri*	8. *R. candeiana*	8. *F. labiosa*
	9. *Cor. antillarum*		9. *L.* cf. *karreri*
	10. *F. labiosa*		10. *C. tobagoensis*
	11. *R. candeiana*		11. *H. cribrostoma*

Table 6. Rank abundance of the most common species identified at each collecting site in February 2002.

Boston Bay	Sponge Haven	Dock	'Cuda Cut
1. *I.* cf. *diaphana*	1. *P. acervalis*	1. *Rhizonubecula* sp.	1. *P. acervalis*
2. *Cor. antillarum*	2. *I.* cf. *diaphana*	2. *P. acervalis*	2. *Rhizonubecula* sp.
3. *P. acervalis*	3. *Rhizonubecula* sp.	3. *I.* cf. *diaphana*	3. *I.* cf. *diaphana*
4. *S. dominicensis*	4. *C. cushmani*	4. *S. dominicensis*	4. *S. dominicensis*
5. *C.* cf. *cushmani*	5. *S. dominicensis*	5. *R. candeiana*	5. *H. palabunda*
6. *F. labiosa*	6. *F. labiosa*	6. *P. goesi*	6. *R. candeiana*
7. *R.* cf. *adherens*	7. *Cor. antillarum*	7. *H. palabunda*	7. *Cos. antillarum*
8. *Rhizonubecula* sp.	8. *R.* cf. *adherens*	8. *F. labiosa*	8. *P.* cf. *squamiformis*
9. *Triloculina* sp. *I.* cf. *diaphana*	9. *C. tobagoensis*		
10. *P. goesi*	10. *C. planorbis*		
11. *C. tobagoensis*	11. *P.* cf. *squamiformis*		
	12. *Tretromphalus* sp. *H. palabunda*		
	13. *N.* cf. *terquemi*		

Table 7. Bray-Curtis Measure of Dissimilarity for sites sampled within Twin Cays, Belize, in June 2001.

	Boston Bay	Sponge Haven	Dock	'Cuda Cut
Boston Bay	0	0.3300	0.5272	0.1659
Sponge Haven	-----	0	0.2435	0.1686
Dock	-----	-----	0	0.3958
'Cuda Cut	-----	-----	-----	0

Table 8. Bray-Curtis Measure of Dissimilarity for sites sampled within Twin Cays, Belize, in February 2002.

	Boston Bay	Sponge Haven	Dock	'Cuda Cut
Boston Bay	0	0.1139	0.5601	0.4640
Sponge Haven	-----	0	0.4740	0.5601
Dock	-----	-----	0	0.1298
'Cuda Cut	-----	-----	-----	0

DISCUSSION

Even though the seagrass *T. testudinum* exhibits minimal seasonal variation in the vicinity of Twin Cays, Belize, the epiphytic foraminiferal communities living attached to the leaf blades show distinct seasonal differences in mean density per unit blade area (N/cm^2), species richness (S), and evenness (E), between June 2001 (the warmer, wet season) and February 2002 (the cooler, dry season) (Tables 1, 2).
These differences are potentially attributable to any number of biotic and abiotic factors that affect both the foraminiferal populations and their phytal substratum. Biotic factors, such as the life-history characteristics of individual species, competition, and predation. The benthic foraminiferal response to organic carbon flux is well documented (Loubere and Fariduddin, 1999; Murray, 2001). Benthic foraminiferans have been shown to feed on bacteria, diatoms and other microalgae (Bernhard and Bowser, 1992, Lipps, 1983; Schwab and Hofer, 1979), all of which are components of the microbial biofilm that coats the seagrass blades (Pl. 1, figs. a-g). A few foraminiferal species are also known to utilize directly dissolved organic material (Delaca et al., 1981). In low-nutrient, are all known to influence the species composition of communities (Krebs, 1994). Abiotic factors, such as temperature, salinity, light, nutrients, and the water-flow regime (currents and tides), have been observed to vary on a seasonal basis in Belize which has a subtropical climate and a pronounced wet and dry season (Koltes et al., 1998, Rützler and Ferraris, 1982). Some of the factors that potentially impact the epiphytic foraminiferal populations off Twin Cays include an influx of nutrients from the surrounding mangroves during the wet season, an incursion of open-water species into the main channel during the dry season, and the thermal tolerances of individual species.
The benthic foraminiferal response to organic carbon flux is well documented (Loubere and Fariduddin, 1999; Murray, 2001). Benthic foraminiferans have been shown to feed

on bacteria, diatoms and other microalgae (Bernhard and Bowser, 1992, Lipps, 1983; Schwab and Hofer, 1979), all of which are components of the microbial biofilm that coats the seagrass blades (Pl. 1, figs. a-g). A few foraminiferal species are also known to utilize directly dissolved organic material (Delaca et al., 1981). In low-nutrient, oligotrophic regimes, mangroves represent a source of organic carbon and nutrients for the surrounding nutrient-limited marine ecosystems (Robertson et al., 1992). Mangrove-derived nutrients are exported primarily in the form of leaf litter, detritus, particulate organic matter, and dissolved organic material, the interchange being facilitated by tidal rhythms (Hemminga et al., 1995; Marguillier et al., 1997; Mohammed et al., 1999). Although the influence of nutrient enrichment is most pronounced in the seagrass beds that are in closest proximity to the mangrove fringe, mangrove-derived nutrients may be exported for considerable distances from their source (Hemminga et al. 1995, Marguillier et al., 1997; Mohammed et al., 1999; Moran et al., 1991). Mangrove-derived nutrient enrichment may result in increased growth rates, shoot densities, and biomass of nearby seagrass beds, as well as increased epiphyte levels on the seagrass blades (Frankovich and Fourqurean, 1997; Koch and Madden, 2001; Koltes et al., 1998; Tomasko and Lapointe, 1991; van Tussenbroek, 1995). For example, in the Bahamas Koch and Madden (2001) recorded epiphyte loads that were 5 to 36 times higher on seagrasses in close proximity to the mangrove fringe of Sweeting's Cay than those recorded on seagrasses growing in the central lagoon or channel.

February 2002 also saw the incursion of predominantly open-water species into the mangrove habitats. The relative abundance of *Planorbulina acervalis* more than doubled in February 2002 at all sites and the species *Planogypsina* cf. *P. squamiformis* and *Tretomphalus* sp. were found in slightly higher abundances at some of the sites' sampled. In February 2002, *Cornuspiramia antillarum* was the second most common species in Boston Bay, comprising 17.41±1.36% of the epiphytic population, a dramatic increase in the population compared to June 2001 when it comprised only 0.53±0.53% of the population in Boston Bay. *Cornuspiramia antillarum* is a dendritic, encrusting milioline species that dominates epiphytic foraminiferal populations in open-water habitats subject to high nutrient influx. Off Man O'War Cay, a small mangrove island approximately 5 km to the north of Twin cays that hosts a breeding population of frigate birds, *C. antillarum* comprised 91.1±1.01% and 89.79±0.03% of the epiphytic foraminiferal populations in June 2001 and February 2002, respectively (S. Richardson, unpublished data).

Very little information is known about the thermal tolerances and/or growth optima of most species of benthic foraminiferans (Murray, 2001). In the Twin Cays region, water temperatures range from a monthly mean low of 23.6°C in January to a monthly mean high of 37.7°C in August (Koltes et al., 1998). Several of the epiphytic foraminiferal species observed living on *T. testudinum* in Belize (*Cornuspiramia antillarum*, *Hemidiscella palabunda*, *Iridia* cf. *I. diaphana*, *Planogypsina squamiformis*, *Rhizonubecula* cf. *R. adherens*, *Rosalina candeiana*, and *Sorites dominicensis*) appear to have broader thermal tolerances than they experience in Belize as they have their northernmost distributional limits in the Indian River Lagoon, Florida (Buzas and Severin, 1977; Culver and Buzas, 1980, 1982; S. Richardson, unpublished data). *Thalassia testudinum* in the latter region exhibits pronounced seasonal variation in leaf production, leaf elongation, and biomass (Gacia, 1999), and the epiphytic foraminiferal

populations in this region exhibit pronounced seasonality as well (S. Richardson, unpublished data). On 02 February 2001, a total of nine foraminiferal species (S=9, H=1.5267, E=0.5114) were identified among the 386 individuals surveyed on 20 seagrass blades (44.13 cm^2 mean blade area, σ=8.77, σ^2=76.93) collected from Jupiter Sound, Florida; and, on 26 May 2001, a total of 12 foraminiferal species (S=12, H=1.6348, E=0.4274) were identified among the 2,945 individuals surveyed on 20 seagrass blades (50.16 cm^2 mean blade area, σ=6.18, σ^2=38.15) collected from the same site (S. Richardson, unpublished data). Buzas and Hayek (2000) also reported strong seasonality in the densities of the sediment-dwelling foraminiferal biota in the Indian River Lagoon with densities highest in the summer months (June-August) and lowest in the winter months (December-February).

Values of Shannon's H generally range between 1.5 and 3.5 (Magurran 1988) and can vary according to the evenness, or distribution, of individuals within each species used to calculate H (Hayek and Buzas, 1997). This effect was seen in the Twin Cays data in June 2001 when a higher value of H was obtained for the Sponge Haven site (H=2.1965) than the Dock site (H=1.4699) even though Sponge Haven had a lower value of species richness (S=25) than the Dock (S=27). Evenness was higher at Sponge Haven (E=0.6824) than the Dock (E=0.4460) due to the higher dominance of the species *Rhizonubecula* sp. at the latter site. The low value of H seen at the Dock site (H=1.1521) in February 2002 is due to the low evenness (E=0.1666), and corresponding high dominance, of the encrusting milioline species *Rhizonubecula* sp. (Tables 3, 4).

High dominance has been shown to be characteristic of marine communities that are subject to stress from eutrophication and pollution (Lambshead et al., 1983; Tomascik and Sander, 1987). The dominance of *Rhizonubecula* sp. in June 2001 at the Sponge Haven, Dock, and 'Cuda Cut sites, where it was observed to be the most abundant species, and the continuing dominance of this species at the Dock site in February 2002 may indicate that *Rhizonubecula* sp. can be used as a marker for increased nutrients in the water column of mangrove habitats. Calem and Pierce (1993) measured moderately high concentrations of organic suspended material in the Main Channel of Twin Cays compared to the open lagoon during the dry season (February and March), although no published data is available for the wet season at these localities.

Iridia cf. *I. diaphana* is a single-chambered agglutinated foraminiferan that appears to be tolerant of fluctuating salinities in nearshore, tropical to subtropical marine ecosystems (S. Richardson, unpublished data). The dominance of this species in Boston Bay during June 2001 may reflect increased freshwater runoff from Hidden Lake into Boston Bay during the wet season (Calem and Pierce, 1993). *Sorites dominicensis*, the second most abundant species in Boston Bay in June 2001, has been observed to flourish in semiprotected embayments with low-water movement (Kloos and MacGillavry, 1984). *Crithionina* cf. *C. cushmani*, a single-chambered agglutinated foraminiferan that builds its dome-shaped test with sponge spicules and sediment grains, exhibited increased abundances at both Sponge Haven and Boston Bay in February 2002. This increase may reflect higher suspended sediment loads in the water column during the times that these sites were sampled (Calem and Pierce, 1993).

It is difficult to ascertain whether the differences observed in the relative abundance of the various species identified in this study truly reflect seasonal responses to changing environmental conditions without first conducting a long-term study that

extends over several years and involves more closely spaced sampling intervals. Buzas and Hayek (2000) analyzed data collected over a 20-year period from the Indian River Lagoon, Florida and found that both the population densities and the relative abundance of the five most common species of sediment-dwelling foraminiferans, varied considerably from season-to-season, as well as from year-to-year. Their results emphasize the importance of long-term monitoring programs for understanding regional trends in the population dynamics of specific taxa (Buzas and Hayek, 2000). Continued long-term monitoring of the epiphytic foraminiferal populations at Twin Cays will be important for establishing base-line data that can be used to assess any future changes that might result from both human-induced and climate-related environmental stresses.

Tilman et al. (2001) predict that expansion of agriculture over the next 50 years will result in 2.4-2.7-fold increases in the nitrogen- and phosphorus-driven eutrophication of near-shore marine ecosystems. In Belize, continued development is expected to result in increased erosion and sedimentation in coastal areas leading to the subsequent decline of nearshore mangrove, seagrass and coral reef habitats (Heyman and Kjerve, 1999). It is hoped that the substantial body of knowledge on the Twin Cays mangrove ecosystem that has been assembled over the past 20 years will serve as a foundation for implementing a more comprehensive and coordinated multispecies monitoring effort that will better enable us detect and respond to these threats.

SUMMARY

In contrast to their phytal substrate, the epiphytic foraminiferal biotas living attached to the leaf blades of *Thalassia testudinum* in the vicinity of Twin Cays, Belize exhibit distinct seasonal differences in mean density per unit blade area (N/cm^2), species richness (S) and evenness (E). The most pronounced seasonal differences observed were in the mean densities per unit blade area (N/cm^2), which were higher at all sites in June 2001 (the warmer, wet season) relative to February 2002 (the cooler, dry season). The values of S recorded at Sponge Haven, the Main Channel near the Dock, and 'Cuda Cut, were higher in June 2001 than in February 2002. Likewise, values of E calculated for these sites were higher in June 2001 than in February 2002. The Boston Bay site had a lower values of S and E in June 2001 compared to February 2002. No significant differences were noted in the values of Shannon's H and the mean species richness per blade (N/cm^2) calculated for each site. These seasonal differences are suggested to result from a combination of biotic and abiotic factors, which may include the influx of nutrients from the adjacent mangrove fringe during the wet season, an incursion of open-water species into the mangrove habitats during the dry season, and the thermal and salinity tolerances of individual species.

Rhizonubecula sp. is identified as a potential indicator species of increased nutrients in mangrove habitats. This encrusting, milioline foraminiferan was observed to be the dominant species in June 2001 at the Sponge Haven, Dock, and 'Cuda Cut sites and again in February 2002 at the Dock site. Continued long-term monitoring of the epiphytic foraminiferal populations living in the vicinity of the Twin Cays mangrove island will be important for establishing baseline data that can be used to detect changes

due to increased anthropogenic activities, such as the eutrophication of coastal waters, and global climate change.

ACKNOWLEDGMENTS

REFERENCES

Archibald, J.M., D. Longet, J. Pawlowski, and P.J. Keeling
  2003. A novel polyubiquitin structure in Cercozoa and Foraminifera: Evidence for a new eukaryotic supergroup. *Molecular Biology and Evolution* 20 (1):62-66.
Banner, F.T., C.P.G. Pereira, and D. Desai
  1985. "Tretomphaloid" float chambers in the Discorbidae and Cymbaloporidae. *Journal of Foraminiferal Research* 15 (3):159-174.
Bernhard, J.M., and S.S. Bowser
  1992. Bacterial biofilms as a trophic resource for certain benthic foraminifera. *Marine Ecology Progress Series* 83 (2-3):263-272.
Bock, W.D.
  1968. Two new species of Foraminifera from the Florida Keys. *Contributions from the Cushman Foundation for Foraminiferal Research* 19:27-29.
  1971. A handbook of the benthonic Foraminifera of Florida Bay and adjacent waters. In: Bock, W.D., Lynts, G.W., Smith, S., Wright, R., Hay, W.W., and Jones, J.I., eds. *A Symposium of Recent South Florida Foraminifera.* Miami Geological Society, Memoir 1:1-72.
Brady, H.B.
  1870. Part II. Analysis and description of Foraminifera. *Annals and Magazine of Natural History* 4:279-309.
Brönnimann, P.
  1949. Pflanzenbewohnende tropische Foramniferen nebst Beschreibung von *Cymbalopora tobagoensis* n. sp. *Verhandlungen der Naturforschenden Gesellschaft* in Basel 60:179-185.
Buzas, M.A., and L.-A. Hayek
  2000. A case for long-term monitoring of the Indian River Lagoon, Florida: Foraminiferal densities, 1977-1996. *Bulletin of Marine Science* 67 (2):805-814.

22

Buzas, M.A., and K.P. Severin
  1982. Distribution and systematics of foraminifera in the Indian River, Florida. *Smithsonian Contributions to Science* 36:1-38.
Buzas, M.A., R.K. Smith, and K.A. Beem
  1977. Ecology and Systematics of Foraminifera in two *Thalassia* habitats, Jamaica, West Indies. *Smithsonian Contributions to Paleobiology* 31:1-139.
Calder, D.R.
  1991. Abundance and distribution of hydroids in a mangrove ecosystem at Twin Cays, Belize, Central America. *Hydrologia* 216-217:221-228.
Calem, J.A., and J.W. Pierce
  1993. Distributional control of seagrasses by light availability, Twin Cays, Belize, Central America. *Atoll Research Bulletin* 387:1-13.
Culver, S.J.
  1990. Benthic Foraminifera of Puerto Rican mangrove-lagoon systems: potential for paleoenvironmental interpretations. *Palaios* 5:34-51.
  1991. Early Cambrian foraminifera from West Africa. *Science* 254:689-69.
Culver, S.J., and M.A. Buzas
  1980. Distribution of Recent benthic Foraminifera off the North American Atlantic Coast. *Smithsonian Contributions to the Marine Sciences* 6:512 pp.
  1982. Distribution of Recent benthic Foraminifera in the Caribbean region. *Smithsonian Contributions to the Marine Sciences* 14:382 pp.
  1998. Patterns of occurrence of benthic Foraminifera in time and space. In: Donovan, S.K., and C.R.C. Paul, eds. *The Adequacy of the Fossil Record.* John Wiley and Sons, Chichester, UK.
  2000. Response of shallow water foraminiferal palaeocommunities to global and regional environmental change. In: Culver, S. J., and Rawson, P. F., eds. *Biotic Response to Global Change: The Last 45 Million Years.* Cambridge University Press, Cambridge, UK, pp. 122-134.
Cushman, J.A.
  1922. Shallow-water Foraminifera of the Tortugas region. *Carnegie Institution of Washington, Marine Biology* 17 (311):1-85.
  1946. The genus *Hauerina* and its species. Contributions from the Cushman Laboratory for Foraminiferal Research 22:8-12.
Frankovich, T.A., and J.W. Fourqurean
  1997. Seagrass epiphyte loads along a nutrient availability gradient, Florida Bay, USA. *Marine Ecology Progress Series* 159:37-50.
Fujita, K., and P. Hallock
  1999. A comparison of phytal substrate preferences of *Archaias angulatus* and *Sorites orbiculus* in mixed macroalgal-seagrass beds in Florida Bay. *Journal of Foraminiferal Research* 29:143-151.
Hallock, P., and M.W. Peebles
  1993. Foraminifera with chlorophyte endosymbionts: habitats of six species in the Florida Keys. *Micropaleontology* 20 (3/4):277-292.
Hansen, H.J., and S.A. Revets
  1992. A revision and reclassification of the Discorbidae, Rosalinidae, and Rotaliidae. *Journal of Foraminiferal Research* 22(2):166-180.

Hayek, L.-A., and M.A. Buzas
  1997. *Surveying Natural Populations.* Columbia University Press, New York, 563 pp.
Hemminga, M.A., and C.M. Duarte
  2000. *Seagrass Ecology.* Cambridge University Press, Cambridge, England, 298 pp.
Hemminga, M.A., P. Gwada, F.J. Slim, P. de Koeyer, and J. Kazungu
  1995. Leaf production and nutrient contents of the seagrass *Thalassodendron ciliatum* in the proximity of a mangrove forest (Gazi Bay, Kenya). *Aquatic Botany* 50:159-170.
Heyman, W.D.
  1999. Hydrological and oceanographic considerations for integrated coastal zone management in Southern Belize. *Environmental Management* 24 (2):229-245.
Hofker, J.
  1972. *Primitive Agglutinated Foraminifera.* E. J. Brill, Leiden, The Netherlands, 95 pp., 27 pls.
Høglund, H.
  1947. Foraminifera in the Gullmar Fjord and the Skagerak. *Zoologiska Bidrag från Uppsala* 26:328 pp., 32 pls.
Hottinger, L., E. Halicz, and Z. Reiss
  1993. Recent Foraminifera from the Gulf of Aqaba, Red Sea. *Academia Scientiarum et Artium Slovenica, Ljubljana, Opera* 33:1-179
Kloos, D.P., and H.J. MacGillavry
  1985. Variability and population dynamics of *Sorites orbiculus* (Foraminifera, Soritidae). Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen. Series B: Palaeontology, Geology, Physics, Chemistry, Anthropology 88 (2):199-209.
Keeling, P.J.
  2001. Foraminifera and Cercozoa are related in actin phylogeny: two orphans find a home? *Molecular Biology and Evolution* 18:1551-1557.
Koch, M.S., and C.J. Madden
  2001. Patterns of primary production and nutrient availability in a Bahamas lagoon with fringing mangrove. *Marine Ecology Progress Series* 219:109-119.
Koltes, K., J.J. Tschirky, and I.C. Feller
  1998. Carrie Bow Cay, Belize. In: Kjerfve, B. (ed.), CARICOMP--Caribbean coral reef, seagrass and mangrove sites. *Coastal region and small island papers* 3:79-94, UNESCO, Paris.
Krebs, C.J.
  1994. *Ecology: The Experimental Analysis of Distribution and Abundance.* Harper Collins, New York, 800 pp.
Langer, M.R.
  1993. Epiphytic foraminifera. *Marine Micropaleontology* 20:235-265.
Lambshead, P.J.D., and H.M. Platt
  1983. Detection of differences among assemblages of benthic species based on an assessment of dominance and diversity. *Journal of Natural History, London* 17:859-874.
Le Calvez, J.
  1935. Sur quelques Foraminifères de Villefranche et de Banyuls. *Archives de Zoologie Expérimentale et Générale* 77 (2):79-98.

24

Le Calvez, Y.
   1977. Révision des Foraminifères de la collection d'Orbigny. II. Foraminifères de l'île de Cuba—Tome I. *Cahiers de Micropaléontologie* 1, 127 pp.
Lipps, J.H., and A.Y. Rozanov
   1996. The Late Precambrian-Cambrian agglutinated fossil *Platysolenites*. *Paleontological Journal* 30 (6):679-687.
Loeblich, A.R., Jr., and Tappan, H.
   1964. Sarcodina, chiefly "Thecamoebians" and Foraminiferida. *Treatise on Invertebrate Paleontology, Part C, Protista 2, Volume 1,* 511 pp.
Loubere, P., and M. Fariduddin
   1999. Benthic Foraminifera and the flux of organic carbon to the seabed. In: Sen Gupta, B.K., ed. *Modern Foraminifera.* Kluwer Academic Publishers, Great Britain, pp. 181-199.
Magurran, A.E.
   1988. *Ecological Diversity and its Measurement.* Princeton University Press, Princeton, New Jersey, 179 pp.
Marguillier, S., G. Van der Velde, Dehairs, F., Hemminga, M.A., and Rajagopal, S.
   1997. Trophic relationships in an interlinked mangrove-seagrass ecosystem as traced by $\partial^{13}C$ and $\partial^{15}N$. *Marine Ecology Progress Series* 151 (1-3):115-121.
McIlroy, D., O. Green, and M. Brasier
   2001. Palaeobiology and evolution of the earliest agglutinated Foraminifera: *Platysolenites, Spirosolenites* and related forms. *Lethaia* 34 (1):13-29.
Mohammed, S.M., R.W. Johnstone, B. Widen, and E. Jordelius
   2001. The role of mangroves in the nutrient cycling and productivity of adjacent seagrass communities, Chwaka Bay, Zanzibar. In: Richmond, M. D., and Francis, J., eds. The 20th Anniversary Conference on Advances in Marine Science in Tanzania, Zanzibar (Tanzania), 28 June-1 July 1999. Marine Science Development in Tanzania and Eastern Africa 1:205-226.
Moran, M.A., R.J. Wicks, and R.E. Hodson
   1991. Export of dissolved organic matter from a mangrove swamp ecosystem: evidence from natural fluorescence, dissolved lignin phenols, and bacterial secondary production. Marine Ecology Progress Series 76:175-184.
Murray, J.W.
   1973. *Distribution and Ecology of Living Benthic Foraminiferids.* Heinemann Educational Books, London.
   1991a. *Ecology and Paleoecology of Benthic Foraminifera.* John Wiley, New York and Longman Scientific and Technical, Harlow, U.K.
   1991b. Ecology and distribution of benthic foraminifera. In: Lee, J.J., and Anderson, O.R., eds. *Biology of Foraminifera.* Academic Press, London and San Diego, pp. 221-368.
   2001. The niche of benthic foraminifera, critical thresholds and proxies. *Marine Micropaleontology* 41:1-7.
Orbigny, A.D. d'
   1839. Foraminifères. In: de la Sagra, M.R., *Histoire Physique, Politique et Naturelle de L'Île de Cuba,* 223 pp.

Pawlowski, J., M. Holzmann, C. Berney, J. Fahrni, A.J. Gooday, and T. Cedhagen
  2003. The evolution of early Foraminifera. *Proceedings of the National Academy of Sciences* 100 (20):11494-11498.
Phillips, R.C., and E.G. Meñez
  1988. *Seagrasses. Smithsonian Contributions to the Marine Sciences* 34:104 pp.
Richardson, S.L.
  2000. Epiphytic Foraminifera of the Pelican Cays, Belize: Diversity and Distribution. In: Macintyre, I. B., Rützler, K., eds. *The Natural History of the Pelican Cays*. Atoll Research Bulletin 475:208-228.
Robertson, A.I., D.M. Alongi, and K.G. Boto
  1992. Food chains and carbon fluxes. In: Robertson, A.I. and Alongi, D.M., eds. *Tropical Mangrove Ecosystems*. Coastal and Estuarine Studies 41:293-326. American Geophysical Union, Washington, DC.
Rützler, K., and I.C. Feller
  1996. Caribbean mangrove swamps. *Scientific American* 1996 (March):94-99.
Rützler, K., and J.D. Ferraris
  1982. Terrestrial environment and climate, Carrie Bow Cay, Belize. In: Rützler, K., and Macintyre, I.G., eds. *The Atlantic Barrier Reef Ecosystem at Carrie Bow Cay, Belize. I. Structure and communities*. Smithsonian Contributions to the Marine Sciences No. 12:77-91.
Schwab, D., and H.W. Hofer
  1979. Metabolism in the protozoan *Allogromia laticollaris* Arnold. *Zeitschrift für mikroskopisch-anatomische Forschung*, Leipzig 93:715-727.
Semeniuk, T.A.
  2000. Spatial variability in epiphytic Foraminifera from micro- to regional scale. *Journal of Foraminiferal Research* 30 (2):99-109.
  2001. Epiphytic Foraminifera along a climatic gradient, Western Australia. *Journal of Foraminiferal Research* 31 (3):191-200.
Sen Gupta, B.K.
  1999. Foraminifera in marginal marine environments. In: Sen Gupta, B. K., ed. *Modern Foraminifera*. Kluwer Academic Publishers, Great Britain, pp. 141-159.
Tilman, D., J. Fargione, B. Wolff, C. D'Antonio, A. Dobson, R. Howarth, D. Schindler, W. H. Schlesinger, D. Simberloff, and D. Swackhamer
  2001. Forecasting agriculturally driven global environmental change. *Science* 292:281-284.
Tomascik, T., and F. Sander
  1987. Effects of eutrophication on reef building corals. II. Structure of scleractininan coral communities in fringing reefs, Barbados, West Indies. *Marine Biology* 94:53-75.
Tomasko, D.A., and B.E. Lapointe
  1991. Productivity and biomass of *Thalassia testudinum* as related to water column nutrient availability and epiphyte levels: field observations and experimental studies. *Marine Ecology Progress Series* 75:9-17.
Tussenbroek, I.B. van
  1995. *Thalassia testudinum* leaf dynamics in a Mexican Caribbean coral reef lagoon. *Marine Biology*, Berlin, 122 (1):33-40.

Wantland, K.F.
    1975. Distribution of Holocene benthonic Foraminifera on the Belize Shelf. In: Wantland,
        K.F., and Pusey, W.C., eds. *Belize Shelf--Carbonate Sediments, Clastic Sediments,
        and Ecology.* American Association of Petroleum Geologists, Tulsa, Oklahoma, pp.
        332-399.
Williams, S.L., and K.L. Heck, Jr.
    2001. Seagrass community ecology. In: Bertness, M.D., Gaines, S.D., and Hay, M.E., eds.
        Marine Community Ecology. Sinauer Associates, Sunderland, MA, pp. 317-337.
Williamson, W.C.
    1858. On the Recent Foraminifera of Great Britain. Ray Society, London, 107 pp.
Wilson, B.
    1998. Epiphytal foraminiferal assemblages on the leaves of the seagrasses *Thalassia
        testudinum* and *Syringodium filiforme. Caribbean Journal of Science* 34 (1-2):132-
        136.
Zieman, J.C.
    1975. Quantitative and dynamic aspects of the ecology of turtle grass, *Thalassia
        testudinum.* Estuarine Research 1:541-562.
Zieman, J.C., R.T. Zieman
    1989. The Ecology of the Seagrass Meadows of the West Coast of Florida: A
        Community Profile. Biological Report 85(7.25), U.S. Fish and Wildlife Service,
        Washington DC, USA, 155 pp.

APPENDIX 1

List of species

1. *Ammonia* cf. *A. parkinsoniana* (d'Orbigny, 1839) (not figured). *Ammonia beccarii* (Linné, 1758): WANTLAND, 1975, p. 395, text-figs. 3 l, 4 a-d, 5 a.

2. *Amphistegina gibbosa* d'Orbigny, 1839 (not figured). *Amphistegina gibbosa* d'Orbigny, 1839: LE CALVEZ, 1977b, p. 6, text-figs. 4, 5.

3. *Androsina* cf. *A. lucasi* Lévy, 1977 (Pl. 1, fig. f). *Archaias angulatus* (Fichtel and Moll, 1803): WANTLAND, 1975, p. 392, text-figs. 9 o-r, 12 b, 16 j, k, m-0); *Androsina lucasi* Lévy, 1977. HALLOCK AND PEEBLES, 1993, p. 278, pl. 1, fig. 1-4.

4. *Articulina antillarum* Cushman, 1922 (Pl. 2, fig. e). *Articulina antillarum* CUSHMAN, 1922, p. 71, pl. 12, fig. 5. *Articulina sagra* d'Orbigny, 1839: BRADY, 1884, p. 184, pl. 12, figs. 17, 18; BOCK, 1971, p. 33, pl. 13, fig. 7. *Articulina lineata* Brady, 1884: WANTLAND, 1975, p. 391, text-fig. 9 j.

5. *Articulina mucronata* (d'Orbigny, 1839) (Pl. 2, fig. b). *Vertebralina cassis* var. *mucronata* d'Orbigny, 1839: CUSHMAN, 1922, p. 63; CUSHMAN, 1929, p. 96, pl. 22, fig. 5. *Vertebralina cassis* d'Orbigny, 1839: WANTLAND, 1975, p. 389, text-fig. 9 i.

6. *Bolivinita rhomboidalis* (Millet, 1899) (Pl. 2, fig. h). *?Bolivina compacta* (Sidebotton, 1905): CUSHMAN, 1922, p. 26, pl. 1, fig. 10. *?Bolivina lowmani* Phleger and Parker, 1951: BOCK, 1971, p. 46, pl. 16, fig. 14. *Bolivinita rhomboidalis* (Millet, 1899): WANTLAND, 1975, p. 393, text-fig. 10 s. BUZAS, SMITH AND BEEM, 1977, p. 74, pl. 2, figs. 3, 4.

7. *Cornuspira planorbis* Schultze, 1854 (Pl. 2, fig. a). *Cornuspira planorbis* Schultze, 1854: WANTLAND, 1975, p. 387.

8. *Cornuspiramia antillarum* (Cushman, 1922) (Not figured). *Nubecularia antillarum* CUSHMAN, 1922, p. 58, text-figs. 7, 8.

9. *Coscinospira antillarum* (d'Orbigny, 1839) (Pl. 3, fig. h). *Dendritina antillarum* d'Orbigny, 1839: LE CALVEZ, 1977, p. 39, pl. 3, figs. 1-7. *Peneroplis pertusus* (Forskål, 1775): WANTLAND, 1975, p. 391.

10. *Cribroelphidium poeyanum* (d'Orbigny, 1839) (Pl. 4, fig. g). *Cribroelphidium poeyanum* (d'Orbigny, 1839): WANTLAND, 1977, p. 396, text-figs. 3m, 4i, 5b, c, 6g, h, 7h, 13a, 16i.

11. *Crithionina cushmani* Hofker, 1972 (Not figured). *Crithionina cushmani* HOFKER, 1972, p. 69, pl. 20, figs. 15, 16; pl. 21, figs. 1-3.

12. *Crithionina* sp. (Pl. 3, fig. c).

13. *Cymbaloporetta squammosa* (d'Orbigny, 1839) (Not figured). *Cymbaloporetta squamosa* (d'Orbigny, 1839): WANTLAND, 1975, p. 391, text-fig. 9 l. *Rosalina squammosa* d'Orbigny, 1839: LE CALVEZ, 1977b, p. 100, text-figs. 1-14 (p. 101).

14. *Cymbaloporetta tobagoensis* (Brönnimann, 1949) (Pl. 2, fig. i). *Cymbaloporetta bradyi* Cushman, 1915: WANTLAND, 1975, p. 398. *Cymbaloporetta tobagoensis* BRÖNNIMAN, 1949, p. 183, text-fig. 1a, b.

15. *Flintinoides labiosa* (d'Orbigny, 1839) (Pl. 4, fig. c). *Triloculina labiosa* d'Orbigny, 1839: CUSHMAN, 1922, p. 77, pl. 12, fig. 1. *Miliolinella labiosa* (d'Orbigny, 1839): WANTLAND, 1975, p. 390, text-figs. 9 a-g.

16. *Hemidiscella palabunda* Bock, 1968 (Pl. 3, fig. e). *Hemidiscella palabunda* BOCK, 1968, p. 27, pl. 4, figs. 3-9; WANTLAND, 1975, p. 385, text-figs. 10 i, j.

17. *Heterillina* cf. *H. cribrostoma* (Heron-Allen & Earland, 1915) (Not figured). *Heterillina cribrostoma* (Heron-Allen & Earland, 1915: WANTLAND, 1975, p. 391, text-figs. 8 r, 14 a-m.

18. *Iridia* cf. *I. diaphana* Heron-Allen & Earland, 1914 (Pl. 1, figs. f, g; Pl. 3, fig. a). *Iridia diaphana* Heron-Allen & Earland, 1914: CUSHMAN, 1922, p. 18.

19. *Laevipeneroplis* cf. *L. karreri* Wiesner, 1923 (Pl. 1, fig. a; Pl. 2, fig. d). *Peneroplis bradyi* Cushman, 1930: WANTLAND, 1975, p. 391, text-fig. 9 m.

20. *Miliammina fusca* (Brady, 1870) (Not figured). *Quinqueloculina fusca* BRADY, 1870, p. 286, pl. 11,figs. 2, 3.

21. *Neoconorbina* cf. *N. terquemi* (Rzehak, 1888) (Pl. 2, fig. g). *Neoconorbina terquemi* (Rzehak, 1888): WANTLAND, 1975, p. 394, text-figs. 6 k, 12 k.

22. *Patellina corrugata* Williamson, 1858 (Not figured). *Patellina corrugata* Williamson, 1858: CUSHMAN, 1931, p. 11, pl. 2, fig. 7.

23. *Planorbulina acervalis* Brady, 1884 (Pl. 3, fig. d). *Planorbulina acervalis* Brady, 1884: WANTLAND, 1975, p. 397, text-fig. 11 d.

24. *Planorbulina mediterranensis* d'Orbigny, 1826 (Not figured). *Planorbulina mediterranensis* d'Orbigny, 1826: WANTLAND, 1975, p. 397, text-fig. 11 b.

25. *Planogypsina* cf. *P. squamiformis* (Chapman, 1901) (Pl. 3, fig. f). *Planogypsina* cf. *P. squamiformis* (Chapman, 1901): HOTTINGER, HALICZ, AND REISS, 1993, p. 126, pl. 171, figs. 1-9.

26. *Pseudohauerina occidentalis* (Cushman, 1946) (Pl. 4, fig. e). *Hauerina occidentalis* Cushman, 1946: WANTLAND, 1975, p. 391, text-fig.8 q.

27. *Pseudowebbinella goësi* (Höglund, 1947) (Pl. 3, fig. b). *Webbinella hemispherica* (Jones, Parker and Brady, 1865): LE CALVEZ, 1935, p. 88, text-fig. 7a, b. *Crithionina goësi* HÖGLUND, 1947, p. 36, pl. 3, figs. 1-6; text-fig. 8. *Pseudowebbinella goësi* (Höglund, 1947): HOFKER, 1972, p. 70, pl 21, figs. 7-15.

28. *Quinqueloculina bicostata* d'Orbigny, 1839 (Not figured). WANTLAND, 1975, p. 387, text-fig. 3a. b. 13n.

29. *Quinqueloculina* sp. (Not figured). *Quinqueloculina seminulum* (Linné, 1758): WANTLAND, 1975, p. 389, text-fig. 3f, g.

30. *Rhizonubecula adherens* Le Calvez, 1935 (Not figured). *Rhizonubecula adherens* LE CALVEZ, 1935, p. 96, text-fig. 11 a, b.

31. *Rhizonubecula* sp. (Not figured).

32. *Rosalina candeiana* d'Orbigny, 1839 (Pl. 3, fig. i). *Rosalina candeiana* d'Orbigny, 1839: WANTLAND, 1975, p. 394, text-figs. 10 c, d, 12 n; LE CALVEZ, 1977b, p. 83, text-figs. 1-8 (p. 85).

33. *Rosalina* sp. (Not figured). *Rosalina* sp. WANTLAND, 1975, p. 395, text-figs. 10 f, g.

34. *Rotorbis auberii* (d'Orbigny, 1839) (Pl. 4, figs. a, b). *Rosalina auberii* D'ORBIGNY, 1839, p. 94, pl. 4, figs. 5-8. *Discorbis mira* CUSHMAN, 1922, p. 39, pl. 6, figs. 10, 11: WANTLAND, 1975, p. 394, text-figs. 10 a, b. *Discorbis auberii* (d'Orbigny,

1839): LE CALVEZ, 1977, p. 77, pl. 9, figs. 1-8. *Rotorbis mira* (d'Orbigny, 1839): HANSEN AND REVETS, 1992, p. 175, pl. 3, figs. 1-3.

35. *Sagenina* frondescens (Brady, 1879) (Not figured). *Sagenina frondescens* (Brady, 1879): LOEBLICH & TAPPAN, 1964, p. C205, text-fig. 117-4.

36. *Sigmoihauerina atlantica* (Cushman, 1946) (Pl. 4, fig. h). *Hauerina bradyi* Cushman, 1917: CUSHMAN, 1922, p. 71; BOCK, 1971, p 30, pl. 12, fig. 9; WANTLAND, 1975, p. 391. *Hauerina atlantica* CUSHMAN, 1946, p. 8, pl. 1, figs. 25-26.

37. *Sorites dominicensis* Ehrenberg, 1839 (Pl. 1, fig. c, d; Pl. 4, fig. i). *Amphisorus hemprichii* Ehrenberg, 1839: WANTLAND, 1975, p. 392, text-fig. 11 c.

38. *Textularia agglutinans* d'Orbigny, 1839 (Pl. 4, fig. d). *Textularia agglutinans* D'ORBIGNY, 1839, p. 144, pl. 1, figs. 17, 18, 32-34; WANTLAND, 1975, pl. 385, text-figs. 3 h, 11 g.

39. *Tretomphalus* sp. (Pl. 3, fig. g). *Tretomphalus bulloides* (d'Orbigny, 1839): WANTLAND, 1975, text-figs. 13 r, s. *Neoconorbina (Tretomphaloides) concinna* (Brady, 1884): BANNER, PEREIRA, AND DESAI, 1985, p. 166, pl. 1, figs. 6-10, pl. 2, figs. 1-3.

40. *Triloculina bicarinata* d'Orbigny, 1839 (Pl. 4, fig. f). *Triloculina bicarinata* d'Orbigny, 1839: WANTLAND, 1975, p. 389, text-fig. 8 l.

41. *Triloculina* cf. *T. trigonula* (Lamarck, 1804) (Pl. 2, fig. c). *Triloculina trigonula* (Lamarck, 1804): CUSHMAN, 1929, p. 56, pl. 12, figs. 10, 11; pl. 13, figs. 1, 2; BOCK, 1971, p. 28, pl. 12, figs. 3, 4.

31

PLATES

31

Plate 1. Scanning electron micrographs of *Thalassia testudinum* blade from Twin Cays, Belize. Scale bar=100 µm (Figs. a, f); 10 µm (Figs. b, g); 5 µm (Figs. c-e). **a** Specimen of *Laevipeneroplis* cf. *L. karreri* attached to seagrass blade. Note pseudopodia and feeding cyst along periphery of outermost chamber; **b** Close-up of pennate diatoms and dinoflagellate on blade surface; **c, d** View of pseudopodia emerging from apertures in test of *Sorites dominicensis*; **e** Close-up of microbial biofilm on blade surface; **f** Specimen of *Iridia* cf. *I diaphana* attached to seagrass blade. Note network of reticulated pseudopodia emanating from test periphery; **g** Enlarged view of *I.* cf. *I. diaphana* pseudopodia. Note diatom frustule being engulfed by pseudopodia in upper center of image.

Plate 1

34
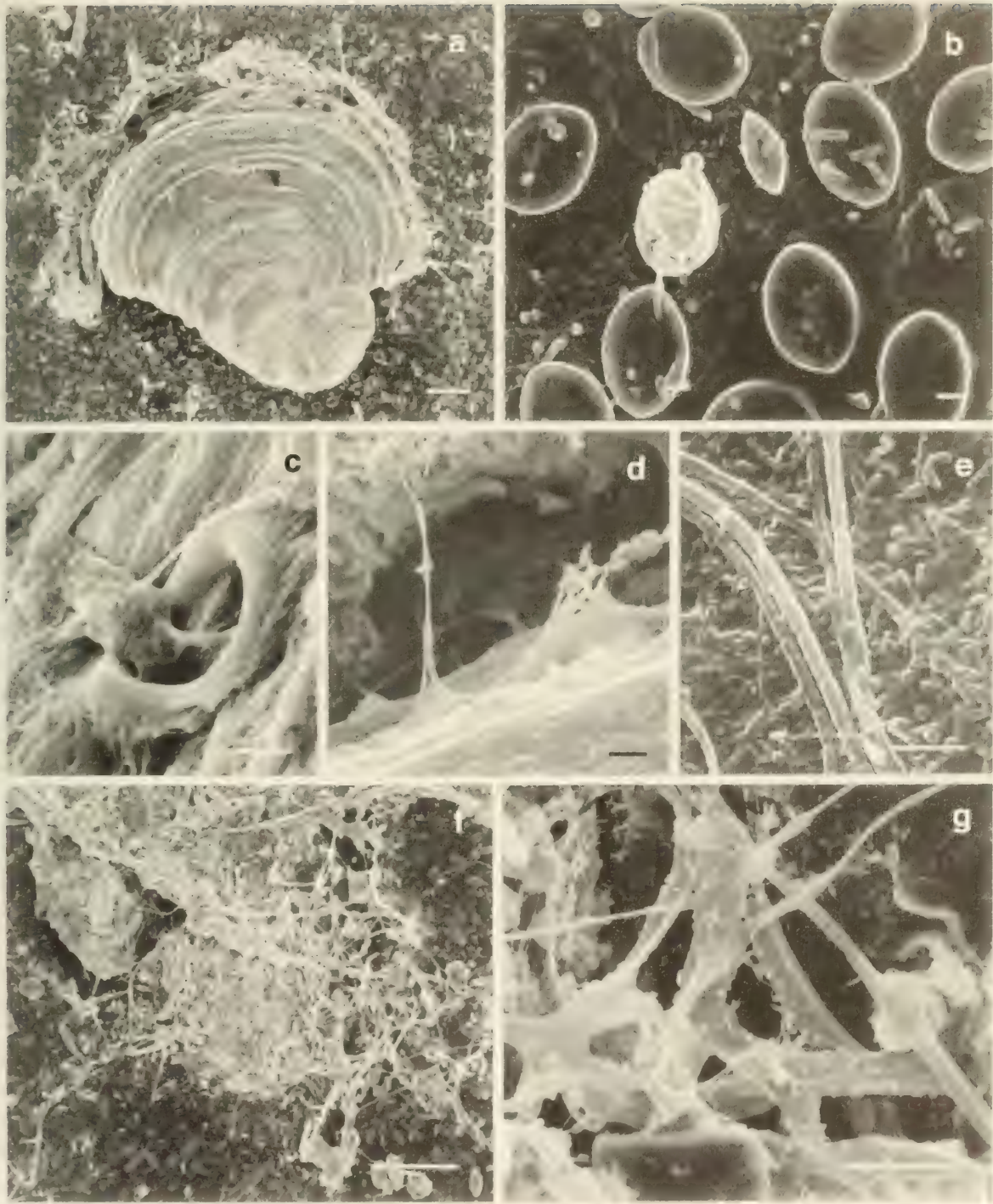
Plate 2. Scanning electron micrographs of epiphytic foraminiferans from Twin Cays, Belize. Scale bar=100 μm (Figs. a-g, i); 50 μm (Fig. h). **a** *Cornuspira planorbis*; **b** *Articulina mucronata*; **c** *Triloculina* cf. *T. trigonula*; **d** *Laevipeneroplis* cf. *L. karreri*; **e** *Articulina antillarum*; **f** *Androsina* cf. *A. lucasi*; **g** *Neoconorbina* cf. *N. terquemi*; **h** *Bolivinita rhomboidalis*; **i** *Cymbaloporetta tobagoensis*.

Plate 2

36

Plate 3. Scanning electron micrographs of epiphytic foraminiferans from Twin Cays, Belize. Scale bar=110 μm (Figs. a, d); 100 μm (Figs. b, c, e-i). **a** *Iridia* cf. *I. diaphana*; **b** *Pseudowebbinella goësi*; **c** *Crithionina* sp.; **d** *Planorbulina acervalis*; **e** *Hemidiscella palabunda*; **f** *Planogypsina* cf. *P. squamiformis*; **g** *Tretomphalus* sp.; **h** *Coscinospira antillarum*; **i** *Rosalina candeiana*.

Plate 3

38
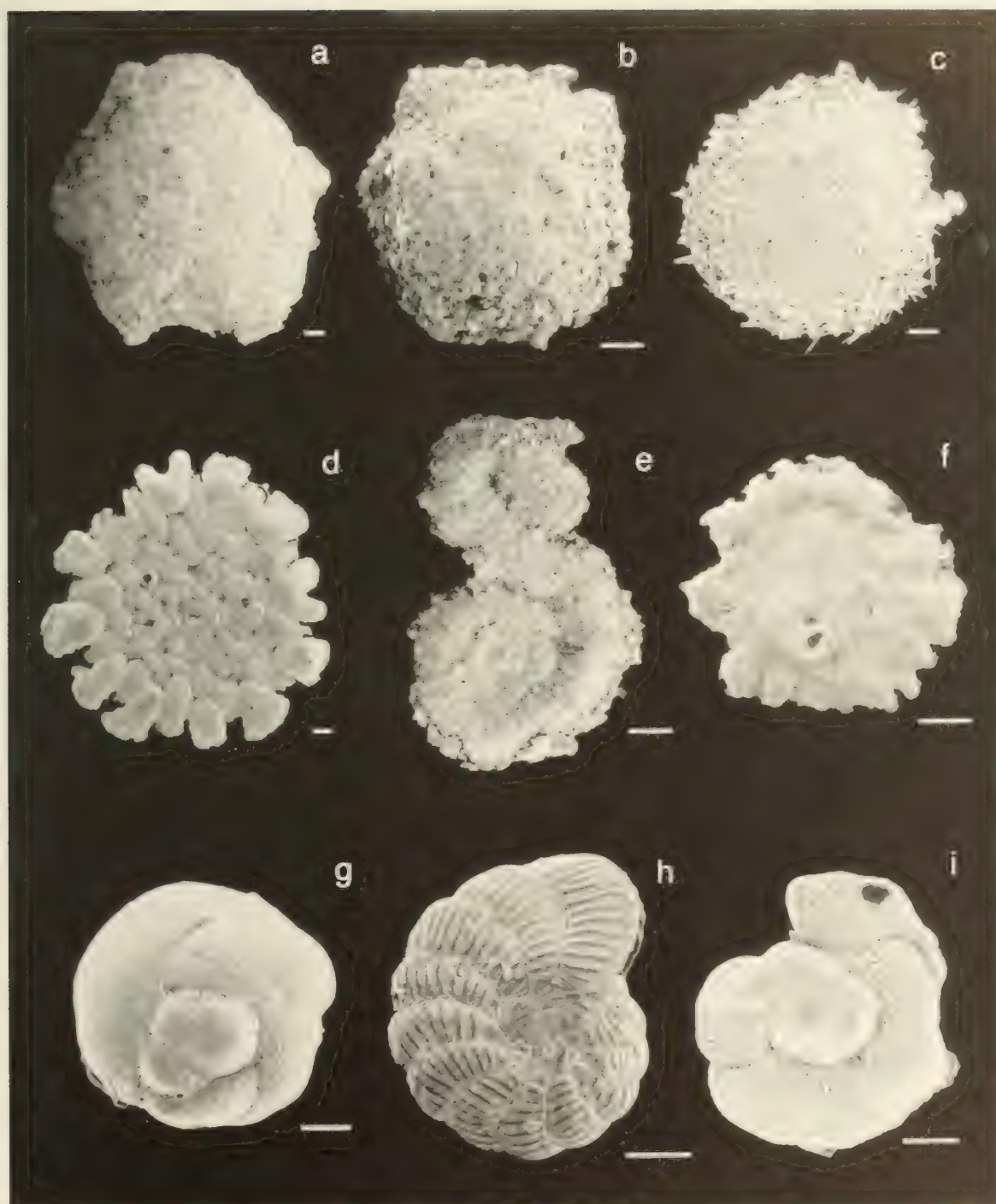
Plate 4. Scanning electron micrographs of epiphytic foraminiferans from Twin Cays, Belize. All scale bars=100 μm. **a, b** *Rotorbis auberii*; **c** *Flintinoides labiosa*; **d** *Textularia agglutinans*; **e** *Pseudohauerina occidentalis*; **f** *Triloculina bicarinata*; **g** *Cribroelphidium poeyanum*; **h** *Sigmoihauerina atlantica*; **i** *Sorites dominicensis*.
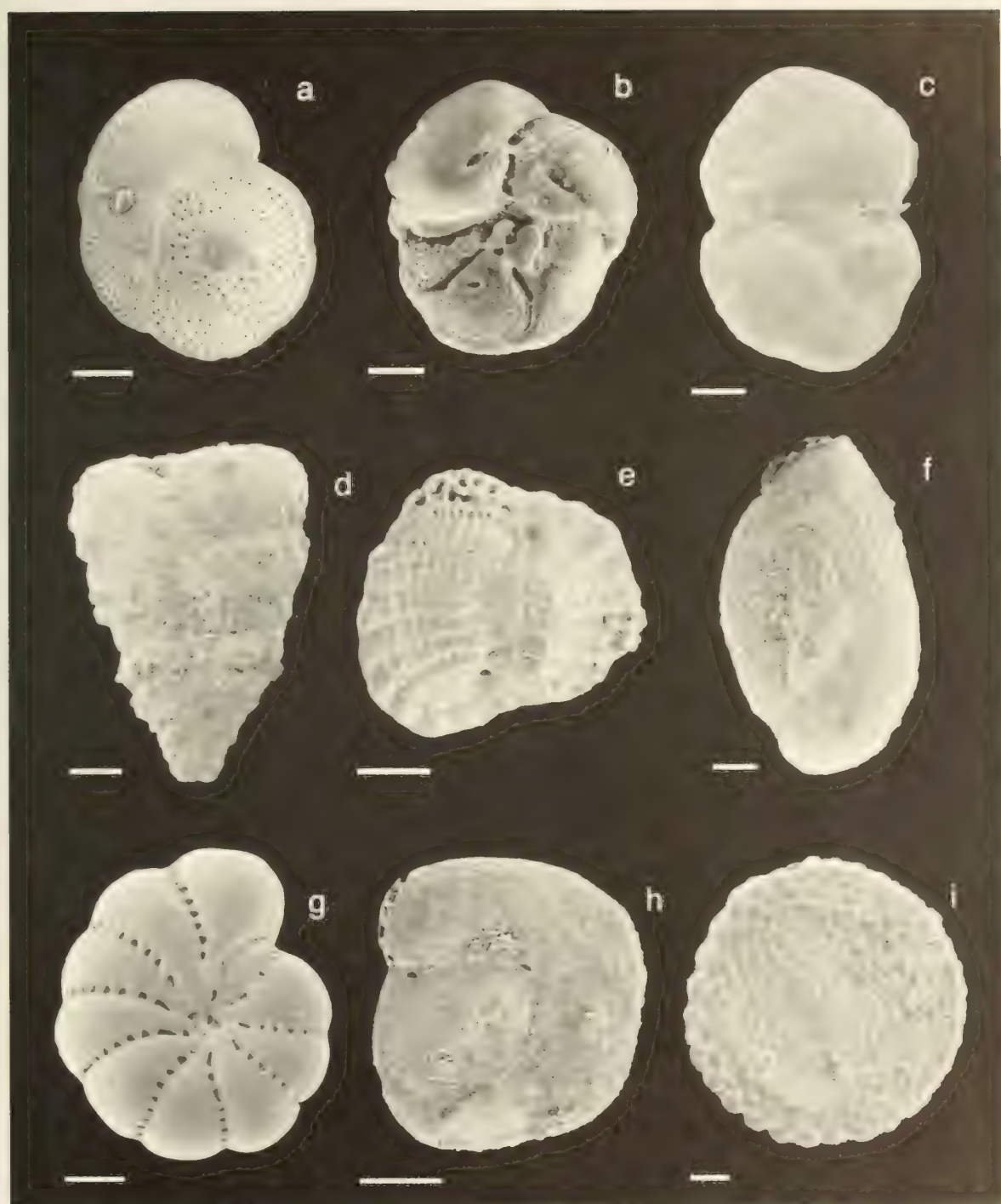
Plate 4

SPONGE SPECIES RICHNESS AND ABUNDANCE AS INDICATORS OF MANGROVE EPIBENTHIC COMMUNITY HEALTH

BY

MARIA C. DIAZ, KATHLEEN P. SMITH, AND KLAUS RÜTZLER
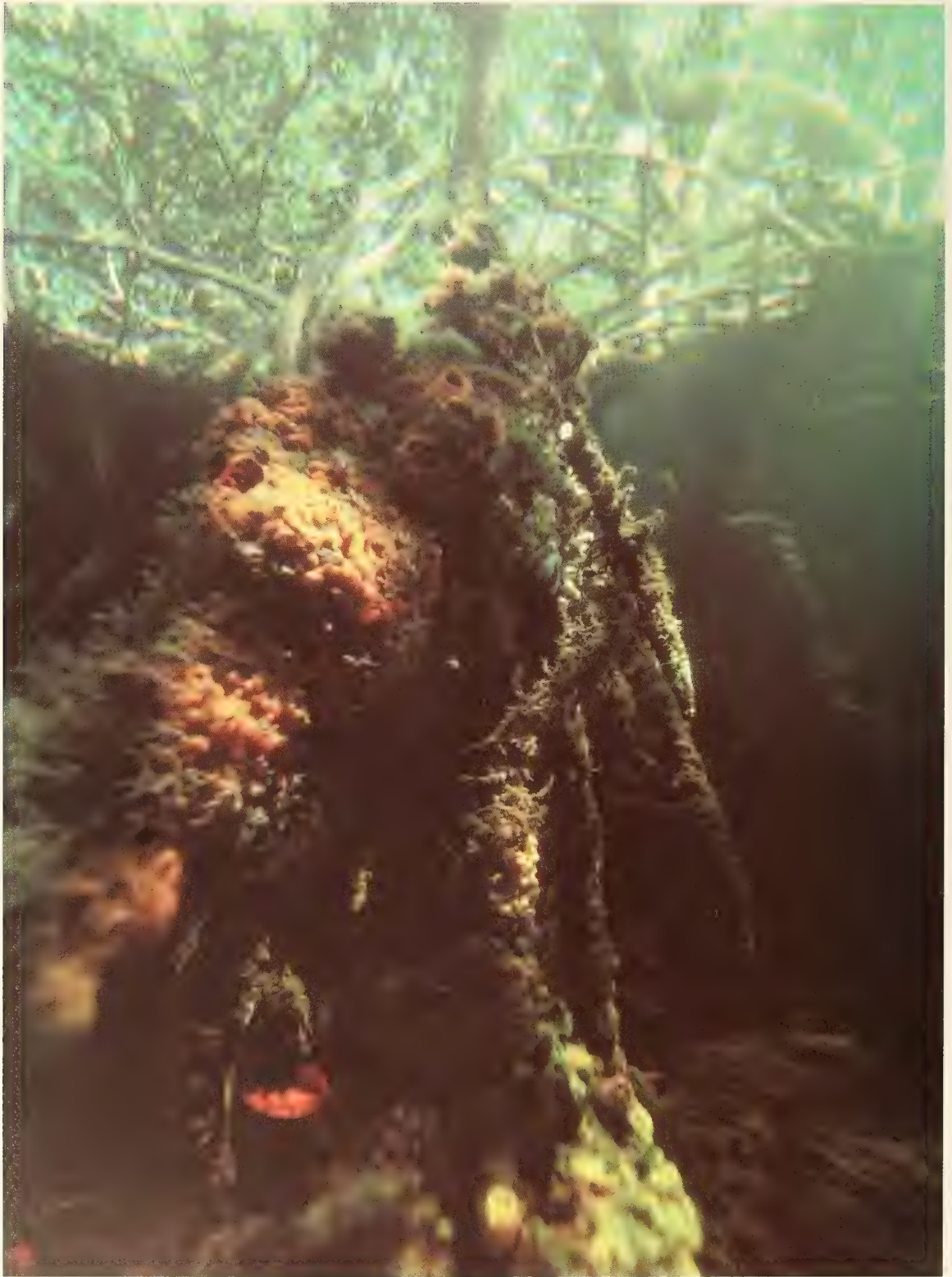
The Caribbean fire sponge (*Tedania ignis*) is a prominent member of the red-mangrove root community at Twin Cays and throughout the region. It contains a toxin that may cause severe skin irritation in humans but does not affect a rich community of invertebrates, such as entoprocts, polychaetes, crustaceans, and ophiuroid brittle starts which populates its surface and interior spaces. (Photo, Chip Clark.)

SPONGE SPECIES RICHNESS AND ABUNDANCE AS INDICATORS OF MANGROVE EPIBENTHIC COMMUNITY HEALTH

BY

MARIA C. DIAZ[1, 2], KATHLEEN P. SMITH[3], AND KLAUS RÜTZLER[1]

ABSTRACT

In the Caribbean Sea, sponges are diverse and common colonizers of subtidal mangrove substrates such as aerial roots and peat banks. On the other hand, few species are widely distributed, whereas the majority is rare. Biodiversity studies should therefore cover appropriately sized survey areas to allow the encounter with species that have low population densities. In the characterization of sponge population structure at specific sites, it is preferable to use a large number of short transects rather than a few long ones. Trials conducted at mangrove islands on the southern Belize barrier reef platform show that surveying multiple transects of 15-20 m length along the fringe of tidal channels (covering 50-70 stilt roots) reveal more than 90% of the epibiont species present at each site. We found that the majority of the widely distributed species are among the most frequent colonizers and their abundance, with the exception of a few, is maintained over at least a six-year period. Sponge species richness can serve as a bioindicator of subtidal community health as long as there are baseline data to determine its variation over time. However, this method is not suitable for comparing geographically distant mangrove systems. Common and widely distributed mangrove species, such as *Haliclona manglaris*, *H. curacaoensis*, *H. implexiformis*, *Mycale magniraphidiphera*, *Clathria venosa*, and *Geodia papyracea*, and other generalist species, notably *Tedania ignis*, *Hyrtios proteus*, *Spongia tubulifera*, *Chondrilla nucula*, *Mycale microsigmatosa*, and *Scopalina ruetzleri*, may best reflect changes in the environmental conditions at particular sites. The families Chalinidae, order Haplosclerida (six *Haliclona* spp. and two *Chalinula* spp.), and Mycalidae, order Poecilosclerida (four *Mycale* spp.), include the most diversified taxa among mangrove sponge populations. Up to 20 percent of mangrove roots at Twin Cays harbored two or three *Haliclona* species each, whereas *Mycale* species were common but rarely two co-occurred on the same root. These families, in particular, are being investigated for their suitability as bio-indicators of mangrove health by evaluating changes in their population dynamics and responses to natural and anthropogenic ecological stress conditions.

---

[1] Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560-0163.

[2] Museo Marino de Margarita, Boulevard El Paseo, Boca del Rio, Peninsula de Macanao, Nueva Esparta, Venezuela.

[3] 2517 CR 600E, Dewey, IL 62840.

INTRODUCTION

Mangroves are among the most imperiled marine ecosystems since they are subjected to extreme anthropogenic environmental pressures, such as organic run-off from land, disturbances from suspended sediment, and damages from clear-cutting. The rich assemblage of species found associated with subtidal mangrove habitats (aerial roots, peat walls) offer the potential to serve as indicators of the biological and ecological status of this ecosystem. Among the most conspicuous and abundant epibionts of these habitats in the Caribbean we found algae, sponges, ascidians, anemones, and hydroids (Littler et al., 1985; Rützler and Feller, 1987, 1996; Calder, 1991; Ellison and Farnsworth, 1992; Rützler et al., 2000; Goodbody, 2000; Macintyre et al. 2000). In this work we summarize our understanding of the diversity and relative abundance of sponge epibionts on aerial roots of mangrove cays in southern coastal Belize, and we compare it with data generated from other Caribbean mangrove systems.

Until recently, knowledge about the biodiversity of Caribbean mangrove sponges was restricted to a few locations and was dispersed in regional taxonomic monographs, for instance, on the Dry Tortugas, Florida (de Laubenfels, 1936); Bermuda (de Laubenfels, 1950); Port Royal, Jamaica (Hechtel, 1965); Bahamas (Wiedenmayer, 1977); Lesser Antilles (van Soest, 1980, 1984); north-east Colombia (Zea, 1987); and Los Roques, Venezuela (Diaz et al., 1993). The largest research initiative focused on the diversity of mangrove sponges so far has taken place in Belize and was carried out as part of the Smithsonian Institution's Caribbean Coral Reef Ecosystems Program based at the Carrie Bow Marine Field Station on the barrier reef of Belize (Rützler and Feller, 1987; 1996; Rützler, 1995; De Weerdt, et al., 1996; Hadju and Rützler, 1998; Rützler et al., 2000). We have learned that poriferan epibionts in Caribbean mangroves represent an untapped source of biological diversity, with distinct supra-generic composition, and with new species or sub-species to be discovered at every site or geographic locality examined in detail (Rützler et al., 2000).

Ecologically speaking, sponges in Caribbean mangrove systems are known to be dominant in abundance and species diversity, to offer their mangrove-root substratum direct protection from damaging isopod borings, and to stimulate root development by increasing nutrient availability through microbial symbionts (Rützler, 1969; Sutherland, 1980; Toffart, 1983; Alvarez, 1989; Bingham, 1992; Ellison et al., 1996; Farnsworth and Ellison, 1996; Diaz and Ward, 1997; Diaz et al., in press). Sponge distribution shows a vertical zonation that is determined by the frequency of air exposure (Rützler, 1995). Farnsworth and Ellison (1996) report that massive sponges dominate the fouling community, both in terms of numbers of roots occupied (35%) and percent of space covered (30%) on red mangrove prop-roots which on average have 90% of their subtidal portion covered by epibionts. Competitive abilities, such as growth rates and chemical defenses against predation play an important role in sponge abundances (Wulff, 2000, 2002). Successful colonization of roots appears to be controlled primarily by the supply of larvae (Farnsworth and Ellison, 1996) that are known, among tropical sponges, to have poor dispersal and recruitment ability (Zea, 1993, 1996; Rützler et al., 2000).

We have recently initiated a multi-site monitoring effort to evaluate the status of sponge populations at two Caribbean sites: Bocas del Toro in Panama, and Twin Cays and Manatee Cay in Belize. By studying the structure and dynamics of sponge populations at these sites we expect to extract patterns of distribution and abundance that may serve as an indicator measure of epibiont community health. In the present work, we will analyze data published and new data on the diversity and abundance of sponges on mangroves in southern Belize, and compare them with observations obtained elsewhere in the Caribbean. We will discuss the potential use of sponges in the evaluation of mangrove systems health.

LOCALITIES AND METHODS

Five sites, four at Twin Cays, and one in the Pelican Cays were selected as study locations. Table 1 lists their geographic coordinates, and habitat conditions. At Twin Cays, two sites (Sponge Haven South and Sponge Haven North) were established along the main channel on the leeward side of the West Island, and two sites (Hidden Creek and The Lair) were determined along deep channels that branch off the main channel (see map in Rützler et al., 2004). These latter two channels are less exposed to waves from wind and passing boats, the most common water disturbances in the islands. Hidden Creek and The Lair both are contiguous to internal ponds and lakes and therefore strongly influenced by twice-daily tidal changes that empty and fill these lagoons. The site in the Pelican Cays is located in lagoon C of Manatee Cay, and is characterized by low water turbidity, low wave exposure, and proximity to coral reefs (Macintyre et al., 2000).

Transects (30 m long) were placed along the red-mangrove fringe at each site. On each root within a transect, we counted the presence of all sponge species and nine other

Table 1. Location of sites in mangrove islands on the southern Belize barrier reef surveyed during August 2003, and some of their physical characteristics.

Site	Coordinates	Depth (m)	Wave exposure	Turbi-dity	No. roots/m	Mangrove habitat
Lair channel[1]	16° 49' 46.3" N 88° 06' 06.1" W	1.5–1.8	Very low	Low	3.5	Internal channel
Sponge Haven South[1]	16° 49' 40.5" N 88° 06' 16.5" W	1–1.8	Low	Mid	3.3	Main channel
Sponge Haven North[1]	16° 49' 43.3" N 88° 06' 17.1" W	1–1.2	Low	Mid	4	Main channel
Hidden Creek[1]	16° 49' 33.7" N 88° 06' 11.3" W	1.8–2	Very low	Mid	2	Internal channel
Manatee Lagoon[2]	16° 40' 03.3" N 88° 11' 32.4" W	1–1.5	Very low	Low	4	Pond

[1]Twin Cays; [2]Pelican Cays

of the most conspicuous functional groups (grouped taxa): filamentous cyanobacteria, fleshy green algae, calcareous green algae, fleshy red algae, calcareous red algae, macroalgal turf (mixture of various algal species), anthozoans, bivalves, and ascidians. The sponge species were photographed, and if their identity was unclear, a small fragment was collected, fixed in a solution of formalin in seawater (10%), and transferred into ethanol 70% within a week. At the field station, a compound microscope was used to examine skeleton structure in dried hand sections cleared in Permount medium (Fisher Scientific). Spicule types were determined after dissolving samples in concentrated household bleach (5% sodium hypochlorite).

Abundance estimates were expressed as a percentage of number of roots that were occupied for each species or group. To allow comparison of abundance of sponge species between this study and Rützler et al. (2000), we distinguished three categories of abundance: 1= rare, for species found in 1-2% of roots; 2= common, for species found in 3-19% of roots; and 3= abundant, for species found in >20% of roots.

RESULTS

Sponge Species Richness at Twin Cays

A total of 35 species were found among the four sites studied at Twin Cays, with 19-23 species detected per site within each transect (Tables 2, 3). Farnsworth and Ellison (1996) reported 20 sponge species at five Twin Cays sites, whereas Rützler et al. (2000) recorded 54 species with approximately 26-42 species per locality (Table 4). In the present study, four species were found only at the Manatee Cay lagoon site: *Chondrilla nucula*, *Placospongia intermedia*, *Mycale "americana"*, and. *Amphimedon erina*.

The cumulative number of sponge species per roots surveyed at each study site is expressed in a graph to demonstrate the species area relationship in these communities (Fig. 1). The typical asymptotic curve is obtained for all sites. Overall, the number of species increases until 60-70 roots have been surveyed, stabilizing and changing but slightly after that. This number of roots corresponds to a 15–20 m distance. The number of sponge species per root ranged from 1–8, with a mean species richness value of 1.8–3 (Table 4). Hidden Creek presents the highest species richness per root, despite showing the lowest total number of species.

Table 2. Sponge diversity on mangrove aerial roots at five sites surveyed during August 2003.

Quantitative parameters	Lair channel	Sponge Haven South	Sponge Haven North	Hidden Creek	Manatee Lagoon
Species per root (mean, SD)	2 ± 2	1.8 ± 1.5	2.4 ± 1.6	3 ± 2	1.9 ± 1.4
Species number (range)	1–8	1–6	1–6	1–8	1–5
Species number (total)	22	23	21	19	28

Table 3. Sponge species distribution and frequency of occurrence (%) on roots observed along transect lines at five sites in Central Belize during a survey in August 2003.

Species	The Lair	Sponge Haven South	Sponge Haven North	Hidden Creek	Manatee Lagoon
Oscarella sp.	0	1	0	0	1
Cinachyrella apion	0	0	0	5	0
Geodia papyracea	0	0	1	0	0
Chondrilla nucula	0	0	0	0	19
Placospongia intermedia	0	0	0	0	1
Spirastrella mollis	0	0	0	0	2
Terpios manglaris	0	0	1	0	0
Tethya aff. *actina*	1	0	0	0	0
Lissodendoryx aff. *isodicyialis*	10	10	13	24	0
Biemna caribaea	21	2	13	34	1
Clathria schoenus	0	1	20	0	2
C. microchelus	4	15	7	2	3
C. venosus	2	0	5	5	6
Mycale microsigmatosa	2	6	20	0	2
M. magniraphidifera	5	2	7	2	1
M. carmigropila	0	1	0	0	2
M. "americana"	0	0	0	0	3
Iotrochota birotulata	0	0	0	0	2
Tedania ignis	11	27	33	34	26
Scopalina ruetzleri	0	1	1	3	4
Amorphinopsis sp.	1	0	0	14	0
Halichondria magniconulosa	0	20	17	25	21
Haliclona implexiformis	21	2	20	36	0
H. curacaoensis	27	11	34	44	0
H. manglaris	22	47	39	9	0
H. tubifera	9	3	1	5	0
H. vermeulensis	0	0	0	0	1
Haliclona sp.	0	0	0	0	1
Chalinula pseudomolibta	2	0	0	3	0
Amphimedon erina	0	0	0	0	9
Hyrtios proteus	11	13	6	20	6
Ircinia felix	1	7	0	0	0
Spongia tubulifera	5	6	12	10	6
S. pertusa	3	0	0	0	3
Dysidea etheria	14	0	2	0	0
Chelonaplysilla erecta	0	0	0	0	2
Pleraplysilla sp.	0	0	0	0	3
Halisarca sp.	3	0	0	7	0
Clathrina aff. *coriacea*	16	1	13	10	16
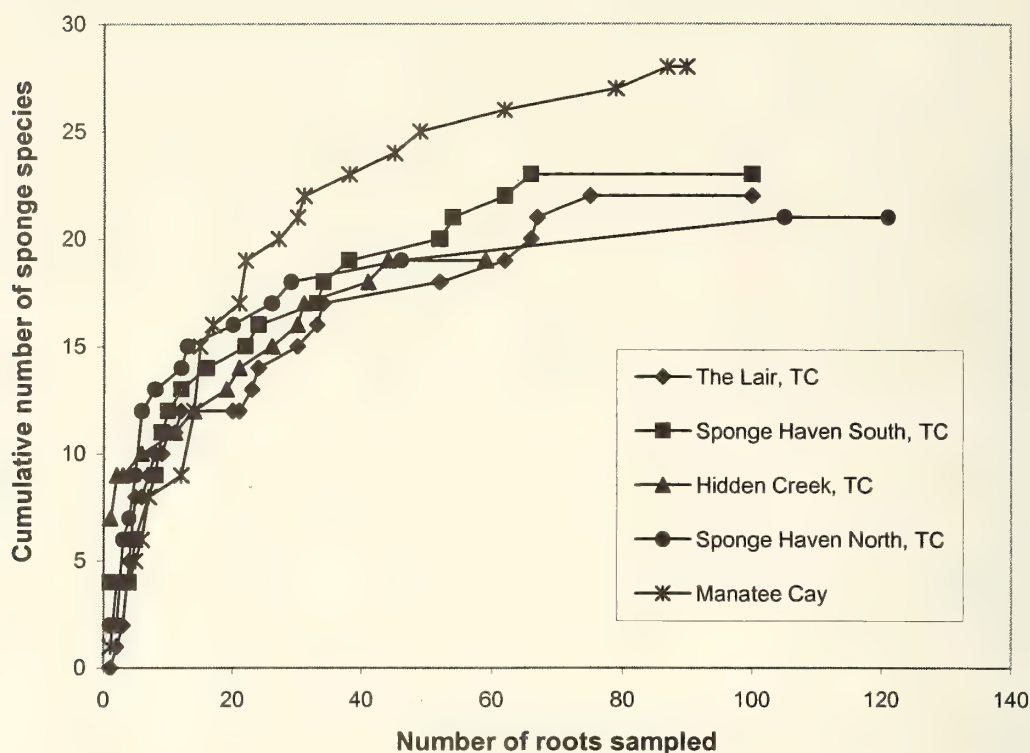
Figure 1. Cumulative number of sponge species on roots surveyed along fringe transects át four Twin Cays (TC) sites and one Pelican Cays site (Manatee Cay); survey of August 2003.

Trends in Sponge Species Richness in Caribbean Mangroves

The data on sponge diversity at geographically distant Caribbean sites (Table 4) shows that sponges can represent a moderate (8–15%) to high (>50%) portion of mangrove-root epifaunal diversity. An extremely diverse mangrove epibiont community was found at the Pelican Cays in southern Belize, with a sponge richness that equaled the taxonomic diversity of macroalgae, 147 species versus 148 species for each group, respectively (Littler et al., 2000; Rützler et al., 2000). The highest diversity occurs in mangrove channels, or lagoons, with depths >1m, and water of low turbidity (Alcolado, in prep; Rützler et al., 2000). The best studied area in the Caribbean, in terms of the biodiversity of marine sponges associated with mangroves, includes several Cays spread over a 50 km section behind the barrier reef of southern Belize (Rützler et al., 2000). The authors found that mangrove sponge species richness may be highly variable within a relatively small geographic scale, such as the difference between low-diversity Twin Cays and Blue Ground Range (54 and 57 species) and rich Pelicans Cays (147 species), located 12–24 km away. Various studies have shown that the Pelican Cays present particular biological and physical conditions that promote epibiont diversity (Table 5). Farnsworth and Ellison (1996) found that this high variability of species composition and richness may also occur between contiguous roots, channels, and lagoons at any one mangrove island.

Table 4. Numbers of sponge and mangrove epifaunal species reported from various Caribbean localities. (NA= not available.)

Reference	Locality	Number of sponge species	Number of Epifauna Species
Rützler, 1969	Bahamas	13	NA
Sutherland, 1980	Morrocoy, Venezuela	16	32
Toffart, 1981[1]	Guadalupe	10	70
Toffart, 1981[1]	Puerto Rico	8	75
Toffart, 1981[1]	Trinidad	4	50
Toffart, 1981[1]	Florida	4	32
Diaz et al., 1985	Morrocoy, Venezuela	25	NA
Orihuela et al., 1991	La Restinga, Venezuela	18	35
Bingham and Young, 1992	Indian River, Florida	3	25
Farnsworth and Ellison, 1996	Wee Wee, Spruce, Peter Douglas, and Twin Cays	24	59
Rützler et al., 2000	Twin Cays, Belize	54	NA
Rützler et al., 2000	Pelican Cays, Belize	147	217[2]
Alcolado (unpubl.)	North & S.W. Cuba	48	NA
Diaz et al. 2003	La Restinga, Venezuela	42	NA
Diaz (in prep)	Bocas del Toro, Panamá	60–70	NA

[1]See Toffart (1981) for sources of data from each site; [2]Number of sponge and ascidian species

Table 5. Comparison of sponge species richness, biological traits, and turbidity level of the subtidal environment at three geographically contiguous sets of mangrove islands behind the barrier reef in southern Belize.

Biological and physical traits	Twin Cays	Blue Ground	Pelican Cays
Sponge species richness (Rützler et al., 2000)	57	54	147
Open-reef taxa (Rützler et al., 2000)	None	Low	High
Spongivorous predators (Wulff, 2000)	Low	NA	High
Roots/ m	2.2	3.6	4-6
Turbidity	Mid	High	Low

Sponge Species Distribution

Figure 2 depicts the diversity of sponge epibionts on mangrove substrates at seven sites in five southern Belize cays from a survey in 1997 (Rützler et al., 2000). Forty four percent of the species (80 spp.) where present only at one site, Cat Cay, whereas less than 4% (7 spp.) were found to be common to all seven localities studied; these were,

8

Chondrilla nucula, Clathria venosa, Tedania ignis, Scopalina ruetzleri, Haliclona manglaris, Hyrtios proteus, and *Spongia tubifera.* We should note that *C. nucula, T. ignis, S. ruetzleri,* and *H. proteus* are species that can also be found inhabiting seagrass beds and shallow reefs habitats nearby. *C. venosa, H. manglaris,* and *S. tubifera* seem to be the only mangrove-specific species that are found across the mangrove cays of southern Belize.
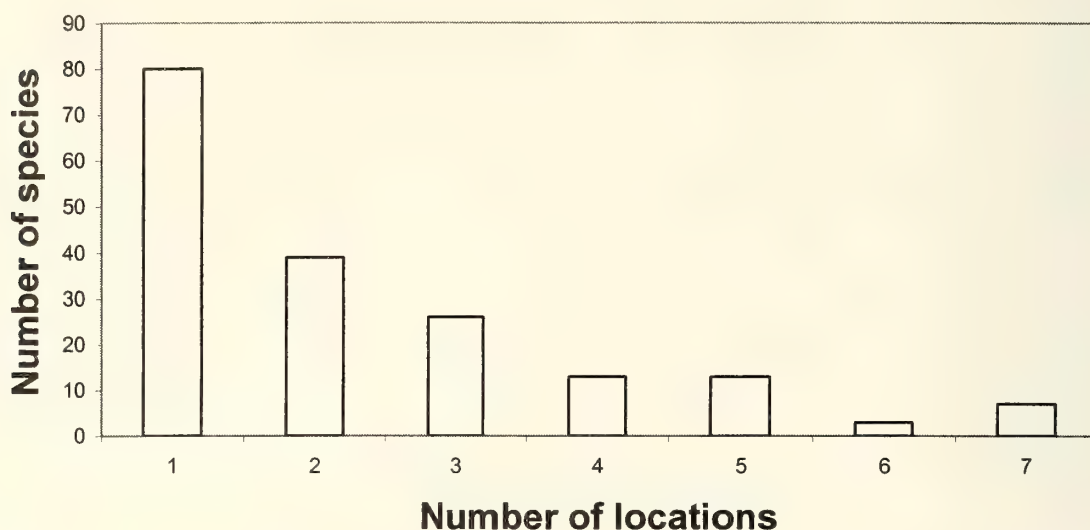

Figure 2. Frequency of sponge species shared between seven locations at five mangrove cays in southern Belize during a survey in 1997 (data from Rützler et al., 2000).

The presence and frequency of occurrence of sponge species on stilt roots determined during the present study are shown in Table 3. Seven species were present at all five study sites: *Biemna caribaea, Clathria microchelus, Mycale magniraphidifera, Tedania ignis, Hyrtios proteus, Spongia tubulifera,* and *Clathrina* aff. *coriacea.* Comparing with the 1997 survey we find that *T. ignis, H. proteus* and *S. tubulifera* maintained their wide distribution, but *Haliclona manglaris, Clathria venosa,* and *Scopalina ruetzleri* were found at only four sites. *C. nucula* seemed to have disappeared from the Twin Cays sites whereas in 1997 it was found to be either common (Sponge Haven South) or rare (Hidden Creek). *M. magniraphidiphera* and *Clathria microchelus* which were, respectively, rare or absent in 1997, are found at all sites in the present survey. We should also note the absence of four typical mangrove species of *Haliclona,* that is, *H. curacaoensis, H. implexiformis, H. manglaris, and H. tubulifera* from the recently surveyed area at Manatee Cay Lagoon C.

Sponge Species Relative Abundance

The most abundant species at the Twin Cays sites are: *Haliclona manglaris* (9-47%), *H. curacoensis* (11-44%), *H. implexiformis* (2-36%), *Biemna caribea* (2-34%), *Tedania ignis* (11-34%), *Lissodendoryx* aff. *issodyctialis* (10-24%), *H. magniconulosa* (17-25%), *Hyrtios proteus* (6-20%), and *Clathrina* aff. *coriacea* (1-16%). Comparing these results with the 1997 survey, we find that *M. magniraphidifera* changed its

abundance from rare to common, and that *G. papyracea* decreased its high abundance (in Hidden Creek, and Sponge Haven South) to an apparent absence. In Manatee Cay we found that the most abundant species were *T. ignis* (26%), *Halichondria magniconulosa* (21%), *C. nucula* (19%), *C.* aff. *coriacea* (16%), *A. erina* (9%), *Hyrtios proteus* (6%), and *S. tubulifera* (6%). *H. magniconulosa* was absent in Manatee Cay during the 1997 survey but became an abundant species in 2003.

Grouped Taxa Relative Abundance

In the present work, the frequency of occurrence of grouped taxa is used as an indicator of their abundance (Table 6). Sponges were the most common root colonizer (67-88%) at all Twin Cays sites, ascidians were second (27-47%). At Manatee Cay,

Table 6. Frequency (%) of occurrence of the 10 most common epibiont taxa on mangrove aerial roots at five sites on the southern barrier reef of Belize during a survey in August 2003.

Root numbers and taxa	The Lair	Sponge Haven South	Sponge Haven North	Hidden Creek	Manatee Lagoon
No. of roots surveyed	105	99	121	59	89
Cyanophytes (filamentous)	31	36	37	12	16
Chlorophytes (fleshy)	23	8	17	15	25
Chlorophytes (calcareous)	1	5	1	2	0
Rodophytes (fleshy)	18	9	16	25	20
Rodophytes (calcareous)	0	15	5	0	2
Macroalgae turf	8	10	0	14	0
Sponges	67	79	88	75	87
Cnidarians (*Aiptasia* sp.)	10	20	24	8	0
Bivalves (mangrove oyster)	12	7	5	5	3.4
Ascidians	34	47	27	41	90

sponges were second (occupying 87% of roots) after ascidians (90%). At Twin Cays sites, ascidians were followed by filamentous cyanobacteria (12-36%), fleshy chlorophytes (8-25%), and the anthozoan *Aiptasia* sp. (8-24%).

DISCUSSION

Sponge Diversity

Data presented here and elsewhere show that sponges constitute one of the most diverse single groups of animals among the epibiont community of mangrove aerial roots. Species richness data from different Caribbean localities reveal sponge diversities from a few up to 147 species (Table 4), depending apparently on a combination of physical parameters, such as water turbidity, salinity, wave exposure, root density, and tidal ranges, and biological factors, such as competition, larval supply, and predation (Calder, 1991; Littler et al., 1985; Taylor et al., 1986; Ellison and Farnsworth, 1992; Rützler, 1995; Rützler et al. 2000; Wulff, 2000). Calm, clear, open-ocean-influenced deeper lagoons or channels seem to favor sponge colonization and growth.

The variability of species richness reported from Caribbean sites may be the result of different sampling methods. For example, in the current study we recorded 28 sponge species when surveying a 30 m transect along the fringe at Manatee lagoon. Previously, in 1997, seven sponge experts collected 95 species while swimming along the entire 638 m perimeter of the same lagoon (Rützler et al., 2000). A similar example is a study of red mangrove root epifauna at La Restinga National Park (Venezuela) where Orihuela et al. (1988) reported 18 species at five study sites. Subsequently, one of us involving 12 students found 33 species, including two new records for Venezuela, while swimming along the fringe for one hour at each of three sites (Diaz et al., 2003). This discrepancy in results is mostly due to the patchiness of species distribution and to the fact that 60% of the species are rare, that is, they are found only once or twice in a survey. It is therefore necessary to determine optimal transect length and numbers to determine an accurate measure of species richness.

Three surveys carried out at Twin Cays over the past 10 years resulted in three different estimates of sponge species richness: 20 species from three sites (Farnsworth and Ellison, 1996), 54 species from four sites (Rützler et al., 2000), and 35 species from four sites (this study). Two major factors might have contributed to these differences. First, the area covered by the surveys, which for Rützler et al. (2000) included the entire fringe, for Farnsworth and Ellison (1996) and our own study was restricted to discontinuous 50 m and 30 m transects, respectively. Farnsworth and Ellison (1996) surveyed 50 roots within each transect whereas we examined 59–121 roots per site, thus registering a larger species number despite the shorter transects. Another possible factor contributing to these differences is the level of sponge expertise of the surveyor. It is probable that more species can be differentiated by a person who is familiar with the morphological variations in this group. Considering the different methods, efforts, and skill levels employed in the various studies of sponge diversity, we believe that the richness of sponge species in Caribbean mangroves is far underestimated. We conclude that an accurate estimate of sponge biodiversity in mangroves, as in other habitats, requires large survey areas or long transects (for instance, along the fringe of the forest) and inclusion of several topographically representative sites at each island or location, rather than short random transects.

The most diversified sponge taxa recorded in our study belong to the family Chalinidae (6 *Haliclona* spp., 2 *Chalinula* spp.) and the Mycalidae (4 *Mycale* spp). Three to 21 percent of the roots supported 2–3 chalinid species (Sponge Haven South 3%, The

Lair 17%, Hidden creek 11 %, Sponge Haven North 21 %). In contrast, although only second in importance, rarely is more than one mycalid species found on one root.

Sponge Distribution and Abundance

About half of the sponges from mangrove roots in Twin Cays are rare or low in abundance, that is, occurring only at one or two of the various study sites (20 of 40 spp. in 2003). The comparison between the two surveys carried out in southern Belize (Rützler et al.; 2000; and present study) allows to conclude that the majority of the species that are widely distributed across the region (*Clathria venosa, Scopalina ruetzleri, Tedania ignis, Haliclona manglaris, Hyrtios proteus, Spongia tubifera*) maintained a wide geographic distribution during this six year period. Few species increased their geographic distribution, such as *Biemna caribaea, Clathria microchelus, Mycale magnirhaphidiphera, Halichondria magniconulosa*, and *Clathrina* aff. *coriacea*, and others, such as *Geodia papyracea* and *Chondrilla* aff. *nucula*, decreased it. Farnsworth and Ellison (1996) found that the rank a major species had in frequency of occurrence (number of roots occupied by a species) was maintained during their two year study. However, the relative importance and dominance (% area coverage) of individual species and species groups varied substantially between two years of sampling. In general, it seems that widely distributed species tend to be frequent colonizers, and this status tends to be maintained through time. On the other hand, there are other less common species that might be restricted to one island or locality but occur there in very high abundance. This is the case for *Mycale microsigmatosa* and *Clathria schoenus* (20% each at Sponge Haven North) and *Dysidea etheria* (15% at The Lair). Table 7 lists the species that have been reported in various studies as the most frequent or abundant mangrove root colonizers. As can be seen, *Haliclona* spp, *T. ignis, Lissodendoryx isodyctialis, S. ruetzleri, Dysidea janiae*, and *H. magniconulosa* represented the most conspicuous species on the mangroves in at least two Caribbean localities. Among these species, some are mangrove specialist species (*L. isodyctialis, H. magniconulosa*, and *Haliclona* spp.), whereas others are generalists found also in seagrass beds and on some coral reefs (*T. ignis, S. ruetzleri*).

Sponges as Biological Indicators of Mangrove Health

Owing to the high natural variability of sponge diversity among mangrove islands, we do not recommend the use of species numbers as indicators of the health status of a particular mangrove system without a baseline study that allows interpretation of changes over time. On the other hand, certain sponge species could serve as biological indicators of mangrove health. Sponges have been recognized as good indicators of organic and oil pollution in subtidal reef habitats (Alcolado and Herrera, 1987; Muricy et al., 1989). Some of the requisites are that selected species are normally abundant in the study area, easy to sample in quantity, and show clear responses to the stress factors affecting the community (Linton and Warber, 2003). The first two requirements are easily satisfied by several species inhabiting Belizean mangroves. However, among these common species, one must distinguish two groups: Generalists with wide habitat preference that inhabit mangrove roots and peat walls, seagrass, and coral reefs, and mangrove specialists that inhabit predominantly mangrove roots. In Belizean mangrove

Table 7. Sponge species recognized as the most frequent or abundant in Caribbean mangrove systems. (MS= mangrove specialist; G= generalist found in mangroves, seagrass beds, and coral reefs; U= preference not known.)

Species	Rützler (1969)	Diaz et al. (1985)	Alcolado (unpublished data)	Farnsworth & Ellison, 1996	Present study	Habitat preference
Myriastra kalitetilla	X					MS
Chondrilla nucula	X					G
Lissodendoryx isodictyalis		X	X		X	MS
Biemna caribaea					X	MS
Liosina monticulosa			X			U
Desmacella janiae		X	X			U
Clathria (as *Raphidophlus*) *schoenus*		X				G
Mycale microsigmatosa[1]			X			G
Tedania ignis	X		X	X	X	G
Scopalina ruetzleri			X		X	G
Halichondria magniconulosa		X	X		X	MS
Haliclona implexiformis				X	X	MS
H. curacaoensis				X	X	MS
H. manglaris					X	MS
Haliclona sp.	X					G
Amphimedon viridis		X				G
Hyrtios proteus					X	G
Ircinia felix	X		X			G
Spongia spp.		X			X	G
Dysidea etheria			X			G
Clathrina aff. *coriacea*					X	G

[1]This species has been found by one of us (CMD) growing profusely on pilings and other artificial substrates near Bocas del Toro, Panamá.

islands, the first group includes, in order of importance, *Tedania ignis*, *Hyrtios proteus*, *Spongia tubulifera*, *Chondrilla nucula*, and *Scopalina ruetzleri*. The second group is made up primarily by *Haliclona manglaris*, *H. curacaoensis*, *H. implexiformis*, *Mycale magniraphidiphera*, *M. microsigmatosa*, *Clathria venosa*, and *Geodia papyracea*. Knowing the life histories of these species in terms of growth and recruitment rates, longevity, microhabitat preference, competitive abilities, and chemical toxicity allows us to interpret responses to possible stressors, such as changes in distribution and abundance. Muricy et al., (1989) demonstrated this concept among common inhabitants of rocky subtidal habitats in Brazil that have been subjected to organic and oil pollution,

that is, *T. ignis*, *C. nucula*, *S. ruetzleri* and *M. microsigmatosa*. An increase of generalist species (such as, *T. ignis*) on the mangrove roots at the expense of the disappearence of a mangrove specialist could be a sign of alarm that physical or biological conditions are dropping to levels that only ecologically resilient species might be able to confront and survive. Among the specialist species encountered in our study, members of the families Chalinidae and Mycalidae have great potential owing to their high diversification and abundance in mangrove ecosystems. In Belizean mangroves, the best candidates to serve as biological indicators of mangrove health are *H. manglaris*, *H. curacaoensis*, *H. implexiformis*, *M. magniraphidiphera*, and *M. microsigmatosa;* they have widespread distribution and high colonization frequency on mangrove roots.

Relative Abundance of Functional Groups

Our data on the abundance of major functional groups on roots along the fringe of Twin Cays channels reflect Farnsworth and Ellison's (1996) descriptions of leeward sites of islands where sponges and ascidians are the most frequent colonizers whereas algae and hydroids dominate windward locations. Toffart (1983) examining mangrove sites in Guadalupe found two major communities: one in the tidal canals where roots are covered mainly by annelid tube worms and amphipod crustaceans, the other along the lagoonal shoreline characterized by ascidians and sponges. Alcolado (personal comm.) reports that sponge populations are absent in areas close to river inputs and in very shallow water with high suspended-sediment content, but abound in locations that are influenced by the sea, with dense foliage shading the habitats, and depths exceeding 1 m. Similar epibiont community structure on mangrove roots has also been noted elsewhere in the Caribbean (Rützler, 1969; Sutherland, 1980; Toffart, 1983; Alvarez, 1989; Bingham, 1992). Roots on the wind-ward side of islands, in shallow water (<1 m), or touching the ground tend to have the lowest species numbers. Toffart (1983) differentiated three types of roots in the mangrove fringe according to the abundance of sponges and tunicates: the outermost roots (type I) where sponges and tunicates covered >30% of the root surface, the middle roots (type II) where sponges and tunicates cover < 30% of the root surface, and the inner root (type III) where sponges and tunicates are absent. We conclude that the relative abundance of functional groups is a good biological parameter to monitor because each group responds differently to physico-chemical parameters (temperature, salinity, sedimentation, light, nutrients). For instance, we noted an increase of filamentous-cyanobacterial coatings and mats at some sites at Twin Cays that may be worth monitoring because this group is always present but not known for having large abundances in healthy Caribbean mangroves.

14

REFERENCES

Alcolado, P.M., and A. Herrera
  1987. Efectos de la contaminación sobre las comunidades de esponjas en el litoral de la Habana, Cuba. *Academia de Ciencias de Cuba, Reportes de Investigación del Instituto de Oceanología* 68:1-17.

Alvarez, A.I.
  1989. *Establecimiento, desarrollo y mantenimiento de una comunidad epibentónica tropical.* Dissertation. Universidad Central de Venezuela, Caracas, Venezuela.

Bingham, B.L.
  1992. Life histories in an epifaunal community: coupling of adult and larval processes. *Ecology* 73:2244-2259.

Bingham, B.L., and C.M. Young
  1995. Stochastic events and dynamics of mangrove root epifaunal community. *Marine Ecology* 16:145-163.

Calder, D.R.
  1991. Abundance and Distribution of Hydroids in a mangrove ecosystem at Twin Cays, Belize, Central America. *Hydrobiologia* 216/217:221-228.

Díaz, H., M. Bevilacqua, and D. Bone.
  1985. Esponjas en manglares del Parque Nacional Morrocoy. *Fondo Editorial, Acta Científica Venezolana*, Caracas, 62p.

Diaz, M.C., Akob, D., and S.C. Cary
  2004. Detection and phylogenetic affinities of nitrifying microbial symbionts among epibiont sponges of nutrient limited *Rhizophora mangle* stands. *Proceedings of the 6th International Conference on Sponge Biology*. Rapallo, Italy, October 2002 (in press).

Diaz, M.C., S. Pauls, E. Villamizar, A. Alvizu, M.E. Amaro, M. Cellamare, S. Grune, I. Hernandez, S. Narciso, A. Perez, J. Perez, I. Ramirez, R. Ramos, M.P. Romero, and P. Young
  2003. *Porifera Biodiversity in Nueva Esparta, Venezuela: Common Species from "La Restinga" and Cubagua Island.* Abstract. The Twin Cays Mangrove Ecosystem, Belize: Biodiversity, Geological History, and Two Decades of Change. Smithsonian Institution Conference, December, 2003. Fort Pierce, Florida.

Diaz, M.C., R.W.M van Soest, and S.A. Pomponi
  1993. A systematic revision of the Central Atlantic Halichondrida (Demospongiae, Porifera). Part III: Description of valid species. In: Recent Advances in Ecology and Systematics of Sponges. Uriz, M.J. and K. Rützler (eds.). *Scientia Marina* 57 (4):283-306.

Diaz, M.C., and B.B.Ward
  1997. Sponge-mediated nitrification in tropical benthic communities. *Marine Ecology Progress Series* 156:97-107.

Ellison, A.M., and E.J. Farnsworth
  1992. The ecology of Belizean mangrove-root fouling communities: patterns of epibiont distribution and abundance, and effects on root growth. *Hydrobiologia* 247:87-98.

Ellison A.M., E.J. Farnsworth, and R.R. Twilley
  1996. Facultative mutualism between red mangroves and root-fouling sponges in
    Belizean mangal. *Ecology* 77 (8):2431-2444.
Farnsworth, E.J., and A.M. Ellison
  1996. Scale dependent spatial and temporal variability in biogeography of mangrove-
    root epibiont communities. *Ecological Monographs* 66:45-66.
Goodbody, I.
  1995. Ascidian communities in Southern Belize-a problem in diversity and
    conservation: Aquatic conservation. *Marine and Freshwater ecosystems* 5:355-
    358.
  2000. Diversity and distribution of ascidians (Tunicata) in the Pelican Cays, Belize.
    Atoll Research Bulletin 480:301-326.
Hadju, E., and K. Rützler
  1998. Sponges, genus Mycale (Poecilosclerida: Demospongiae: Porifera), from a
    Caribbean mangrove and comments on subgeneric classification. *Proceedings of
    the Biological Society of Washington* 11(4):737-773.
Hechtel, G.J.
  1965. A systematic study of the Demospongiae of Port Royal, Jamaica. *Bulletin of the
    Peabody Museum of Natural History* 20:1-103.
Linton, L M, and F. Warner
  2003. Biological indicators in the Caribbean coastal zone and their role in integrated
    coastal management. *Ocean & Coastal Management* 46: 261–276.
Laubenfels, M.W. de
  1936. A discussion of the sponge fauna of the Dry Tortugas in particular and the West
    Indies in general, with material for a revision of the families and orders of the
    Porifera. Papers of the Tortugas Laboratory 30:1-22.
  1950. The Porifera of the Bermuda Archipelago. *Transactions of the Zoological Society
    of London* 27:1-154, plates 1-2.
Littler M.M., P.R. Taylor, D.S. Litter, R.H. Sims, and J.N. Norris
  1985. The distribution, abundance, and primary productivity of submerged
    macrophytes in a Belize barrier-reef mangroves system. *Atoll Research Bulletin*
    289:1-20.
Littler D.S, M.M. Littler, and B.L. Brooks.
  2000. Checklist of Marine Algae and seagrasses from the ponds of the Pelican Cays,
    Belize. *Atoll Research* Bulletin 474:151-206.
Macintyre I.G., I. Goodbody, K. Rützler, D.S.Litter, and M.M.Litter
  2000. A general biological and geological survey of the rims of ponds in the major
    mangrove islands of the Pelican Cays, Belize. *Atoll Research Bulletin* 467:13-34.
Muricy, G.
  1989. Sponges as pollution-biomonitors at Arraial do Cabo, Southeastern Brazil.
    Revista Brazileira de Biologia 49(2): 347-354.
Orihuela B., H. Diaz, and J.E. Conde,
  1990. Mass Mortality in a mangrove roots fouling community in a hypersaline tropical
    lagoon. *Biotropica* 23(4b): 592-601.

16

Pearson, T.H., and R. Rosenberg
  1987. Feast and famine: structuring factors in marine benthic communities. In: *Organization of Communities: Past and Present,* edited by J. R. H. Gee, and P. S. Guillen, 373-398. Oxford: Blackwell Scientific Publications.
Rützler, K.
  1969. The mangrove community: Aspects of its structure, faunistics, and ecology. *Memorias, Simposio Internacional Lagunas Costeras, 1967* 515-536. Mexico, D.F.: UNAM.
  1995. Low-Tide exposure of sponges in a Caribbean Mangrove community. *Marine Ecology* 16:165-179.
Rützler, K., M.C. Diaz, R.W.M. van Soest, S. Zea, K.P. Smith, B. Alvarez, and J.L. Wulff
  2000. Diversity of sponge fauna in mangroves ponds, Pelican Cays, Belize. *Atoll Research Bulletin* 476:231-248.
Rützler, K., I. Goodbody, M.C. Diaz, I. C. Feller, and I.G. Macintyre
  2004. The aquatic environment of Twin Cays, Belize. *Atoll Research Bulletin* 512:1-49.
Rützler, K., and I.C. Feller
  1987. Mangrove swamp communities. *Oceanus* (Woods Hole) 30(4): 16-24.
  1996. Caribbean Mangrove Swamps. *Scientific American* 274 (3): 94-99.
Soest, R.W.M. van
  1980. Marine Sponges from Curaçao and other Caribbean localities. Part II: Haplosclerida. *Studies on the Fauna of Curaçao and other Caribbean Islands* 191:3-132.
  1984. Marine Sponges from Curaçao and other Caribbean localities. Part III: Poecilosclerida. *Studies on the Fauna of Curacao and other Caribbean Islands* 199:1-160.
Sutherland, J.P.
  1980. Dynamics of the epibenthic community on roots of the mangrove *Rizophora mangle* at Bahia de Buche, Venezuela. *Marine Biology* 58:75-84.
Taylor P.R., M.M. Littler, D.S. Littler
  1986. Escapes from hervibory in relation to the structure of mangrove island macroalgal communities. *Oecologia* 69:481-490.
Toffart, J.L.
  1983. Peuplement des racines de palétuviers en Guadeloupe (Antilles Francaises): I-Analse floristique et faunistique, méthodologie et premier résultat. *Bulletin Ecologique* 14 (4):227-239.
Weerdt, W.H., K. Rützler, and K.P. Smith
  1991. The Chalinidae (Porifera) of Twin Cays, Belize, and adjacent waters. *Proceedings of the Biological Society of Washington* 104:189-205.
Wiedenmayer, F.
  1977. Shallow-*water sponges of the Western Bahamas.* Birkhäuser Verlag, Basel, 287 pp., 43 pls.
Wulff, J.L.
  2000. Sponge predators may determine differences in sponge fauna between two sets of Mangrove Cays, Belize Barrier Reef. *Atoll Reseach Bulletin* 477:250-263.

2002. *How much can competition and predation influence sponge community composition?* Abstract. Bolletino dei Musei e degli Istituti biologici dell'Universitá di Genova, 66-67:222.

Zea, S.

1987. *Esponjas del Caribe Colombiano.* Catálogo Científico, Colombia, 286 pp.

1993. Recruitment of Demosponges (Porifera, Demospongiae) in rocky and coral reef habitats of Santa Marta, Colombian Caribbean. *Marine Ecology* 14:1-21.

1996. Random patterns of sponge distribution in remote, oceanic reef complexes of the Southwestern Caribbean. Abstracts, *8th International Coral Reef Symposium, Panamá,* June 24-29, 1996, p.215.

SPONGES ON MANGROVE ROOTS, TWIN CAYS, BELIZE:
EARLY STAGES OF COMMUNITY ASSEMBLY

BY

JANIE WULFF

Figure 1. Index map of Twin Cays showing Sponge Haven and Hidden Creek study sites.

SPONGES ON MANGROVE ROOTS, TWIN CAYS, BELIZE: EARLY STAGES OF COMMUNITY ASSEMBLY

BY

JANIE WULFF

ABSTRACT

A combination of transplant experiments and provision of recruitment surfaces has been initiated to determine the degree to which differences in sponge diversity and species composition at two Twin Cays sites, Hidden Creek and Sponge Haven, reflect differences in abiotic factors, ecological interactions, and recruitment history. This report contains the first stages of this project. The Hidden Creek sponge fauna differs from the Sponge Haven fauna largely by deletions. To determine if the cause might be stressful abiotic factors in Hidden Creek, five sponge species common at Sponge Haven, but absent from Hidden Creek, were transplanted to Hidden Creek. After 12 months, all but two transplanted individuals had died (97% mortality), suggesting distribution constraint by episodically unfavorable abiotic factors. To follow recruitment and community development from a start on bare substrata, pipes of pvc, each 25 cm long and 2.2 cm outside diameter, were suspended among prop roots in Hidden Creek. After 20 months, 69 sponge individuals representing 11 species were living on eight pipes. Differences in species composition and relative abundance between pipes and roots underscore the importance of postrecruitment processes in community development.

INTRODUCTION

Effects of abiotic environmental factors, interactions with other organisms, and historical events combine to determine the assortment of species that co-occur at a site. Lurking within this simple and obvious statement are the complexities of actually learning the relative importance of these factors in a particular system. Consistent correlations of environmental factors with distribution patterns can provide hints about physical and chemical requirements of species, but manipulative experiments and long-term studies often provide surprisingly additional insights and are required for understanding dynamics.

Sponges are one of the dominant groups inhabiting submerged portions of prop roots of Caribbean mangroves with respect to numbers of species, numbers of individuals, and total biomass (e.g. Rützler and Feller, 1996). Some species are found on mangrove roots and not in other habitats with sufficient predictabililty that they can be referred to as "typical mangrove sponge species" (e.g., summary of other faunal studies in Wulff, 2000), but variations on this typical fauna have been documented. A particularly striking variation is found in the Pelican Cays where sponge species typically found on

---

shallow coral reefs inhabit prop-roots (Rützler et al., 2000). Overlap in species composition between the Pelican Cays prop-root sponge fauna and the more typical assortment at Twin Cays, 18 km distant, is only 22% of a total of 166 species and forms (Fig. 2a). Species inhabiting both sets of cays tend to be rare in one or the other set, e.g., only three species were designated with the highest abundance rank of "3" (on a 1-2-3 scale; Rützler, et al., 2000) at sites in both sets of cays. The large differences in species composition and diversity (Rützler et al., 2000) between the Pelican Cays (146 species) and the more typical mangrove-root sponge fauna at Twin Cays (56 species) may reflect differences in processes influencing community composition (Wulff, in press). Faster-growing typical mangrove sponge species can prevent typical reef species from inhabiting Twin Cays mangrove roots by outcompeting them. Diversity is relatively low as expected in a system in which competitive dominants eliminate some species. By contrast, spongivorous fishes can prevent some typical mangrove species from inhabiting Pelican Cays mangrove roots by consuming them. This may prevent competitive exclusion, allowing an especially high diversity of typical reef species to coexist on the roots.

Comparisons among sites within Twin Cays reveal species composition and diversity differences as well. This study reports on the first stages of an investigation into differences between Hidden Creek and Sponge Haven, which are only separated by 330 m across a shallow bay and the channel between the cays (map on page facing title page). While differences in species composition between the Pelican Cays and Twin Cays reflect the low number of shared species, the Hidden Creek sponge fauna differs from those in Sponge Haven primarily by deletions (Fig. 2b). Species shared by these two sites constitute 39% of the combined species and those shared species are 73% of the Hidden Creek sponge fauna suggesting that periodically stressful abiotic conditions in the tidally influenced creek (Rützler, 1995) might inhibit some typical mangrove species from living there.

A combination of transplant experiments and provision of recruitment surfaces have been initiated to determine the degree to which differences in diversity and composition of these two Twin Cays sites reflect chance historical differences that are maintained by limited larval dispersal, ecological interactions that differ between sites and abiotic factor differences that favor some species over others. In a more general context, these data address the influence of trade-offs among growth, recruitment, predator defenses, and ability to cope with environmental stresses on community development in systems characterized by discrete substrata.

METHODS

Recruitment and Community Development

Lengths of pvc pipe, 2.2 cm outside diameter and 25 cm long, were suspended among the mangrove roots in Hidden Creek in June, 2001. By March, 2003 (i.e., 20 months after deployment) the pipes were covered by a variety of sessile organisms, chiefly sponges. All dimensions of every individual on each of the eight pipes were measured for nondestructive determinations of volume. The pipes were left undisturbed
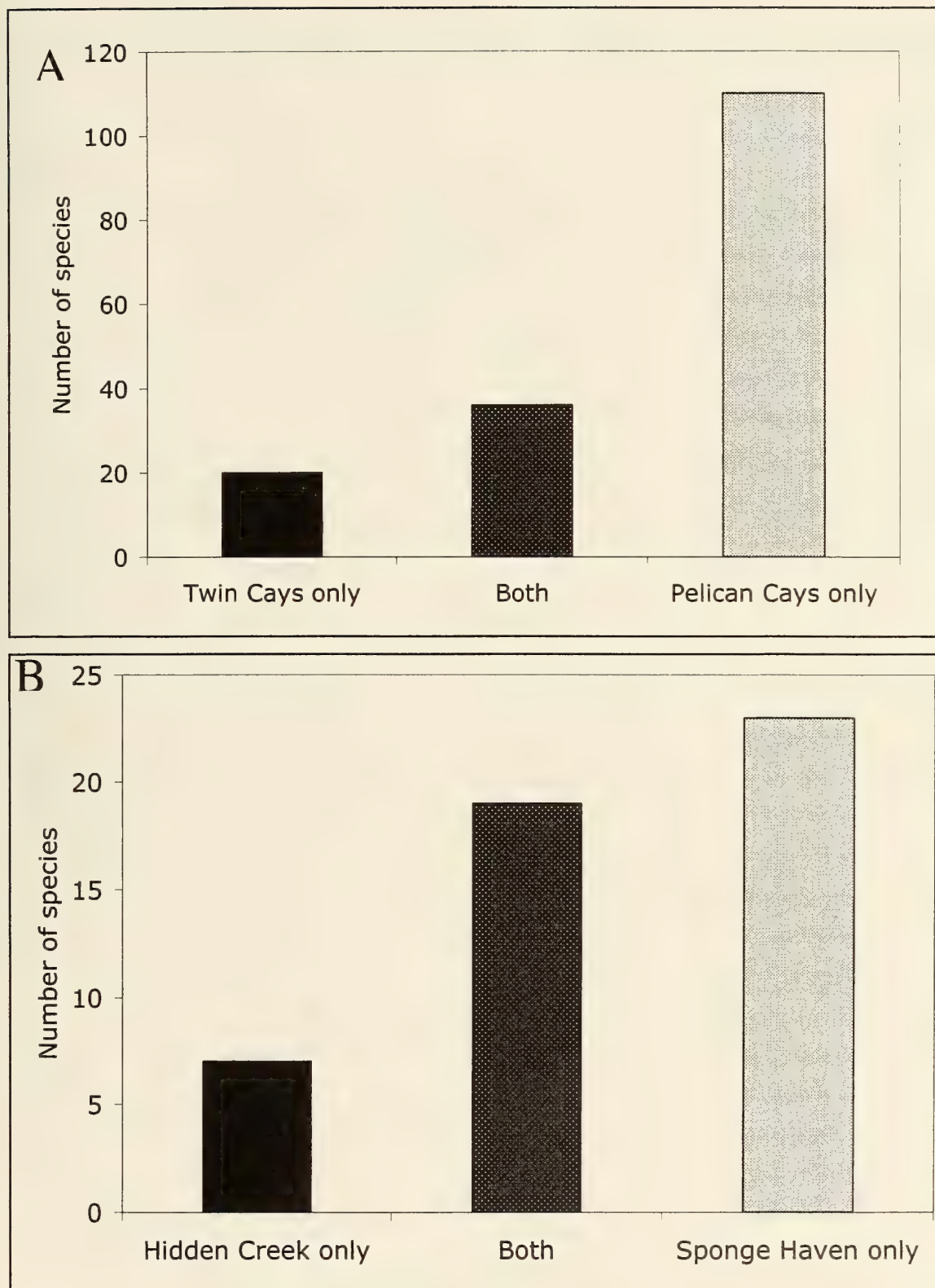
Figure 2 A, B. Comparison of the patterns of shared and not-shared species at: A) two sets of mangrove cays, Pelican Cays and Twin Cays; and B) two sites within Twin Cays, Sponge Haven and Hidden Creek.

4

so community development could be followed but tiny pieces of individuals to which names could not be applied confidently in the field were collected for identification in the lab.

Transplanted Sponges

In March 2003, 12-13 individuals of each of five sponge species commonly inhabiting prop-roots, and sometimes the peat, at Sponge Haven, *Mycale microsigmatosa*, *Calyx podatypa*, *Spirastrella mollis*, *Halichondria* cf. *poa*, and aff. *Tedania ignis* (noticeably different in overall morphology and color from *Tedania ignis* and treated consistently differently by predators but with the same spicule complement), across the Twin Cays channel from Hidden Creek were transplanted to roots in Hidden Creek where they had not been found. Immediately after the sponges were collected at Sponge Haven they were transported in a bucket of seawater (transferred into and out of the bucket underwater) to Hidden Creek. Volume, initially 2-8 cm^3, was determined for each individual by immersion in a graduated cylinder and the measured sponges were then attached to bare spots on the roots with small (1 mm in diameter) labeled cable ties. Transplants were checked daily for 10 days and again after one year.

RESULTS

Recruitment and Community Development

After 20 months, all eight pipes were nearly completely covered with sessile organisms with bare space ranging from 0 to 21.2 cm^2 out of a total surface area of 175 cm^2 for an individual pipe (i.e., <12.1% bare space on any particular pipe and only 4.3% of the total). In addition to sponges, macroscopic colonists included colonial ascidians, algae, polychaete worms, a few anemones and one spiny oyster. Sponges were by far the most abundant with respect to both surface area covered and total volume of live tissue.

Eleven sponge species were identified on the pipes (Figure 2 a,b,c). Abundance on the pipes (with respect to number of individuals or total volume), or even presence or absence, was not consistently related to abundance on the roots, either negatively or positively (Figure 3a,b,c). Four of the 11 species were among those designated as "abundant" (i.e., 3 on a scale of 1-2-3) by a team of Caribbean sponge experts (Rützler et al., 2000), three species had been given an abundance rank of 2, and two species a rank of 1. The other three species inhabiting the pipes could not be assigned confidentally to described species but are in genera found in Hidden Creek (*Clathria, Lissodendoryx,* and *Haliclona*). Conversely, three of the seven species that were given the high abundance rank of 3 on the Hidden Creek roots were not found on the pipes at all (*Tedania ignis, Haliclona implexiformis*, and *Amorphinopsis* sp.). It is very possible that additional species were represented on the pipes as individuals that were too small to be seen in the field. Because the time course of community development was an important focus for this study, leaving the pipes relatively undisturbed in the field was deemed more important than risking losses by bringing them to the lab for microscopic evaluation.

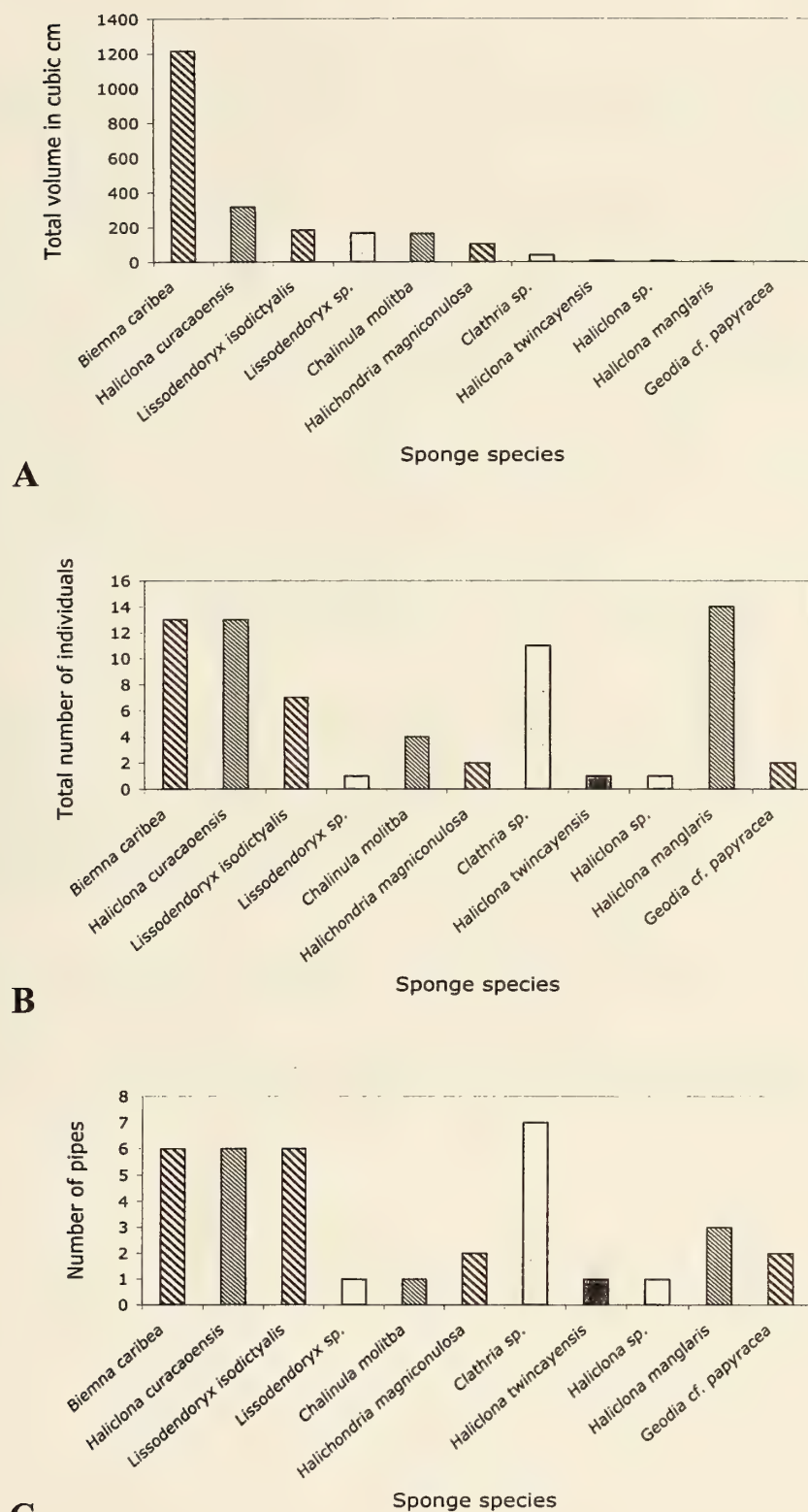**Figure 3 A,B,C.** Sponges inhabiting eight pvc pipes suspended among mangrove prop roots at Hidden Creek, Twin Cays, for 20 months from June 2001 to March 2003. Relative abundance of these species on unmanipulated mangrove roots as designated by a team of Caribbean sponge experts (Rützler, et al., 2000) is indicated as follows: "abundant" = course diagonals; "common" = fine diagonals; "rare" = dark grey; "not present" = light grey.

6

Four orders of Demospongiae were represented on the pipes: five species in Haplosclerida (all in the genus *Haliclona*), four in Poecilosclerida (two in the genus *Lissodendoryx*); one in Astrophorida; and one in Halichondrida. Abundance on the pipes was not correlated with taxon on the order or genus level. For example, the five *Haliclona* species had abundance ranks (with respect to total volume) among the 11 species of 2, 5, 8, 9, 10 (Fig. 3a).

Growth-form variation of the sponges inhabiting the pipes was limited, relative to the full range of possibilities among demonsponges, but still varied from encrusting (*Clathria* sp.) to broad-based massive-fistulose (*Lissodendoryx isodictyalis*, *Lissodendoryx* sp., *Halichondria magniconulosa*, and *Geodia* cf. *papyracea*), to clusters of fistulose branches that are relatively loosely substratum-bound (*Biemna caribea*, *Chalinula molitba*), to clusters of low mounds (*Haliclona curaçaoensis*), encrusting cushions, sometimes with thin stolons (*H. manglaris*) and very thin branches (*H. twincayensis*). No patterns in abundance on the pipes were related to growth form with the exception that the species that was represented by the most individuals and on the most pipes (*Clathria* sp.) was an encrusting species, a growth form rarely seen on the roots.

Growth rate did not appear to be related to abundance on pipes. Growth rates had been measured over seven months for six of the most common species in Hidden Creek (Wulff in review). One of the species that increased in volume most rapidly (*Biemna caribea*) was also the species that was most abundant by volume on the pipes and among the most abundant with respect to numbers of individuals and numbers of pipes colonized. But another species that grew rapidly (*Tedania ignis*) did not appear on the pipes at all and the third rapid grower (*Halichondria magniconulosa*) was represented at intermediate levels by all three abundance measures (Fig. 3 a,b,c).

Species on the pipes fell into two categories with respect to how evenly they were represented among the pipes. *Clathria* sp., *Biemna caribea*, *Haliclona curaçaoensis*, and *Lissodendoryx isodictyalis* were each found on six-to-seven of the eight pipes; but the other species were each on three or fewer pipes and four of them were on only one pipe (Fig. 3c).

Transplanted Sponges

Most individuals of *Calyx podatypa*, *Spirastrella mollis*, *Halichondria* cf. *poa*, and aff. *Tedania ignis* that were transplanted from Sponge Haven to Hidden Creek appeared to be reattached and healthy by the end of 10 days. Several individuals of one species, *Mycale microsigmatosa*, developed necrotic patches where the cable ties held them onto the mangrove roots but the undamaged portions attached within a day and began to grow so quickly that encrusting portions extended as far as 1.5 cm from the transplant by 10 days.

After one year, however, only two of the 63 transplanted individuals survived. Specific growth rates of these two individuals, both of which were *Calyx podatypa*, were high (6.8 and 15 in a year; starting sizes were 3.5 and 6 cm^3, and ending sizes were 27.3 and 96.1 cm^3). A few transplants and their labels were missing because entire roots had been lost, especially near the mouth of Hidden Creek, but most of the labeled cable ties were found indicating the clear demise of the transplants.

DISCUSSION

Distribution Constraints by Abiotic Factors

Intolerable abiotic factors are often accepted as explanations of constraints on distributions of species because of consistent correlations of distribution patterns with particular abiotic factors. Sometimes transplant experiments give surprising results, demonstrating that the actual constraints are interactions with other organisms or recruitment patterns (e.g., Wulff, in press). In this study, however, the hypothesis that some common Sponge Haven species are inhibited from living in Hidden Creek because of unfavorable abiotic factors is not rejected by the data. Transplants reattached and grew for at least the first 10 days suggesting that conditions sufficiently unfavorable to kill the Sponge Haven species are episodic. The other possibility that was not tested is that a smooth pufferfish, *Testudineus sphoeroides*, which is common in Hidden Creek but which I have never observed at Sponge Haven, may have consumed the transplanted species. The two *C. podatypa* individuals that were thriving had both become partially covered by dense mats of *Halimeda* suspended from roots, possibly protecting them from predators.

If more transplants had survived, the possibility would have to be considered that these species are missing from the Hidden Creek fauna due to lack of larval dispersal into Hidden Creek. But the mortality of 97% of the transplants suggests that conditions would not be reliably favorable in Hidden Creek for at least some of the species that are common at nearby Sponge Haven even if their larvae were able to travel over to Hidden Creek.

Intermediate Stages of Sponge Species Assembly on Twin Cays Prop-Roots

Although most bare space on the pipes was filled by sessile organisms at 20 months, this time period appears to have been insufficient for development of the usual prop-root community for Hidden Creek. On the other hand, the crowded conditions on the pipes made it clear that 20 months is long enough that much post-recruitment sorting may have already occurred.

Results from the few studies that have focussed specifically on recruitment of Caribbean sponges provide insights that may aid interpretation. In Zea's (1993) study of sponge recruitment on acrylic plates in six reef and rocky-shore sites in the Colombian Caribbean, recruitment was on the whole low and highly variable among plates. Zea concluded that proximity to abundant adult sponges was the best predictor of abundance of recent recruits although recently settled sponges were not identified by species. Zea's point that even his bimonthly sampling scheme allowed some invisible recruitment, i.e., sponges which both settled and died between sampling periods, cautions against interpreting the assortment of species on pipes after 20 months in this study as a full record of recruitment.

Two of the most common Hidden Creek species, *Tedania ignis* and *Halichondria magniconulosa*, were included in a study of recruitment of four species on shallow (< 2m) boulders and cobbles in the Indian River Lagoon, FL by Maldonado and Young (1996). They distinguished four microhabitats by light and current flow conditions and

determined that adult abundance of each of the four species was associated with a different set of water flow and light conditions. However, in all four microhabitats *T. ignis* was by far the most abundant recruit after 35 days although it dropped in abundance by 96 days. For three of the four species, adult abundance had no statistical association with larval recruitment.

Sutherland (1980) also noted the lack of relationship between adult abundance and larval recruitment for sponge species inhabiting mangrove roots in Venezuela. He evaluated recruitment of mangrove sponges by suspending asbestos panels, 20 cm by 122 cm, among the prop roots for 18 months and comparing communities that developed on the panels with those on the roots. None of the five most abundant (by % cover) species on the roots were among the top five species in recruitment. The discrepancy between abundance on roots and panels was so extreme in some cases that the ranks for recruitment rates of the three most abundant sponge species (*Tedania ignis*, *Mycale microsigmatosa*, and *Sigmadocia* cf. *caerulea*) were 24, 7, and 10 (out of a total of 25). These results led Sutherland to conclude that accumulation of species in this community reflects results of competition with neighbors after settlement.

The importance of the size and shape of substrata provided for settlement was stressed by Sutherland (1980). Communities on his panels were more similar to each other than those on roots reflecting two differences between panels and roots: 1) each of the much larger panels was more likely to sample all possible larvae available for settlement; and 2) the continuity of the panel substrata allowed superior competitors to continue growing over large areas instead of having to recruit onto each small patch. Thus dissimilarity in species composition among roots results from the low rate of recruitment and the discontinuity of the substrata. Sutherland pointed out how this combination increases species diversity by slowing the elimination of inferior competitors from the system, a point that had also been made by Jackson and Buss (1975) based on their studies of communities on undersurfaces of foliaceous corals.

Seasonality of recruitment was not observed by Sutherland (1980) in Venezuela, but Zea (1993) found recruitment to be influenced strongly by seasonal temperature changes associated with upwelling at Santa Marta, Colombia. In Rützler's (1987) study of settlement on acrylic plates among mangrove roots at Twin Cays, recruitment onto the plates occurred throughout the year but with variations among species depending on the season and prior recruits. This result raises the interesting possibility that differences among sponge species in timing of larval release could increase diversity of mangrove root inhabitants by allowing different species to be the initial colonists of roots entering the water at different times of the year.

The importance of acceptable microhabitats for recruitment is demonstrated by consistent reports of higher rates of settlement by sponge larvae in darker microhabitats (e.g., Zea, 1993; Maldonado and Young, 1996) for Caribbean species. Although materials used for experimentally provided settlement surfaces appear to have minimal impact (Zea, 1993; Reiswig, 1973; Rützler, this volume), the lack of microtopographic features and dark areas that might inspire increased recruitment may have decreased recruitment on smooth pvc pipes. However, the pipes do mimic the prop roots well as the roots are also cylindrical and smooth when they first enter the water. Depth may be an important aspect of microhabitat. Rützler's (1995) study of influence of tidal fluctuations in water level on mangrove sponges demonstrated differences in the depths at which

some common Hidden Creek species live on the roots. The pipes in the present study were suspended so that the tops were always at least 20 cm below the lowest tide marks, possibly decreasing representation on them of species such as *Haliclona implexiformis* and *Lissodendoryx isodictyalis* that can tolerate emersion better than other species (Rützler, 1995) although these species also grow on deeper portions of roots.

At Hidden Creek, differences in species composition and relative abundance between the pipes and roots that are still clear and consistent after 20 months indicate that development of the communities on roots is a longer-term process involving sorting of the species that recruit initially. Positive correlation between specific growth rate and survival of six of the most abundant species in Hidden Creek (Wulff, in press) suggest that competition, mediated by growth rate, influences species composition. There are too few published studies to be confident of the pattern yet, but it is intriguing that Zea's (1993) study of sponge recruitment on coral reefs demonstrated a positive relationship between adult abundance and recruitment, whereas studies on habitats with discrete substrata, mangrove roots and cobbles, showed no relationship (Maldonado and Young, 1996; Sutherland, 1980; this study). Interplay between competition and recruitment success is of especially great importance in systems characterized by discrete substrata. In this system, provision of new substrata as roots grow and enter the water for the first time ensures that there are chances for inferior competitors to inhabit roots that are not colonized by superior competitors if they can balance their lack of competitive ability with recruitment success.

ACKNOWLEDGMENTS

REFERENCES

Jackson, J.B.C., and L.W. Buss
  1975. Allelopathy and spatial compeititon among coral reef invertebrates. *Proceedings of the National Academy of Science, USA*, 72:5160-5163.
Maldonado, M., and C.M. Young
  1996. Effects of physical factors on larval behavior, settlement and recruitment of four tropical demonsponges. *Marine Ecology Progress Series*, 138:169-180.
Reiswig, H.M.
  1973. Population dynamics of three Jamaican Demospongiae. *Bulletin of Marine Science*, 23:191-226.

Rützler, K.

1987. *Recruitment of sponges and their space competitors to settling plates in Twin Cays mangrove habitats.* Pp. 23-24 in Caribbean Coral Reef Ecosystems Program Report 1987, National Museum of Natural History, Washington, D.C.

1995. Low-tide exposure of sponges in a Caribbean mangrove community. P.S.Z.N.I.: *Marine Ecology*, 16:165-179.

Rützler, K., M.C. Diaz, R.W.M. van Soest, S. Zea, K.P. Smith, B. Alvarez, and J. Wulff
2000. Diversity of sponge fauna in mangrove ponds, Pelican Cays, Belize. *Atoll Research Bulletin*, 476:230-248.

Rützler, K., and I.C. Feller
1996. Caribbean mangrove swamps. *Scientific American*, March 94-99.

Sutherland, J.P.
1980. Dynamics of the epibenthic community on roots of the mangrove *Rhizophora mangle*, at Bahia de Buche, Venezuela. *Marine Biology*, 58:75-84.

Wulff, J.L.
2000. Sponge predators may determine differences in sponge fauna between two sets of mangrove cays, Belize barrier reef. *Atoll Research Bulletin* 477:250-263.

In press. Trade-Offs in resistance to competitors and predators, and the diversity of tropical marine sponges. Journal of Animal Ecology.

ATOLL RESEARCH BULLETIN

NO. 520

GNATHOSTOMULIDA FROM THE TWIN CAYS, BELIZE, MANGROVE
COMMUNITY

BY

WOLFGANG STERRER

Figure 1. Species-rich sampling site for gnathostomulids at West Bay ("Candy's Trail") with *Thalassia testudinum* in fine-to-medium sand among roots of *Rhizophora mangle*.

GNATHOSTOMULIDA FROM THE TWIN CAYS, BELIZE, MANGROVE COMMUNITY

BY

WOLFGANG STERRER[1]

ABSTRACT

Gnathostomulida, a phylum of microscopic, interstitial marine worms, are well represented in detritus-rich sandy sediments that are usually found between coral reefs, seagrasses and mangroves. Of 25 species encountered in more than 100 sediment samples collected in southern Belize between 1974 and 2004, 18 species were found in the vicinity of Twin Cays.

INTRODUCTION

Gnathostomulida are small, unsegmented, acoelomate worms that live in the interstices of marine sand. Similar in habitus to free-living Turbellaria, as they were first described (Ax,1956), they are now considered a phylum (Riedl, 1969; Sterrer, 1972) on the basis of their unique features, particularly a monociliary epidermis (each epidermal cell carries only a single cilium), and a bilaterally symmetric pharynx equipped with complex cuticular mouth parts. Found exclusively in shallow marine sand, Gnathostomulida are thought to graze on the bacterial and fungal microflora which coats sand grains. Their preference for detritus-rich sand, in which they occur at the boundary between reduced and oxygenated sediments, suggests that in addition to very low oxygen requirements they may have mechanisms for sulfide detoxification. Only 94 species, in 25 genera, are currently known worldwide (Sterrer, 2001), many with cosmopolitan distribution. Gnathostomulida may be among the most primitive living Bilateria (Ax, 1985; Sterrer et al., 1985), with possible phylogenetic affinities to Rotifera and Micrognathozoa (Giribet et al., 2004).

During nine visits to the Carrie-Bow Cay Field Station in southern Belize (Rützler and Macintyre, 1982) between 1974 and 2004, I collected more than 100 sediment samples which yielded a total of 25 species of Gnathostomulida (including seven species and two genera new to science), the largest number from any area in the world (Sterrer, 1998). While most samples came from the immediate vicinity of Carrie-Bow Cay, two came from the Pelican Cays (Sterrer, 2000); the nearby mangrove island of Twin Cays was sampled repeatedly.

---

[1] Bermuda Natural History Museum, Flatts FLBX, Bermuda; e-mail wsterrer@bbsr.edu.

2

METHODS

Collecting and specimen extraction are detailed in Sterrer (1998). Using snorkeling or scuba, the upper 5 centimeters of sediment are scooped into a bucket by hand until the latter is full; a primary sample thus consists of about 10-15 liters of sand with a little overlying seawater. In the lab, this primary sample is periodically subsampled by scooping the superficial layer of sand (about 500 ml) into a flask and shaking it in an isotonic magnesium sulfate solution. The floating meiofauna is then poured through a 63-μm sieve and allowed to recover before it is sorted into species and analyzed under the phase-contrast microscope. Extraction ends when the sample ceases to produce gnathostomulids, usually after 7-12 days.

RESULTS

Sediment samples taken from the vicinity of Twin Cays contained 18 out of a total of 25 species of Gnathostomulida recorded in the greater Carrie Bow Cay region (Table 1). None of the species were unique to Twin Cays. Whereas the muddy bottoms in mangrove channels never contained gnathostomulids, the sampling program confirmed the preference of this phylum for fine-to-medium, even coarse, sand but always with a high admixture of marine (not terrigenic) detritus as is typically found among and between seagrasses, mangroves, and coral reefs. In this preferred environment, diversity may reach a dozen species per sample and gnathostomulids may outnumber all other meiofauna including nematodes. Two sites off West Bay were sampled repeatedly being particularly productive and together producing all of the 18 species: the first ("Candy's Trail") in the intertidal to 0.5 m among roots of Red Mangrove where fine-to-medium sand is interspaced with short *Thalassia testudinum* (Fig. 1); and the second about 50 meters offshore from the first, where *Thalassia* grows in medium-to-coarse sand at 1-2 m depth with *Clypeaster* and *Oreaster* as conspicuous macrobenthos.

ACKNOWLEDGMENTS

REFERENCES

Ax, P.
  1956. Die Gnathostomulida, eine rätselhafte Wurmgruppe aus dem Meeressand. *Abhandlungen der Akademie der Wissenschaften und Literatur Mainz, mathematisch-naturwissenschaftliche Klasse.* 8:1-32.

Ax, P.
    1986. The position of the Gnathostomulida and Platyhelminthes in the phylogenetic
        system of the Bilateria . In: S. Conway Morris, J.D. George, R. Gibson, and
        H.M. Platt (eds.), *The origins and relationships of lower invertebrates.* The
        Systematics Association, Clarendon Press, Oxford, pp. 168-180.
Giribet, G.; Sørensen, M.V.; Funch, P.; Kristensen, R.M.; and Sterrer, W.
    2004. Investigations into the phylogenetic position of Micrognathozoa using four
        molecular loci*: Cladistics.* 20:1-13.
Riedl, R.J.
    1969. Gnathostomulida from America. *Science.* 163:445-442.
Rützler, K. and Macintyre, I.G. (eds.)
    1982. *The Atlantic Barrier Reef Ecosystem at Carrie Bow Cay, Belize, I. Structure
        and Communities.* Smithsonian Institution Press, Washington DC, 539 p.
Sterrer, W.
    1971. Gnathostomulida: problems and procedures. *Smithsonian Contributions to
        Zoology.* 76:9-15.
Sterrer, W.
    1972. Systematics and evolution within the Gnathostomulida. *Systematic Zoology.*
        21:151-173.
Sterrer, W.
    1992. Clausognathiidae, a new family of Gnathostomulida from Belize. *Proceedings
        of the Biological Society of Washington.* 105:136-142.
Sterrer, W.
    1998. Gnathostomulida from the (sub)tropical northwestern Atlantic. *Studies on the
        Natural History of the Caribbean Region.* 74:1-178.
Sterrer, W.
    2000. Gnathostomulida in the Pelican Cays, Belize. *Atoll Research Bulletin,* 478:
        266-272.
Sterrer, W.
    2001. Gnathostomulida from Australia and Papua New Guinea. *Cahiers de Biologie
        Marine.* 42:363-395.
Sterrer, W., Mainitz, M., and Rieger, R. M.
    1985. Gnathostomulida: enigmatic as ever. In: S. Conway Morris, J.D. George,
        R. Gibson, and H.M. Platt (eds.), *The origins and relationships of lower
        invertebrates.* Oxford, Clarendon Press, p. 183-199.

4

Table 1. Gnathostomulida from the greater Carrie-Bow Cay region
(species recorded in Twin Cays are in bold)

Genus	species	Author
Order Filospermoidea		
Family Haplognathiidae		
Haplognathia	*asymmetrica*	Sterrer, 1991
Haplognathia	*belizensis*	Sterrer, 1998
Haplognathia	*lunulifera*	(Sterrer, 1969)
Haplognathia	**rosea**	**(Sterrer, 1969)**
Haplognathia	**ruberrima**	**(Sterrer, 1966)**
Family Pterognathiidae		
Cosmognathia	**aquila**	**Sterrer, 1998**
Cosmognathia	**arcus**	**Sterrer, 1991**
Cosmognathia	*manubrium*	Sterrer, 1991
Pterognathia	*alcicornis*	Sterrer, 1998
Pterognathia	*crocodilus*	Sterrer, 1991
Pterognathia	**ctenifera**	**Sterrer, 1969**
Pterognathia	*swedmarki*	Sterrer, 1966
Pterognathia	**ugera**	**Sterrer, 1991**
Order Bursovaginoidea		
Suborder Scleroperalia		
Family Clausognathiidae		
Clausognathia	**suicauda**	**Sterrer, 1992**
Family Mesognathariidae		
Labidognathia	**longicollis**	**Riedl, 1970**
Tenuignathia	**rikerae**	**Sterrer, 1976**
Family Paucidentulidae		
Paucidentula	**anonyma**	**Sterrer, 1998**
Family Onychognathiidae		
Onychognathia	**rhombocephala**	**Sterrer, 1998**
Family Gnathostomulidae		
Gnathostomula	**axi**	**Kirsteuer, 1964**
Gnathostomula	**peregrina**	**Kirsteuer, 1969**
Suborder Conophoralia		
Family Austrognathiidae		
Austrognathia	*christianae*	Farris, 1977
Austrognathia	*microconulifera*	Farris, 1977
Austrognatharia	*medusifera*	Sterrer, 1998
Austrognatharia	*sterreri*	(Kirsteuer, 1969)
Austrognatharia	*strunki*	Farris, 1973

SIPUNCULAN DIVERSITY AT TWIN CAYS, BELIZE
WITH A KEY TO THE SPECIES

BY
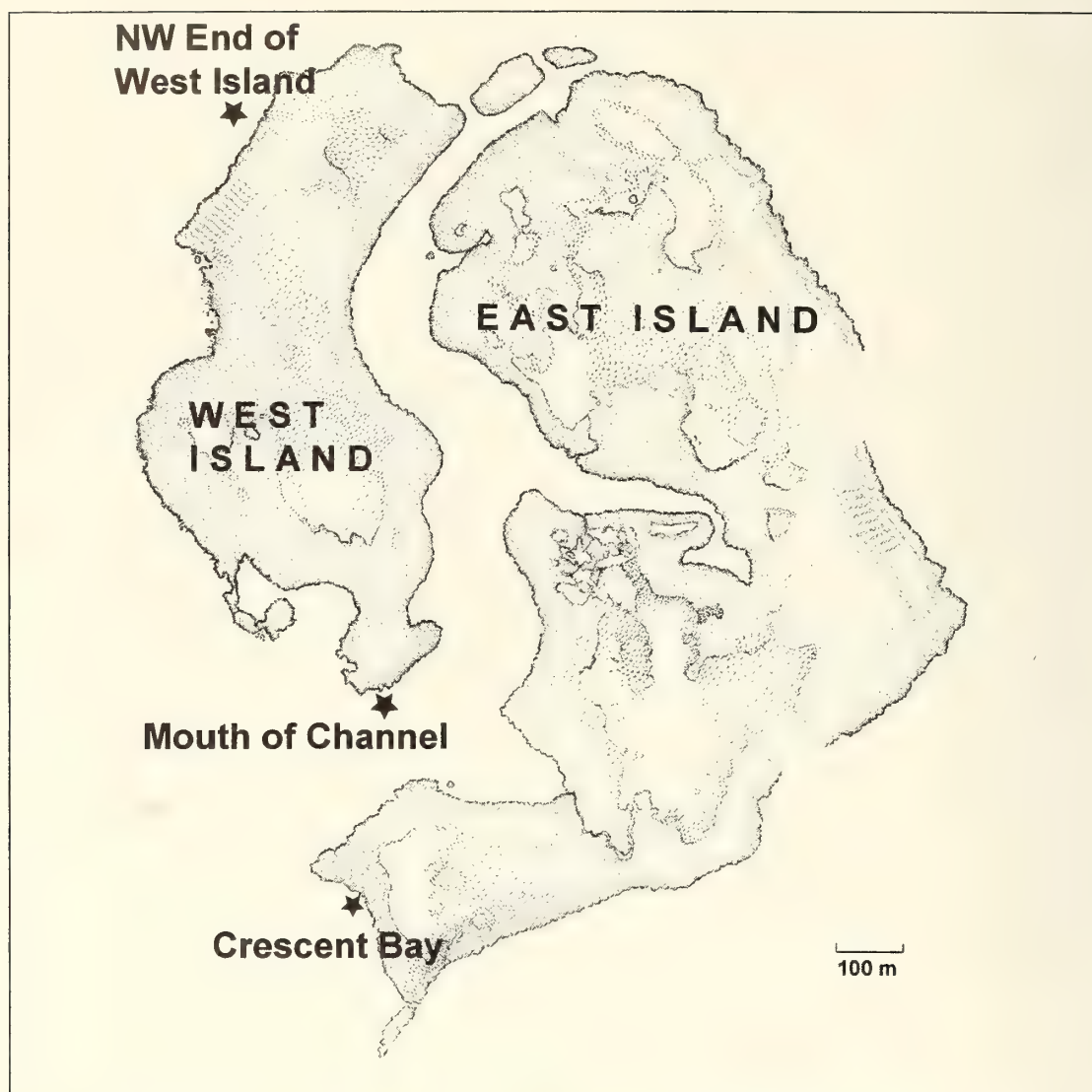
ANJA SCHULZE AND MARY E. RICE

Figure 1. Index map showing the location of collection sites

SIPUNCULAN DIVERSITY AT TWIN CAYS, BELIZE WITH A KEY TO THE SPECIES

BY

ANJA SCHULZE[1] and MARY E. RICE[1]

ABSTRACT

We collected the following six species of Sipuncula in three intertidal and shallow subtidal sites around Twin Cays from April 18-24, 2003: *Golfingia elongata* (Keferstein, 1862); *Siphonosoma cumanense* (Keferstein, 1867); *Sipunculus nudus* Linnaeus, 1766; *Aspidosiphon (Paraspidosiphon) fischeri* ten Broeke, 1925; *Aspidosiphon (Paraspidosiphon) laevis* de Quatrefages, 1865; and *Aspidosiphon (Paraspidosiphon) parvulus* Gerould, 1913. Of these, *Golfingia elongata* and *Aspidosiphon parvulus* were the most common ones, both collected from mangrove and *Thalassia* root mats. The distribution of *Golfingia elongata* seemed patchy. *Sipunculus nudus* and *Siphonosoma cumanense* were found in low density in consolidated sand flats. In total, 14 sipunculan species are reported from Carrie Bow Cay and surrounding cays. We include a key to all the species found in the area.

INTRODUCTION

Sipuncula are common inhabitants of tropical shallow waters but due to their cryptic habitats are often underrepresented in faunal surveys. They inhabit coral rubble, mangrove and seagrass roots, sand and occasionally mollusc shells. Morphologically, they are recognizable by an unsegmented trunk region and a retractable introvert, usually bearing an array of tentacles. They range in size from a few millimeters to about 30 cm.

We here report 14 species from the surroundings of Carrie Bow Cay and provide a key to their identification. Six of these species have been collected around Twin Cays and are described in more detail. This paper complements a previous account of Sipuncula from the Carrie Bow Cay area which focused on hard-substrate species (Rice and Macintyre, 1979).

COLLECTING METHODS

Between April 18 and 24, 2003, we sampled three areas around Twin Cays to study sipunculan diversity (Fig. 1). All sample sites were in the intertidal or shallow subtidal zone (up to 1 m depth) within approximately 20 m of the mangroves. Owing to

---

[1] Smithsonian Marine Station, 701 Seaway Drive, Fort Pierce, FL 34949.

evaporation, rainfall and freshwater runoff from the island, water temperature and salinity fluctuate greatly at these sites (Ferraris et al., 1994).

Sipunculans were retrieved by digging as deep as 30 cm into the substrate and subsequent sieving of the substrate using a sieve with 3 mm-mesh size. Sipunculan worms were separated from the roots and other fauna, collected in sealed containers and kept alive for up to one week in clean containers with regular water changes.

The following three types of substrate were sampled:

Substrate 1: Dense mangrove root mats with algal cover of variable thickness (*Caulerpa, Cladophora, Halimeda*).

Substrate 2: *Thalassia* stands with small interspersed sandy patches, *Thalassia* roots usually intertwined with mangrove root mats and some algal cover.

Substrate 3: Patches of consolidated sand; algal cover negligible.

SPECIES ACCOUNTS

Family Sipunculidae

Sipunculus nudus Linnaeus, 1766 (Fig. 2A)

Diagnosis. Distinct bands of circular and longitudinal musculature (24-34 longitudinal muscle bands). A short introvert without hooks but with large papillae and lamellate tentacles. Four introvert retractor muscles. Brain with sponge-like anterior processes.

Comments. The only recovered specimen was approximately 7.5 cm in length (*S. nudus* is commonly larger, up to 30 cm).

Distribution and habitat. Cosmopolitan in temperate, subtropical and tropical waters, mostly less than 30 m deep. The only other known occurrence of *S. nudus* from the direct vicinity to mangroves is in Puerto Rico (pers. obs. M. E. Rice). Substrate type at Twin Cays: 2.

Siphonosoma cumanense (Keferstein, 1867) (Fig. 2B)

Diagnosis. Large, elongate species (up to 40 cm long) with anastomosing longitudinal and circular muscle bands. Number of longitudinal muscle bands: 18-24. Typically forms several tight constrictions along its body, or breaks into pieces, when retrieved from its substrate. Short introvert with pronounced papillae. Tentacles long and digitiform.

Comments. We only retrieved three specimens around Twin Cays. In comparison, in the Aldabra Atoll, Indian Ocean, *S. cumanense* represents up to 8.6% of the total biomass in intertidal soft substrates with algal and/or *Thalassia* cover (Hughes and Gamble, 1977).

Distribution and Habitat. Widespread in the Caribbean and Western Atlantic. Also common in Western Pacific, Indian Ocean and Red Sea. The other known occurrence in the vicinity of mangroves is in Puerto Rico (pers. obs. M. E. Rice). Substrate type at Twin Cays: 2, 3.

Family Golfingiidae

Golfingia elongata (Keferstein, 1862) (Fig. 2C).

Golfingia cylindrata. - Ferraris et al. 1994: Figs. 5, 6, 7 d, pp. 397-406.

Diagnosis. Slender worm with short introvert and simple crown of tentacles. Body wall musculature smooth, not divided into bands. 8-10 rows of hooks on introvert. No prominent papillae.

Comments. At Twin Cays, *G. elongata* inhabits the upper layers of the mangrove root mats. Specimens are similar to the roots in color and texture and often difficult to detect. Twin Cays specimens were 20-30 mm in trunk length. The occurrence of the species was patchy.
G. elongata is an osmoconformer that remains unaffected by large and repeated changes in salinity but apparently can only tolerate relatively short exposure to increased water temperatures (Ferraris et al, 1994). The only other report of a *Golfingia* species (unidentified) in the vicinity of mangroves is from the Pacific coast of Columbia (Cantera et al., 1999).
Few morphological features distinguish *G. elongata* from its congeners. It is possible that the Twin Cays specimens actually represent a different species than the ones recorded from deep, cold water. Comparisons of DNA sequence data are desirable.

Distribution and Habitat. Widespread in Atlantic and Pacific from arctic to tropical waters from intertidal to 590 m depth. Substrate type at Twin Cays: 1, 2.

Family Aspidosiphonidae

Aspidosiphon (Paraspidosiphon) fischeri ten Broeke, 1925 (Fig. 3A).

Paraspidosiphon fischeri. - Rice and Macintyre, 1979: Table 22.

Diagnosis. The dorsal anal shield, a characteristic feature of the genus, is round and has indistinct borders. Longitudinal body wall musculature in bands. *A. fischeri* has approximately 18 strongly anastomosing longitudinal muscle bands.

Distribution and habitat. Widespread in the Caribbean, usually in rock. Also known from the Pacific coast of Panama, Ecuador, James and Hood Islands and the Galápagos Islands. Substrate type at Twin Cays: 2.

Aspidosiphon (Paraspidosiphon) parvulus Gerould, 1913 (Fig. 3B).

4

Diagnosis. The anal shield is indistinctly set off from the trunk and bears spine-like papillae around its edge. Anal and caudal shields are dark.

Comments. The Twin Cays specimens are consistent with the description of *Aspidosiphon spinososcutatus* Fischer, 1922, a species that was later fused with *Aspidosiphon (Paraspidosiphon) parvulus* by Cutler (1994). Visual comparisons of the Twin Cays specimens with specimens from the Indian River lagoon, however, suggest that there are subtle differences. As in *Golfingia elongata*, comparison of DNA sequence data is desirable.

Distribution and Habitat. Western Atlantic and Caribbean. Generally inhabitant of rocks. Substrate type at Twin Cays: 1, 2.

Aspidosiphon (Paraspidosiphon) laevis de Quatrefages, 1865 (Fig. 3C)

Paraspidosiphon speciosus. – Rice and Macintyre, 1979: Table 22, p. 317.

Diagnosis. Solid anal shield with 10-15 longitudinal grooves; 25-35 longitudinal muscle bands can usually be seen through the body wall.

Distribution and habitat. Widespread in the Caribbean, Atlantic, Central and Indo-West Pacific; generally inhabitant of rocks.
Substrate type at Twin Cays: 1, 2.

Additional sipunculan species from the vicinity of Carrie Bow Cay:

Antillesoma antillarum (Grübe and Oersted, 1858).
Apionsoma misakianum (Ikeda, 1904).
Aspidosiphon elegans (Chamisso and Eysenhardt, 1821).
    Aspidosiphon brocki. – Rice and Macintyre, 1979: Figs. 138, 139 b, c, pp. 311, 313-319, Table 22; Rice, 1970: Figs. 1, 2, pp. 1618-1620.
Aspidosiphon (Paraspidosiphon) steenstrupii Diesing, 1859.
    Paraspidosiphon steenstrupi. – Rice and Macintyre, 1979: Figs. 138, 139 e, pp. 311, 313-319, Table 22.
Lithacrosiphon cristatus Sluiter, 1902.
    Lithacrosiphon alticonus. – Rice and Macintyre, 1979: Figs. 138, 139 a; pp. 311, 313-320, Table 22.
Phascolion gerardi Rice, 1993.
Phascolosoma nigrescens Keferstein, 1865.
    Phascolosoma varians. – Rice and Macintyre, 1979: pp. 316-317, Table 22
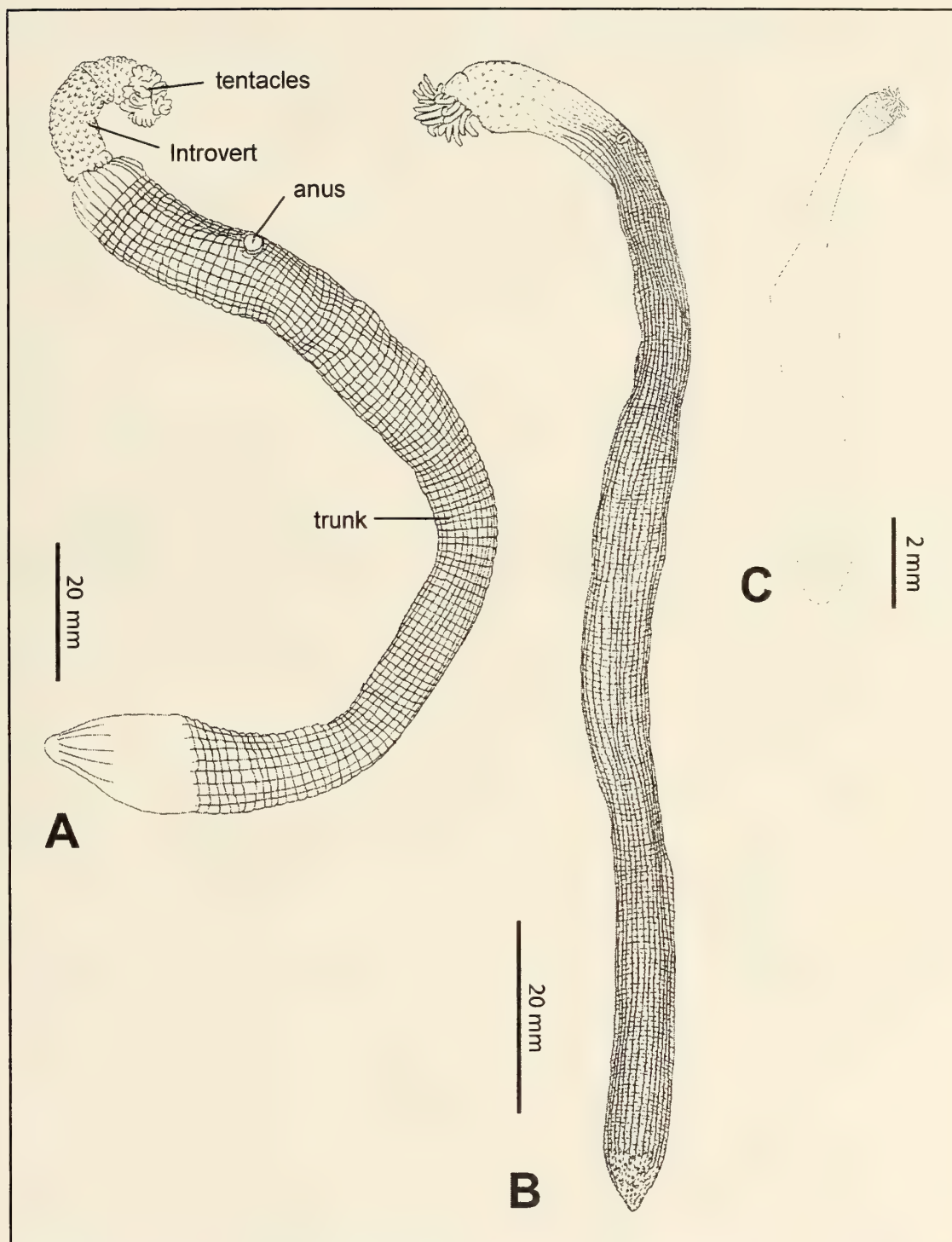*Phascolosoma perlucens* Baird, 1868.

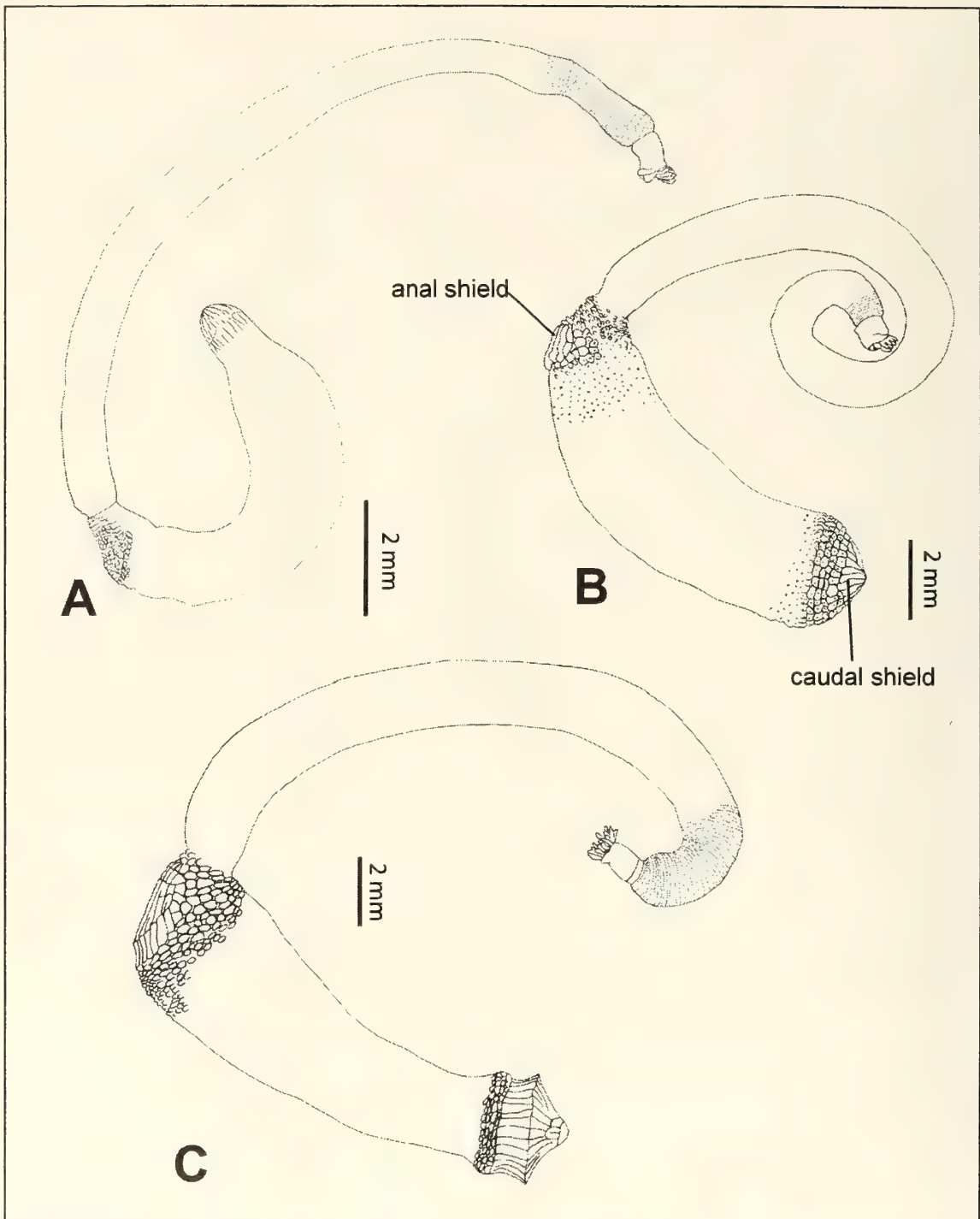**Figure 2**. Sipuncuncula from Twin Cays. A) *Sipunculus nudus*. B) *Siphonosoma cumanense*. C) *Golfingia elongata*.

6

Figure 3. Sipuncula from Twin Cays. A). *Aspidosiphon (Paraspidosphon) fischeri*. B). *Aspidosiphon (Paraspidosiphon) parvulus*. C). *Aspidosiphon (Paraspidosiphon) laevis*.

KEY TO THE SIPUNCULA IN THE VICINITY OF CARRIE BOW CAY

1. Large (usually > 50 mm); longitudinal and circular musculature in bands; hooks absent.....2
- longitudinal musculature in bands or continuous; circular musculature continuous, hooks present or absent.....3
2. Body wall with 24-34 longitudinal muscle bands; lamellate tentacles; introvert with triangular papillae pointing posteriorly.....*Sipunculus nudus*
- body wall with less than 24 longitudinal muscle bands, digitiform tentacles.....*Siphonosoma cumanense*
3. Anal shield present.....4
- anal shield absent.....9
4. Anal shield distinctly cone-shaped, often overgrown with coralline algae.....*Lithacrosiphon cristatus*
- anal shield more or less flat.....5
5. Longitudinal musculature in bands.....*Paraspidosiphon*, 6
- Longitudinal musculature continuous.....*Aspidosiphon elegans*
6. Anal and caudal shield distinct and with regular grooves.....*Aspidosiphon (P.) laevis*
- Anal shield without longitudinal grooves.....7
7. Anal shield with spinelike papillae around its edge.....*Aspidosiphon (P.) parvulus*
- Anal shield without spinelike papillae.....8
8. Anal shield round with indistinct borders; caudal shield indistinct.....*Aspidosiphon (P.) fischeri*
- Anal and caudal shields distinct; caudal shield with irregular grooves; numerous papillae on trunk, especially in anterior and posterior regions, composed of multiple polygonal plates.....*Aspidosiphon (P.) steenstrupii*
9. Longitudinal muscle bands absent.....10
- longitudinal muscle bands present.....12
10. Body wall smooth; dark, hooks present in 8-10 rows.....*Golfingia elongata*
- Trunk with papillae.....11
11. Trunk with numerous, small papillae, especially at posterior end; hooks present and in about 60 rows.....*Apionsoma misakianum*
- hooks scattered; introvert about 3x as long as trunk; numerous, prominent papillae on trunk; anus midway on the introvert.....*Phascolion gerardi*
12. Hooks absent; long, digitiform tentacles, body covered with conical papillae.....*Antillesoma antillarum*
- Hooks present, trunk usually with darkly pigmented bands.....13
13. Introvert with 100 or more rows of hooks.....*Phasolosoma nigrescens*
- 15-25 rows of hooks; reddish, conical, posteriorly directed preanal papillae.....*Phascolosoma perlucens*

DISCUSSION

All six sipunculan species found at Twin Cays are widespread throughout the Caribbean and beyond. All of them have been reported from shallow, warm water but

8

seem to be opportunistic with respect to suitable habitat. The ability to withstand large fluctuations in temperature, salinity and oxygen concentration probably enables them to live in close vicinity to mangroves and in the mangrove root mats. However, if the *Golfingia elongata* specimens from Twin Cays prove to be genetically distinct from the deep-water *G. elongata*, they might represent a separate species associated with the dense mangrove root mats.

Sipunculus nudus and *Siphonosoma cumanense* generally inhabit semipermanent burrows in consolidated sand banks. Accordingly, they were found in the more sandy areas with less algal cover around Twin Cays. The *Aspidosiphon (Paraspidosiphon)* species are generally inhabitants of rocks. The dense network of mangrove roots, *Thalassia* roots and algal cover may provide a similarly protected habitat.

Surveys of mangrove-associated invertebrate fauna often include sampling of the prop roots of the trees but not the extensive fine root mats in the immediate vicinity. Sipunculan worms seem to have a patchy occurrence in the root mats, often resulting in unsuccessful sampling attempts. More extensive sampling of similar habitats throughout the Caribbean will reveal whether the sipunculan fauna of the Twin Cays mangroves is representative of the region.

It is notable that *Phascolosoma arcuatum* (Gray, 1828), a species that commonly inhabits brackish mangrove swamps around the high tide line in the Indo-West Pacific and the Indian Ocean (Green, 1975a, b; Green and Dunn,1976, 1977), has never been reported from the Caribbean.

ACKNOWLEDGMENTS

REFERENCES

Cantera, K.J.R., B.A. Thomassin, and P.M. Arnaud
    1999. Faunal zonation and assemblages in the Pacific Colombian mangroves.
        Hydrobiologia 413:17-33.
Cutler, E.B.
    1994. *The Sipuncula. Their systematics, biology and evolution.* Ithaca, NY, Cornell
        University Press.
Ferraris, J.D., K. Fauchald, K., and B. Kensley
    1994. Physiological responses to fluctuation in temperature or salinity in
        invertebrates. Adaptations of *Alpheus viridari* (Decapoda, Crustacea),
        Terebellides parva (Polychaeta) and *Golfingia cylindrata* (Sipunculida) to the
        mangrove habitat. *Marine Biology* 120:397-405.
Green, W.A.
    1975. The annual reproductive cycle of *Phascolosoma lurco* (Sipuncula). Pages 161-
        168 in Rice, M.E. & M. Todorovic (eds). *Proceedings of the international*

symposium on the biology of the Sipuncula and Echiura. Belgrad, Naucno Delo Press.

1975. *Phascolosoma lurco*: a semi-terrestrial sipunculan. Pages 267-280 in Rice, M. E. & M. Todorovic (eds). *Proceedings of the international symposium on the biology of the Sipuncula and Echiura*. Belgrad, Naucno Delo Press.

Green, J., and D.F. Dunn

1976. Chloride and osmotic balance in the euryhaline sipunculid *Phascolosoma arcuatum* from a Malaysian mangrove swamp. *Biological Bulletin* 150:211-221.

1977. Osmotic and ionic balance in the mangrove sipunculid, *Phascolosoma arcuatum*. *Marine Research in Indonesia* 18:61.

Hughes, R.N., and J.C. Gamble

1977. A quantitative survey of the biota of intertidal soft substrata on Aldabra Atoll, Indian Ocean. *Philosiphical Transactions of the Royal Society B* 279:327-355.

Rice, M.E.

1970. Asexual reproduction in a sipunculan worm. *Science* 167:1618-1620.

Rice, M.E., and I.G. Macintyre

1979. Distribution of Sipuncula in the coral reef community, Carrie Bow Cay, Belize. *Smithsonian Contributions to Marine Science* 12:311-320.

MOLECULAR GENETIC AND DEVELOPMENTAL STUDIES ON MALACOSTRACAN CRUSTACEA
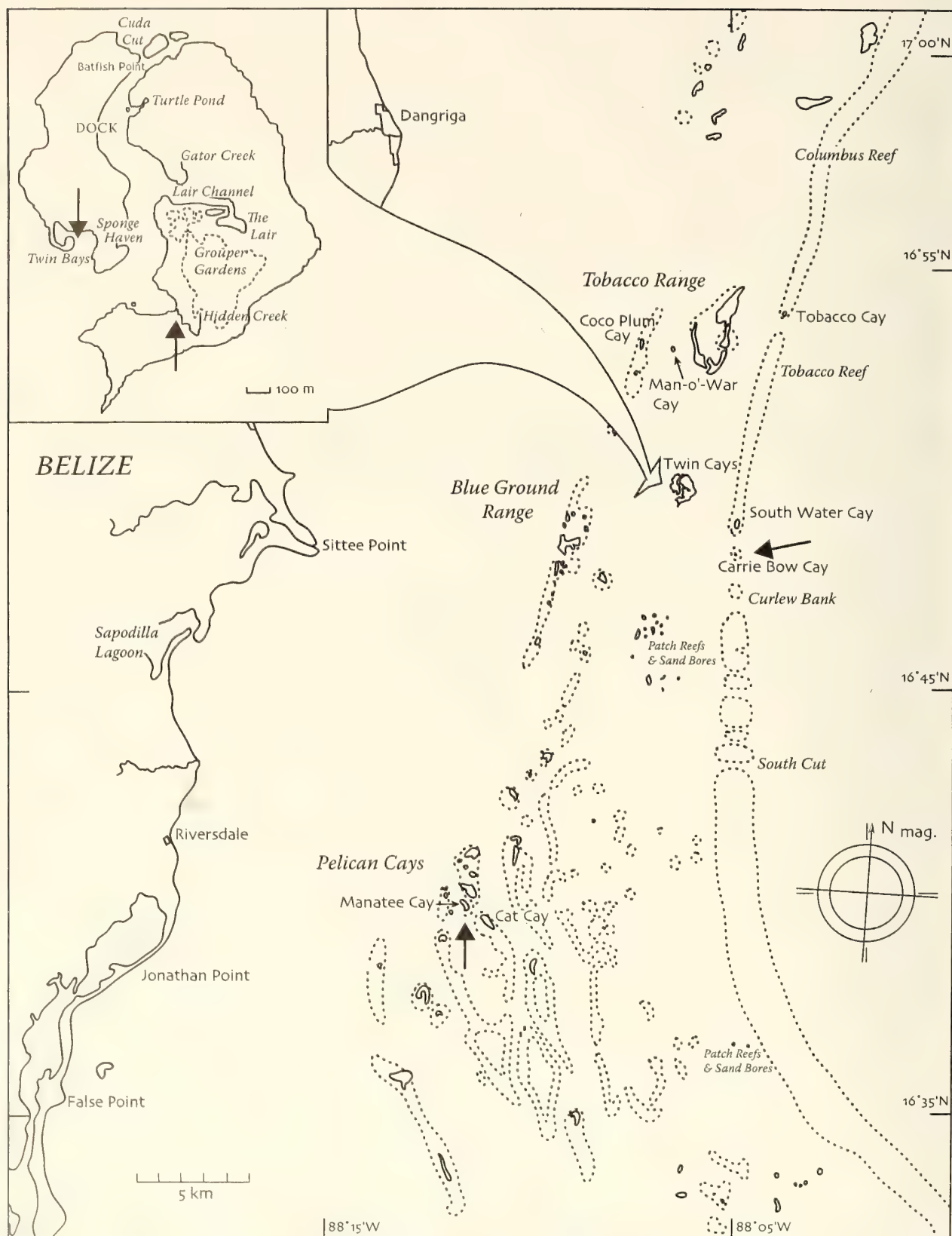
BY

WILLIAM E. BROWNE

Figure 1. Collecting localities (black arrows) at Twin Cays, off Carrie Bow Cay, and in the Pelican Cays

MOLECULAR GENETIC AND DEVELOPMENTAL STUDIES ON MALACOSTRACAN CRUSTACEA

BY

WILLIAM E. BROWNE

ABSTRACT

Arthropods dominate our seas, land, and air and have done so for hundreds of millions of years. Among the arthropods the crustaceans present us with an extremely rich history of morphological change, much of which is still represented among extant forms (morphological disparity among the crustaceans is much higher than in any other group of arthropods). With regard to the Crustacea, several characteristics of the amphipod crustacean embryo make it particularly well suited to embryological manipulations. These include early holoblastic (complete) cleavage coupled with early cell division asymmetries that facilitate microinjection. The high diversity of crustacean taxa near Carrie Bow Cay presents a unique opportunity to extend previous findings in laboratory strains of the amphipod *Parhyale hawaiensis*. In addition, the exploration of standing genetic variation in natural populations may yield important clues in the search for mechanisms by which genes influence organismal development and sculpt morphology through time. The principal collection sites are at south Twin Cays (Twin Bays, Hidden Creek), Manatee Cay (Pelican Cays), and outside the barrier reef near Carrie Bow Cay.

INTRODUCTION

Our ongoing studies of crustacean development and molecular genetics depend greatly on field observations and new samples for laboratory analysis. The Carrie Bow Cay surroundings offer a multitude of habitats from bluewater to coral reefs and mangrove islands within a radius of a few kilometers. The collection sites that we visit regularly include Twin Bays located on the south-west tip of Twin Cays, Hidden Creek on the south end of East Twin Cays, Ctenophore Ridge located off the south tip of Manatee Cay (Pelican Cays), and the water column outside the barrier reef, one-half mile east of Carrie Bow Cay where we make plankton tows at night (Fig. 1).

Kewalo Marine Lab/PBRC, University of Hawaii, 41 Ahui St., Honolulu, HI, 96813.
email: wbrowne@hawaii.edu

Crustacean Diversity

A conservative estimate of the number of extant arthropod species is 1,097,289 (85% of described extant invertebrates). Crustaceans, which (again conservatively) currently number 68,171 extant species, are second only to hexapods in metazoan species diversity. However, the total number of crustaceans, both described and undescribed, is estimated to be 5-10x higher than the current species count (Brusca and Brusca, 2003). For example, peracaridan crustaceans occupying coral reefs alone are thought to number ~54,500 species (Kensley, 1998). Clearly identification of extant crustaceans is far from saturation, and thus the observed diversity of morphological form among crustaceans can only continue to expand.

Several recent studies examining the evolutionary relationships among the major groups of arthropods suggest two possible relationships between the Hexapoda (including insects) and the Crustacea (Fig. 2). One possibility is that the two groups are sister taxa (Boore et al., 1995; Friedrich et al., 1995; Eernisse, 1997; Boore et al., 1998; Giribet et al., 2001) (Fig. 2B). The other possibility is a 'Pancrustacea' clade in which the insects branch from within a paraphyletic Crustacea (Reiger and Shultz, 1997; Hwang et al., 2001) (Fig. 2A). In this scenario, insects would represent a terrestrialized branch of crustaceans.

Under either of these two hypotheses of insect-crustacean relationships, the Crustacea bear the closest affinity to insects among the arthropods. Thus exploration of evolutionary transformations within the crustaceans, and between the crustaceans and insects, should be a high priority for biologists interested in understanding the connections between development and evolution within and between these two clades.
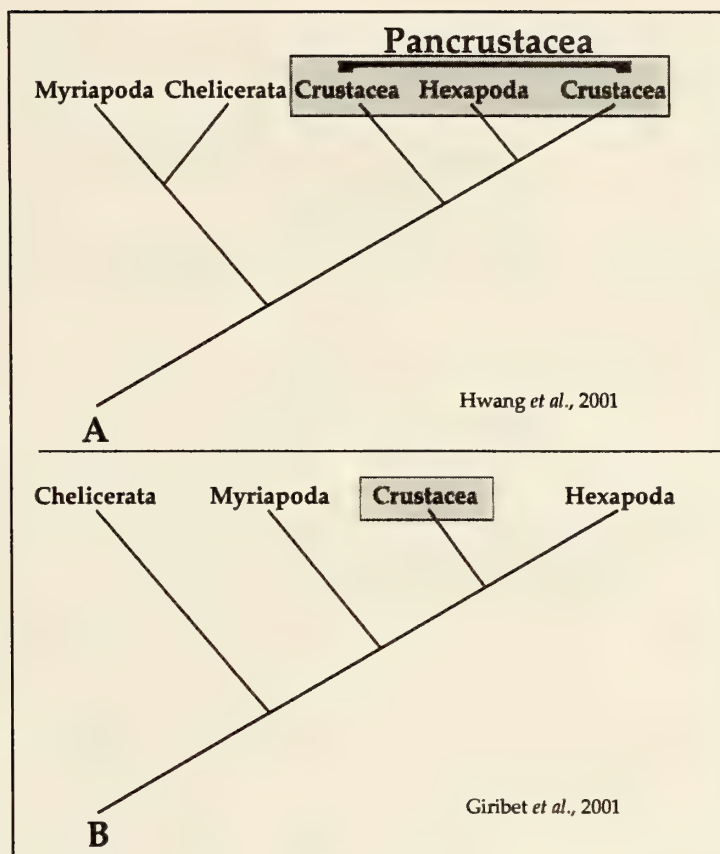
Figure 2. Current hypotheses regarding the Crustacean-Insect relationship.
(A) The 'Pancrustacea' hypothesis places the hexapod lineage within the Crustacea. Under this scenario current morphologic classification schemes of the Crustacea are paraphyletic and are grades. The monophyletic Pancrustacea presents the hexapods as a terrestrialized branch of crustaceans. Data from Hwang et al., 2001 suggests that the more basal Myriapoda and Chelicerata are sister taxa. (B) The competing hypothesis suggests current classification schemes correctly identify a monophyletic Crustacea. Data from Giribet et al., 2001 suggests that the Crustacea and Hexapoda are sister taxa. Their work places the Myriapoda + Crustacea + Hexapoda in a monophyletic clade with chelicerates as the basal outgroup.

Crustacean Appendages: comparative morphology meets comparative gene expression

The Crustacea largely interact with their environment via their appendages; thus vast amounts of variation exist between the different appendages of a single individual as well as between appendages from different species. Comparative studies of crustacean appendage development present an important story regarding the evolution of morphology over both relatively short (a few million years) and relatively long (a few hundred million years) evolutionary time scales. Comparisons of appendage development utilizing molecular and genetic data garnered from *Drosophila* appendage development have been a recurrent theme in recent comparative work in an attempt to understand the molecular basis for some of the variation seen in crustacean limbs (e.g. Williams, 1998; Nulsen and Nagy, 1999; Abzhanov and Kaufman, 2000; Browne and Patel, 2000; Williams et al., 2002).

4

Generally crustacean limbs fall between two morphological extremes (Fig. 3). At one extreme is the lobed phyllopodous appendage composed of limb branches that are broad and laterally compressed (e.g. *Artemia*, *Eubranchipus*, *Triops*) (Fig. 3A). At the other extreme is the seemingly uniramous appendage which appears to be one multiarticulated rod (all other limb branches have been eliminated or greatly reduced) (e.g. *Stenorhynchus*) (Fig. 3C). The ancestral state of the crustacean limb most likely was neither a strictly phyllopodous limb nor a strictly uniramous limb but a biramous limb composed of two primary branches (Fig. 3B) (Schram, 1986).

Despite the variation seen in crustacean limbs, a consistent nomenclature allows us to compare the different limb morphologies (Fig. 3). The region of the limb most proximal to the body wall is termed the coxopodite (historically termed the 'protopod') (blue shading in Fig. 3). The coxopodite may consist of up to three articulating elements (Fig. 3B, 3C) or be a simple fused structure (Fig. 3A). Distal to the coxopodite is the telopodite (lighter shading in Fig. 3). The telopodite includes the main limb branches termed the endopod and exopod (Fig. 3B). The principal ventral branch is the endopod. The principal dorsal branch is the exopod. Additional cuticular structures may be present on the coxopodite; however, they are not multi-jointed structures. Cuticular structures arising ventral and medial to the endopod are termed endites (Fig. 3A); for example, the crustacean gnathobase is often thought to be an elaborated endite. Cuticular structures arising dorsal and lateral to the exopod are termed exites (Fig 3A and 3B). A common exite structure is the epipod that usually serves a respiratory function (Schram, 1986; Manton, 1977; McLaughlin, 1982; Williams and Nagy, 1996).

Crustacean limbs also can be grouped according to their organization along the A-P axis of the body. Different regions (tagmata) of the body possess characteristic types of limbs with characteristic functions. Cephalic appendages typically include two pairs of antennae (an1 and an2) involved in sensory, and often motor, functions. The gnathal region contains the mandibles (mn) and two pairs of maxillary appendages (mx1 and mx2) that are primarily associated with feeding functions. Thoracic and abdominal appendages are of variable numbers and morphologies and are variably involved in feeding, respiration, and locomotion.
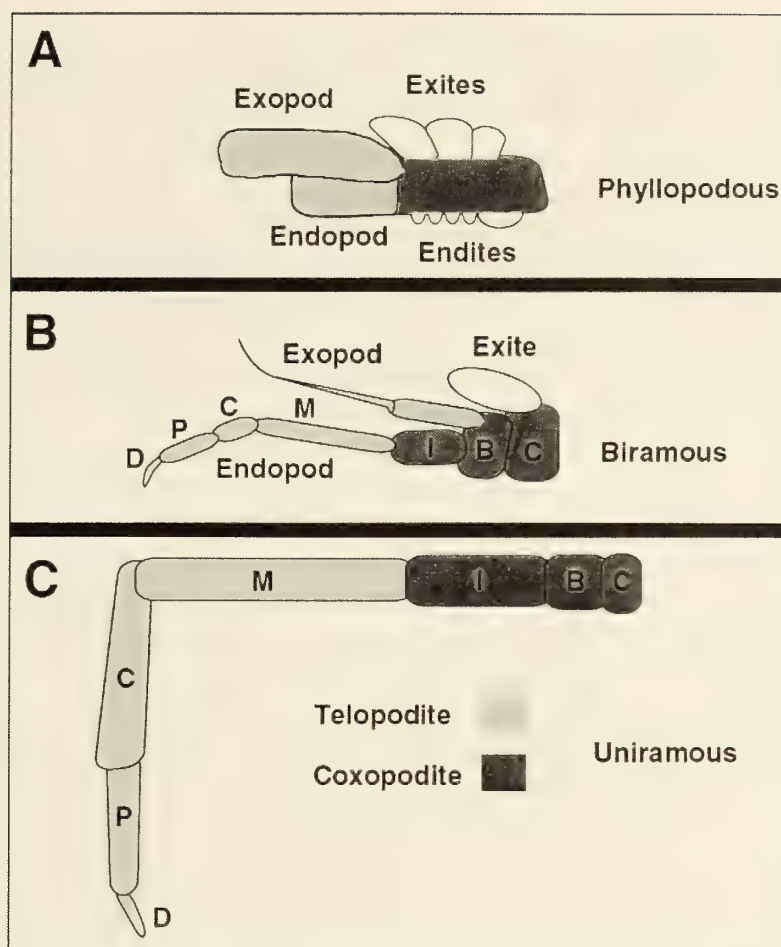
Figure 3. Crustacean appendage morphology.
Ventral is down, dorsal is up. Dark shading indicates the coxopodite, which can be a fused structure (A) or composed of up to three jointed, articulating elements (B and C). Proximal to distal, the three elements are: C-coxa, B-basis, and I-ischuim. Light shading indicates the telopodite, which can be unbranched (C) or include two major distal branches emanating from the coxopodite (A and B). The ventral-most branch is the endopod; the dorsal-most branch is the exopod. While these branches can exhibit considerable variation, the endopod typically constists of four jointed, articulating elements. Proximal to distal, these four elements are: M-merus, C-carpus, P-propodus, and D-dactyl. The coxopodite may also possess a number of cuticular projections that may articulate at the junctions with the coxopodite but are non-jointed. Projections arising ventral and medial to the endopod are endites (A). Projections arising dorsal and lateral to the exopod are exites (A and B).

A crustacean 'model' system for the study of embryonic development and evolution

The use of model systems in developmental biology has played a crucial role in advancing our understanding of biological phenomena in complex multi-cellular organisms such as the metazoans. The six major metazoan model systems in use (the fly *Drosophila melanogaster*, the nematode *Caenorhabditis elegans*, the frog *Xenopus laevis*, the chicken *Gallus domestica*, the zebrafish *Danio rerio* and the mouse *Mus musculus*) share several experimental characteristics that have made them workhorses for developmental and genetic investigation. In all six, the use of forward and reverse

genetic techniques can be employed to alter normal gene expression both temporally and spatially. In addition, techniques for cell lineage analysis and methods of micromanipulation have been developed including microinjection, transplantation, cell explantation, and cell ablation. The wide breadth of experimental techniques available in these systems allows for complex developmental questions regarding gene function, cell fate, and pattern formation to be explored. The results from these studies can be used as starting points for broader investigation of metazoan pattern formation and changes in both morphology and gene function through evolutionary time.

There are currently ~1.33 million described species of metazoans. Each, of course, bears a unique genome shaped by a unique evolutionary history. Of this number, the invertebrate grade represents 96% of metazoan species. Vertebrates represent the remaining 4% of metazoans (Brusca and Brusca, 2003). Of the six major model systems, four are vertebrates. A realistic understanding of biological diversity is further hindered by the fact that identification of extant invertebrates is far from saturation, whereas identification of new vertebrate species has slowed and is likely close to complete. While significant data has been obtained from each of the major model systems in use, comparisons to other non-model taxa are necessarily constrained by the current limited, and skewed, sample size. In particular, comparative data to date has largely been informative strictly with regard to issues of conservation of gene expression and/or gene function. This is due to the vast evolutionary distances that exist between the current model systems. Extrapolations from model system data sets can be problematic. For example the two invertebrate model systems, *C. elegans* and *Drosophila*, share a common ancestor well over 550 million years ago, time enough to mask the evolutionary transitions that have crafted nematodes in one case and flies in the other case.

In the past 15 years the number of non-model taxa in which descriptive analyses of gene expression have been made has steadily increased. Thus far, the interpretable data has largely served to reinforce concepts related to conservation of expression (e.g. Patel et al., 1989). Again this is due, in large part, to the paucity of data reported in non-model organisms. Among these non-model taxa currently being utilized, a small number can now be considered as 'minor' model systems in which techniques for reverse genetics are beginning to be successfully applied and some micromanipulations have proven to be feasible (for example, the long history of the sea urchin, *Strongylocentrotus purpuratus*, as a developmental system and the more recent history of the flour beetle, *Tribolium castaneum*, as a developmental and potential genetic system).

Evolution acts on species at the level of the population and, as barriers to gene exchange arise, independent lineages are generated. Each isolated lineage, or species, can then be described by virtue of unique characters not shared with other lineages or species (Harrison, 1998; de Queiroz, 1998; Shaw, 1998). Evolution by the process of lineage splitting generates differences between extant species and the relationships between groups of extant species are largely assessed via extrapolation from comparative observations between living representatives of a given lineage. This is particularly the case with regard to recent examinations of embryological phenomena. While this type of observation, in particular of gene expression patterns, in non-model systems provides suggestive data for conservation and, more recently, the divergence or convergence of specific characters, processes, and/or mechanisms (e.g. Patel et al., 1989; Davis et al.,

2001; Abouheif and Wray, 2002), it is only functional data generated in the small number of major and minor model system taxa that allow for secure and robust interpretations regarding any observed changes in gene expression.

Thus, we are currently presented with many critical unanswered questions regarding the tempo (rate or pace) and mode of evolutionary change over time, as well as how evolution has generated the full range of extant biological diversity, that cannot be addressed with the current complement of model systems for embryonic development. This problem is being addressed by 'gap-filling' with new systems more closely related to model systems currently in use by researchers. Optimally these new systems are taxa in which functional studies can be feasibly designed and implemented. In this way we are beginning to identify important differences between species that can be shown, by functional experimentation, to have evolutionary significance.

The Amphipoda

The amphipods [Peracarida; Malacostraca; Crustacea] are commonly referred to as beachhoppers or scuds. Within the Crustacea, amphipods rank as one of the most ecologically successful and speciose extant orders and occur in nearly all known marine, fresh, and brackish water environments as well as in high-humidity terrestrial ecosystems (such as tidal zones, coastal flood plains, and forest leaf litter) (e.g. Barnard and Karaman, 1991; Vinogradov et al., 1996; Lindeman, 1991; Sherbakov et al., 1999; Kamaltynov, 1999; Vainola and Kamaltynov, 1999; Sheader et al., 2000; Poltermann et al., 2000). They have predominately exploited scavenging niches and thus an apt description for the group would be 'the flies of the sea'. The ecological diversity represented in the group is reflected in similarly high levels of morphological disparity. Several thousand amphipod species have been described (>7000), and the current rate of several new species descriptions per year suggests that the upper limit of extant amphipod species is far higher than the current species count. Phylogenetic relationships among amphipods remain poorly resolved with the current suites of morphological characters in use by systematists (Fig. 4) (Martin and Davis, 2001; Kim and Kim, 1993). However there are distinct characters that unite amphipods as a natural, monophyletic group. Most recognizable among these characters are lateral compression of the body, sessile compound eyes, and the orientation of the thoracomere appendages (periopods) to the body axis (periopods 1-2 orient anteriorly, periopods 3-5 orient posteriorly, thus the name for the group, amphipod) (Plate 1, A-B). Additionally amphipod thoracic appendages bear two dorsal branches of interest, large coxal plates that have become flattened, heavily cuticularized, protective sheets attached dorsally to the base of thoracic appendages (Plate 1, A-B) and the gills that are also laterally compressed but have a highly complex internal network of branching tubes used for gas exchange. The cephalon has a unique organization in which thoracomere 1 (t1), bearing the maxillipeds, is fused to the head. This fusion is accompanied by a close arrangement of the gnathal appendages, including the maxillipeds, in a basket shape around the mouth to form a highly compact buccal mass (Plate 1, B). The Amphipoda are a monophyletic, species-rich, assemblage and amphipod groups contain a web of complex relationships between members. These are the hallmarks of a highly successful evolutionary lineage.

8

The marine amphipod, *Parhyale hawaiensis* (Dana, 1853), is well suited for both mechanistic and functional genetic studies within crustaceans (Browne, 2003; Browne and Patel, 2000; Gerberding et al., 2002). Several aspects of *Parhyale* embryological development are derived when compared to other peracaridian species such as isopods and mysids (cell lineage and early cell-cleavage patterns, as well as later gene expression correlating with the development of specific morphological structures). Examples of the types of characters we are actively exploring are variations in early embryonic cleavage patterns and associated cell lineages (Gerberding et al., 2002) and changes in the expression patterns of genes involved in embryonic segmentation, limb patterning, and nervous system patterning (Browne, 2003; Browne and Patel, 2000; Duman-Scheel and Patel, 1999; Averof and Patel, 1997).
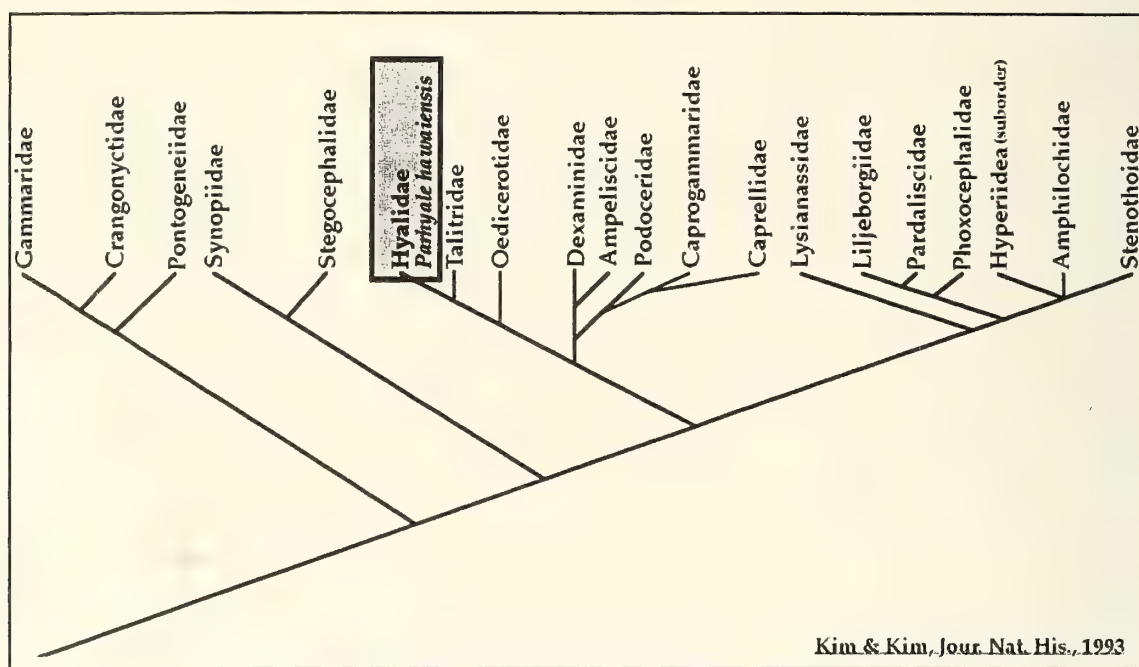
Figure 4. Proposed relationships among some of the major groups of amphipods.
The Kim and Kim, 1993 phylogenetic analysis of several morphological characters propose a monophyletic Hyalidae (indicated by red box). *Parhyale hawaiensis* is a member of this large family of amphipods. The Talitridae are considered sister taxa to the hyalids. The talitrids include *Orchestia cavimana*, an amphipod in which cell lineage and gene expression analyses have been reported (e.g. Wolff et al., 2002; Scholtz et al., 1994). It is important to note that the affinities among most amphipod groups are far from resolved and this represents only a first approximation of relationships within the Amphipoda.

FIELD WORK AND LABORATORY STUDIES

How to explain the circumtropical distributions of *Parhyale hawaiensis* and *Stenopus hispidus*: Ecotypes or Species Complexes?

Both *Parhyale hawaiensis* and the coral-banded shrimp, *Stenopus hispidus,* are present in both Atlantic and Pacific Oceans. Both have largely overlapping circumtropical distributions based on their respective morphological descriptions. Their

life histories, however, are vastly different from one another. Ecologically, *Parhyale hawaiensis* is a detritovour that has a circumtropical, worldwide, intertidal, and shallow-water marine distribution (Shoemaker, 1956; Barnard, 1965) (Fig. 5), possibly existing as a species complex (Myers, 1985).
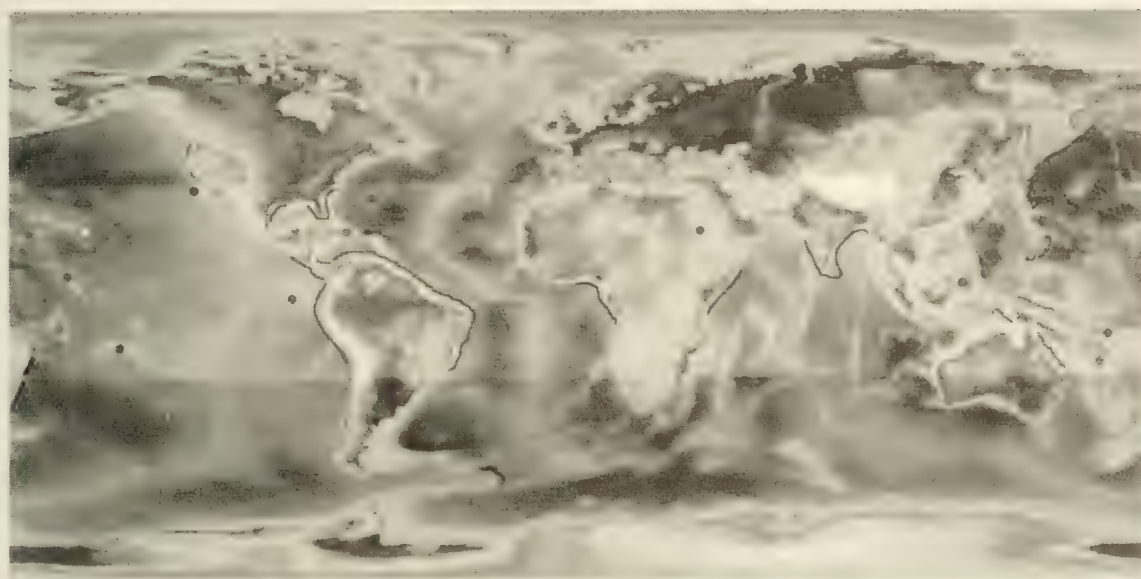

Figure 5. Geographical distribution of *Parhyale hawaiensis*.
Light-shaded region indicates the approximate north-south boundaries capable of supporting *Parhyale hawaiensis*. The range of *P. hawaiensis* is extensive; they inhabit shallow water environments and are found associated with continental coastlines (including bays and estuaries), mangrove forests, shallow reefs, marine atolls, seamounts, etc. Black markings indicate known ranges [Atlantic: Texas, Florida, North Carolina, Bermuda, Haiti, Dominican Republic, Puerto Rico, St. Croix, Curacao, Bonaire, Panama, Colombia, Venezuela, Brazil, Democratic Republic of the Congo] [Pacific: Lower California, Costa Rica, Panama, Ecuador, Galapagos Islands, Hawaii, Johnston Island, throughout Oceanica, Polynesia, Micronesia, Bay of Bengal, India, Arabian Sea, Red Sea, East Africa]. Type locality, Maui, is indicated with a black/white bull's-eye.

In the case of *Parhyale hawaiensis*, though widely distributed, they inhabit shallow waters and are a benthic species. In addition, embryonic development occurs in a protected environment, is direct, and the juveniles are benthic. Thus, dispersal across large bodies of water for this species is presumably a significant problem. The broad distribution of *Parhyale hawaiensis* in the face of this dispersal problem suggests that many populations of *Parhyale hawaiensis* may be relatively isolated from one another. Gene-flow analysis is a useful tool to employ to attempt to determine the degree of genetic exchange between population 'islands'. A possible scenario would be that *Parhyale hawaiensis* exists as groups of loosely connected ecotypes and/or as a species complex. Importantly, if morphological variation were found to correlate with observed population structure, the connection between genetic change and morphological change within a single species could be addressed (or very closely related species, if a species flock exists). Work is in progress regarding population genetics in *Parhyale hawaiensis*.

In contrast to *Parhyale*, *Stenopus hispidus* (Plate 1, C) possesses a benthic adult reproductive phase coupled with a pelagic larval phase. In this case a potential mechanism for wide dispersal of *Stenopus hispidus* would appear to exist. Interestingly observations of adult populations at locations in both the Atlantic and Pacific suggest that juveniles are often settling at depths in excess of 35-40 ft along less protected outer-reef walls, while larger reproductive adults in pairs predominate in less exposed, shallow, inner reefs. In habitats that are exposed to significant disturbance for extended periods no stratification of *Stenopus hispidus* by age is observed. This is suggestive of a recruitment regime that could be in part deciphered with robust gene-flow information regarding the connections between populations. Currently several *Stenopus hispidus* genetic loci are being assayed to address these questions including the CO1, ITS-1, 12S, and cytb genes.

Parhyale hawaiensis: Early Development and Lineage Analysis

Observations of cell lineages resulting from holoblastic cell cleavage have been made in just a few animals. These observations of the differential movement of cell populations relative to one another have been made utilizing simple visual discrimination techniques as well as by injection of tracers designed to label a specific cell and its resulting progeny. Invariant cell-lineage patterns have been described in the nematode *C. elegans* (Sulston et al., 1981), the ascidian *H. roretzi* (Nishida, 1987), and the annelid *H. triserialis* (Weisblat et al., 1984).

Among the arthropods, most insects examined, such as *Drosophila*, have superficial cleavage early in development and thus appear to lack invariant cell lineages during early development. Early cell fates in *Drosophila* embryogenesis appear to be governed in large part by the regulation of positional information cascades arrayed along the embryonic anterior-posterior and dorsal-ventral axes (summarized in Fig. 6). The presence of identifiable, invariant, cell lineages in insects, such as *Drosophila*, are restricted to specific tissues such as the nervous system later in development.
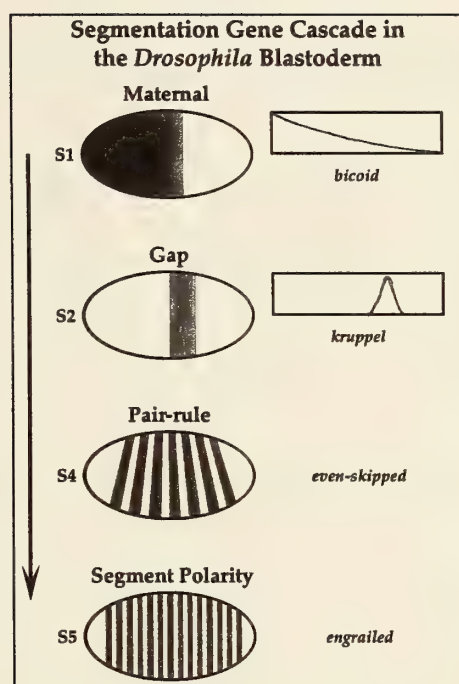
**Segmentation Gene Cascade in
the *Drosophila* Blastoderm**

Maternal

S1

bicoid

Gap

S2

kruppel

Pair-rule

S4

even-skipped

Segment Polarity

S5

engrailed

Figure 6. Schematic Representation of the Segmentation Gene Cascade in *Drosophila*.
The arrow indicates the time axis. Embryos are oriented anterior to the left and dorsal up. The metameric organization of *Drosophila* is generated via deployment of a hierarchical gene cascade. First the anterior-posterior axis is established by opposing maternal gradients (for example the *bicoid* gene) in stage 1 embryos (S1). The relative concentrations of these maternal gradients (schematized in block form to the right of the embryo) regulate zygotic expression of the next group of genes in the cascade, gap genes (for example *hunchback, kruppel, giant, knirps*, etc.). This group of genes is deployed in stage 2 (S2) embryos. The wide gap gene expression domains are used as cues for the next group of genes to be expressed in the cascade, pair-rule genes (for example *hairy, fushi tarazu, even-skipped, paired, runt*, etc.). This group of genes is deployed in the syncytial blastoderm of stage 4 (S4) embryos. The pair-rule genes pattern the A-P axis with a two-segment periodicity. This two-segment periodicity of gene expression is followed by expression of segment polarity genes (for example *engrailed, gooseberry, patched, wingless*, etc.). This group of genes is deployed in the cellular blastoderm of stage 5 (S5) embryos. Segment polarity genes define compartment boundaries within each developing parasegment. The parasegment unit serves as the reiterated metamer upon which morphological segments are patterned.

A number of crustaceans have total cleavage in early embryogenesis; however, only a small number of studies have attempted to determine whether invariant cell lineages occur (Bigelow, 1902; Hertzler et al., 1992; Hertzler et al., 1994; Gerberding et al., 2002, Wolff et al., 2002). The unique early blastomere arrangement in amphipods has been well described (Langenbeck, 1898; Weygoldt, 1958; Scholtz, 1990) but only two lineage studies have been completed (Gerberding et al., 2002; Wolff et al, 2002). Historically, the literature has suggested that crustaceans exhibit spiral cleavage (e.g. Shiino, 1957; Anderson, 1969; Anderson, 1973; Nielsen, 2001).

To the contrary, in *Parhyale* we find a very clear radially based early cleavage program. At the eight-cell stage, *Parhyale* embryos have four macromeres and four micromeres and each blastomere lineage is restricted to a single germ layer. There is no obvious resemblance between lineage patterns observed in *Parhyale* and those described

among spiralians, nematodes, and deuterostomes. In addition, the *Parhyale* lineage maps unexpectedly differ substantially from the few partial lineage maps described for most other crustaceans (Gerberding et al., 2002).

Head Gap Gene Ortholog Expression and Function in *Parhyale hawaiensis* Neurogenesis

Disparity of form within the crustacean is intimately associated with the ability of the crustacean nervous system to interface with the local environment, integrate information, and respond to changing conditions. A large body of work exists regarding the neuroanatomy of the Crustacea (e.g. Sandeman et al., 1992; Harzsch et al., 1999; Harzsch, 2001; Harzsch and Glotzner, 2002). In addition, recent comparative studies between crustaceans and insects have suggested both strong similarities and notable differences in neuronal morphology (Whitington et al., 1993; Whitington, 1996). These initial studies have been extended to suggest the homology between a small number of specific neuronal identities by correlating similarities in neuronal morphology with the expression of molecular markers (Duman-Scheel and Patel, 1999).

The crustacean brain possesses a great deal of variation that would seem to correlate with changes in the degree of terrestrialization, dependence on visual stimuli, and feeding habits (Schmitz, 1992; Thompson et al., 1994). Clearly anterior head development is quite different between *Parhyale* and the fly *Drosophila*. Current work exploring the dynamics of gene expression in the head and brain of *Parhyale* (Plate 1, D) seeks to explore the role of these regulatory genes in crustacean brain and nervous system development. The natural outgrowth of this data, in an evolutionary and developmental context, is to look at these patterning mechanisms in different, but related, head/brain and nervous systems. Within the Amphipoda the hyperiids demonstrate dramatic changes in head morphologies (Vinogradov et al., 1996).

Relationships Among and Within the Amphipoda, Pelagic Hyperiids: 'Cracking' the Amiphipod Code

As things stand now the Amphipoda are generally organized into two groups, the largely benthic gammarids (to which *Parhyale hawaiensis* belongs) and the exclusively pelagic hyperiids (it is highly likely that the hyperiids, as currently recognized, are a polyphyletic assemblage). Phylogenetic resolution among the Amphipoda is currently poor. Notably, gammarid and hyperiid amphipods have very sharp differences in the organization of their heads and anterior nervous systems, which are most likely due to constraints imposed by their very different respective life histories. Detailed studies of hyperiids are very few due to their exclusively pelagic life history.

Fairly stable populations of the hyperiid *Glossocephalus milneedwardsi* and its host ctenophore, *Mnemiopsis sp* (Plate I, E) can be found at shallow depths along the submerged ridge (Ctenophore ridge) extending from the southern tip of Manatee Cay (Pelican Cays). This area is somewhat sheltered from open water by Cat Cay to the east; however, a strong upwelling current here brings large numbers of ctenophores to the surface. The vast majority of the ctenophore swarms are composed of *Mnemiopsis*

with some *Beroe*. Approximately 10-15% of the *Mnemiopsis* individuals carry the associated *Glossocephalus*. The availability of large numbers of *Glossocephalus* adults, juveniles, and embryos, with their host *Mnemiopsis* along the shallow ridges in the Pelican Cays represents a unique and rare opportunity to observe hyperiid behavior *in situ*, and to obtain high quality embryonic material for molecular work. The Pelican Cay population of *Glossocephalus* is extremely compelling in this regard and continued work on this species can fill a void in current knowledge regarding hyperiid amphipods.

Glossocephalus appears to have a non-parasitic relationship with *Mnemiopsis*, involved perhaps with cleaning the host surface at regular intervals (observations from both the field and animals observed in holding tanks at Carrie Bow Cay). The adult, juvenile, and embryonic stages of *Glossocephalus* have the same optically transparent properties of the *Mnemiopsis* host. Interestingly, newly fertilized single-celled *Glossocephalus* embryos appear to have a large lipid droplet sequestered within the yolk. Light microscopy examination of the first few cell cleavage events in live embryos allows tracking of the lipid droplet as it is progressively compartmentalized to one side of the embryo (the transparent quality of the embryo precludes direct observation of cell cleavage planes). Later in development the droplet is sequestered in the developing midgut. As the midgut begins digesting remaining yolk reserves, the droplet is observed breaking down in the digestive ceacum and anterior region of the maturing midgut.

Three lines of investigation are currently being undertaken in *Glossocephalus*. As their head morphology is radically different from that of *Parhyale*, I am interested in embryonic patterning events during early head ectoderm development and brain development that differ between the two species. Formal lineage analysis in *Glossocephalus* will provide an important contrast with that of *Parhyale* (Gerberding et al., 2002) and *Orchestia* (Wolff et al, 2002) regarding the evolution of the invariant cell lineage observed in these two species of gammarid amphipods. Finally the hyperiid amphipod life history and behavioral aspects of host interaction make the population of *Glossocephalus* found near Carrie Bow Cay an important study group, particularly since there is a marked paucity of data in the literature on this group. A number of modifications to various limb appendages appear to directly support their interactions with host ctenophores. Additional behavioral documentation along with morphological analysis of appendage morphology should shed light on the host/symbiont relationship.

CONCLUDING REMARKS

The intense examination of laboratory strains of *Parhyale hawaiensis* in combination with comparative studies will yield important clues in the search for mechanisms by which genes influence organismal development and sculpt morphology. Ongoing comparative investigations of crustacean brain/nervous system development, appendage development, cell lineage analysis, and population structure in related taxa will provide invaluable information regarding how these patterning mechanisms change through time. The unique mangrove Cay/barrier reef environment near Carrie Bow Cay, in combination with the field station facilities, provides easy access to a number of crustacean species important to these comparative investigations.

ACKNOWLEDGMENTS

REFERENCES

Abouheif, E., and G.A. Wray
    2002. Evolution of the Gene Network Underlying Wing Polyphenism in Ants. *Science* 297:249-252.

Anderson, D.T.
    1969. On the embryology of the cirrepede crustaceans Tetraclita rosea (Krauss), Tetraclita purpurascens (Wood), Chthamalus antennatus (Darwin), and Chamaesipho columna (Spengler) and some considerations of crustacean phylogenetic relationships. *Philosophical Transactions of the Royal Society of London, Series B* 256:183-235.

Anderson, D.T.
    1973. Embryology and Phylogeny in Annelids and Arthropods. Oxford: Pergamon Press.

Averof, M., and N.H Patel.
    1997. Crustacean appendage evolution associated with changes in Hox gene expression. *Nature* 388:682-686.

Abzhanov, A., and T.C. Kaufman
    2000. Homologs of Drosophila Appendage Genes in the Patterning of Arthropod Limbs. *Developmental Biology* 227:673-689.

Barnard, J.L.
    1965. Marine Amphipoda of Atolls in Micronesia. *Proceedings of the United States National Museum* 117:459-551.

Barnard, J.L., and G.S. Karaman
    1991. The Families and Genera of Marine Gammaridean Amphipoda (Except Marine Gammaroids). *Records of the Australian Museum Supplement* 13:1-866.

Bigelow, M.A.
    1902. The early development of Lepas. *Bulletin of the Museum of Compartive Zoology* 40:61-144.

Boore, J.L., T.M. Collins, D. Stanton, L.L. Daehler, and W.M. Brown
  1995. Deducing the pattern of arthropod phylogeny from mitochondrial DNA
        rearrangements. *Nature* 376:163-165.
Boore, J.L., D.V. Lavrov, and W.M. Brown
  1998. Gene translocation links insects and crustaceans. *Nature* 392:667-668.
Browne, W.E.
  2003. Ph.D. Thesis. The embryonic development of *Parhyale hawaiensis*. The
        University of Chicago.
Browne, W.E., and, N.H. Patel
  2000. Molecular genetics of crustacean feeding appendage development and
        diversification. *Seminars in Cell and Developmental Biology* 11:427-435.
Brusca, R.C., and G.J. Brusca
  2003. Invertebrates: Sinauer Associates, Inc.
Dana, J.D.
  1853. Crustacea. Part II. *United States Exploring Expedition* 14:689-1618.
Davis, G.K., C.A. Jaramillo, and N.H. Patel
  2001. Pax group III genes and the evolution of insect pair-rule patterning.
        Development 128:3445-3458.
de Queiroz, K.
  1998. The General Lineage Concept of Species, Species Criteria, and the Process of
        Speciation: A Conceptual Unification and Terminological Recommendations. In
        Endless Forms: Species and Speciation, (ed. D. J. Howard and S. H. Berlocher),
        pp. 57-75. Oxford: Oxford University Press.
Duman-Scheel, M., and N.H. Patel
  1999. Analysis of molecular marker expression reveals neuronal homology in
        distantly related arthropods. *Development* 126:2327-2334.
Eernisse, D.J.
  1997. Arthropod and annelid relationships re-examined. In *Arthropod Relationships*,
        (ed. R.A. Fortey and R.H. Thomas), pp. 43-56. London: Chapman and Hall.
Friedrich, M., and D. Tautz
  1995. Ribosomal DNA phylogeny of the major extant arthropod classes and the
        evolution of myriapods. *Nature* 376:165-167.
Gerberding, M., W.E. Browne, , and N.H. Patel
  2002. Cell lineage analysis of the amphipod crustacean Parhyale hawaiensis reveals an
        early restriction of cell fates. *Development* 129:5789-5801.
Giribet, G., G.D. Edgecombe, and W.C. Wheeler
  2000. Arthropod phylogeny based on eight molecular loci and morphology. *Nature*
        413:157-161.
Harrison, R.G.
  1998. Linking Evolutionary Pattern and Process: The Relevance of Species Concepts
        for the Study of Speciation. In *Endless Forms: Species and Speciation*,
        (ed. D.J. Howard and S. H. Berlocher), pp. 19-31. Oxford: Oxford University
        Press.
Harzsch, S., J. Miller, J. Benton, and B. Beltz,
  1999. From Embryo to Adult: Persistent Neurogenesis and Apoptotic Cell Death

16

Shape the Lobster Deutocerebrum. *Journal of Neuroscience* 19:3472-3485.

Harzsch, S.
  2000. Neurogenesis in the crustacean ventral nerve cord: homology of neuronal stem cells in Malacostraca and Branchiopoda? *Evolution and Development* 3:154-169.

Harzsch, S., and J. Glotzner
  2001. An immunohistochemical study of structure and development of the nervous system in the brine shrimp Artemia salina Linnaeus, 1758 (Branchiopoda, Anostraca) with remarks on the evolution of the arthropod brain. *Arthropod Structure and Development* 30:251-270.

Hertzler, P.L., and W.H. Clark, Jr.
  1992. Cleavage and gastrulation in the shrimp *Sicyonia ingentis*: invagination is accompanied by oriented cell division. *Development* 116:127-140.

Hertzler, P.L., S.W. Wang, and W.H. Clark Jr.
  1993. Mesendoderm Cell and Archenteron Formation in Isolated Blastomeres from the Shrimp Sicyonia ingentis. *Developmental Biology* 164:333-344.

Hwang, U.W., M. Friedrich, D. Tautz, C.J. Park, and W. Kim
  2002. Mitochondrial protein phylogeny joins myriapods with chelicerates. *Nature* 413: 154-157.

Kamaltynov, R.M.
  1999. On The Evolution of Lake Baikal Amphipods. *Crustaceana* 72:921-931.

Kensley, B.
  1998. Estimates of species diversity of free-living marine isopod crustaceans on coral reefs. *Coral Reefs* 17:83-88.

Kim, C.B., and W. Kim
  1994. Phylogenetic relationships among gammaridean families and amphipod suborders. *Journal of Natural History* 27:933-946.

Langenbeck, C.
  1898. Formation of the germ layers in the amphipod *Microdeutopus gryllotalpa* costa. *Journal of Morphology* 14:301-336.

Lindeman, D.
  1991. Natural history of the terrestrial amphipod *Cerrorchestia hyloraina* Lindeman (Crustacea: Amphipoda; Talitridae) in a Costa Rican cloud forest. *Journal of Natural History* 25:623-638.

Manton, S.M.
  1977. The Arthropoda: Habits, Functional Morphology, and Evolution: Clarendon Press.

Martin, J.W., and G.E. Davis
  2001. An Updated Classification of the Recent Crustacea. In *Natural History Museum of Los Angeles County, Science Series*, (ed., pp. 1-123. Los Angeles.

McLaughlin, P.A.
  1982. Comparative Morphology of Crustacean Appendages. In *The Biology of Crustacea: Embryology, Morphology, and Genetics*, vol. 2 (ed. L. G. Abele), pp. 197-256. New York: Academic Press.

Myers, A.A.
   1985. Shallow-water, Coral Reef and Mangrove Amphipoda (Gammaridea) of Fiji. *Records of the Australian Museum Supplement* 5:1-143.

Nielsen, C.
   2001. Animal Evolution: Interrelationships of the Living Phyla: Oxford University Press.

Nishida, H.
   1987. Cell lineage analysis in ascidian embryos by intracellular injection of a tracer enzyme. III. Up to the tissue restricted stage. *Developmental Biology* 121:526-541.

Nulsen, C. and Nagy, L.M.
   1999. The role of wingless in the development of multibranched crustacean limbs. *Development, Genes, and Evolution* 209:340-348.

Patel, N.H., T.B. Kornberg, and C.S. Goodman
   1989a. Expression of engrailed during segmentation in grasshopper and crayfish. *Development* 107:201-212.

Patel, N.H., E. Martin-Blanco, K.G. Coleman, S.J. Poole, M.C. Ellis, T.B. Kornberg, and C.S. Goodman
   1989b. Expression of engrailed Proteins in Arthropods, Annelids, and Chordates. *Cell* 58:955-968.

Poltermann, M., H. Hop, and S. Falk-Peterson
   2000. Life under Arctic sea ice - reproduction strategies of two sympagic (ice-associated) amphipod species, *Gammarus wilkitzkii* and *Apherusa glacialis*. *Marine Biology* 136:913-920.

Regier, J.C., and J.W. Shultz
   1997. Molecular Phylogeny of the Major Arthropod Groups Indicates Polyphyly of Crustaceans and a New Hypothesis for the Origin of Hexapods. *Molecular Biology and Evolution* 14:902-913.

Sandeman, D., R. Sandeman, C. Derby, and M. Schmidt
   1992. Morphology of the Brain of Crayfish, Crabs, and Spiny Lobsters: A Common Nomenclature for Homologous Structures. *Biological Bulletin* 183:304-326.

Schmitz, E.H.
   1992. Chapter 10: Amphipoda. In *Microscopic Anatomy of Invertebrates*, vol. 9: Crustacea (ed. F. W. Harrison), pp. 443-528. New York: Wiley-Liss, Inc.

Schram, F.R.
   1986. Crustacea: Oxford University Press.

Scholtz, G.
   1990. The formation, differentiation and segmentation of the post-naupliar germ band of the amphipod *Gammarus pulex* L. *Proceedings of the Royal Society of London Series B* 239:163-211.

Scholtz, G., N.H. Patel, and W. Dohle
   1994. Serially homologous engrailed stripes are generated via different cell lineages in the germ band of amphipod crustaceans (Malacostraca, Peracarida). *International Journal of Developmental Biology* 38:471-478.

18

Shaw, K.L.

    1998. Species and the Diversity of Natural Groups. In *Endless Forms: Species and Speciation*, (ed. D. J. Howard and S. H. Berlocher), pp. 44-56. Oxford: Oxford University Press.

Sheader, M., C.L.Van Doverand , and T.M. Shank

    2000. Structure and function of *Halice hesmonectes* (Amphipoda: Pardaliscidae) swarms from hydrothermal vents in the eastern Pacific. *Marine Biology* 136: 901-911.

Sherbakov, D.Y., R.M. Kamaltynov, O.B. Ogarkov, R.Vainola, J.K. Vainio, and E.Verheyen

    1999. On the Phylogeny of Lake Baikal Amphipods in the Light of Mitochondrial and Nuclear DNA Sequence Data. *Crustaceana* 72:911-919.

Shiino, S.M.

    1957. Crustacea. In *Invertebrate Embryology*, (ed. M. Kume and K. Dan), pp. 333-338. Tokyo: Bai Fu Kan Press.

Shoemaker, C.R.

    1956. Observations on the amphipod genus Parhyale. *Proceedings of the United States National Museum* 106:345-358.

Sulston, J.E., E. Schierenberg, , J.G. White, and J.N. Thomson

    1981. The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Developmental Biology* 100:64-119.

Thompson, K.S.J., M.P. Zeidler, and J.P. Bacon

    1994. Comparative Anatomy of Serotonin-Like Immunoreactive Neurons in Isopods: Putative Homologues in Several Species. *Journal of Comparative Neurology* 347:553-569.

Vainola, R., and R.M. Kamaltynov

    1999. Species Diversity and Speciation in the Endemic Amphipods of Lake Baikal: Molecular Evidence. *Crustaceana* 72:945-956.

Vinogradov, M.E., A.F. Volkov, and, T.N. Semenova

    1996. Hyperiid Amphipods (Amphipoda, Hyperiidea) of the World Oceans. Washington, D.C.: Smithsonian Institution Libraries.

Weisblat, D.A., S.Y. Kim, and G.S. Stent

    1984. Embryonic origins of cells in the leech *Helobdella triserialis*. *Developmental Biology* 104:65-85.

Weygoldt, P.

    1958. Die Embryonalentwicklung des Amphipoden Gammarus pulex pulex (L). *Zool. Jb. Anat.* 77:51-110.

Whitington, P.M.

    1996. Evolution of neural development in the arthropods. *Seminars in Cell & Developmental Biology* 7:605-614.

Whitington, P.M., D. Leach, and R. Sandeman

    1993. Evolutionary change in neural development within the arthropods: axonogenesis in the embryos of two crustaceans. *Development* 118:449-461.

Williams, T.A.
    1998. Distalless expression in crustaceans and the patterning of branched limbs. *Development, Genes, and Evolution* 207:427-435.

Williams, T.A., and L.M. Nagy
    1996. Comparative limb development in insects and crustaceans. *Seminars in Cell and Developmental Biology* 7:615-628.

Williams, T.A., C. Nulsen, and L.M. Nagy
    2002. A Complex Role for Distal-less in Crustacean Appendage Development. *Developmental Biology* 241:302-312.

Wolff, C., and G. Scholtz
    2002. Cell Lineage, Axis Formation, and the Origin of Germ Layers in the Amphipod Crustacean *Orchestia cavimana*. Developmental Biology.

PLATE I

A-B. The *Parhyale hawaiensis* body plan.
(A) Schematic of adult body plan. The cephalon (head) is in white and consists of the first six segments plus the first segment of the pleon (thoracomere 1). All segments from the second cephalic segment posterior bear a pair of appendages. For the cephalon these appendages from anterior to posterior are; antennae 1 (an1), antennae 2 (an2), mandibles (mn), first maxillaries (mx1), second maxillaries (mx2), and the maxillipeds of thoracomere 1 (t1). The pereon, composed of thoracomeres 2-8 (t2-t8), is coded red. Each thoracomere of the pereon possesses paired appendages. The proximal most element of each appendage, the coxa, has a dorsal branch which is compressed and expanded into a structure called the coxal plate which closely follows the margin of its associated thoracomere body wall. The appendages of thoracomeres 2 and 3 are distinctly subchelate in form and termed gnathopods. Thoracomeres 4-8 possess appendages termed periopods. The first two pairs of periopods are oriented anteriorly whereas periopods on thoracomeres 5-8 are oriented posteriorly. The first three segments of the abdomen (a1-a3) are grouped into the pleon. Each bears a pair of appendages termed pleopods. The final three segments of the abdomen (a4-a6) are grouped into the urosome. Each urosome segment bears a pair of uropods. The animal terminates along its anterior-posterior axis with a telson, which is a cleft flap of cuticle posterior and dorsal of the anus. (B) Sexually mature animals possess a number of dimorphic characters. Males are larger than females. The second pair of gnathopods (t3) is enlarged in males. Females possess a ventral brood pouch in which they incubate eggs until hatching (arrowhead). All amphipods retain a highly compressed arrangement of mouthparts into a compact basket termed the buccal mass (arrow).

C. The coral banded shrimp, *Stenopus hispidus*.
Stenopus hispidus typically occupies obstructed overhang habitats such as mangrove prop root junctions and spaces between and under plate corals. The figure shows the typical upside down posture. This individual is a mature female. The yellow arrow indicates developing embryos held ventrally by the swimmerets. The turquoise arrowhead indicates the dorsal position of the ovaries, in this case full of developing oocytes.

D. Expression of *Ph otd1* in *Parhyale hawaiensis*.
Anterior is up, blue staining is the fluorescent marker DAPI and indicates the position of each cell nucleus, red staining is digoxogenin labeled probe to *Ph otd1* mRNA and indicates cells expressing the *Ph otd1* gene. This particular embryo is in the germband stage of development. During this stage of embryonic development in *Parhyale* anterior *Ph otd1* expression has resolved into two ectodermal bilateral clusters that will become the future anteriormost brain neuromere, the protocerebrum. The single, more posterior and medial, column of *Ph otd1* expressing cells mark cells fated to become the ventral midline.

E. The hyperiid amphipod, *Glossocephalus milneedwardsi* and host ctenophore, *Mnemiopsis*.
Animals in this photo are in holding tanks at Carrie Bow Cay. *Glossocephalus milneedwardsi* is an exclusively pelagic amphipod that is known to associate with the ctenophore *Mnemiopsis*. The photo shows the typical types of positions *Glossocephalus* occupies on the outer surface of the ctenophore host. Red arrows indicate male *Glossocephalus*. The lower male is in a 'cleaning' position with the ventral aspect of the head in close proximity to the host. The white arrows indicate female *Glossocephalus*. Embryos in the ventral brood pouch are visible as opaque, white light scatter in this photo.
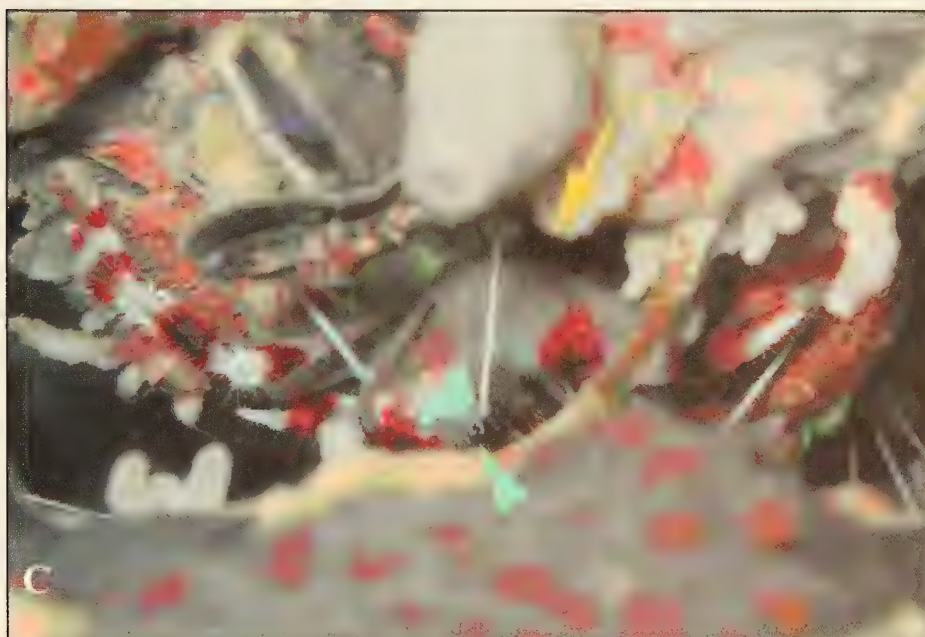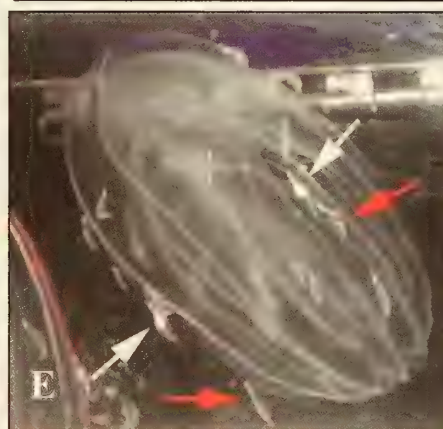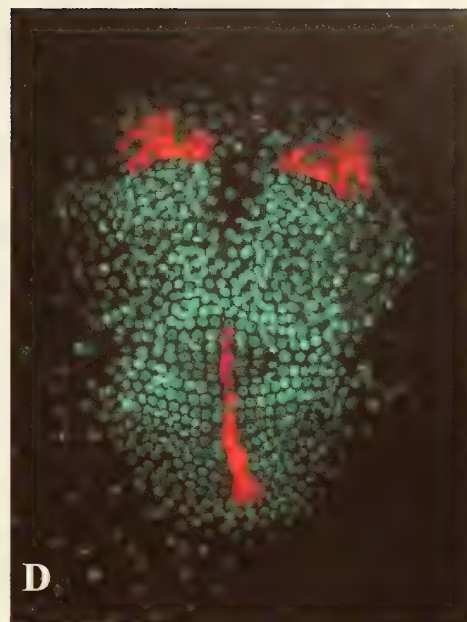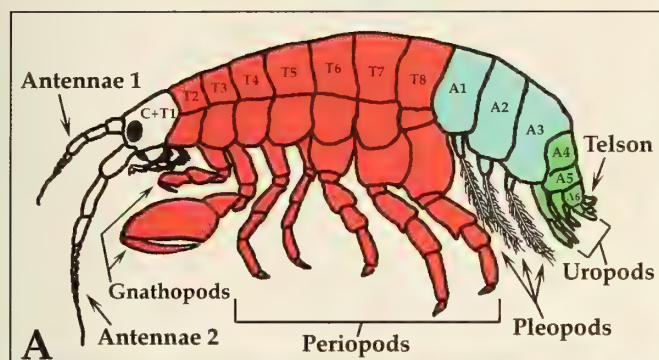
21

A

B 5mm

C

D

E

PLATE 1

BRYOZOANS FROM BELIZE

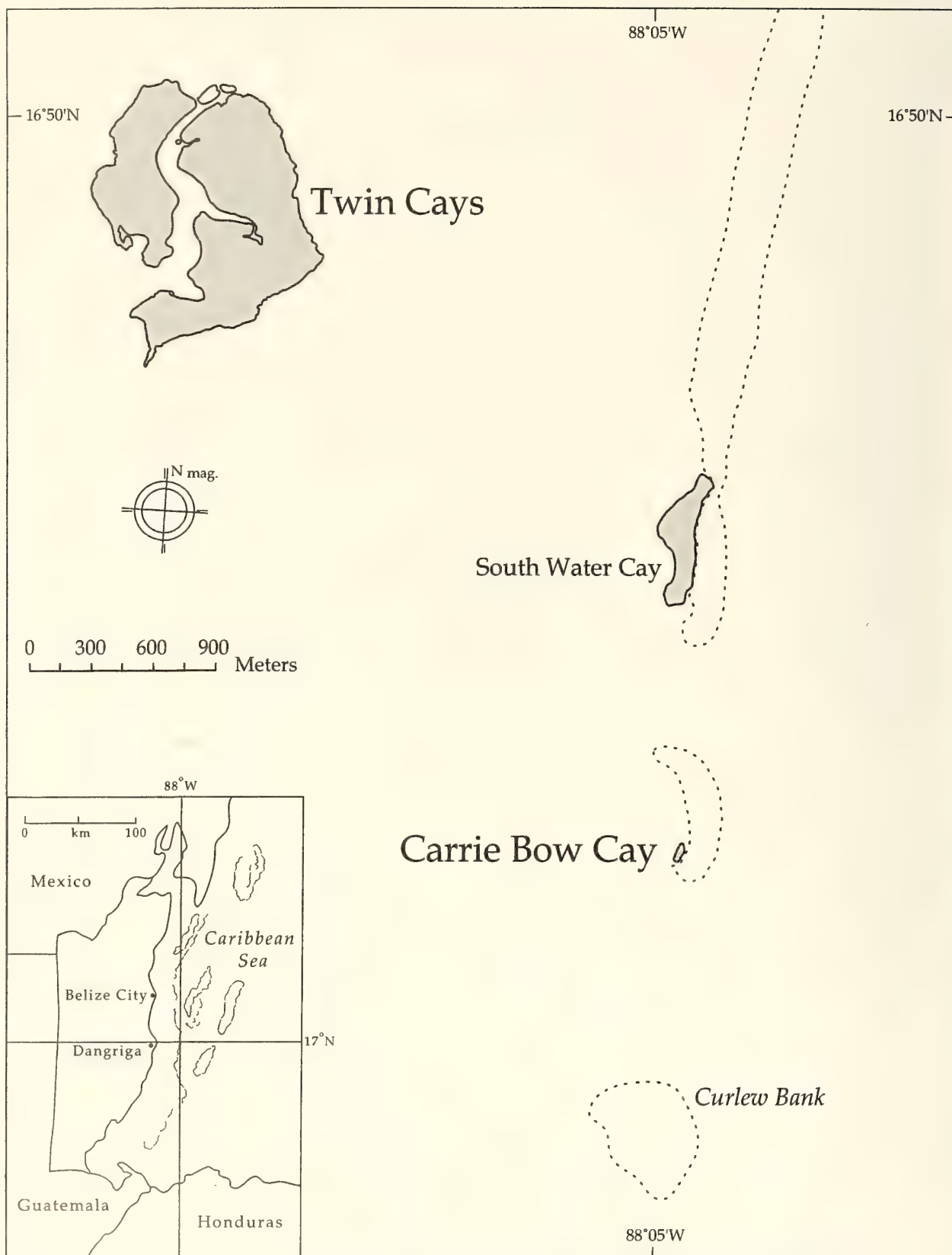BY

JUDITH E. WINSTON

Figure 1. Index map showing locations of Twin Cays and Carrie Bow Cay.

BRYOZOANS FROM BELIZE

BY

JUDITH E. WINSTON[1]

ABSTRACT

The two studies carried out at Carrie Bow Cay and Twin Cays on bryozoans from reef and mangrove habitats have focused on ecology, behavior, and basic taxonomic description. The first visit by the author in November 1980 was primarily for the purpose of carrying out a preliminary survey of the bryozoans from the vicinity. Thirty-six species of bryozoans, including one new genus and four new species, were found in this survey. During the 1980 visit, pilot studies of avicularian behavior of reef-dwelling species were also made.

A second visit was made in October-November 1984 in order to carry out further observations on avicularia, as well as to study the distribution of bryozoans in the Twin Cays mangrove ecosystem, and to make additional observations on living colonies of reef bryozoans at Carrie Bow Cay. One unusual new cheilostome species, *Synnotum circinatum*, was also discovered at Twin Cays during this trip and is described here.

INTRODUCTION

With increasing human pressure on coastal environments, including mangroves and reefs, documentation of coastal faunas becomes ever more vital. Bryozoans are components of coral-reef and mangrove communities world-wide, but the taxonomic composition of such bryozoan faunas is well known in only a very few areas. A 1980 visit to Carrie Bow Cay resulted in a descriptive taxonomic publication on the shallow water (20 m or less) bryozoan fauna of the reef off Carrie Bow Cay and in mangrove habitats at Twin Cays (Winston, 1984). Thirty-six species of bryozoans, including one new genus and four new species, were collected and described. It was the first report on marine bryozoans from Belize waters, the nearest published bryozoan collections previous to that having been made in deep water off the Yucatan Peninsula in the 19th century by the U.S. Fisheries Commission steamer *Albatross* (Canu and Bassler, 1928). Preliminary studies of the behavior of bryozoan avicularia of the common reef-dwelling species were also carried out during this first visit. A return two week visit to Carrie Bow Cay in 1984 to carry out additional studies on behavior of bryozoan avicularia also allowed opportunities for more collections in the area as well as observations on living colonies from reef and mangrove habitats. One new species, *Synnotum circinatum*, found at Twin Cays is described in this paper.

[1]Virginia Museum of Natural History, 1001 Douglas Avenue, Martinsville, VA 24112, U.S.A.

STUDY AREA AND METHODS

Description of Sites

Carrie Bow Cay, located on the barrier reef of Belize, has been the site of studies by scientists from the Smithsonian National Museum of Natural History for about 30 years (Fig. 1). The small cay which houses the field station is surrounded by five types of reef habitat: lagoon, back reef, reef crest, inner fore-reef and outer fore-reef (Rützler and Macintyre, 1982). Most of the bryozoans studied came from the "Outer Ridge" on the outer fore-reef where masses of broken *Acropora cervicornis* accumulated following a 1964 hurricane.

Twin Cays (16°48.6'N, 88°08.9'W) are a pair of mangrove islands located northwest of Carrie Bow Cay (Fig. 1). The site is characterized by winding channels and embayments, areas of sea grass, and a diversity of organisms encrusting the red mangrove stilt roots that, in this location, stay mostly submerged rather than being exposed by tidal fluctuations as in some other areas.

General Methods

Bryozoans were collected by scuba diving and snorkeling and taken to the lab where they were maintained for up to six hours in aerated-seawater holding tanks for observation, avicularia experiments and photography. Photographs were taken on a Wild M-5 microscope equipped with an adapter for a Nikon 35 mm camera and strobe setup. Voucher specimens were fixed in formalin and either wet-preserved in 70% ethanol, or washed in fresh water and air-dried for identification and taxonomic study. Specimens for SEM were later ultrasonically cleaned and allowed to dry, or bleached with Clorox ® to remove all tissue, leaving the details of the calcareous zooid skeleton visible. They were then coated with gold or palladium and studied and photographed using SEM.

Avicularian Behavior

Bryozoans are a phylum of colonial invertebrates with three living marine orders: Ctenostomata, Cyclostomata and Cheilostomata. Their colonies are made up of several to several thousands of physically connected microscopic individuals (**zooids**). The basic feeding zooid (**autozooid**) of a cheilostome colony is a box-like structure filled with body fluids, cells, muscles and internal organs belonging to the **polypide**. The polypide consists of a funnel of ciliated tentacles called a **lophophore**, a mouth, and a U-shaped gut, all of which are retracted into the protective zooid box when not in use. In cheilostome bryozoans, the dominant group in most marine habitats, zooid walls are variously reinforced with chitin and calcium carbonate, but all zooids retain some membranous frontal wall area either on the frontal surface, or in some form of in-pocketing from the frontal surface, which allows the zooid hydrostatic system to operate. Toward the distal end of the frontal wall is a hinged and chitinized trap door called the

operculum. Muscles contract and increase fluid pressure inside the zooid, slowly pushing the compressed lophophore and introvert region of the polypide out of the zooid through the opening operculum, so that the ciliated tentacles of the lophophore can expand to feed. Disturbance, a sudden water current or the movement of a trespassing organism on the colony surface, for example, results in the polypide's rapid retraction back into the zooid.

Avicularia are polymorphic zooids found in many species of cheilostome bryozoans. The most primitive type of avicularia have been usually been considered to be the **B zooids** found in some species, which may be larger than **A zooids** (autozooids), and have a feeding polypide like those of A zooids, but have an enlarged or thickened operculum. Most avicularia are smaller than zooids, and may either replace them in budding sequence in the colony (**vicarious** or **interzooecial avicularia)** or be budded from the frontal wall of a supporting autozooid (**frontal avicularia**). Avicularian zooids have an modified operculum called a **mandible**. It is enlarged compared to an autozooid operculum, often covering most of the frontal wall of the avicularium, and is usually more rigidly chitinized, strengthened, and modified, sometimes edged with a sharp pointed tip (e.g, Fig. 2), rows of chitnous teeth, or shaped into a bristle or paddle form. The avicularium zooid may have enlarged muscles to operate the heavy mandible and retains some membranous frontal wall area, but most of it is essentially a reinforced socket for the closed mandible. There is no functional polypide, although sometime a **polypide rudiment** tipped with sensory cilia is present (Fig. 2), especially in the **bird's head** or **pedunculate** avicularia that are usually considered the most derived type.

Figure 2. Pedunculate avicularium of *Synnotum circinatum* with open mandible. PR= opening of polypide rudiment. SC=cluster of sensory cilia.

For the avicularian behavior experiments (Table 1) the goal for each species was to have a timed period for general observation of one or more to determine if avicularia had a species-specific behavior pattern, whether any innate rhythm of activity could be detected in an undisturbed colony. When possible this period was followed by testing for response to mechanical disturbances, by jarring the dish or colony, probing the avicularia directly (with fine insect pin or pig's eyelash tipped probe), and by producing a rapid current of seawater with a syringe. If time permitted, further testing of the colony was carried out by introducing a chemical or particle-based stimuli: crab juice, oyster juice, a solution of mixed amino acid powder dissolved in seawater (from health food store capsules), milk, Spirulina (dried blue-green algae) powder in seawater, mud in seawater, and tiny plastic beads in seawater.

Table 1. Observations on avicularia of Belize bryozoans

Species [Type of avicularia]	Innate behavior *# of avicularia in 6X view shutting in timed observation on undisturbed colony	Response to mechanical stimuli	Response to chemical stimuli
Synnotum circinatum [pedunculate]	avicularia open *2/15"	Almost any current makes branches coil; avicularia do not respond	Possible response to amino acid solution
Hippopodina feegeensis [frontal]	0/10"	Hard to trigger closure Had to probe at hinge line No response to current jet	_____
Reptadeonella costulata [frontal]	1/10",0/10"	When lophophores retracted all avicularia closed and reopened Brushing causes closure and reopening; jarring caused a few to close	No response to amino acid solution Polychaete caught by colony, remained shut several hours. 2 more syllid polychaetes caught by 2d colony
Rhynchozoon verruculatum [frontal]	avic. open almost immediately open when auto-zooids shut	Probing mandibles>shut, but immediately reopen Jarring> no reaction	No response to chemical cues or to salinity change
Celleporaria albirostris [interzooecial]	1/15", palate surface sticky[1]	Will close on direct mech. stimulation by probe, esp. at hinge line. Did not close On small polychaete making Slow exploratory movement	Oyster juice caused closure, trapped polychaete still trapped (dead) 11 hrs later. Jet of water caused closure
Stylopoma spongites [interzooecial]	1/10"	Close at jarring or strong current application, reopen immediately No response to sediment, lie open under mud	Possible response to Amino acid solution
Smittipora levinseni [interzooecial]	0/10"	Current > closure No response to mud No response to exploratory Movement by polychaete Sharp vibration> closure	Amino acid solution causes partial closure

[1]Sediment sticks to palate surface, but not to frontal surface of autozooids.

Table 1 (cont'd)

Cribrilaria flabellifera [interzooecial]	Mandibles paddle-shaped	Jet of water>flip direction Prodding> flip from one direction to other rather than open/shut rx	Amino acid solution consistently causes reversal of direction Not just particles, as neither Micronic beads or Spirulina had that effect
Labioporella granulosa [interzooecial]	0/10" most shut 25" before most open 2d colony, avic Opened sooner	Jarring, some opened Probing on hinge caused Slow closure and Reopening Jet of water> closure	No response to amino acid solution and crab juice
Crassimarginatella tuberosa [B zooids]	Gape open in testing position like A-zooids	Don't react like avicularia. Polypide scans with 1 tentacle = male zooid?	No rx to amino acid solution by B zooids A zooids try to feed on it
Steginoporella magnilabris [B zooids]	Bs open before As., open wide	Jet of water causes closure. Closure can affect all Bs in area	Jet of crab juice, Bs opened wide, or open and shut without affecting A zooids Amino acid solution > open wide or open, then shut. Nematode caught by A-zooid operculum
Trematooecia aviculifera [frontal and interzooecial]	Small frontal immed.open Large interzoo-ecial slow to open, after 30" most open.	Jet of water makes them open	Possible response to amino acid solution

RESULTS

Avicularian Behavior

The 1980 visit to Carrie Bow Cay had shown that species with diverse types of avicularia, including B zooids, and pedunculate avicularia were abundant there. It had also shown that it was possible to maintain the colonies in good condition in the lab long enough for behavioral studies to take place. However, compared to studying other aspects of bryozoan biology like feeding, working with avicularia proved challenging for two reasons. One reason was the small size (less than 50 μm) of the avicularia of many species. The other was the long periods of inactivity characteristic of all but the bird's

6

head type, resulting in many periods of observation in which no activity was detected. In a freshly collected bryozoan colony, brought into the lab and observed in a dish of seawater under the microscope, there will be periods of feeding. At those times autozooid opercula first open to a testing position, then, if no threat is detected, polypides protrude lophophores completely and expand them to feed, only retracting if jarred or disturbed by sharp currents or activities of other animals in the dish. At other times autozooid opercula remain closed and the colony quiescent. The mandibles of avicularia, on the other hand, open almost immediately and remain open even when autozooid opercula are shut unless they are provoked to close by some stimulus. In only a few species did there appear to be an innate pattern of movement in unstimulated colonies.

Table 1 summarizes the results of observations of avicularia of bryozoans from Carrie Bow and Twin Cays. Although, due to limited numbers of specimens and inadequate time for experimental work (microscope lighting in the field lab at that time depended upon a generator being run for electricity and was limited in duration) the experiments were not complete for each species, results were still valuable. Most striking was the clear demonstration that avicularia of both the larger interzooecial (*Celleporaria*) and smaller frontal (*Reptadeonella*) types had the ability to capture small predators like syllid polychaetes (Winston, 1986). Capture often resulted in damage to avicularia and both the damage and the remains of animals caught were visible on colonies for some time. Slow exploratory movements by colony trespassers did not generate avicularian closure; rapid or violent movements were triggers.

It was also clear that avicularia otherwise responded primarily to mechanical stimuli, such as quick or jarring movements of the colony substratum, or a rapid and intense water current.

There were some indications that chemical stimuli might sometimes be involved in triggering closure or opening. Although the substances used in testing were not very sophisticated, responses by several species suggested that proteinaceous substances (amino acids or body fluids from other invertebrates) were possible triggers for avicularia movements.

Twin Cays Bryozoan Community

At Twin Cays bryozoans were found living attached to *Thalassia* blades, as drift in the seagrass beds adjacent to the mangrove stands and on the mangrove roots themselves (Fig. 3). Three of the most common species belonged to the Ctenostomatida. These soft-bodied forms, tolerant of lowered or changing salinities, often dominate bryozoan communities in harbors and estuaries in temperate and tropical waters.
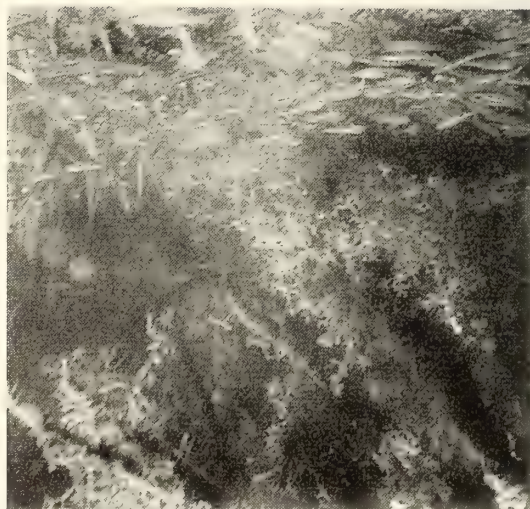
Figure 3. *Zoobotryon verticillatum* in Twin Cays seagrass bed
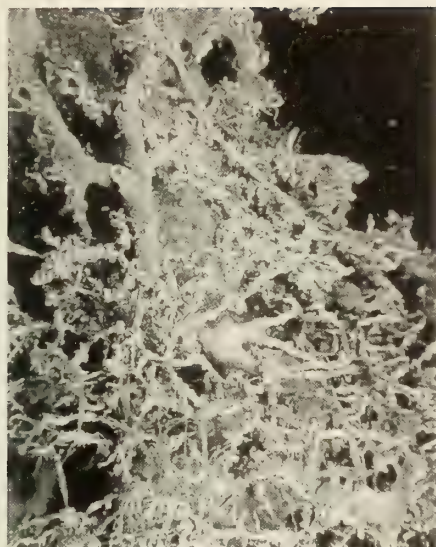
Figure 4. *Zoobotryon* clumps attached to mangrove roots.

Zoobotryon verticillatum (Delle Chiaje, 1828) (Fig. 3 &4). This massive stoloniferan ctenostome, whose colonies resemble dirty strands of cellophane noodles, was the most common bryozoan at Twin Cays. It was found both attached to mangrove roots and freely drifting or attached to grass blades in the seagrass beds. The species is now found world-wide in warm seas, but was originally described from Trinidad (as an alga); perhaps it is actually native to the Caribbean.

Amathia vidovici (Fig. 5). Colonies of *Amathia vidovici* (Heller, 1867) were common at Twin Cays. This species has been reported from fouling and mangrove communities at a number of sites around the world, including the Indian River Lagoon in Florida.

Bowerbankia maxima (Fig. 6). *Bowerbankia maxima* Winston, 1982 is a robust white pigmented ctenostome whose colonies formed festoons of stolons and clustered zooids on bare space on submerged roots. Described from the Atlantic coast of Florida, it has so far been found in Jamaica and South Carolina as well as Belize.

Figure 5. *Amathia vidovici,* colonies growing on mangrove root.

Figure 6. *Bowerbankia maxima*, colonies attached to mangrove root.

8

The other two common bryozoans at Twin Cays are members of the order Cheilostomatida.

Schizoporella pungens (Canu and Bassler, 1928) (Fig. 7). *Schizoporella pungens* was the most common encrusting cheilostome bryozoan. It formed massive iridescent purple crusts with orange-rimmed growing edges. Where large tube worms were attached to the roots, the *Schizoporella* colonies often developed tubular branches around their tubes. This species was identified in Winston (1984) as *Schizoporella ?serialis* Heller 1867, following Banta and Carson (1977). At that time bryozoan workers understood

Figure 7. *Schizoporella pungens* encrusting mangrove root at Twin Cays. Fuzziness of picture is due to expanded lophophores of zooids.
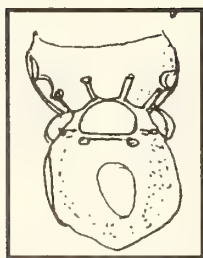

Figure 8. *Schizoporella pungens,* sketch of ancestrula from colony recruited at Twin Cays.

that there were a least two species complexes of fouling or ecologically opportunistic *Schizoporella,* a *unicornis* group and an *errata* group, and *serialis* was considered to be the oldest name for the *errata*-like species. Later SEM studies of material from the Adriatic, W. Africa, Caribbean and Brazil by various authors have clarified the situation further. Caribbean *errata*-like material appears to be morphologically distinct from E. Atlantic, Mediterranean, and W. African "*violacea-serialis*", a cluster which may also include Brazilian material (d'Orbigny's *S. isabelleana* from Rio de Janeiro). The Caribbean and E. Pacific (e.g. Costa Rican and Hawaiian specimens) material differs slightly in characters of avicularia and orifice. The Caribbean name, *Schizoporella pungens,* should probably be used for Belize and other Caribbean specimens, at least until molecular studies are completed for the entire *errata* complex.

The morphology of the ancestrula (the initial zooid developed from the settled and metamorphosed larva) and the pattern of budding leading from it, may also be useful in distinguishing bryozoan species. Therefore, during the two-week 1984 visit, bryozoan "traps" consisting of screened microscope slide arrays were wired in several spots among mangrove roots near potential parent colonies of *Schizoporella.* Although the time period for recruitment was short (<13 days), a few *S. pungens* larvae did settle, of which the largest had an ancestrula and 7 zooids at the time of collection. A sketch of the ancestrula of *S. pungens* is shown in Figure 8. It has 8 oral spines, a semi-circular operculum and a frontal wall with an ovoid membranous area. The ancestrular polypide has 14 tentacles and a yellow orange color. The growth rate of *Schizoporella* is much greater than that of reef species from the area. The 1980 study had shown that colonies 6 X 6 cm in diameter grew on panels deployed for 6 months. In contrast, reef panels placed out for the same time period had no bryozoan colonies larger than 1 cm^2.

The most interesting discovery at Twin Cays was of a new species of *Synnotum* (Fig. 9) attached to mangrove roots among ascidians and sponges. Morphologically this delicate branching species is similar to the wide-spread tropical species *Synnotum aegypticum*, but the free ends of the branches of the new species writhe and twist into knots when touched, with a mobility that is completely lacking in its congener. Movements by zooids and branches of colonies are not unknown in bryozoans but most have been reported from ctenostomes, e.g., *Bowerbankia, Mimosella, Triticella.* The stalks of the avicularia of members of the cheilostome genus

Figure 9. *Synnotum circinatum*, new species. Erect branching colonies attached to mangrove root.

Camptoplites are in almost constant motion (Winston 1991), but this report may be the first record of whole branch movements in a cheilostome. A formal description of the new species is given below.

<div align="center">

Order Cheilostomatida
Family Epistomiidae Gregory, 1893
Genus *Synnotum* Pieper, 1881

Synnotum circinatum, New Species
Figures 2, 9-11.

</div>

Diagnosis: *Synnotum* with evenly bifurcating branches spaced along a delicate stolon. Branches perform coiling motion when disturbed. Zooids about 1/3 larger than those of *Synnotum aegyptiacum* and more triangular in shape; their avicularia larger and with a more clearly demarcated peduncle than those of *aegyptiacum.*

Etymology: circinatus (Latin) = coiled, curled away from an apex.

Type Material: Holotype, USNM no. 1026612, Paratype VMNH nos. 3173 and 3174 .

Description: Colony is erect, glassy white in color, consisting of evenly bifurcating candalabra-like branches attached along a delicate transparent stolon. Each uniserial branch has two faces, made up of back-to-back chains of zooids (Fig. 10). Zooids are elongate (about .30 mm L X .12 mm W), broader distally and narrowing

10

proximally, with a membranous walled ovoid opesia taking up most of the frontal wall (about 27L X .10 mm W) and with lateral walls delicately calcified. In partially decalcified specimens, a tubular chitinous joint-like region is visible at the proximal end of each zooid, each zooid back-to-back pair thus forming an internode. The operculum is semicircular, about 0.5 mm H by 0.10 mm W, and large relative to zooid size (Fig. 11). On one side of the distal wall of the zooid is a short-stalked cat's ear-shaped pedunculate avicularium with a hooked triangular mandible (Figs. 2, 11). On each face of the branch these avicularia oppose each other so that at a low magnification (Fig. 10), a zooid appears to have two. Polypides are transparent white, with 10 asymmetrical, slightly campylonemidan tentacles. Ovoid white embryos with large apical tufts and a dark-red C-shape of pigmented cells surrounding them, are brooded in enlarged gonozooids which they grow to fill almost entirely, leaving only space for the tiny brown body, remains of the degenerated polypide.

Figure 10. [left] *Synnotum circinatum*, SEM of colony branches.

Figure 11. [right] *Synnotum circinatum*, close up of zooid and avicularia..

Discussion: Unlike those of the very similar *Synnotum aegyptiacum*, the branches of *Synnotum circinatum* respond to a touch of forceps or a strong current of water, by coiling and writhing from their free ends. The uncalcified joints between each zooid may provide the means for this movement, although it has not been observed in Florida colonies of *Synnotum aegyptiacum* which have a similar structure. Marcus (1941) studied colony development and reproduction in *Synnotum agyptiacum*. He found that zooids with functional polypides occur only near the growing tips of branches. Those

further down the branch contain embryos and proximal-most zooids with only brown bodies. This seems to be the case also in *Synnotum circinatum*. However, Marcus also noted that in *S. aegyptiacum* rootlets produced from proximal zooids were able to regenerate new zooids and branches. He pointed out that, although the lack of ability to regenerate polypides might be disadvantageous, the ability of *Synnotum* colonies to regenerate new branches from rootlets might actually provide an ecological advantage — enhanced dispersal by fragmentation and regeneration. The ability of *Synnotum circinatum* branches to wrap around narrow objects might also be advantageous for dispersal. Colony fragments could be carried to new sites on crustacean legs for example, or if transported by currents, can readily attach themselves to substrata in a new location.

Distribution: So far known only from Twin Cays, Belize.

Observations on Carrie Bow Cay Reef Species

During the 1984 visit, additional collections were made on various parts of the reef, and a number of species were observed alive so that colony color, embryo color, and polypide morphology could be noted. Most reef-dwelling bryozoans are found in cryptic situations: caves, crevices or under surfaces of corals and rubble. As had been noted previously, the most diverse bryozoan assemblages occurred in *Acropora* coral rubble areas on the Outer Ridge. Bryozoans did encrust the interstices of the leafy *Agaricia* coral that dominante the spur-and-groove zone. Species found there included *Cribrilaria flabellifera, Hippopodina feegeensis, Labioporella granulosa, Puellina* sp., *Rhynchozoon verruculatum, Stylopoma spongites, Trematooecia aviculifera,* and *Steginoporella* sp. Since collecting bryozoans in this zone usually necessitated destroying live coral substrata, most collecting for behavioral work was done in the outer-ridge rubble. In the shallow back-reef area only *Hippopodina feegeensis, Trematooecia aviculifera,* and *Steginoporella* sp. were noted.

Gemelliporidra belikina Winston, 1984. Living colonies of this small mound-shaped species had not been seen alive in the first study, but were collected from the outer ridge in 1984. They had a glassy translucent calcification and an transparent orange coloration of polypides.

Trematooecia aviculifera (Canu and Bassler, 1923). *Trematooecia aviculifera* (Fig. 12) was the only bryozoan able to grow exposed on vertical and upper surfaces of reef substrata at Carrie Bow Cay and one of the few Caribbean reef bryozoans large and brightly colored enough to be noticed by nonbryozoologist divers. Underwater, living colonies look fluorescent green but they are salmon pink at the surface. The embryos in their ovicells are a shiny maraschino cherry red. The tentacles, 18-19 in number, are translucent pink. Unlike some bryozoans, in *Trematooecia* ovicelled zooids brooding embryos retain feeding polypide.

12

Figure 12. *Trematooecia aviculifera* colony growing on side of coral head, Carrie Bow reef.

***Crassimarginatella tuberosa* (Canu and Bassler, 1928)**. *Crassimarginatella tuberosa* (Fig. 13) colonies were collected for observations of their B zooids. While examining these colonies, it was noted that fouled frontal membranes in this bryozoan species, like those of cupuladriid bryozoans (Winston and Håkansson, 1989) and many others, underwent a molting process. The frontal walls of many zooids in older colony regions had become fouled with calcareous algae and algal films. Yet in patches, even in old regions, there were areas with clean greenish yellow walls. It was noticed during the avicularia observations that frontal walls of some fouled zooids had peeled partly off, and new frontal walls, transparent and shiny, lay beneath them. Other zooids had swollen frontal walls such as had been observed in molting cupuladriids. Peeling away the old frontal membrane exposed a new frontal wall, complete with new operculum, that had formed beneath the old one.
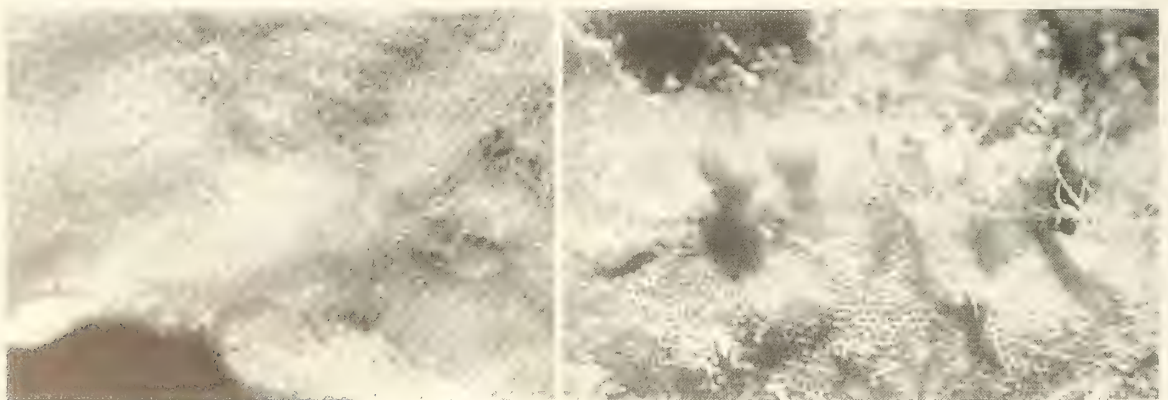

Figure 13. Colony of *Crassimarginatella tuberosa*, Carrie Bow reef, fouled and clean areas of colony.

DISCUSSION

Avicularian Behavior Studies

The function of avicularia in cheilostome bryozoan colonies has been a mystery fascinating biologists since Darwin's time. Recent observations and video studies at Carrie Bow Cay (e.g., Winston, 1984, 1986, 1991) suggest that one function may be to clean colony surfaces. Avicularia may also be the "ears" of the colony in sensing the movement of water and of trespassing organisms. Another function may be to act as defenders of the colony by capturing trespassers and potential predators. Through their ability to hold decaying remains of captures indefinitely, they may even provide a supplementary food source. More sophisticated experiments would help to clarify their response to physical and chemical stimuli. Although time-consuming and difficult, comparative studies would aid in determining the amount of predation on colonies in different habitats and depths. One avenue of research along these lines would be to quantify the damage to avicularia or the remains of trespassers still caught by them.

Taxonomic Studies

Considering that 2 brief surveys, a small amount of collecting by just one person, resulted in 5 new species being described, the 37 species described so far from the Carrie Bow and Twin Cays probably represent only a small fraction of the actual bryozoan diversity of this world's second largest barrier-reef system. A larger survey, carried out at a number of sites along the barrier reef and in other mangrove areas, would easily triple the number of bryozoans known from Belize.

ACKNOWLEDGMENTS

REFERENCES

Banta, W.C., and R.J.M. Carson
   1977. Bryozoa from Costa Rica. *Pacific Science.* 31:381-424.
Marcus, E.
   1941. Sobre o desenvolvimento do briozoario *Synnotum aegyptiacum. Arquivos de Cirurgia Clínica e experimental.* 5:227-234.
Rützler, K. and I.G. Macintyre
   1982. Habitat distribution and community structure of the barrier reef complex near Carrie Bow Cay. Pages 9-43 in K. Rützler and I.G. Macintyre (eds.) *The*

14

Atlantic Barrier Reef Ecosystem at Carrie Bow Cay, Belize 1: Structure and Communities. Smithsonian Contributions to the Marine Sciences.

Winston, J.E.
1984. Why bryozoans have avicularia -- a review of the evidence. *American Museum of Natural History Novitates.* 2789:1-26.
1986. Victims of avicularia. P.S.Z.N.I. *Marine Ecology.* 7:193-199.
1991. Avicularian behavior -- a progress report. *Soc. Sci. Nat. l'Ouest France. Mém. hors ser.* 1:531-540.

Winston, J.E. and E. Håkansson
1989. Molting by *Cupuladria doma*, a free-living bryozoan. *Bulletin of Marine Science.* 44:1152-1158.

ATOLL RESEARCH BULLETIN

NO. 524

DIVERSITY AND DISTRIBUTION OF ASCIDIANS (TUNICATA)
AT TWIN CAYS, BELIZE

BY

IVAN GOODBODY

Figure 1. Twin Cays index map

DIVERSITY AND DISTRIBUTION OF ASCIDIANS (TUNICATA) AT TWIN CAYS, BELIZE

BY

IVAN GOODBODY

ABSTRACT

Forty species of ascidian in nine Families have been recorded from Twin Cays during a ten-year period of study. Twin Cays is the type locality for three of these species. Colony-forming species, notably those in the families Didemnidae, Polycitoridae and Perophoridae, predominate over solitary species which are poorly represented. The distribution in Twin Cays and habitat requirements of these different species are considered and the composition of the fauna is compared with that of other well documented mangrove environments in the Caribbean Basin. It is suggested that several other species of ascidian may colonize Twin Cays in years to come if the environment alters significantly. Notable among these are two species of *Ecteinascidia* which have breeding populations within a few kilometers distance from Twin Cays.

INTRODUCTION

Twin Cays, Belize is a mangrove-covered cay of about 75 ha., divided into two islands by a north-to-south channel opening at either end to the open sea. Detailed descriptions of the system are given elsewhere in this issue of the Bulletin. (Rützler et al., 2004; Rodriguez and Feller, 2004).

Forty species of ascidian have been identified from the area. Most species are found growing on the adventitious hanging roots of the red mangrove tree (*Rhizophora mangle*), while a few species are characteristic of bottom sediments. The *Rhizophora* roots provide an excellent growing surface for a variety of sessile marine invertebrates. As this community matures it becomes a fiercely competitive environment in which sponges and ascidians are the dominant living organisms. One attribute of the environment is that new space is constantly being created by growth of the root tips. Under favorable circumstances, growth of an individual root tip may exceed 0.2 to 0.4 mm per day *(pers. obs.)*. Secondary space is also important in the form of bivalve (*Isognomon alatus*) shells, algal filaments and benthic seagrasses (*Thalassia testudinum*). Another feature of the environment is that most parts of the system are regularly flushed by diurnal tidal rhythms producing fast water flows in narrow channels (e.g. Hidden

Department of Life Sciences and Centre for Marine Sciences, University of the West Indies, Kingston 7, Jamaica

Creek, Gator Creek) and more sluggish transport in broader channels (e.g. Lair Channel). These currents, fast or slow, renew supplies of suspended particulate matter on which the sessile community depends for food. In addition, when the tide rises it floods across the swamp floor and during neap tide the water carries decomposed organic matter back into the channels and ponds further replenishing food resources. All of these attributes of the environment are exploited by ascidians according to their individual needs resulting in a species-rich assemblage over a relatively small linear distance. The total linear measure of mangrove fringe along which sessile communities grow on *Rhizophora* roots is in the order of 4.63 kilometers. (Rodriguez and Feller, 2004).

METHODOLOGY

During a 10-year period between 1984 and 1994, I made 20 visits to Belize to work from the Carrie Bow Cay laboratory. Each visit lasted 2-to-3 weeks and during each visit daily trips were made to Twin Cays for the purpose of surveying and studying the ascidian fauna. All seasons of the year were covered during the overall period of study. Observations were made by free swimming with a mask and snorkel and all observations were recorded on an underwater slate to be transferred to written notebooks after return to the laboratory at Carrie Bow. Where continuous observation of individual ascidians or colonies was required, the site was marked by attaching a small plastic numbered label to the relevant mangrove root with a tie-wrap. To follow growth and change in colonial species a photographic record was made using Nikonos II and III underwater cameras with flash heads. Every accessible site in Twin Cays was covered by this survey with particular attention paid to the Main Channel, Twin Bays, Hidden Creek, Lair and Lair Channel, Turtle Pond and Channel, Candy's Pond, Grouper Gardens and Gator Creek. A few observations were also carried out in the open-water shallow areas to east and west of the two Cays. To the east the sea floor is mostly covered by *Thalassia* beds growing in soft sediments while to the west is a mixture of *Thalassia,* coarse sands and broken coral rock (Figure 1).

SPECIES COMPOSITION

A total of 40 species of ascidian have now been recorded from Twin Cays (Table 1) and of this number three species have been described as new taxa during the course of the study. It will be seen from the table that there is a preponderance of clonal species belonging to a few Families, notably Didemnidae, Polycitoridae Perophoridae and Styelidae. To simplify the presentation of information I have chosen to treat the fauna family-by-family rather than species by species. General taxonomic information on most species will be found in Van Name (1945) and additional information on individual families will be found in the literature cited with each family. A previous account of ascidian diversity elsewhere on the Barrier Reef in Belize is given in Goodbody (2000). Eleven species at Twin Cays are sufficiently common and prominent in the sessile community to warrant designation as key species. These are marked with an asterisk (*)

in Table 1. Voucher specimens of all species referred to as occurring at Twin Cays have been deposited in the U.S. National Museum of Natural History. The habitat distribution of different species at Twin Cays is summarized in Table 2. See Plate 1.

Family **Polyclinidae** (F. Monniot 1972, 1983b)   Unlike at many other sites in the Caribbean this family is poorly represented in Twin Cays. A single specimen of *Aplidium antillense* was found overgrowing the test of *Ascidia interrupta* in Twin Bays on November 17, 1984 and on several occasions an algal-bearing polyclinid was seen on the peat bank near the north end of the Main Channel. No specimen survived collecting and the identity of the animal is unknown. The presence of algae in a polyclinid is unusual. *Aplidium exile* occurs on *Rhizophora* roots at the nearby Blue Ground Range and is of regular occurrence in lagoons at the Pelican Cays and on nearby reefs (Goodbody, 2000). Hence it is possible that it may colonize Twin Cays in the future.

Family **Didemnidae** (F. Monniot, 1983a)   The Didemnidae are an abundant family in most marine environments throughout the tropics. Twin Cays is no exception. By virtue of their ability to replicate rapidly and form sheet-like colonies, they are found overgrowing most types of substrate. The inclusion of white calcareous spicules within the test substance makes many species conspicuous members of sessile communities. For a detailed account of the family characteristics the reader is referred to Kott's (2001) excellent monograph on Australian species.

Of the seven species recorded from Twin Cays (Table 1), only two species can be regarded as Key species in the sessile community. *Didemnum conchyliatum* (Plate 1a) is one of the most common didemnids throughout the Caribbean. In Twin Cays it is ubiquitous, functioning as a primary colonizer on fresh root tips, panel surfaces and other bare space. It is recorded from most habitats (Table 2) but seems to exhibit a preference for relatively quiet environments such as those along the margins of the Lair Channel and in Twin Bays. Throughout the Caribbean *D. conchyliatum* occurs in several color morphs, orange, white, gray (Goodbody, 2000). At Twin Cays a bright orange morph predominates.

Diplosoma glandulosum (Plate 1b) is another key species forming massive gelatinous colonies in many parts of the system. It is particularly abundant in the North Channel close to Cuda Cut and Batfish Point. In this area it hangs as drapes from the roots of *Rhizophora*. It is also abundant in the Lair Channel where it flourishes on the upper surface of the blades of *Thalassia* plants. In contrast, it is absent from Hidden Creek and Gator Creek where water flow is fast at times. Colonies occur in a number of different color morphs including marbled black and white, gray, green, white, brown. Many colonies have very large common cloacal openings on the upper surface and sometimes along the colony margin. Frequently, colonies are found which appear to be clasping a root from the back suggesting a tendency for larvae to settle on the back of the root where light intensity will be least. *D. glandulosum* releases larvae around the noon-day meridian (Goodbody, 1995) thus providing the larva with opportunity to select the most favorable conditions of shade for settlement. The related species *Diplosoma listerianum* occurs throughout Twin Cays but is neither as common nor as conspicuous as *D. glandulosum*. *D. listerianum* is usually a drab grey color with orange spotting due to

4

the bright orange stomach of individual zooids showing through the thin test substance. The species forms smaller colonies than its congener and often occurs filling spaces between other sessile organisms in the community.

The remaining four species of didemnid at Twin Cays are less common than those mentioned above. *Lissoclinum fragile* is usually snowy white growing in flat sheets spreading over roots or shell surfaces in a very few places, notably in Twin Bays where it is relatively common. The related, but poorly known, *Lissoclinum abdominale* seems confined to a single location in the North Channel close to Cuda Cut where it grows as gelatinous sheets on the vertical face of the peat bank at a depth of about one meter. *L. abdominale* is one of a number of ascidians on the Barrier Reef which contain symbiotic algae in the cloacal canals. While *Prochloron* has been identified as the symbiont in *Diplosoma virens* (Goodbody, 2000), the symbiont in *L. abdominale* has not been investigated; nevertheless it gives a greenish tinge to the otherwise gray-colored colony. *Didemnum psammathodes* forms small flat colonies, muddy brown in color. The color is due to the accumulation of faecal pellets in the test substance; white spicules are thinly distributed in the test but insufficient to give the colony the usual white color found in other species of the genus. The species is rare in Twin Cays; colonies are recorded from the Main Channel (Dock), Hidden Creek, Twin Bays and the Lair. The only other didemnid found at Twin Cays is a single record of *Trididemnum cyanophorum*, another algal-bearing species collected by Klaus Rützler in about 0.5m depth off the northwest tip of the island on May 14, 1989.

Family **Polycitoridae** (F. Monniot, (1972, 1983c) This important family is represented by four species. *Eudistoma olivaceum* (Plate 1d) is widespread throughout the system but is not particularly conspicuous. The species tends to frequent the shaded portions of *Rhizophora* root clusters usually close to the peat bank and within half a meter of the water surface. In this habitat, it is common along the northern shore of the Lair Channel and in many locations along the main channel. The species occupies a similar habitat in the Port Royal mangrove, Jamaica (Goodbody, 2003). At Twin Cays it is also abundant in portions of Candy's Pond close to areas of drainage from the swamp. Electron-micrographs of the gut contents of this species collected from Candy's Pond suggest that it may feed largely on bacteria. Both heterotrophic bacteria and cyanobacteria are recognizable in the micrographs of the gut contents. The preference shown by this species for settling in sites close to the peat bank may be a mechanism for exploiting a situation rich in bacteria draining off the swamp floor. The gut contents are not exclusively bacterial and larger photosynthetic organisms such as dinoflagellates, diatoms and single-cell chlorophytes are visible in the micrographs.

Two other species, *Eudistoma capsulatum and Eudistoma obscuratum*, have been recorded in the Main Channel at Twin Cays. These species are usually reef-dwelling species and have previously been found in mangrove ponds elsewhere on the Barrier Reef at Pelican Cays (Goodbody, 2000). Unlike colonies of the species found at North Lagoon Cay, colonies *of Eudistoma obscuratum* in Twin Cays are relatively small and except for their intense black coloration, are inconspicuous elements of the sessile community. Most specimens were seen at the north end of the Main Channel close to Cuda Cut and Batfish Point. The fourth member of the Family Polycitoridae occurring in

Twin Cays is *Distaplia corolla* (Plate 1c) which forms conspicuous orange-colored colonies all through the system. There are two focal points in its distribution in Twin Cays, one at the eastern end of the Lair Channel on the southern shore just before the junction of the channel with the Lair. The other focus is in the eastern half of Twin Bays where it was common along the northern shore. While these sites are foci of abundance, the species is widely distributed along the Main Channel but is absent from areas of fast-moving water as in Hidden Creek, Gator Creek or Turtle Channel. *D. corolla* occurs in two color morphs at Twin Cays, one bright orange and the other purple. The orange morph is more common than the purple. It is of particular interest to note at this point that there is no confirmed record of the occurrence at Twin Cays of *Cystodytes delle chiajei,* a polycitorid common throughout the mangrove ponds at Pelican *Cays.*

Family **Pycnoclavellidae** (Goodbody, 1996)   A single species, *Pycnoclavella belizeana,* is the only member of this genus known to occur in the Western Atlantic. Originally described from specimens collected near Batfish Point in the Main Channel, the species subsequently has been found to be of fairly widespread distribution on the edge of the peat bank along both sides of the northern end of the Main Channel and in Grouper Gardens. Zooids are only about 1.0 mm in length and hence are difficult to discern unless the bank is carefully studied. The species has also been found at Fisherman's Cay in the Pelican Cays (Goodbody, 2000) and it is likely that it will be found to be widespread along the Barrier Reef in Belize wherever suitable habitat exists.

Family **Perophoridae** (Goodbody, 1994; Goodbody & Cole (*in press*)   Based on abundance this exclusively colonial family is the most successful group of ascidians in Twin Cays. Four dominant species in the genus *Perophora* and two species in the genus *Ecteinascidi*a occur throughout the ponds and channels. Twin Cays is the type locality for two species of *Perophora*, *P. regina* (Plate 1e) and *P. carpenteria* (Plate 1f), which were first described during the course of this study. *P. regina* is a large and active species exhibiting strong muscular activity and a constant dancing motion throughout the colony. This suggests that the animal may have high metabolic demands for food and oxygen and, in keeping with this, the species is most frequently encountered in those parts of Twin Cays where there is strong-to-moderate water flow such as in Hidden Creek, Turtle Pond and Turtle Channel and in parts of the northern end of the Main Channel. *P. carpenteria* is one of a group of three very similar small species which are difficult to separate from one another in the field. *P. carpenteria* is the most abundant of these small species, so much so that it is here considered as a key species. Colonies overgrow other elements of the sessile community, the peat bank itself and the species is common everywhere growing over the upper surface of the blades of Turtle Grass (*Thalassia testudinum*).

   Perophora viridis is less common and is usually found growing along the stems of Bryozoan colonies or on macroalgal stems. I have never found *P. viridis* growing on the peat bank or on grass blades as does *P. carpenteria* and it is suggested that in habitat selection *P. viridis* may seek a location raised above the substratum or above the sessile community in general.

   The fourth species in this group is *Perophora bermudensis* which tends to grow in loose clusters of zooids often hanging down into a stream of relatively fast moving water.

A colony of this sort has been recorded frequently at the western side of the entrance to the Lair where it is exposed to the flow of water passing this point at tidal oscillations. An almost identical situation exists in the Port Royal mangroves in Jamaica where colonies of this species hang into a similar tidal flow (Goodbody, 2003). *P. bermudensis* does not always grow in these loose hanging colonies and may also be found growing as a creeping sessile colony along the margins of the peat bank. For further discussion of these growth forms see Goodbody (1994). Another small species, *Perophora multiclathrata*, occasionally occurs in mangrove environments in the Caribbean and has once been recorded at Twin Cays: a small colony was collected from a mangrove root at the Big Dipper on May 8, 1986. *P. multiclathrata* also occurs at Carrie Bow Cay where it is found under slabs of coral rock.

There are two species of *Ecteinascidia* occurring at Twin Cays. *E. styeloides* (Plate 1g), is an erect form growing usually as clusters of grey-green zooids. It is found throughout the system but is only common in the Lair and Hidden Creek. The second species, *E. minuta* (Plate 1h), is much smaller, more like a species of *Perophora* in external appearance but growing either as recumbent colonies on mangrove roots or other substrata or growing as diffuse colonies of small erect zooids. The recumbent form is the dominant form in Twin Cays and is found throughout the system but particularly in the inner more stressed areas such as in Candy's Pond, the Lair, Turtle Pond and in Hidden Lake and the upper reaches of Hidden Creek (Fig. 1). It frequently makes use of secondary space for settlement and in Hidden Creek is specially abundant on the shells of the oyster *Isognomon alatus*. Two other species of *Ecteinascidia, E. turbinata* and *E. conklini* occur elsewhere in the Southwater Reserve and it is surprising that neither has colonized Twin Cays. For further information on these species of *Ecteinascidia* see Goodbody and Cole (*in press*) and the discussion at the end of this paper.

Family **Ascidiidae** (C. Monniot, 1973, 1983a.) Animals in this large and important family usually grow as solitary zooids, not colonies. Nevertheless, under favorable conditions clusters of solitary zooids may grow close together as occurs with *Ascidia curvata* in Jamaican mangrove lagoons (Goodbody, 2003). At Twin Cays the family is represented by six species, none of which is common. *Phallusia nigra* has been found occasionally on roots in the Main Channel close to Batfish Point and also on roots in Twin Bays. By virtue of its glossy black color, *P. nigra* is readily recognized in the field whereas other species that are less conspicuous may often be overlooked. *Ascidia interrupta* is another large (5-10 cm) solitary form common throughout the Caribbean on reefs and in mangroves. At Twin Cays *A. interrupta* was occasionally found in Twin Bays and Lair Channel (Fig. 1), usually as isolated zooids and not in clusters. *Ascidia tenue* is a small (2-3 cm) poorly known species. The only record from Twin Cays is of four zooids collected from a settlement panel at the north end of the Main Channel on May 8, 1986. *Ascidia correloides* is another small species (1-2 cms) normally associated with reef environments. A single specimen was collected from the peat bank at the north end of the Main Channel in Twin Cays on May 23, 1986. *Ascidia curvata* is a medium-sized species in which the test is often very transparent. It has only once been recorded from Twin Cays: a single zooid collected on February 16, 1984 from the peat bank on the east side of the Main Channel. *Ascidia sydneiensis* is the largest species in the genus

found on the Belize Barrier Reef. It is usually benthic in habitat living buried in soft sediments with two greenish-yellow siphons protruding above the sediment surface. Twin Cays is an ideal environment for this species but it has only once been recorded--- a 9-cm long specimen collected in the Main Channel on March 30, 1981.

Family **Styelidae** (Sloot, 1969; C. Monniot, 1983b)   This is another large family which contains both clonal and aclonal species some of which are very common at Twin Cays. *Botrylloides nigrum* (Plate 1i) is a colony-forming species growing as bright-orange to brick-red sheets covering mangrove roots, overgrowing other sessile organisms and very occasionally growing on the peat bank itself. It is abundant throughout the Main Channel but less common in other parts of the system. Among these colonies are other very similar colonies which I have assigned to the Indo-Pacific species *Botrylloides perspicuum* that is characterized by the presence of vascular ampullae arranged in rows in the test between the rows of zooids. Colonies assigned to this species were also found commonly at Pelican Cays in southern Belize (Goodbody, 2000). This apparent overlap of two such closely related species (Kott and Goodbody, 1982) deserves more intensive research than has been possible during this project. *Botryllus tuberatus* (formerly known as *Botryllus primigenus*) has only been recorded once at Twin Cays on *Thalassia* leaves in Hidden Lake in February 1984. This species is widespread but nowhere common on the Barrier Reef in both mangrove and reefal environments. *Botrylloides magnicoecum* and *Botryllus planus* have only been found rarely (Table 2).

Polyandrocarpa tincta is a colony-forming species normally associated with reefal communities rather than mangroves. Nevertheless, colonies are occasionally found in mangrove areas and the species is recorded once from Twin Cays. A colony was collected from a *Rhizophora* root on the east side of the Main Channel just north of Crescent Bay in February 1984. This area of the channel is not otherwise heavily populated by ascidians or other sessile community growth. It is possible, therefore, that special ecological conditions prevail along this shoreline which promote survival of a normally reef-dwelling species but do not support growth of the usual mangrove-root community. These ecological conditions have not been investigated.

Styela canopus is a solitary species found commonly throughout Twin Cays in most habitats. It is frequently found as erect zooids amongst other elements of the sessile community, easily recognized by characteristic yellow and brown stripes on the inner surface of the siphons. An alternative growth form is squat and rounded found frequently on the shells of the bivalve *Isognomon alatus*. *Styela plicata* is a solitary species only rarely encountered in Caribbean mangroves. Two specimens were recorded growing on oyster shells in the North Channel in March 1981.

The remaining species in the Family Styelidae all belong to the genus *Polycarpa*. *Polycarpa spongiabilis* is one of the largest (5cm) members of the genus found in the Caribbean. It is often abundant in mangrove lagoons living either attached to mangrove roots or buried in sediments with only the siphons visible at the surface. At Twin Cays I have found it only occasionally within the mangrove system. Specimens have been recorded from the Main Channel and Turtle Pond; small specimens have been found embedded among roots of *Thalassia testudinum* in West Bay. *Polycarpa arnoldi* and *Polycarpa cartilaginea* are both small (2cm) reef-dwelling species that have occasionally

been found in West Bay but never in the mangrove system. It is also of interest that *Polycarpa aurita*, which is common in some lagoons at Pelican Cays (Goodbody, 2000), has never been seen at Twin Cays, and no species in the genus *Symplegma* has ever been seen at Twin Cays whereas two species *S. brakenhielmi and S. rubra* are found in lagoons at the Pelican Cays.

Family **Pyurida**e (C. Monniot, 1983c) *Microcosmus exasperatus* is a large (3 to 5cm) species common in mangrove lagoons throughout the Caribbean. It has only rarely been seen at Twin Cays, records including specimens in Twin Bays, Lair Channel and several sites in the Main Channel. There are no confirmed records of either *Pyura vittata* or *Herdmania momus* at Twin Cays although both species are normally common in mangrove systems of this sort.

 Family **Molgulidae** (C. Monniot, 1983c) *Molgula occidentalis* is the only member of this family recorded from the Barrier Reef. Although Van Name (1945) records specimens of 6 cm in length, most specimens collected in Belize are about 2 cm in maximum length. The species is common in parts of the Pelican Cays (Goodbody, 2000) but has seldom been seen at Twin Cays. Specimens have been collected from among *Thalassia* roots at West Bay and from settlement panels in the Main Channel. Because the animal is often embedded in other elements of the sessile community, and sometimes embedded in the peat bank, it is difficult to see and hence may be more common at Twin Cays than appears to be the case.

DISCUSSION

In terms of species richness, the diversity of ascidians at Twin Cays is not especially notable. Table 3 shows the number of ascidian species recorded in various mangrove habitats throughout the Caribbean. Pond A at the Pelican Cays in Belize (42 species) is comparable to Twin Cays (40 species) but Twin Cays and Pond A are both richer than the Fort Rocky Lagoon at Port Royal, Jamaica (25 species). These differences are merely differences in number; species composition is of much more interest from an ecological point of view. The outstanding feature at Twin Cays is the relative paucity of solitary species of ascidian and the abundance of colony-forming species. At Port Royal and at Pelican Cays, colony-forming species are important but solitary species, especially in the Families Ascidiidae, Styelidae, and Pyuridae, are conspicuous elements of the ascidian fauna. In all of these locations, three families of colony-forming ascidian (Didemnidae, Polycitoridae and Perophoridae) contribute significantly to the fauna.

Attention should be focussed on the Perophoridae. Twin Cays is the type-locality for *Perophora regina*, a conspicuous and large member of the genus considered here to be a key species at Twin Cays. Up to the time of writing, this species has only been recorded from two other locations in the Caribbean: Pond B in the Pelican Cays, 15 kilometers south of Twin Cays, and in an isolated lagoon at Blue Ground Range a few kilometers southwest of Twin Cays. No comparable species has been recorded from any other biogeographical region and one is led to speculate as to whether *P. regina* evolved

in Twin Cays and, if so, what were the ecological forces that induced such a development. Wherever the species evolved, it appears to be endemic to the Meso-American Region. Another significant point about the Perophoridae at Twin Cays is the complete absence of *Ecteinascidia turbinata* and *Ecteinascidia conklini*. *E. turbinata* is a widely distributed and common species throughout the Caribbean and is a characteristic inhabitant of eutrophic environments such as Fort Rocky Lagoon, Jamaica and Piscadeera Baai in Curaçao. *E. turbinata* has been found in submerged peat hollows close to Tobacco Range a few kilometers from Twin Cays and has also been recorded at 100 meters depth on the fore-reef at Carrie Bow Cay, hence opportunity for colonization of Twin Cays exists. Similarly, the rare *Ecteinascidia conklini* occurs in some abundance at Blue Ground Range close to Twin Cays but the species has not colonized Twin Cays. It is to be expected that it may do so at some time in the future.

Considering the Caribbean as a whole, it is apparent that members of the Family Perophoridae are a characteristic element of the fauna of mangrove lagoons throughout the region. They are more abundant in mangrove environments than they are in reefal environments. This apparent linkage between mangroves and perophorids is probably associated with two significant features of most members of the family. They are stress resistant, particularly with reference to extremes of salinity and temperature, and they have an ability to regenerate entire colonies or groups of zooids from undifferentiated material residing in stolonic networks or fragments of such networks (cf. Goodbody and Cole, *in press* and Por and Dor, 1975). At Twin Cays the species of this family that are present have a wide distribution occupying almost all habitats (Table 2). It is significant that, as a group, they are especially abundant in Hidden Creek which is a stressed environment subject to strong water flow and considerable fluctuations in temperature (Rützler et al., 2004). In contrast, in the environment of Candy's Pond where changes in temperature and salinity are the important stress factors, only one species in the family, *Ecteinascidia minuta,* has been observed. In this instance it is of further interest that *Perophora regina*, which is abundant in nearby Turtle Pond does not occur in Candy's Pond. It is not possible to throw further light on this pattern of distribution until such time as we learn a great deal more about the ecophysiology of these species.

In the Family Polycitoridae two species, *Distaplia corolla* and *Eudistoma olivaceum*, are considered to be key species in Twin Cays. *Distaplia corolla* was originally described from the Azores (F. Monniot, 1974). Until recently the species was poorly recognized in the Caribbean fauna. It now transpires that it is widely distributed in mangrove environments along the Belize Barrier Reef. I have also found the species in reefal environments in Belize and in Jamaica, but only as small colonies (1 to 2 cm) whereas in some mangrove environments (e.g. pond H at Pelican Cays) it forms large colonies (c.10 cm) and dominates the sessile community. The other key species in the Polycitoridae, *Eudistoma olivaceum,* is common in mangrove environments throughout the Caribbean. It has already been noted above that the species shows a preference for settling in shaded areas at the back of the hanging root complex where it is also close to drainage from the floor of the swamp. It is further suggested that bacteria may be an important element in their diet and that water draining from the swamp floor may provide a rich source of such bacteria. Notwithstanding any preference for shaded areas close to the peat bank, *Eudistoma olivaceum* is widely distributed throughout Twin Cays and at

times has been found to be abundant in the stressed environment of Candy's Pond which appears to provide a reservoir of constantly breeding populations. Although quantitative data are not available, it is apparent that the populations of *E. olivaceum* in Candy's Pond fluctuate and change rapidly from time-to-time. Frequently, mangrove roots in the pond are densely populated by large colonies in which individual heads are large, long and tightly packed with larvae. On other occassions only small populations of newly settled, nonbreeding animals are present. One such occasion occurred in February 1988 after a period of heavy rain storms, thus suggesting that freshwater flooding and reduced salinity may have caused a mass mortality in the population followed by a new settlement of larvae.

Settlement and growth in these populations is maximal in the upper half meter of the water column. As a consequence, the populations will be particularly vulnerable to surface conditions such as freshwater or warm-water influx from the swamp. These populations must be sustained by some particular food supply derived from the enclosed nature of the pond. In the light of what has been recorded earlier about the presence of bacteria in the food cord of *E. olivaceum,* it seems reasonable to postulate that the high level of organic detritus in the pond may support high levels of bacterial production which in turn provide a food source for the ascidians. Whatever the truth is, it is abundantly clear that Candy's Pond is an environment that deserves close attention from future researchers. The channel leading in and out of Candy's Pond and connecting it with Turtle Pond is also an area of interest as very large colonies of *E. olivaceum* occur at either end and occasionally large colonies occur along the length of the channel. Why should such large colonies, 100cm² and more in area, develop at either end of the channel? Presumably it is a function of food supply and water flow. In February 1988 in Turtle Pond there was a relatively rich growth of *E. olivaceum* gradually decreasing in size from the mouth of the channel leading to Candy's Pond toward the entrance to Turtle Channel on the western side of Turtle Pond. In the series of colonies in Turtle Pond, large colonies were replaced by smaller colonies around the margin of the pond further suggesting that the strong flow of food-rich water from Candy's Pond is an important ingredient in encouraging growth and replication of *E. olivaceum*. Its effectiveness is rapidly dissipated as the water mixes with water in Turtle Pond which is influenced also by inflow from The Main Channel of Twin Cays.

At a site in the Lair Channel, which is 65 meters to the east along the north shore from the entrance to Gator Creek, growth of *E. olivaceum* was relatively similar to that in Turtle Pond. There were a lot of medium-sized colonies sheltered on roots with overhanging canopy and on roots hanging away from the peat bank. These colonies were in the size range of 5 to 20 heads each. It is of interest that only a few very small colonies of *E. olivaceum* have been found in the Lair itself so that this pond does not appear to be a focal point for the species in the way that Candy's Pond is a focus. Why? The Lair receives direct drainage from the swamp and is semienclosed. The difference between it and Candy's Pond is the size of the channel through which the pond is flushed. The Lair is less isolated than Candy's Pond and, in consequence, may have less opportunity to develop bacterial populations than seems to be the case in Candy's Pond. In Gator Creek, colonies grow in fairly large numbers on the peat walls of the creek as is also the case in Hidden Creek. Throughout the remainder of the Twin Cays channel system *E. olivaceum*

occurs only sporadically here and here on roots, usually where other sessile organisms do not have a strong presence, often occurring towards the tops of roots and always in small colonies, usually 5 to 10 heads. A site just north of the dock on the west bank of the main channel is representative of this situation. Formerly I considered that competition with other sessile organisms was the main factor controlling the distribution and abundance of *E. olivaceum* but the situation in Turtle Pond (and Candy's Pond) leads me to believe that food is the most important factor. If food is an important limiting factor, the abundance and status of the Lair Channel population may be explained by food resources entering the channel from Gator Creek close to the site in question. We may conclude, therefore, that *E. olivaceum* is an adaptable species and is one which deserves much closer study from an ecological and physiological point of view.

It is of interest at this point to notice that no species in the Family Clavelinidae has been found at Twin Cays, whereas at Pelican Cays, 15 km further south, two species, *Clavelina picta* and *C. puertosecensis,* are abundant in many of the lagoons. *C. picta* also occurs at Blue Ground Range a few kilometers southwest of Twin Cays and *C. puertosecensis* has been found on the fore-reef slope at Carrie Bow Cay, so there is potential for both species to colonize Twin Cays at some time in the future. The closely related Family Pycnoclavellidae is represented at Twin Cays by *Pycnoclavella belizeana* which forms colonies of tiny zooids along the edge of the peat bank in the Main Channel. By virtue of size and abundance, the species can have little ecological significance in the sessile community but nevertheless its presence is of biogeographical interest since no other member of the family is recorded from the Caribbean.

At present there is a fairly comprehensive knowledge of the diversity and distribution of ascidians in the Caribbean as a result of the work of many specialists (Traustedt, 1882, 1883; Sluiter, 1898; Van Name, 1921, 1945; Monniot and Monniot, 1984; Millar, 1962; Millar and Goodbody, 1974; Goodbody, 2000, 2003). Only a few local details need to be resolved. At the time of this writing, we can confirm the presence of 117 species in 39 genera in the Caribbean representing about 5% of known species worldwide. It is time to seek explanations for this diversity and there are some compelling questions for which we need answers. At the community level we should be asking how it is that so many different species of filter-feeding organisms are able to compete for similar resources in the mangrove environment and continue to survive. The same question arises again at the species level and is well illustrated in the genus *Perophora*. Three very closely related species, *P. viridis*, *P. carpenteria*, and *P. bermudensis*, live in close proximity to one another in the sessile communities of Twin Cays apparently competing for the same resources. Ecological theory suggests that there must be significant differences in their requirements--- otherwise one or more species would be eliminated by competition. It is to questions like this that we should now be directing research through study of single-species ecology. This information is needed to understand the complexity of the whole sessile community. Attention should be focused on other aspects of population growth and regulation in these communities. Apart from competition, the question of predation arises as a force controlling ascidian populations in mangrove environments. My observations at Twin Cays have thrown little light on sources of predation. Species of the gastropod *Diodora* are occasionally seen grazing on colonial ascidians as are also the Polyclad Turbellarians *Thysanozoon nigrum* and

12

Pseudoceros crozieri. An unidentified nudibranch and a species of *Thais* have been seen grazing on Dis*taplia corolla.* With a few exceptions fishes seem uninterested in feeding on ascidians. Angelfish *(Pomacanthus sp)* and the Spadefish (*Chaetodipterus faber*) have been seen grazing on sessile communities and include small perophorids in their diet.

CONCLUSION

On the basis of my observations in mangrove environments throughout the Caribbean I believe we may generalize and make a number of postulates about the factors that control the distribution and abundance of ascidians in Twin Cays and other mangrove systems.

- Ascidians are hard substratum limited. In mangrove systems this restricts them to living on mangrove roots and other biota such as oyster shells. In the few cases (e.g. *Polycarpa spongiabilis*) in which they live in bottom sediments, it will usually be found that there is the remnant of a shell or other hard object attached to the posterior of the zooid; this probably is the original substrate on which the larva settled.

- Ascidians are inhibited by high sediment loading in the water column. In consequence ascidians survive best where water exchange is good and suspended sediments are removed by water flow before they can resettle on and suffocate the sessile community.

- Long roots hanging in shallow water often reach to the bottom sediments; during windy conditions such roots drag on the bottom disturbing the sediment. In consequence ascidians are usually found growing where roots hang clear of the bottom sediments.

- Ascidians are inhibited by close proximity of certain sponges, e.g. *Tedania ignis.*

- Most ascidians, but not all, are sensitive to stress, particularly lowered salinity (Goodbody, 1961). Mangrove swamps seasonally collect large volumes of rainwater which drains laterally through the root fringe giving rise to a surface layer of low-salinity water that inhibits ascidian growth in the upper portion of the roots. This upper zone becomes dominated by the oyster *Isognomon alatus*, which is apparently resistant to low-salinity stress. When normal salinity is restored, fast growing opportunist species of ascidian, such as *Ecteinascidia minuta*, subsequently use the oyster shell as a substrate for settlement.

- Temperature may not be important in defining local distribution but may affect reproductive processes. At Twin Cays, warm-water fluxes due to daytime heating periodically drain through the root system, e.g. in Hidden Creek (Rützler et al., 2004), and several species of ascidian survive in this environment (See Table 2).

The distribution of ascidians at Twin Cays may be explained in terms of these general postulates. A main focus of ascidian growth occurs at the north end of the Main Channel where water exchange is good, driven into the channel by the prevailing northeast wind. Water flow at this north end has caused deep erosion of the peat bank in this area so that mangrove roots are long and hang well clear of the peat bank. A similar but less obvious situation occurs at the southern end of the channel in the vicinity of Sponge Haven.

The lower end of Hidden Creek has rich communities of ascidians, especially members of the Perophoridae. This may be attributed to the strong flow of water that keeps sedimentation to a minimum and has eroded the peat bank in such a way as to enable mangrove roots to hang free from the bank. A negative factor in Hidden Creek is the temperature fluctuation already referred to above.

Twin Bays is different from any other environment at Twin Cays because of its sheltered position at the leeward end of the island. It nevertheless seems to experience sufficient flushing to prevent the accumulation of high levels of organic detritus such as occur in the closed environment of Candy's Pond. The most significant ascidian presence in Twin Bays was the population of *Distaplia corolla* but there is insufficient information on the ecology of this species to suggest why it should thrive at this site.

In general we may postulate that, at Twin Cays and elsewhere, the drainage of organic material from the swamp floor into the aquatic system must be a major element in maintaining sessile communities on the mangrove roots. Finally, the population structure of ascidians at Twin Cays should not be regarded as stable. The proximity to reefal communities elsewhere in the Southwater Reserve provides ample opportunity for opportunistic colonization in Twin Cays by species normally associated with reef environments. Such species (e.g. *Ascidia corelloides)* may colonize and survive but fail to reproduce and form stable populations in Twin Cays. Nevertheless, once a species does establish a breeding population, opportunity exists for that species to spread throughout the ecosystem and colonize wherever suitable habitat exists. In consequence, the diversity and distribution of species as described in this paper may alter from time-to-time as new colonists invade and existing colonists change their distribution with the passage of time and changing ecological conditions.

REFERENCES

Goodbody, I.
   1961. Mass mortality of a marine fauna following tropical rains. *Ecology* 42:150-155.
Goodbody, I.
   1994. The tropical Western Atlantic Perophoridae (Ascidiacea): The genus
       Perophora. Bull. mar. Sci. 55 (1):176-192.
Goodbody, I.
   1995. The timing of larval release in colonial acidians. *Caribbean Mar. Studies.* 4:3-
       10.
Goodbody, I.
   1996. *Pycnoclavella belizeana*, a new species of ascidian from the *Caribbean. Bull.
       mar. Sci* .58 (2):590-597.
Goodbody, I.
   2000. Diversity and distribution of ascidians (Tunicata) at the Pelican Cays, Belize.
       Atoll Res. Bull. 480 24 pp.
Goodbody, I.
   2003. The ascidian fauna of Port Royal, Jamaica I. harbor and mangrove dwelling
       species. *Bull. mar. Sci.* 73 (2):457-476.
Goodbody, I. and L. Cole.
   In press. The tropical Western Atlantic Perophoridae (Ascidiacea): II. The genus
       Ecteinascidia. Bull. mar. Sci.
Kott, P.
   2001. The Australian Ascidiacea Part 4, Aplousobranchia (3) Didemnidae. *Memoirs of
       the Queensland Museum* 47(1):1-407.
Kott, P. and I. Goodbody.
   1982. The Ascidians of Hong Kong. pp 503-504. *In* Morton, B. S. & C.K. Tseng (*Eds.*)
       Proceedings of the first International Marine Biological Workshop: the flora and
       fauna of Hong Kong and Southern China. Vol.1. Hong Kong University Press.
Millar, R.H.
   1962. Some ascidians from the Caribbean. *Studies on the fauna of Curacao and other
       Caribbean Islands.* XIII:61-77.
Millar, R.H., and I. Goodbody
   1974. New species of ascidians from the West Indies. *Studies on the fauna of Curacao
       and other Caribbean islands.* XLV:142-161.

Monniot, C.
   1973. Ascidies Phlebobranches des Bermudes. *Bull. Mus. natn. Hist. nat., Paris* 3e
ser.,
            82, Zool. 61:939-948.
Monniot, C.
   1983a. Ascidies littorales de Guadeloupe II. Phlebobranches. *Bull. Mus. natn. Hist.*
            nat., Paris 4e sér., 5, Séction A. No.1:51-71.
Monniot, C.
   1983b. Ascidies littorales de Guadeloupe IV Styelidae. *Bull. Mus. natn. Hist. nat.,*
            Paris 4e sér., 5, Séction A. No.2:423-456.
Monniot, C.
   1983c. Ascides littorales de Guadeloupe VI Pyuridae et Molgulidae. *Bull. Mus. natn.*
            Hist. nat., Paris 4e sér., 5, Séction A. No.4:1021-1044.
Monniot, C. and F. Monniot.
   1984. Ascidies littorales de Guadeloupe VII. Espèces nouvelles et complementaires a
            l'inventaire. *Bull. Mus. natn. Hist. nat., Paris* 4e sér., 6, Section A. No. 3:567-
            582.
Monniot, F.
   1972. Ascidies aplousobranches des Bermudes. Polyclinidae et Polycitoridae. *Bull.*
            Mus. natn. Hist. nat., Paris 3e sér., 82, zool. 61:949-962.
Monniot, F.
   1974. Ascidies littorales et bathyales récoltées au cours de la campagne Biaçores:
            Aplousobranches. *Bull. Mus. natn. Hist. nat., Paris* 3e sér., No.251, Zoologie
            173:1287-1325.
Monniot, F.
   1983a. Ascidies littorales de Guadeloupe I. Didemnidae. *Bull. Mus. natn. Hist. nat.,*
            Paris 4e sér., 5, séction. A. 1:5-49.
Monniot, F.
   1983b. Ascidies littorales de Guadeloupe III. Polyclinidae. *Bull. Mus. natn. Hist.*
            nat.,Paris 4e sér., 5, séction A. 2:413-422.
Monniot, F.
   1983c. Ascidies littorales de Guadeloupe V. Polycitoridae. *Bull. Mus. natn. Hist. nat.,*
            Paris 4e sér., 5, séction A. 4:999-1019.
Por, F. D. and I. Dor.
   1975. Ecology of the metahaline pool of Di Zahav, Gulf of Eilat with notes on the
            Siphonocladacea and the typology of near-shore marine pools. *Mar. Biol.* 29:
            37-44.
Rodriguez, W. and I. C. Feller.
   2004. Mangrove landscape characterization and change in Twin Cays, Belize
            using aerial photography and IKONOS satellite data. *Atoll Res. Bull.* 512:1-22
Rützler K., I. Goodbody, M.C. Diaz, I.C. Feller, and I.G. Macintyre
   2004. The aquatic environment of Twin Cays, Belize. *Atoll. Res. Bull.* 513:1-49
Sloot, C. J. van der
   1969. Ascidians of the Family Styelidae from the Caribbean. *Studies Fauna Curaçao*
            30:1-57.

16

Sluiter, C.P.
    1898. Tuniciers recueillis en 1896 par la Chazallie ans la mer des Antilles. *Mém Soc. Zool. Fr.* 11:5-34.
Traustedt, M. P. A.
    1882. Westindiske Ascidiaesimplices, Forste Adfeling Phallusiadae. *Vidensk Meddr dansk naturh. Foren* 1881:257-288.
Traustedt, M. P. A.
    1883. Westindiske Ascidiae simplices, Anden Adfeling Molgulidae og Cynthiadae. *Vidensk Meddr dansk naturh Foren* 1882:108-134.
Van Name, W.G.
    1921. Ascidians of the West Indian region and south-eastern United States. *Bull. Amer. Mus. nat. Hist.* 44:283-294.
Van Name, W.G.
    1945. The North and South American Ascidians. *Bull. Amer. Mus. nat. Hist.* 84:1-476.

Table 1. List of Ascidian Species Recorded from Twin Cays
Total recorded species = 40   * = key species

FAMILY: Polyclinida*e*
Aplidium antillense (Gravier, 1955)
FAMILY: Didemnidae
Trididenum cyanophorum Lafargue et Duclaux, 1979
*Didemnum conchyliatum (Sluiter, 1898) (Plate 1a)
Didemnum psammathodes (Sluiter, 1895)
Lissoclinum abdominale F. Monniot, 1983
Lissoclinum fragile (Van Name, 1902)
*Diplosoma glandulosum F. Monniot, 1983 (Plate 1b)
Diplosoma listerianum (Milne-Edwards, 1841
FAMILY: Polycitoridae
*Distaplia corolla Monniot F., 1974 (Plate 1c)
Eudistoma capsulatum (Van Name, 1902)
Eudistoma obscuratum (Van Name , 1902)
*Eudistoma olivaceum (Van Name, 1902) (Plate 1d)
FAMILY: Pycnoclavellidae
Pycnoclavella belizeana Goodbody, 1996 (Type locality)
FAMILY: Perophoridae
*Perophora bermudensis Berrill, 1932
*Perophora carpenteria Goodbody, 1994 (Type locality) (Plate 1f)
Perophora multiclathrata (Sluiter, 1904)
*Perophora regina Goodbody & Cole, 1987 (Type locality) (Plate 1e)
Perophora viridis Verrill, 1871
*Ecteinascidia minuta (=E. tortugensis) Berrill, 1932 (Plate 1h)
*Ecteinascidia styeloides (Traustedt, 1882) (Plate 1g)
FAMILY: Ascidiidae
Ascidia corelloides (Van Name, 1924)
Ascidia curvata (Traustedt, 1882)
Ascidia interrupta Heller, 1878
Ascidia sydneiensis Stimpson, 1855
Ascidia tenue Monniot, 1983
Phallusia nigra (=Ascidia nigra) Savigny, 1816
FAMILY: Styelidae
Botrylloides magnicoecum (Hartmeyer, 1912)
*Botrylloides nigrum Herdman, 1886 (Plate 1i)
Botrylloides perspicuum Herdman, 1886
Botryllus planus (Van Name, 1902)
Botryllus tuberatus Ritter et Forsyth, 1917
Polyandrocarpa tincta Van Name, 1902
Polycarpa arnoldi Michaelsen, 1915
Polycarpa cartilaginea (Sluiter, 1898)
Polycarpa spongiabilis Traustedt, 1883

Table 1 cont'd
Styela canopus (=S. partita) Savigny, 1816
Styela plicata   (Lesueur, 1823)

FAMILY: Pyuridae
Pyura munita (Van Name, 1902)
Microcosmus exasperatus Heller, 1878
FAMILY: Molgulidae
Molgula occidentalis Traustedt, 1883

Table 2. The Distribution of Ascidian Species in Different habitats at Twin Cays
a = abundant: + = present: - = absent: o = occasional: r = rare.
Key species are indicated in bold
tb = Twin Bays, c = Main channel, nc = North Channel, lc = Lair Channel
l = Lair, hc = Hidden Creek, hl = Hidden Lake, tp = Turtle Pond, cp = Candy's Pond, gg
= Grouper Gardens, gc = Gator Creek, w = West Bay, op = Outer Perimeter

Species	tb	c	nc	lc	l	hc	hl	tp	cp	gg	gc	w	op
Aplidium antillense	r	-	-	-	-	-	-	-	-	-	-	-	-
Trididemnum cyanophorum	-	-	-	-	-	-	-	-	-	-	-	-	r
Didemnum conchyliatum	+	+	+	+	+	+	-	+	-	o	-	-	-
Didemnum psammathodes	r	r	-	r	r	o	-	r	-	-	-	-	-
Lissoclinum abdominale	-	-	+	-	-	-	-	-	-	-	-	-	-
Lissoclinum fragile	+	-	-	-	-	-	-	-	-	-	-	-	-
Diplosoma glandulosum	-	o	a	a	-	-	-	-	-	-	-	-	-
Diplosoma listerianum	-	+	+	+	+	-	-	-	-	-	-	-	-
Distaplia corolla	a	+	o	a	-	-	-	-	-	o	-	-	-
Eudistoma capsulatum	-	o	r	-	-	-	-	-	-	-	-	-	-
Eudistoma obscuratum	-	o	o	-	-	-	-	-	-	-	-	-	-
Eudistoma olivaceum	o	o	o	+	+	+	-	+	a	-	+	-	-
Pycnoclavella belizeana	-	+	+	-	-	-	-	-	-	+	-	-	-
Perophora bermudensis	-	o	o	+	a	+	-	-	-	o	-	-	-
Perophora carpenteria	+	+	+	+	+	+	-	-	-	+	-	-	-
Perophora multiclathrata	-	-	r	-	-	-	-	-	-	-	-	-	-
Perophora regina	-	o	+	-	-	a	-	a	-	-	-	-	-
Perophora viridis	-	-	-	o	o	+	-	+		r	-	-	-
Ecteinascidia minuta	+	o	o	+	+	+	+	+	+	-	-	-	-
Ecteinascidia styeloides	-	o	o	o	+	a	-	-	-	o	-	-	-
Ascidia corelloides	-	-	r	-	-	-	-	-	-	-	-	-	-
Ascidia curvata	-	r	-	-	-	-	-	-	-	-	-	-	-
Ascidia interrupta	o	-	-	o	-	-	-	-	-	-	-	-	-

Table 2 con'td

Species													
Ascidia sydneiensis	-	r	-	-	-	-	-	-	-	-	-	-	-
Ascidia tenue	-	-	r	-	-	-	-	-	-	-	-	-	-
Phallusia nigra	o	-	o	-	-	-	-	-	-	-	-	-	-
Botrylloides magnicoecum	-	r	-	-	-	-	-	-	-	-	-	-	-
Botrylloides nigrum	-	a	+	-	-	o	-	-	-	-	-	-	-
Botrylloides perspicuum	-	+	+	-	-	-	-	-	-	-	-	-	-
Botryllus planus	-	r	-	o	-	-	-	-	-	-	-	-	-
Botryllus tuberatus	-	-	-	-	-	-	r	-	-	-	-	-	-
Polyandrocarpa tincta	-	r	-	-	-	-	-	-	-	-	-	-	-
Polycarpa arnoldi	-	-	-	-	-	-	-	-	-	-	-	r	-
Polycarpa cartilaginea	-	-	-	-	-	-	-	-	-	-	-	r	-
Polycarpa spongiabilis	-	r	-	-	-	-	-	r	-	-	-	o	-
Styela canopus	-	o	o	o	o	o	-	-	-	-	-	-	-
Styela plicata	-	-	r	-	-	-	-	-	-	-	-	-	-
Pyura munita	-	-	-	-	-	-	-	-	-	-	-	o	-
Microcosmus exasperatus	r	r	r	r	-	-	-	-	-	-	-	-	-
Molgula occidentalis	-	o	-	-	-	-	-	-	-	-	-	r	-

Table 3. The number of Species of Ascidian occurring at different mangrove locations in the Caribbean

Site	# Ascidian Species	Reference
Jamaica, Fort Rocky	25	Goodbody, 1993
Curacao, Pisadera Baai	23	Goodbody, 1984
Bonaire, Lac	15	Goodbody, 1984
Belize, Pelican Cays Pond A	42	Goodbody, 2000
Pond C	39	Goodbody, 2000
Pond H	27	Goodbody, 2000
Twin cays	40	This work

PLATE

Plate 1. Representative species of ascidians at Twin Cays in situ: *a, Didemnum conchyliatum; b, Diplosoma glandulosum; c, Distaplia corolla; d, Eudistoma olivaceum; e, Perophora regina; f, P. carpenteria; g, Ecteinascidia styeloides; h, E. minuta; i, Botrylloides nigrum.*

Plate 1

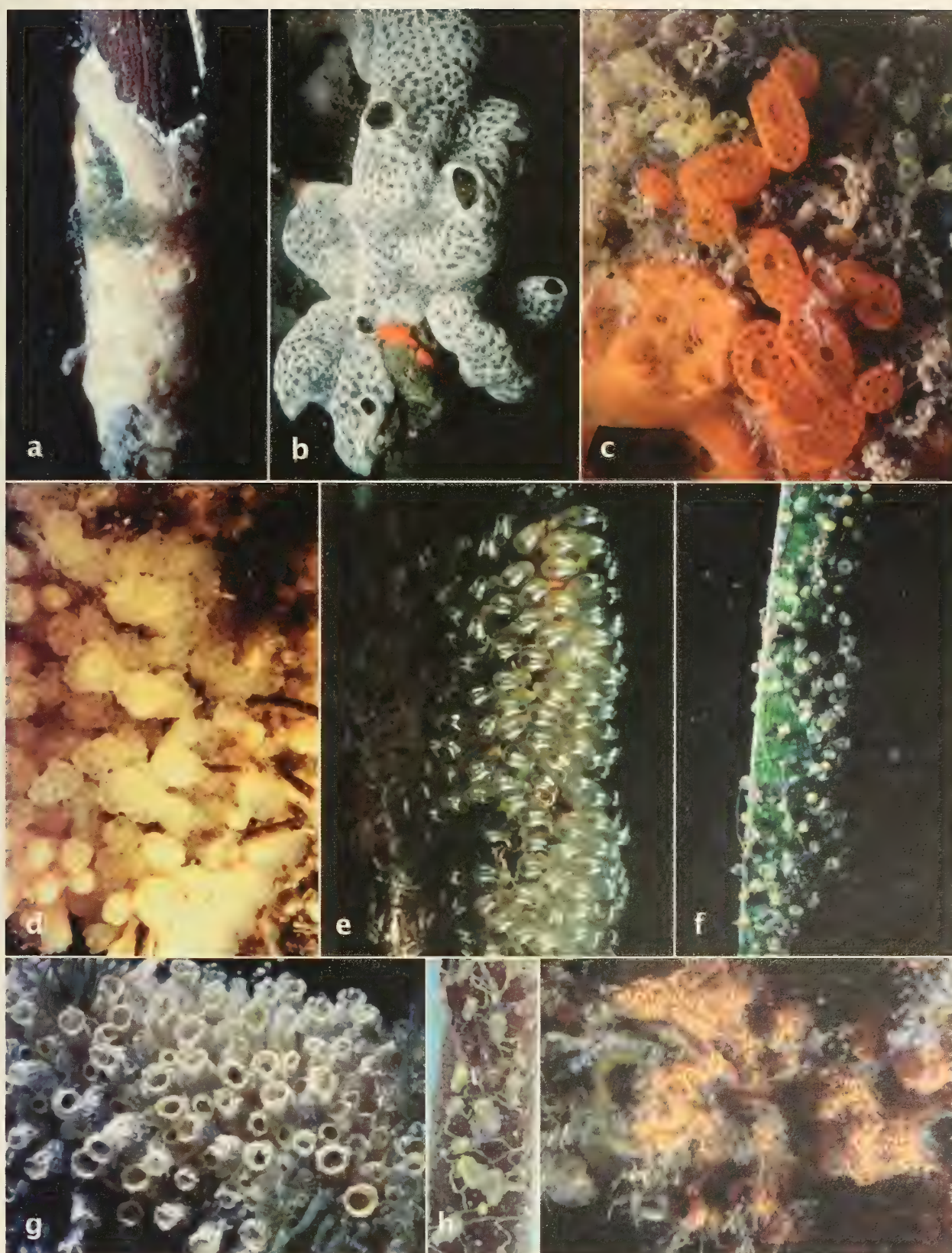GROVELING IN THE MANGROVES: 16 YEARS IN PURSUIT OF THE
CYPRINODONT FISH *RIVULUS MARMORATUS* ON THE BELIZE CAYS

BY

D. SCOTT TAYLOR, WILLIAM P. DAVIS, AND BRUCE J. TURNER
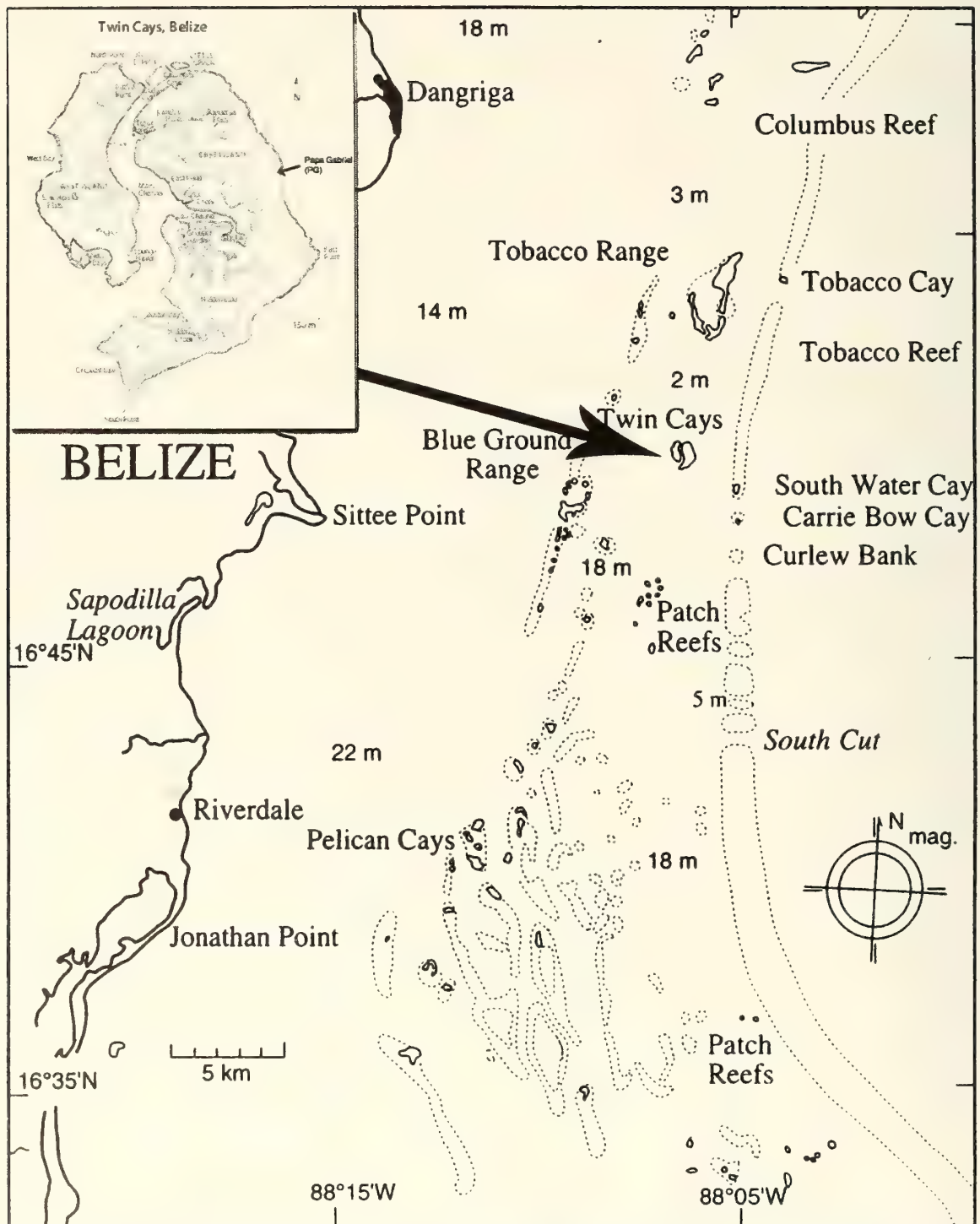
Figure 1. Map of the Belize Cays showing the range of collections for *Rivulus marmoratus*. Glovers Reef is not shown.

GROVELING IN THE MANGROVES: 16 YEARS IN PURSUIT OF THE CYPRINODONT FISH *RIVULUS MARMORATUS* ON THE BELIZE CAYS

BY

D. SCOTT TAYLOR[1], WILLIAM P. DAVIS[2], AND BRUCE J. TURNER[3]

ABSTRACT

The cyprinodont fish *Rivulus marmoratus* is strongly affiliated with the red mangrove *Rhizophora mangle* and distributed throughout the tropical western Atlantic including the Belize Cays. We have studied this animal for 16 years on the cays during six investigations since 1988. We summarize our findings in the field and explore the significance of the continued presence of high numbers of male fish on certain cays, a phenomenon unknown elsewhere. We also present data on mainland (Dangriga) collections where male fish were not found, an as-yet unexplained contrast to the Belize Cays.

INTRODUCTION

The cyprinodont fish (Aplocheilidae) *Rivulus marmoratus* (Poey) is widely distributed from southern Brazil through the Caribbean islands and Central America to the Yucatan and along both coasts of Florida north to central Florida (Lat 26° N) (Taylor, 2000). The fish is strongly affiliated with the red mangrove (*Rhizophora mangle*) and tracks the distribution of this plant in the western Atlantic, thus giving rise to the common name "mangrove rivulus" (Davis et al., 1995). *Rivulus marmoratus* was first described from Cuba by Poey (1880). However, until well into the next century, *R. marmoratus* was thought to be very rare (Harrington and Harrington, 1961; Harrington, 1971) and, until 1988, fewer than 50 specimens were captured in the state of Florida, an area intensely collected by ichthyologists for several decades. At least in some locations, the apparent scarcity of the species appears to be a factor of inappropriate sampling methodology that failed to target specific microhabitats within the mangal.

It is now clear from our combined work in several western Atlantic locales that *R. marmoratus* has been largely overlooked using standard ichthyological methods because of the unique and sometimes "fossorial" nature of its habitats, including crab burrows, transitional pools and even terrestrial refuges, under leaf litter and logs (Davis et al., 1990; Taylor, 2000). Our collection efforts over the last two decades have focused in Florida, the Bahamas, Honduras, and the cays of the Belize barrier reef (Fig. 1). We have developed a search strategy for appropriate *R. marmoratus* habitat as well as specialized

[1]Brevard County Environmentally Endangered Lands Program, 5560 N. US 1, Melbourne, FL 32940 USA.
[2]United States Environmental Protection Agency, Gulf Breeze, FL 32561 USA.
[3]Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 USA.

collection techniques. Our studies demonstrate that *R. marmoratus* can be abundant in appropriate habitat. However, we have also observed that habitat alteration, such as mosquito impoundment construction and destruction of mangroves and saltmarsh, can negatively impact populations (e.g. east coast of Florida) (Davis et al., 1990; Taylor, 2000; Taylor et al., 2003

Rivulus marmoratus has behavioral and physiological adaptations that are novel among fishes in general although perhaps widespread among the approximately 100 species of the genus. *Rivulus marmoratus* survives in water-quality conditions that are adverse for other fishes (high temperature, widely varying salinities, high levels of H_2S and ammonia) with a remarkable suite of physiological and behavioral adaptations. Salinity variation is apparently compensated for with highly efficient chloride cells which allow acclimation in the laboratory to 114 ppt. (King et al., 1989). With this mechanism, *R. marmoratus* has been found in the wild at salinities ranging from 0-68 ppt (Taylor, 2000). Ammonia concentrations can also be quite high in crab burrows (up to 4.7 umol l $^{-1}$) and *R. marmoratus* tolerates these levels (Frick and Wright, 2002). Hydrogen sulfide is acutely toxic to fishes at even very low concentrations and *R. marmoratus* avoids this hazard by leaving the water, a behavior known as "emersion" (Abel et al., 1987). Upon leaving the water, the fish respires in moist environments via an expanded capillary network, absorbing O_2 through the skin and fins (Grizzle and Thiyagarajah, 1987). As long as a damp refuge is available (e.g. mud, rotting logs, leaf litter), *R. marmoratus* can survive emersed in a laboratory setting for at least 66 days. Although considerable weight-loss occurs during a lengthy emersion, fish quickly resume feeding and normal activity when reflooded (Taylor, 1990). Once emersed in the wild, we presume that *R. marmoratus* reenters the water when conditions improve. These remarkable adaptations perhaps explain a common observation of ours: few other fish species are found in most *R. marmoratus* habitats.

Apart from these attributes reminiscent of a salamander, most scientific interest in this species is generated by its unique reproductive system and genetics. *Rivulus marmoratus* is the only vertebrate animal known to reproduce as a self-fertilizing hermaphrodite (Harrington, 1961). The gonad is an integrated ovotestis organ that simultaneously produces eggs and sperm with fertilization in the gonoduct at ovulation (Harrington, 1961). This reproductive strategy results in the fish cloning itself, producing exact genetic duplicates (at the level of multilocus DNA fingerprinting) that are stable for at least 20 generations (Laughlin et al., 1995). This reproductive mode produces homozygous lines in wild populations and most *R. marmoratus* populations are now identified as groups of homozygous clones (Kallman and Harrington, 1964; Harrington and Kallman, 1968; Turner et al., 1992b). However, unlike conventional invertebrate/plant clonal systems, clonal diversity is very high with essentially a 1:1 ratio of fish:clone. This high diversity is enigmatic (Turner et al., 1992b). Further, a more basic question remains about how a homozygous clonal organism, with presumed genetic "limitations", can successfully occupy such a vast geographical range.

This presumably infallible reproductive system is confused by the presence of male fish. Male fish, distinguished by having only testicular gonadal tissue, were first noted in laboratory cultures reared at low temperatures (20° C) (Harrington, 1967). Males

occur in specific wild populations (Taylor, 2000) and show evidence of loss of ovarian tissue. A significant number of male fish were described in the wild from Curacao (Kristensen, 1970) and a few have been found in Florida, Honduras and the Bahamas (Taylor et al., 2001).

The greatest number of males, however, have been observed in the Belize Cay populations and our observations here and subsequent genetic studies have exploded previous concepts of the reproductive/genetic system of *R. marmoratus*. Our first collection in Twin Cays (1988) revealed a large number of males clearly distinct from hermaphrodites by their unique coloration and confirmed by histology (Davis et al., 1990; Turner et al., 1992a) (Fig. 2, 3). These males contain functional testes, often with little or no trace of ovarian tissue. Subsequent genetic investigation revealed almost uniform heterozygosity on some Belize Cays (Lubinski et al., 1995) and this trait has continued for at least several years (Taylor et al., 2001). The most parsimonious way for heterozygosity to occur in this clonal system is via outcrossing. However, the mechanism by which this occurs has not been identified. We hypothesize that, in the presence of males, hermaphrodites suppress internal fertilization and lay unfertilized eggs, that are fertilized externally by males. This is an intriguing mechanism and neither the histology nor physiology have been elucidated nor have functional "females" been identified in the wild.

Figure 3. *Rivulus marmoratus,* male.

Figure 2. *Rivulus marmoratus, hermaphrodite*

A lower percentage of male fish (2%) has been described in populations from Utila, Bay Islands, Honduras and a limited outcrossed, heterozygous population (~2%) has resulted here (Taylor et al., 2001). It is further intriguing to note that the Utila population (135 km distant from Twin Cays) appears to operate under a completely different genetic system with minimal outcrossing.

Thus, the male "issue" continues to be one of the great unanswered questions in *R. marmoratus* research. It may be that although outcrossing is intermittent and infrequent in all populations it appears to have persisted in the Belize Cays for over a

4

decade. The broader question, of course, is the significance of the phenomenon in an apparently highly successful clonal organism that has managed to colonize a huge area of a highly inhospitable environment, the mangal of the western Atlantic.

With this larger question looming, we have persisted in our field investigation of the Belezian mangal and below we summarize our collections of this intriguing animal during six investigations since 1988.

MATERIALS AND METHODS

We sampled *R. marmoratus* populations on Twin Cays and adjacent cays and mainland Belize (Dangriga) during 1988, 1989, 1990, 1992, 2000 and 2003 (Fig. 1). We utilized a number of collection techniques within temporary pools and burrows of the mangrove land crab, *Ucides cordatus*, which have been described elsewhere and are summarized in Table 1 (Taylor, 1990; Davis et al., 1990; Davis et al., 2003). We recorded basic water-quality data including salinity via optical refractometer, temperature, and tidal stages at different times of the year and attempted to correlate these data with behavior of the fish. Each collection period encompassed approximately two weeks. Our collection on Twin Cays focused on the site "Papa Gabriel" (PG) (Fig. 1; Davis et al., 1990). We also have documented extensive clearing of mangroves at this site over the 16-year period.

Table 1. Gear types used for collection of *Rivulus marmoratus* on the Belize Cays.

Gear	Description	Technique
Cup trap/'pipe' trap	8-10 oz. plastic cup fitted w/ plastic funnel or misc. plastic tubular material fitted w/ fiberglass screen funnel	Inserted funnel-down into crab burrows or set in shallow, flooded pools and covered w/ debris to form a 'refuge'
Wire minnow trap (Gee®)	Cylindrical, commercial wire trap w/ funnel on both ends	Set in shallow pools of depth sufficient to flood funnel openings. Covered w/ debris to form 'refuge'
Hook and line	Small 'stick' w/ 25 cm. of light monofilament line and small hook w/ marine polychaete worm bait	Dangle bait in crab burrow. Fish seizes worm end and is lifted out for capture
Dip net	Custom-made wire-frame net (~70 cm dia) w/ 3 mm mesh netting	Thrust rapidly into crab burrows to capture fish

All specimens collected were released except for those retained for ongoing genetic research or for maintenance of a diverse set of clonal lineages in our respective laboratories. Those retained alive were stored after collection and transported in small Whirl-Pac® bags (Davis et al., 1990).

RESULTS

We collected a grand total of 1,271 *R. marmoratus* during our six investigations. We have summarized the collections from PG (n= 986) in Table 2. The following narrative summarizes each collection from PG, surrounding cays and the mainland site.

Table 2: *Rivulus marmoratus* collection data from Twin Cays, Belize Cays for six different years.

Year	Total collected (#herms./#male	% male
1988	121 (85/36)	29.7
1989	183 (161/22)	12.0
1991	252 (240/12)	4.7
1992	265 (246/19)	7.1
2000	134 (111/23)	17.1
2003	31(23/8)	25.8

July 28 to August 10, 1998

Some data on collections for 1988 and 1989, as well as a description of the PG site, were reported by Davis et al. (1990). During this visit, our first, we encountered very wet conditions with heavy rainfall and high tidal stages. A tidal "pool" formed behind the dwelling at PG from rainfall and receding high tides, reaching a maximum depth of 16 cm. Salinity within crab burrows and pools ranged from 13 ppt to 36 ppt and temperature varied from 25° C in burrows to 37° C within the pool in full sun. Many specimens (n=63) were taken from this pool including rarely collected juveniles (5-9 mm TL). We took 33 fish from crab burrows. The first male fish taken in Belize was collected at this PG site. We also took two fish from the Boa Flats area and two from Man 'O War Cay confirming that the species was not confined to Twin Cays. We noted that some hermaphrodites were so gravid that many viable embryos were released into the collection bags.

July 7 to July 16, 1989

By contrast, conditions this year on the Cays were much drier than during 1988 with lower tides and less rainfall. Salinities were as high as 60 ppt in crab burrows and drying pools during this period but had dropped to 17 ppt due to heavy rainfall by the end of the period. Temperature ranged from 24-28° C within burrows and 34-36° C in exposed pools. Extensive clearing of mangroves at PG had occurred during the past year and a swath about 15m x 50m had been clear-cut. In addition to collections on Twin Cays, we expanded our survey to include Tobacco Range (18 hermaphrodites, 1 male) and Blue Ground Range (2 hermaphrodites, 1 male). "Dry" conditions, however, apparently hampered collection efforts as the catch-per-unit-effort (CPUE=1 trap-night) was less during 1989 than 1988 (1988= 0.35 CPUE; 1989= 0.097 CPUE) (Davis et al., 1990). In addition, fish appeared "thin" and no embryos were released in the bags. This year also included our first observation in Belize of what we have termed "sulfured"

R. marmoratus, in which individual fish have patches of white on the epidermis often covering much of the body and fins. We have subsequently observed this phenomenon repeatedly during other drought conditions and believe that it represents growth of sulfur-oxidizing bacteria, possibly *Beggiatoa* sp.

The year 1989 also provided an opportunity to compare collections in the newly cleared site vs. the mangrove areas of PG intact from the previous year. We compared the catch results from 51 traps set in the newly cleared area over a period of 11 days vs. the results of 28 traps set in the uncleared area during the same period. The CPUE for the cleared area was 0.86 vs. 0.61 for the uncleared area.
We are not certain whether this represents a correlative observation, beginning of a trend or the range of variation of field observations.

January 10 to January 23, 1991

This was another "dry" event and resulted in some interesting observations. In addition, at PG further clearing of the site had taken place extending the cleared swath 50 m north of the dwelling and west all the way to Hummingbird Pond. Large piles of cut mangrove were formed for burning and all the crab burrows in the cut shoreline area were gone. Crab burrows that were still present in the newly cleared areas in 1989 were reduced by an estimated 90%. A considerable amount of sand and rock had been placed in the area adjacent to the dwelling and the formerly productive pool area was filled in. Although tides were very low, salinities were not high (ranging from 26 ppt at the inception of the sampling period and dropping to 10 ppt on 1/14/91 following 2.5 cm rainfall). Water temperature fell within a narrow range of 26°-29° C.

The CPUE with cup traps was relatively low (0.27 for a total of 163 trap-nights). Many of the *R. marmoratus* observed within burrows exhibited the "sulfured" condition once again, perhaps more commonly than in 1989. Two new observations were presented to us during this collection. We inadvertently discovered a large number of *R. marmoratus* emersed under some plastic sheeting debris near the shoreline at PG. Further investigation found this to be a common phenomenon and nearly 80 individuals were collected in this fashion from under debris and logs. Apparently, *R. marmoratus* had left the confines of the crab burrows and emersed in these habitats. Emersion under logs and leaf litter was described in Florida (Huehner et al., 1985; Davis et al., 1990), but ours represents the first observation of this behavior for Belize. Another startling example of emersion was found on Peter Douglas Cay. Here we discovered a drying pool (8 m x 3 m: salinity 22 ppt) about 50 m from the shoreline (Fig. 4). It contained several species of dead fish (poeciliids, gerreids) at the water's edge. When we moved a small log (1.5 m long, 9 cm dia) at the edge of the water, a number of *R. marmoratus* fell out. Upon breaking the log open, we found it riddled with termite galleries all full of *R. marmoratus* (Fig. 5). We estimate that over 100 individuals were contained therein and many of these were returned to our stateside laboratory still within a portion of the log. Presumably, this represents yet a further adaptation to poor water quality conditions.

We also found that shallow depressions, usually around the bases of stumps and associated with one or more crab burrows along the intertidal sections of the

Figure 4. Temporary mangrove pool at Peter Douglas Cay. Over 100 Rivulus marmoratus were found emersed inside a galleried log at this site.
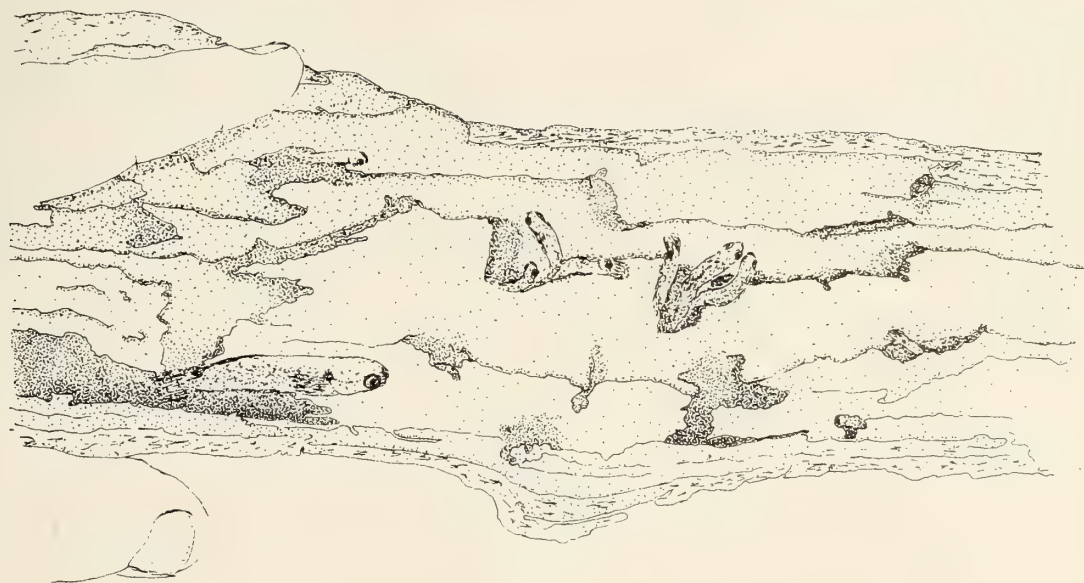

Figure 5. A sketch of the log from Peter Douglas Cay, with emersed Rivulus marmoratus visible.

shoreline, contained large numbers of very large *R. marmoratus*. We collected from 8-21 individuals from three of these sites at PG and most were very large hermaphrodites. Once again, we speculate that these were fish that had moved down the marsh profile due to inhospitable conditions at higher elevations.

8

We sampled other locales this year also. We collected 11 hermaphrodites on Coco Plum Cay, 2 on Elbow and 3 on Pelican Cays and 21 hermaphrodites and 1 male on Ragged Cay. A limited sampling effort at the minimal mangrove habitat on Middle Cay, Glovers Reef produced no *R. marmoratus*.

March 19 to April 1, 1992

This collection period represented yet another dry period, with low tides and little rainfall. At PG we collected *R. marmoratus* at salinities ranging from 34 ppt within burrows to a maximum of 65 ppt in drying pools. This latter represents the highest salinity at which we have collected the fish in any locale. Temperature ranged from 27° in burrows to 37° C in pools. We observed again many "sulfured" fish and considerable emersion inside crab burrows and also noted that the incidence of emersion apparently decreased with flooding tides (we observed tides rise as much as 5 cm in 1.5 h during this period). We completed additional collections at Man 'O War (35 hermaphrodites, 7 males) and Peter Douglas Cays (70 hermaphrodites, 4 males). Another trip to the same mangrove site on Middle Cay, Glovers Reef was again unproductive for *R. marmoratus*.

January 24 to February 6, 2000

We spent two days collecting in the mangroves on the mainland south of Dangriga, Belize. Our goal was to determine the status of mainland populations with a particular focus on a search for male fish not previously reported from mainland sites. We had previously (1991) taken a single hermaphrodite from a site north of Dangriga where we also had found *Rivulus tenuis*, a typically freshwater form, in low-salinity habitats (10 ppt) in the mangroves in shallow pools and crab burrows. The 2000 Dangriga site was located east of the main coastal road, about 12 km south of the center of the city. The site was a heavily shaded, black mangrove forest with numerous burrows and flooded swales and depressions. Salinity was 10 ppt and the water temperature was 24°C and was heavily tannin- stained. These conditions and numerous late-stage salt marsh mosquito larvae present in the pools indicated recent heavy rainfall. We observed frogs throughout the area. Both *R. marmoratus* and *R. tenuis* were collected from crab burrows and pools. We frequently collected both species within the same burrow. A total of 46 *R. marmoratus* was collected and all were hermaphrodites along with 13 *R. tenuis*. We noted that the Dangriga *R. marmoratus* seemed larger than those typically found on the Cays so we compared the SL of this population with those collected at PG during the subsequent week. In fact, the Dangriga population was significantly larger (mean SL= 27.9 mm) than PG (mean SL= 22.9 mm) (*T* test: p< 0.0001). The Dangriga fish were also very robust and several oviposited viable embryos in collection bags.

Conditions on the Cays during this collection period were markedly different than on the mainland with low tides and no recent rainfall apparent. Salinities ranged from 33-41 ppt and water temperature in pools and burrows ranged from 23-26°C during the sampling event. A high incidence of "sulfured" *R. marmoratus* was once again observed and emersion was common. We also found a number of *Ucides* dead within their

burrows, a possible result of hydrogen-sulfide toxicity. As in previous years with low water, we found large numbers of very large *R. marmoratus* (both hermaphrodites and males) along the shoreline areas concentrated in crab burrows associated with stumps. At one such location, we collected 37 hermaphrodites and eight males. The largest hermaphrodite we collected in these habitats was 51 mm SL, one of the largest fish we have collected in the wild. In spite of the preponderance of large hermaphrodites taken, none of these oviposited in collection bags as did Dangriga fish. The concentration of *R. marmoratus* in these intertidal areas led to one of the higher CPUE we have experienced: for 88 trap-nights we collected 87 fish with a CPUE= 0.99.

In 2000, we also collected on Peter Douglas Cay, Cat Cay and Little Lagoon Cay. On Douglas, we relocated the pool where the remarkable occurrence of *R. marmoratus* within a log was found in 1991. The pool was completely dry in 2000 but 13 fish were taken from inside a 5 cm dia log. *Rivulus marmoratus* were also taken on Cat and Little Lagoon Cays (n=4 and 8, respectively).

October 23 to November 5, 2003

Rivulus marmoratus was an incidental part of the focus of this particular trip which focused on a broad investigation of fishes utilizing the mangroves of the Cays. At PG we found further extensive mangrove clearing both north and south, extending several hundred meters south and nearly 100 m north. Crab burrows were completely absent from much of the cleared area and extensive erosion of surface sediments had occurred. Our visit this year was unique in that we encountered the highest water ever in the Cays due to spring tides. We made only a limited trapping effort not specifically targeting *R. marmoratus* in the course of our collections, setting cup traps on the marsh surface only, not in crab burrows. Nevertheless, we did collect 31 *R. marmoratus*. Due to the high tidal inundation, salinities and temperatures were both moderate with salinity very close to 35 ppt and temperatures from 28-30° C. We were able to visit the Peter Douglas site again where the pool site was flooded 12-15 cm deep. Although we did not attempt collection of *R. marmoratus* we observed the following fishes within the pool: *Lutjanus apodus*, *Sphyraena barracuda*, *Poecilia orri* and *Mugil* sp.

DISCUSSION

We have documented that *R. marmoratus* is abundant within the mangal of the Belize Cays and can be collected in appropriate habitats during different seasons and tidal inundation stages. Probably in no other western Atlantic location where we have collected were we able to collect the species in such great numbers and with such consistency. The only exception appears to be at Glovers Reef where we were unable to find the fish. The limited mangrove habitat there may have prevented establishment of the species. There is no question that Glovers should be included within the range of *R. marmoratus* as it has successfully colonized islands, albeit larger islands, more remote that Glovers.

During our trap sampling efforts in Belize we have set hundreds of traps of various types (Table 1) and have experimented with traps constructed of various available materials, including a variety of flotsam (plastic, bamboo) found in the mangroves. None of our traps have been "baited", and we conclude that *R. marmoratus* enters traps set upon the marsh surface (pools, flooded habitats) because they offer "cover". Since we attempt to shield our traps with leaves, branches or sometimes sheets of black plastic, they clearly offer a refuge for the fish. We have observed that fish in flooded habitats dart quickly from one sheltered location (logs, debris) to another and they are rarely exposed for more than an instant. Our practice of setting traps that provide cover, mimic natural shelters, or are set adjacent to existing shelters has proven productive. We also suspect that any single fish entering a trap becomes an enticement for other fish to enter, although in aquaria the fish are normally aggressive toward each other.

Although we did collect many specimens in flooded habitats, the focus of *R. marmoratus* habitat, especially during low-water conditions, is the burrow of the mangrove land crab, *Ucides*. As we have previously described, *R. marmoratus* are orced periodically to the air-water interface in order to respire effectively in the low dissolved-oxygen environment of burrow water (Taylor, 1990). Therefore, when cup traps are properly set bisecting this interface, fish are easily collected within a short period. Other collection techniques, such as dip net and hook and line (Table 1), were of limited importance in our overall collection efforts and were more useful as quick survey tools when assessing crab burrows at a new site. We have observed that Belizean *R. marmoratus* are less likely to be taken consistently by hook-and-line than they are in Florida where fish are more inclined to aggressively attack the worm bait. It would be interesting to determine if this behavior is the result of more limited food resources in the Florida burrow habitat.

Interpretation of water-quality data did not reveal any significant trends. Unsurprisingly, we note that salinities were consistently higher during low-water/ drought conditions, for example, when we encountered a salinity of 65 ppt and were still collecting viable *R. marmoratus*. Salinities this high easily become reduced to nearly 0 ppt after heavy downpours which are common on the Cays.

We frequently observed emersion of *R. marmoratus* in the Belize Cays, especially common during drought or low-water conditions. We observed two patterns of emersion in Belize: 1) when fish were under debris, logs, etc. or inside galleried logs, and 2) emersion onto the sides or near the mouths of crab burrows. We suspect that in case 1, emersion is longer-term, that is, when temporary pools have dried or water-quality conditions within crab burrows are unacceptable. *Rivulus marmoratus* would then often be concentrated in large numbers and frequently be clumped together. We speculate that this may be a moisture-conserving behavior. This aggregating behavior is in strong contrast to behavior in aquatic habitats where the fish are normally quite aggressive to one another. Some of the largest collections were made during 1991 when many fish were emersed under shoreline debris. When emersed fish were exposed by removal of debris they typically would 'flip' away so collectors had to move quickly but if successful, literally handfuls of fish could be collected. Emersion in case 2 is shorter-term, with the fish often flipping in and out of the water several times within an hour. We have

noted that incidence of emersion inside burrows seemed to increase at night (we made several night-time observations), but is reduced with the onset of a flooding tide, day or night. The influence of H_2S on emersion has been well-documented (Abel et al., 1987) but the dynamics of H_2S within crab burrow water or within temporary pools is unknown and an interesting avenue for future research. In addition, there may well be other physiochemical factors that elicit emersion in *R. marmoratus* in the wild (e.g. ammonia).

We have noted consistent clearing of mangroves originating from PG over the years. While our field sampling frequency does not allow specific tracking of the effect of clearing on *R. marmoratus* populations, we suspect that, in the long-term, removal of mangroves is detrimental to the fish. One piece of evidence may be the comparative set of trapping observed in 1989 indicating higher catches in the recently cleared area and perhaps making the fish more vulnerable to capture, We have noted significant erosion of peat substrate (up to several cm) has occurred within a few years after clear-cutting with little or no regrowth of vegetation of any type. Significant reduction from a few to no *Ucides* burrows was found in clear-cut areas after one or two years. Loss of burrows and substrate, together with the lack of any other vegetative cover probably renders these areas unsuitable for sustaining *R. marmoratus* populations.

We have documented carefully the number of phenotypic males collected on Twin Cays during each visit. Although sexing hermaphrodites is relatively easy (the presence of a dark ocellus on the caudal fin representing the distinguishing feature), it is sometimes difficult to determine if smaller presumptive males are in fact male or immature hermaphrodites. In most cases, however, the distinctive reddish-orange tint of males is clearly visible along with the lack of an ocellus, and larger males are spectacularly colored with the distinctive addition of black edges to the caudal and anal fins. The percentage of males varied from 4.7%-29.7% (Table 2) and these ratios are significantly different across years (Chi-square= 62.64; df=5; P<0.001). There appears to be a decreasing trend in number of males from 1988 to 1991, but by 2003 the ratio had increased again (although a small sample size limits assessment). It is unfortunate that the eight-year gap in collections from 1992 to 2000 prevents accurate interpretation of trends. The presence of relatively large numbers of males remains the most interesting aspect of our collections in Belize and, hopefully, monitoring of this aspect will continue along with continued genetic work assessing heterozygosity of Belize Cays populations.

Our collection onshore in Dangriga was motivated by a search for male fish but none were found. While we have documented that males are found along a wide span of the central barrier reef (30 km-from the Pelican Cays to Tobacco Range, Fig. 1), it is curious that they were not observed on the mainland at Dangriga (a distance of about 18 km from the Cays). Other factors contrast these two collection sites: lower salinities in this coastal area (subject to more frequent heavy rainfall and allochthonous run-off) than those encountered on the Cays, at least based on our single collection, and the presence of the sympatric congener *R. tenuis*. Our collection of a few specimens of this species in this oligohaline habitat within crab burrows was previously reported (Davis et al., 1990) but the 2000 collection effort was much more extensive. Other than *Rivulus caudomarginatus* (a sexually dioecious species, as is *R. tenuis*) in coastal areas of Brazil (Huber 1992), we know of no other *Rivulus* species that is sympatric with *R. marmoratus*.

12

There may be as yet undescribed interesting behavioral interactions between these two species at the Dangriga site. Finally, the contrast in size between collections of *R. marmoratus* in Dangriga and Twin Cays is interesting and a long-term study of the Dangriga site would be needed to determine if this is a consistent feature and to identify the ecological factors influencing it.

The continued presence and high percentage of male *R. marmoratus* found on the Belize Cays remains a subject of intense interest. Turner et al. (1992a) and Lubinski et al. (1995) had initially hypothesized that there was probably an ecophenotypic factor inducing male production on the Belize Cays. However, we have yet to identify any environmental factor here which differs from those in other sites where males are not found. In addition, there is no reason to think that Belizean *R. marmoratus* are intrinsically different from other populations. Selfing has been defined as a derived character within the genus *Rivulus* (Huber, 1992) but Weibel et al. (1999) have determined through mtDNA analysis that Belize *R. marmoratus* do not differ from populations in Florida or other Caribbean locations that is, the Belize population is not ancestral to the others.

However, one of us (BJT) has recently demonstrated that "maleness'" may in fact have a genetic basis on Twin Cays. In a "common garden" experiment he has demonstrated that fish from Twin Cays continue to produce a high percentage of males over multiple generations, as compared with lines from Dangriga which produced only the typical low percentage of males commonly seen in laboratory colonies (Turner et al., in prep.). These data add a new element to investigations of this interesting phenomenon.

The past two decades have seen a great body of information added to our knowledge of *R. marmoratus,* and a 16-year span perhaps seems like a long period of time to devote to field research on a single organism in a single locale. But, as the results indicate, observations based upon long-term sample sets lead to more profound understandings. We continue to encounter new phenomena that raise challenging questions. We find the Belize Cays have provided an ideal setting to continue this pursuit.

ACKNOWLEDGMENTS

REFERENCES

Abel, D.C., C.C. Koenig, and W.P. Davis
   1987. Emersion in the mangrove forest fish *Rivulus marmoratus*, a response to hydrogen sulfide. *Environmental Biology of Fishes* 18:67-72.

Davis, W.P., D.S. Taylor, and B.J. Turner
   1990. Field observations on the ecology and habits of the mangrove rivulus (*Rivulus marmoratus*) In Belize and Florida. *Ichthyological Exploration of Freshwaters* 1:123-134.

Davis, W.P., D.S. Taylor, and B.J. Turner
   1995. Does the autecology of the mangrove rivulus fish (*Rivulus marmoratus*) reflect a paradigm for mangrove ecosystem sensitivity? *Bulletin of Marine Science* 57(1):208-214.
   2003. Relevance of mangrove rivulus *Rivulus marmoratus* to ecological and laboratory studies: an encapsulated summary. In: Smith, D. L. and S. Smith, eds., *Proceedings of the 9th Symposium on the Natural History of the Bahamas*, San Salavador, Bahamas pp 91-93.

Frick, N.T., and P.A. Wright
   2002. Nitrogen metabolism and excretion in the mangrove killifish *Rivulus marmoratus* I. The influence of environmental salinity and external ammonia. *The Journal of Experimental Biology* 205:79-89.

Grizzle, J.M., and A.Thiyagarajah
   1987. Skin histology of *Rivulus ocellatus marmoratus*, apparent adaptation for aerial respiration. *Copeia* 1987:237-240.

Harrington, R.W., Jr.
   1961. Oviparous hermaphroditic fish with internal fertilization. *Science* 134:1749-1750.
   1967. Environmentally controlled induction of primary male gonochorists from eggs of the self fertilizing hermaphroditic fish, *Rivulus marmoratus. Biological Bulletin* 132:174-199.
   1971. How ecological and genetic factors interact to determine when self-fertilizing hermaphrodites of *Rivulus marmoratus* change into functional secondary males, with a reappraisal of the modes of intersexuality among fishes. *Copeia* 1971:389-432.

Harrington, R.W., Jr., and E.S. Harrington
   1961. Food selection among fishes invading a high subtropical saltmarsh from onset of flooding through the progress of a mosquito brood. *Ecology* 42:646-666.

Harrington, R.W., Jr., and K.D. Kallman
   1968. The homozygosity of clones of the self-fertilizing hermaphroditic fish *Rivulus marmoratus* (Cyprinodontidae, Atheriniformes). *American Naturalist* 102:337-343.

Huber, J.H.
   1992. Review of *Rivulus*, Ecobiogeography, Relationships. Soc. Franc. d'Ichtyol., Paris. 572 pp.

14

Huehner, M.K., M.E. Schramm, and M.D. Hens
    1985. Notes on the behavior and ecology of the killifish *Rivulus marmoratus* Poey
        1880 (Cyprinodontidae). *Florida Scientist* 48(1):1-6.

Kallman, K.D. and R.W. Harrington, Jr.
    1964. Evidence for the existence of homozygous clones in the self-fertilizing
        hermaphroditic teleost *Rivulus marmoratus* Poey. *Biological Bulletin* 126:101-
        114.

King, J.A.C., A.C. Abel, and D.R. DiBona
    1989. Effects of salinity on chloride cells in the euryhaline cyprinodontid fish *Rivulus*
        marmoratus. *Cell Tissue Research* 257:367-377.

Kristensen, I.
    1970. Competition in three cyprinodont fish species in the Netherlands Antilles.
        Studies on the Fauna of Curacao and other Caribbean Islands 119:82-101.

Laughlin, T.F., B.A. Lubinski, E.-H. Park, D.S. Taylor, and B.J. Turner
    1995. Clonal stability and mutation in the self-fertilizing hermaphroditic fish, *Rivulus*
        marmoratus. *Journal of Heredity* 86(5):399-402.

Lubinski, B.A.,W.P. Davis, D.S. Taylor, and B.J. Turner
    1995. Outcrossing in a natural population of self fertilizing hermaphroditic fish.
        Journal of Heredity 86(6):469-473.

Taylor, D.S.
    1990. Adaptive specializations of the cyprinodont fish *Rivulus marmoratus*. *Florida*
        Scientist 53(3): 239-248.
    2000. Biology and ecology of *Rivulus marmoratus*: new insights and a review.
        Florida Scientist 63(4):242-255.

Taylor, D.S., W.P. Davis , and B.J. Turner
    2003. The status *of Rivulus marmoratus* (Pisces: Aplocheilidae) in the Bahamas. In:
        Smith, D.L. and S. Smith, eds., *Proceedings of the 9th Natural History of the*
        Bahamas, San Salavador, Bahamas, pp. 94-99.

Taylor, D.S., M.T. Fisher, and B.J. Turner
    2001. Homozygosity and heterozygosity in three populations of *Rivulus marmoratus*.
        Environmental Biology of Fishes 61:455-459.

Turner, B.J., W.P. Davis, and D.S. Taylor
    1992a. Abundant males in populations of selfing hermaphrodite fish, *Rivulus*
        marmoratus, from some Belize cays. *Journal of Fish Biology* 40:307-310.

Turner, B.J., J.F. Elder, Jr., T.F. Laughlin, W.P. Davis, and D.S. Taylor
    1992b. Extreme clonal diversity and divergence in populations of a selfing
        hermaphroditic fish. *Proceedings of the National Academy of Sciences U.S.A.*
        89(22):10643-10647.

Weibel, A.C., T.E. Dowling, and B.J. Turner
    1999. Evidence that an outcrossing population is a derived lineage in a hermaphroditic
        fish (*Rivulus marmoratus*). *Evolution* 53(4):1217-1225.

ATOLL RESEARCH BULLETIN

NO. 526

THE SUPRATIDAL FAUNA OF TWIN CAYS, BELIZE

BY

C. SEABIRD McKEON AND ILKA C. FELLER

THE SUPRATIDAL FAUNA OF TWIN CAYS, BELIZE

BY

C. SEABIRD McKEON[1] AND ILKA C. FELLER[1]

ABSTRACT

Previous supratidal surveys of Twin Cays have documented the role of insect diversity in forest structure and dynamics. Information on other supratidal groups is limited. During the winter and summer of 2004, decapods were surveyed using burrow counts, pitfall traps, time-constrained searches and arboreal counts. Lizard populations were measured in quadrat surveys, while snakes and crocodiles were subject to searches of specific habitat types. Crabs numerically dominated the non-insect fauna; the population of *Uca* spp. alone was estimated at over eight million individuals. Questions remain regarding the identification of bats seen at Twin Cays and the impact of a large feral dog population on the islands.

INTRODUCTION

The importance of subtidal mangrove fauna to marine biodiversity has been broadly established (Mumby et al., 2004). The role of the supratidal fauna is less well understood despite demonstrated critical importance to forest structure (Feller, 2002) and regeneration (McKee, 1995; Feller and McKee, 1999). The goals of this paper are: 1) to review literature relevant to our understanding of the supratidal fauna of Twin Cays, Belize; and 2) present new data on groups surveyed during the winter of 2003-2004 and summer of 2004.

The mangrove forest system of Twin Cays has been a study site for biologists since the creation of the Smithsonian Marine Field Station on nearby Carrie Bow Cay in the early 1970s (Rützler and Macintyre, 1982). Previous surveys of the supratidal community have focused primarily on the insect fauna. These studies indicated that insects are the most abundant and species-rich group of animals in the supratidal mangrove habitat (Rützler and Feller, 1988, 1996; Feller, 1995; Feller and Mathis, 1997). Nocturnal and endophytic feeding are common features among many of the mangrove-associated insect species. Because of these cryptic behaviors and the difficult environment for collecting, the complexity of the insect fauna associated with mangroves frequently has been underestimated. Huge arboreal nests (termitaria) and extensive carton-covered foraging trails of the termite *Nasutitermes* sp. are the most conspicuous signs of insect activity in the mangrove. However, ants (Formicidae) are ubiquitous and are clearly the most abundant terrestrial animals on these mangrove and coral cays. At

2

least 20 of the 34 species known from the Belizean cays live in direct association with the mangroves, utilizing hollow twigs and branches as nest sites (Feller, 2002).

The saltwater surface and mudflats on mangrove islands provide habitats for aquatic and semiaquatic insects, including species representing several families of Diptera, Hemiptera, Odonata, and Coleoptera. Comparable habitats in the mainland mangrove support much greater diversity than the cays in this part of the insect fauna. The shore-fly family, Ephydridae, is particularly species-rich in mangrove habitats in Belize. So far, 55 species in this family have been collected along the margins of Twin Cays and other mangrove islands in the Stann Creek District and Turneffe Atoll (Mathis, 1989, 1990, 1991, 1992, 1993). Most species in this family are detritivores and feed on decaying vegetation along the shore. Like other taxa, shore-fly species occupying the cays are a subset of Belize's mainland fauna.

The mangrove canopy provides numerous supratidal habitats for insect herbivores. Each species of mangrove supports a distinctive suite of herbivores, but the damages are more apparent than the herbivores themselves (Feller, 1995). Some species are host specific. For example, *Ecdytophyla* sp., an apical bud moth, feeds exclusively inside the apical buds of *Rhizophora mangle*. The larvae of several moths, including *Megalopyge dyeri* (a puss moth) and *Oiketicus kirbii* (a bagworm), and crickets (Orthoptera) are generalists and feed on all species of mangrove at Twin Cays.

In addition to leaf-feeding herbivores, more than 35 species of wood-feeding insects have been collected from mangrove trees on the cays and mainland in Stann Creek District (Chemsak and Feller, 1988; Feller, 1995; Rützler and Feller, 1999). Although some of these woodborers feed on any available dead wood, several species specialize on living trees of a single species of mangrove. For example, live twigs on *R. mangle* host a feeding guild composed of at least seven species of specialized wood-boring beetles and moths (Feller and Mathis, 1997). Larval stages of these insects feed internally on these twigs, killing them in the process, and creating hollow cylinders of dead wood. Another group of woodborers feed opportunistically on these dead twigs, further modifying trees by constructing galleries and pupal chambers in living and dead woody tissue. These spaces provide critical habitats for ants and numerous other arthropods in the canopy that use them for food, nest sites, prey sites, and diurnal refuge. The animals that use these spaces include ants, spiders, isopods, myriapods, pseudoscorpions, scorpions, crickets, scales, psocopterans, mites, moths, roaches, thrips, buprestids, tenebrionids, anobiids, termites, and cerambycids. More than 70 species of arthropods are associated in some way with twigs in the *R. mangle* canopy (Plate 1).

On Twin Cays, some woodborers kill branches, which contributes more to the total leaf area lost from the canopy than do all of the leaf-feeding herbivores combined (Feller, 2002). For example, the larvae of the beetle *Elaphidion mimeticum* (Cerambycidae) feed on the wood of live trees and deeply girdle wood under the bark. This type of herbivory causes death of branches and boles distal to the girdled area. Frequent attacks by this beetle create numerous small gaps in the *R. mangle* canopy (Feller and McKee, 1999). Thus, insect population dynamics and herbivory have a direct impact on ecosystem-level processes such as forest regeneration, seedling dynamics, nutrient cycling, habitat diversity, and other factors related to light gaps.

Previous studies have suggested that mangroves do not host a characteristic or specialized fauna (Huffaker et al., 1984; Tomlinson, 1986). However, surveys of the

insects at Twin Cays and other mangrove forests along the MesoAmerican Barrier Reef System provide evidence to the contrary. In these forests, *Marmara*, a genus of microlepidopteran tissue miners, is particularly diverse. *Rhizophora mangle* hosts at least four species of *Marmara,* each adapted and restricted to a single type of tissue in this host plant, including the leaf epidermis, propagule periderm, stem periderm, and aerial root periderm (Plate 1; Feller, 1995).

Other occupants of the mangrove supratidal at Twin Cays include two species of small snails: *Melampus coffeus* (the Coffee Bean Snail) and *Littoraria angulifera* (the Mangrove Periwinkle). *Melampus coffeus* is a detritivore, feeding on leaf litter at low tide (Proffitt et al., 1993), and may reach densities of 500 individuals/m^2 (Heard, 1982). Proffitt et al. (1993) found that the percentage of total mangrove leaf litter consumed by *M. coffeus* was unknown, but their data suggested that the snail's effects were considerable. *Littoraria angulifera* can occur in high densities near the mangrove shore, predominantly on *R. mangle* (Gutierrez, 1988). Juvenile snails, more prone to desiccation, are found closer to the waterline, but adults are found in all supratidal levels of the forest. This species is known to feed largely on fungi found just above the mean high water mark on mangrove prop roots and trunks (Kohlmeyer and Bebout, 1987).

Other than the fauna described above, little is known of other animal taxa that occupy the supratidal zone in Neotropical mangroves. To help fill this gap, the purpose of our study was to survey the decapod and reptile fauna of Twin Cays.

MATERIALS AND METHODS

Decapod Surveys

We conducted decapod surveys on Twin Cays during the winter of 2004 in the following habitat types described by Rodriguez and Feller (2004): Fringe, Mixed Woodland, Open Pond Dwarf, *Rhizophora* Dwarf, *Rhizophora/Avicennia* Dwarf, *Avicennia* Basin, Regenerated *Avicennia* Basin, *Avicennia* Orchard, *Avicennia* Scrub, Moribund, and Floc. We surveyed 30 1-m² quadrats with visual counts per habitat type, 10 Pitfall traps within a 25 m² area, and time-constrained searches of a 25 m² area.

Quadrats (1 m^2) were used to count *Uca* spp., *Goniopsis cruentata,* and *Ucides cordatus*, the three most common burrowing crabs on Twin Cays. After an initial study of burrow duration and occupancy, both crabs and crab holes were considered as part of these surveys. For *Uca* spp., burrows could not be distinguished conclusively among species and age classes and were consigned only to genus. Other burrows, most notably those of *G. cruentata* and *U. cordatus,* were distinctive.

Ten pitfall traps (Greenslade, 1964), consisting of 16 oz plastic keg cups, were randomly distributed in a 25 m^2 plot. The cups were sunk into the substrate level to the lip of the cup using a bulb planter. Using this size of pitfall limited the traps to smaller genera, but secured specimens not collected in any other manner. Traps were left in place overnight.

Time-constrained surveys used the same 25 m² plots as the pitfall traps. During a 1-hr time period, terrestrial debris was searched for decapods by overturning fallen branches, removing bark from stumps and snags, and sifting through coarse litter.

4

Aratus pisonii was targeted in species-specific searches of mangrove trees. To determine the density of this crab, a 1-m^2 quadrat was extended vertically from the ground through the top of canopy around the trunk. Two observers, one on either side of the tree, counted visible crabs. When only one observer was available, a plastic bag mounted on a pole was used to flush all crabs to one side of the tree where they were counted.

Reptile Surveys

Reptiles species were individually targeted in different survey types. Anoles and other diurnal lizards were counted within a 25 m^2 quadrat after a 15 min acclimatization period for the lizards to resume activity. Three surveys were conducted for lizards within each of eight dominant terrestrial habitat types. Boas were the focus of 1 hr time-constrained searches targeted on the limited raised sandy banks at the southern end of the East Island near Boa Flats. Crocodiles were surveyed at night along the interior of the Main Channel using a spotlight to reflect eyeshine.

RESULTS

Decapods

Uca spp. were relatively common in all habitat types with highest density in Mixed Woodland, *Avicennia* Orchard, and *Avicennia/Rhizophora* Dwarf stands (Table 1).

Table 1. Burrow surveys from 30 1-m^2 quadrats per habitat type at Twin Cays. Values are means ± 1 SD.

Habitat Type	*Uca* spp.	*Goniopsis cruentata*	*Ucides cordatus*
Mixed Woodland	42.9 ± 9.2	0.0 ± 0.0	0.1 ± 0.0
Fringe	15.8 ± 12.0	0.1 ± 0.0	0.0 ± 0.0
Avicennia Basin	18.3 ± 0.7	0.6 ± 0.0	0.6 ± 0.0
Avicennia Orchard	39.7 ± 10.8	0.0 ± 0.0	0.0 ± 0.0
Scrub	21.6 ± 7.8	0.1 ± 0.0	0.0 ± 0.0
Moribund	11.7 ± 7.1	0.0 ± 0.0	0.0 ± 0.0
Regenerated *Avicennia* Basin	10.8 ± 5.0	0.0 ± 0.0	0.0 ± 0.0
Avicennia/Rhizophora Dwarf	28.9 ± 2.8	0.0 ± 0.0	0.0 ± 0.0

Goniopsis cruentata and *U. cordatus* populations were patchy with highest densities, ~1 crab/m^2, in the *Avicennia* Basin stands (Plate 2). Using the area measurements of Rodriguez and Feller (2004), total crab numbers for habitat areas were extrapolated by multiplying the average density of crabs/m^2 by total area of habitat type. Based on this method, the total estimated *Uca* population of Twin Cays was ~ 8.3 million individuals (Table 2). *Goniopsis cruentata* and *U. cordatus* populations were approximately 23,000 and 20,000, respectively.

Table 2. Estimations of population sizes for *Uca* spp., *Goniopsis cruentata*, and *Ucides cordatus* at Twin Cays. Area for each habitat type is from Rodriguez and Feller (2004).

Habitat Type	Area (m²)	*Uca* spp.	*Goniopsis cruentata*	*Ucides cordatus*
Mixed Woodland	115,102	4,941,725	3,837	15,347
Fringe	99,667	1,574,743	13,289	0
Avicennia Basin	5,842	106,909	3,310	3,505
Avicennia Orchard	12,033	477,309	0	0
Scrub	22,805	493,348	2,281	760
Moribund	3,124	36,645	0	0
Regenerated *Avicennia* Basin	8,174	88,279	0	0
Avicennia/Rhizophora Dwarf	19,166	553,259	0	0

Pitfall traps provided information regarding the distribution of several taxa not recorded in the burrow surveys (Table 3). *Eurytium limosum* occurred broadly within *Rhizophora* habitat types while *Panopeus herbstii* was restricted to the fringe. *Armases ricordi*, a crab common within coastal scrub and clearcut areas (see discussion below), was trapped twice in pitfalls away from these habitat types. *Aratus pisonii*, an arboreal species, was recorded from one pitfall in a Moribund stand where it was being consumed by *E. limosum* (Plate 2).

Table 3. Pitfall trap samples by habitat type. Values are number of animals in 10 pitfall traps per 25 m².

Habitat Type	*Uca rapax.*	*Eurytium limosum*	*Panopeus herbstii*	*Aratus pisonii*	*Armases ricordi*
Mixed Woodland	6	1	0	0	0
Fringe	0	1	4	0	0
Avicennia Basin	10	0	0	0	0
Avicennia Orchard	16	0	0	0	1
Scrub	3	0	0	0	0
Moribund	11	1	0	1	0
Regenerated *Avicennia* Basin	3	0	0	0	0
Avicennia/Rhizophora Dwarf	12	1	0	0	1
Floc Zone	2	0	0	0	0

Three species were added through time-constrained searches: *Coenobita clypeatus* in Regenerated *Avicennia* Basin, *Pachygrapsus transversus* in the Fringe and *Avicennia* Orchards, and *Sesarma curacaoense* in the Fringe.

Arboreal searches for *A. pisonii* revealed a patchy distribution favoring *Rhizophora* habitats, with a total population of approximately 68,000 individuals (Table 4). *Aratus pisonii* was found mainly in the Fringe, and was virtually absent in the Mixed Woodland, *Avicennia* Orchard, Scrub, Moribund, and Dwarf habitat types.

Table 4. *Aratus pisonii* by habitat type with estimates for population size at Twin Cays. Areal coverages of habitat types are given in Table 2. Values for *A. pisonii*/m^2 are means ± 1 SD.

Habitat Type	*Aratus pisonii*/m^2	*Aratus pisonii* per habitat type
Mixed Woodland	0 ± 0	0
Fringe	0.7 ± 1.7	66444
Avicennia Basin	0.1 ± .3	649
Avicennia Orchard	0 ± 0	0
Scrub	0 ± 0	0
Moribund	0 ± 0	0
Regenerated *Avicennia* Basin	0.1 ± 0.3	908
Avicennia/Rhizophora Dwarf	0 ± 0	0

Reptiles

Only *Anolis sagrei* were encountered during the daytime in the 25m^2 quadrat surveys (Table 5). The lizards were most common in the Mixed Woodland habitat type. As with the decapods above, the area measurements of Rodriguez and Feller (2004) were used to extrapolate *A. sagrei* numbers by multiplying the average density of lizards per 25 m^2 by area (m^2) of each habitat type. Approximately 18,600 *A. sagrei* are estimated to inhabit the surveyed habitat types of Twin Cays (Table 5).

Table 5. Number of *Anolis sagrei* per 25 m^2 with estimates for population size at Twin Cays. Areal coverages of habitat types are given in Table 2.

Habitat Type	*Anolis sagrei* /25 m^2	*Anolis sagrei* per habitat type
Mixed Woodland	2.3 ± 0.5	10743
Fringe	1.7 ± 0.5	6644
Avicennia Basin	1.0 ± 0.0	234
Avicennia Orchard	0.3 ± 0.5	160
Scrub	0.7 ± 0.5	608
Moribund	0.7 ± 0.9	83
Regenerated *Avicennia* Basin	0.3 ± 0.5	109
Avicennia/Rhizophora Dwarf	0 ± 0	0

An individual boa (*Boa constrictor*) was found in each of two 1-hr searches of the Boa Flats area of Twin Cays in January. In the July searches, we found no boas. A single, small adult (~2 m) American Crocodile, *Crocodylus acutus,* was observed in the Main Channel during the January survey. Upon our approach, it quickly fled into nearby Gator Creek. July surveys revealed no crocodiles in the main channel, but two individuals were spotted by another observer within Hidden Lake (Stephen Mitten, personal communication).

7

DISCUSSION

Decapods

Decapod crustaceans are the most visually conspicuous element of the supratidal fauna of Twin Cays (Plate 2). Associations of decapods with mangrove forests date from the Cretaceous Period and possibly earlier (Schweitzer et al., 2003). Often found in startling abundance, the group plays crucial roles as (1) consumers of mangrove leaf litter (Camilleri, 1992), (2) meiobenthic predators (Olafsson and Ndaro, 1997), (3) predators of mangrove propagules (McKee, 1995), and (4) aerators of mangrove sediments (Hines and Feller, unpublished data).

Fiddler crabs (*Uca* spp.) dominate the supratidal crustacean fauna of Twin Cays with several hundred individuals often occupying a single square meter of appropriate habitat. The most common species is *Uca rapax*, though a second species, *U. vocator*, is found in small numbers detected through the presence of burrows with chimneys. However, it was not collected in any of the sampling methods in this survey. As crabs of the genus *Uca* frequently have fine niche partitioning (Crane, 1975), it is likely that more species may be found on Twin Cays, particularly in minor habitat types that were not sampled in this study. Other members of the family Ocypodidae found on Twin Cays are the Hairy Land Crab, *Ucides cordatus,* and the Ghost Crab, *Ocypode quadrata*.

Ucides cordatus is a relatively large (≤89 mm carapace width, Warner, 1969) herbivorous/detritivorous crab. It maintains distinctive burrows, which are often found in "villages." These burrows provide haven to other species, including the Mangrove Rivulus, *Rivulus marmoratus*, a tiny hermaphroditic fish (Davis and Taylor, 1987). This crab is rarely seen during the daytime in Belize, coming out at night to feed on fallen leaves.

Ocypode quadrata, a Ghost Crab, is a fast, pallid, predator-scavenger of open beaches. It is uncommon on Twin Cays, being found primarily in areas that have been clearcut, burned, and filled with sandy dredge spoils. In other areas of its range, *O. quadrata* has been identified as a major predator on ground nesting birds and sea turtle hatchlings (Loegering et al., 1995).

The decapod family Grapsidae is also well represented on Twin Cays with five species ranging from arboreal to semifossorial lifestyles. *Aratus pisonii,* the Mangrove Tree Crab, is found throughout the study area though it appears to favor fringe stands dominated by *Rhizophora mangle*. It feeds on *R. mangle* leaves and propagules in the canopy, algae in the intertidal zone, and whatever animal material is available. We have observed *A. pisonii* preying on *Marmara* spp., other insects, and smaller conspecifics.

Goniopsis cruentata, a brightly colored crab with red legs and a midnight blue dorsum, is common in sheltered habitats, particularly among *R. mangle* prop roots and low branches. It occupies terrestrial burrows, which are normally found under debris. Frequently seen above ground, this crab moves very rapidly to shelter when disturbed. A predator of mangrove propagules (McKee, 1995), *G. cruentata* will also consume leaf litter and scavenge animal matter.

Armases ricordi occupies dry wrack lines and upland terraces. Infrequently encountered in the interior of the island, it is more typical of the leeward beaches and

areas filled with dredge spoils. A closely related crab, *Sesarma curacaoense,* was found within coarse woody debris and the leaf litter of the *R. mangle* fringe.

The small grapsid crab *Pachygrapsus transversus* was uncommonly found in leaf litter in fringe zones and *A. germinans* orchards. Ovigerous females were found in the January samples, falling within the breeding period noted by Crane (1947) for Costa Rica. In their study of this species, Abele et al. (1986) noted that "individuals are active at all low tides as soon as their habitat is exposed." This observation also appears to be true for *P. transversus* on Twin Cays.

Mud crabs of the family Xanthidae were represented in the study by two species occupying similar habitats. A relatively large mud crab with white-tipped chelae, *Eurytium limosum,* is a predator-scavenger rarely encountered on the surface during the daytime. In the confines of pitfall traps, it quickly seized and consumed other crabs including conspecifics, *Uca* spp. and *A. pisonii.* When stranded in the pitfall traps, *E. limosum* were eaten by Rufous-Necked Woodrails, based on signs including rail tracks and crab remains. *Panopeus herbstii* is similar in form to *E. limosum,* though lacking the white-tipped chelae, and more restricted to the *R. mangle* fringe.

A common inhabitant of neighboring sandy islands, the terrestrial hermit crab *Coenobita clypeatus* is most frequently encountered in the limited coastal scrub zone of Twin Cays near Boa Flats. This species is a known egg-predator of ground-nesting birds (Meier et al., 1989) and scavenges the wrack line for animal and vegetable matter. Crabs of this genus are host to a commensal springtail of the monogeneric family Coenaletidae (Palacios-Vargas et al., 2000).

We also found the semiterrestrial shrimp *Merguia rhizophorae* in the coarse woody debris along the *R. mangle* fringe of Twin Cays. Largely nocturnal, the species is known from only a few locations throughout the Caribbean.

While more derived reproductive strategies have been observed in some Caribbean supratidal crab faunas (Diesel et al., 2000), all members of the Twin Cays assemblage have a standard life history consisting of a pelagic larval dispersal phase, followed by the adult reproductive form. The evolutionary importance of mangrove areas to the development of terrestriality was discussed by Diesel et al. (2000) who noted "one important key to the invasion of land by crabs is the evolution of reproductive and developmental traits that accompany independence from the marine plankton." It would be of great interest to learn more about the reproductive habits of the terrestrial crabs of Twin Cays, particularly if the assemblage uses the interior ponded areas for breeding.

One striking result of the decapod surveys was the absence of large burrowing crabs, *G. cruentata* and *U. cordatus,* from the Regenerated *Avicennia* Basin, despite relatively high densities of both species in mature stands. The importance of these species as seedling predators (McKee, 1995, Sousa and Mitchell, 1999) and detritivores (McKeon, personal observation) has previously been noted. With the additional commercial importance of *U. cordatus,* the loss of these species becomes an important factor in the discussion of land-use and clearing of mangroves in the Caribbean.

During the January study period, crabs were largely absent from the Floc zone with no burrows present and very limited captures from pitfall traps. Observations of hundreds of *Uca* spp. individuals in the same area in July suggest that during some times of the year these areas may be of greater importance to crabs, *Uca* spp., in particular. The seasonal or hydrological changes involved are unknown but may represent the influx of

new floc material or fresh accumulation of senesced leaves. As this zone forms the leading edge of forest growth in the island interior (Feller, unpublished data), the point at which the floc stabilizes enough for crabs may be an important factor in understanding forest dynamics. The studies of Hines and Feller (unpublished data) demonstrate the importance of *Uca* spp. burrowing to the growth and survival of *Avicennia* in Florida, USA. While results of similar studies in Belize have been less clear, the role of crabs in these transitional zones is of interest for further study.

Sites at Twin Cays that had been altered from the original forest types (cut, burned, and filled), had a different decapod community. *Armases ricordi* was much more abundant in such areas, and the Ghost Crab *Ocypode quadrata*, otherwise absent from Twin Cays, was also common. The addition of these aggressive predator-scavengers to the Twin Cays community may have important ramifications to the island's food webs.

Reptiles

Unlike comparable forests in Southeast Asia and Africa, which are home to numerous aquatic reptile species (Karns et al., 2002; Luiselli and Akani, 2002), the reptile fauna of Belizean mangrove forests is "limited to a few ubiquitous, opportunistic species" (Stafford and Meyer, 2000). Their importance to the supratidal fauna cannot be overstated. With a few subtidal exceptions, reptiles are the dominant predators of the Twin Cays ecosystem, and the adult American Crocodiles and Boa Constrictors feed at high trophic levels. The reptile fauna of Twin Cays is limited to four species in four families: *Anolis sagrei* (Polycroatidae), *Phyllodactylus insularis* (Gekkonidae), *B. constrictor* (Boidae), and *C. acutus* (Crocodylidae).

Easily the most abundant and easily encountered reptile on the island is the Mayan Coastal Anole, *A. sagrei,* which is common throughout the Caribbean as a tramp species, often colonizing coastal areas and inland areas disturbed by humans. Adult males typically maintain a head-down position on vertical trunks for territorial displays of head-bobbing and dewlap extension. We have observed Anoles on Twin Cays eating insects, particularly ants, beetles, and termites. In other parts of their range, these lizards are known to consume many other types of insects, small crabs, and smaller members of the same species. The eggs of *A. sagrei* are deposited in tree holes and other arboreal refugia.

The abundance of *A. sagrei* on Twin Cays is likely to play a major role in the trophic dynamics of the island. Previous researchers have found that removal of lizards of the genus *Anolis* triggers dramatic increases in local arthropod abundance (Pacala and Roughgarden, 1984; Schoener and Toft, 1983). Within the mangrove ecosystem of the Bahamas, the effect of lizard predation on insect herbivores was tested by Schoener (1988). The study demonstrated that leaf damage to *Conocarpus erectus,* a common mangrove associate, was reduced on islands with lizards in comparison to those without.

A pale, large-headed lizard, *Phyllodactylus insularis*, is largely nocturnal at Twin Cays and elsewhere, emerging from under the bark and dead limbs at dusk to feed on insects and other small arthropods. Recorded only from Belizean cays, *P. insularis* is very similar to a closely related species *P. tuberculosus* and often confused with that taxon. Geckos from Twin Cays have keyed out as both species suggesting further study of the taxonomy is warranted. Like other geckos, *P. insularis* has adhesive friction pads on the toes of each foot enabling it to scamper about vertical surfaces with ease. This

species is also quite vocal with individuals emitting a sound much like a high pitched squeak or click. Though counts of this species were not attempted in this study, casual observations by the authors suggest that *P. insularis* may approach the daytime densities of *Anolis sagrei* in some habitat types.

      *Boa constrictor*s are large, heavy-bodied snakes found infrequently on Twin Cays with the exception of the appropriately named "Boa Flats" at the southern end of the East Island. In other parts of their range, boas are generalized predators on vertebrates, consuming lizards, mammals, and birds.

      The threatened *C. acutus* is the only crocodilian to be found in the Twin Cays area. A smaller species, Morelet's crocodile *C. moreletii*, is found at mainland sites on the Yucatan peninsula but is not known from the Belizean cays (Campbell, 1998). American Crocodiles feed largely on fish supplemented with other reptiles, birds, and mammals as opportunity presents. Sexual maturity is reached at a total length of approximately 2.8 m in females-- a size not yet observed by the authors at Twin Cays.

      Colonization of the islands by reptiles has been limited to ovoviviparous species (*B. constrictor*) and lizard species with arboreal egg deposition. Large lizards of the genera *Ctenosaura* and *Iguana* have been known to arrive at Twin Cays and neighboring Carrie Bow Cay but have not established populations. *Crocodylus acutus*, despite healthy breeding populations on other Belizean islands (Platt and Thorbjarnarson, 2000), have yet to be recorded breeding on Twin Cays. Due to a lack of high ground for nesting areas and the absence of fresh or brackish water sources needed by hatchlings (Mazzotti et al., 1986), the authors considered Twin Cays unlikely breeding habitat for the species until a juvenile crocodile, approximately 35 cm in total length, was observed during the July surveys. It is unlikely that a crocodile of such small size would attempt or survive the open water crossing from adjacent breeding populations.

Birds

      As explored by Mitten et al. (2004), mangrove bird communities are poorly studied though authors have made significant recent additions (Sodhi et al., 1997), particularly with regard to Central America (Butler et al., 1997; Lefebvre and Pulin, 1997) and South America (Olmos and Silva-e-Silva, 2001). The role of nesting birds in the diversity of the supratidal fauna is largely unexplored and deserving of mention.

      Rookeries in mangrove forests can affect both biotic and abiotic components of the ecosystem. At the Frigatebird and Brown Booby rookery on nearby Man-of-War Cay, increased availability of nutrients from the bird guano results in higher mangrove productivity and growth rates in comparison with nearby islands that are not rookeries. Unlike the tangled maze of stems and roots that typify the low-stature fringe forests of most of Twin Cays and most other nearby cays, the mangrove trees on Man-of-War Cay are tall (10-15 m) and straight-trunked with few buttressing prop roots. Under the canopy, this forest is relatively open. Although Man-of-War is a very small cay, the insect fauna associated with these tall trees is more species-rich than on much larger, nearby islands (Feller and Mathis, 1997). In addition, insect herbivory of mangrove leaves, stems, and roots on the tall trees at this rookery is greater than is suffered by mangroves on these other islands (Feller and Mathis, 1997). The input of nutrients from the guano also leads to increased decomposition rates in the substrate and affects nutrient-cycling processes.

Adjacent marine communities also respond to this naturally occurring nutrient enrichment. Some marine fungi and algae grow densely in the waters around Man-of-War Cay (Kohlmeyer and Kohlmeyer, 1987). Some of these species of algae, such as *Ulva* sp. and *Enteromorpha* sp., are typically associated with marine environments that are more eutrophic than the oligotrophic waters typical of Belize's coastal zone.

Mammals

Native terrestrial mammals are unknown from Twin Cays. At the time of writing, feral dogs are the only known mammals (other than humans) present on the island. As highly adaptable, social carnivores, they are likely to be second only to crocodiles in an island food chain. Five to 15 dogs are reported from the islands, often near the active dump site at the southern tip of the West Island. The impact of these animals is unknown but is likely to be significant. In the Galapagos, feral dogs have significantly impacted native populations of tortoises, iguanas, and birds (Green and Gipson, 1994). Similar situations have been found in the Hawaiian Islands (van Ripper et al., 2001). Ground nesting birds (including both rail species recorded from Twin Cays) are particularly vulnerable (Fuller, 2001).

An unknown number of bat species hunt above Twin Cays and neighboring islands in the dusk and dawn hours. It is highly likely that these animals reside in the forest during the day. Limited research has been conducted on mangrove bat communities with most research conducted on Australian species (McKenzie and Start, 1989; McKenzie and Rolfe, 1986). Several species known from studies on mainland Belize have strong associations with waterways and coastal habitats *(Noctilio leporinus, Rhynchonycteris naso)* and are likely in the area based on distribution (Reid, 1998).

ACKNOWLEDGEMENTS

REFERENCES

Abele, L.G., P.J. Campanella, and M. Salmon
    1986. Natural history and social organization of the semiterrestrial grapsid crab *Pachygrapsus transversus. Journal of Experimental Marine Ecology* 104:153-170.

12

Butler, R.W., R.I.G. Morrison, F.S. Delgado, R.K. Ross, and G.E.J. Smith
    1997. Habitat associations of coastal birds in Panama. *Colonial Waterbirds* 20:518-
        524.
Camilleri, J.C.
    1992. Leaf-litter processing by invertebrates in a mangrove forest in Queensland.
        Marine Biology 114:139-145.
Campbell, J.A.
    1998. *Amphibians and Reptiles of Northern Guatemala, the Yucatan, and Belize*
        University of Oklahoma Press, Norman.
Chemsak, J.A., and I.C. Feller
    1988. New species of Cerambycidae from Twin Cays, Belize (Coleoptera).
        Proceedings of the Entomological Society of Washington 90:179-188.
Crane, J.
    1947. Intertidal brachygnathous crabs from the west coast of tropical America with
        special reference to ecology. *Zoologica* 32:69-95.
Crane, J.
    1975. *Fiddler Crabs of the World. Ocypodidae: Genus Uca* Princeton University
        Press, Princeton, New Jersey.
Davis, W.P., and D.S. Taylor
    1988. Habitat and behavior of *Rivulus marmoratus*. In *The Twin Cays Mangrove,*
        Belize, and Related Ecological Systems. Results and Summary of Presentations,
        edited by K. Rützler, 31-32. Smithsonian Institution, Washington, DC.
Diesel, R., C.D. Schubart, and M. Schuh
    2000. A reconstruction of the invasion of land by Jamaican crabs (Grapsidae:
        Sesarminae). *Journal of the Zoological Society of London* 250:141-160.
Feller, I.C.
    1995. Effects of nutrient enrichment on growth and herbivory of dwarf red mangrove
        (*Rhizophora mangle*). *Ecological Monographs* 65:477-505.
Feller, I.C.
    2002. The role of herbivory by wood-boring insects in mangrove ecosystems in
        Belize. *Oikos* 97:167-176.
Feller, I.C., and W.N. Mathis
    1997. Primary herbivory by wood-boring insects along an architectural gradient of
        Rhizophora mangle. *Biotropica* 29:440-451.
Feller, I.C., and K.L. McKee
    1999. Small gap creation in Belizean mangrove forests by a wood-boring insect.
        Biotropica 31:607-617.
Fuller, E.
    2000. *Extinct Birds* Oxford University Press, Oxford.
Green, J.S., and P.S. Gipson
    1994. Feral Dogs. In *Prevention and Control of Wildlife Damage*. Cooperative
        Extension Division, Institute of Agriculture and Natural Resources, University
        of Nebraska, Lincoln.
        http://wildlifedamage.unl.edu/handbook/handbook/carnivor/ca_c77.pdf

13

Greenslade, P.J.M.
    1964. Pitfall trapping as a method for studying populations of Carabidae (Coleoptera). *Journal of Animal Ecology* 33:301-310.
Gutierrez, P.C.
    1988. The ecology and behavior of the mangrove periwinkle, *Littorina angulifera*. *Biotropica* 20:352-356.
Heard, R.W.
    1982. *Guide to Common Tidal Marsh Invertebrates of the Northeastern Gulf of Mexico.*, Rep. No. MASGP-79-004. Mississippi Alabama Sea Grant Consortium.
Huffaker, C.B., D.L. Dahlsten, D.H. Janzen, and G.G. Kennedy
    1984. Insect influences in the regulation of plant populations and communities. In *Ecological Entomology*, edited by C.B. Huffaker and R.L. Rabb, 659-695. John Wiley and Sons, New York.
Karns, D.R., H.K. Voris, and T.G. Goodwin
    2002. Ecology of Oriental-Australian rear-fanged water snakes (Colubridae: Homalopsinae) in the Pasir Ris Park mangrove forest, Singapore. *Raffles Bulletin of Zoology* 50:487-498.
Kohlmeyer, J., and B. Bebout
    1986. On the occurrence of marine fungi in the diet of *Littorina angulifera* and observations on the behavior of the periwinkle. *Marine Ecology* 7:333-343.
Kohlmeyer, J., and B. Kohlmeyer
    1987. Marine fungi in the mangal, seagrass, and reef systems of Twin Cays and neighboring islands. In *Caribbean Coral Reef Ecosystems Progress Report for 1987*, edited by K. Rützler, 9-10, Smithsonian Institution, Washington, DC.
Lefebvre, G., and B. Poulin
    1997. Bird communities in Panamanian black mangroves: Potential effects of physical and biotic factors. *Journal of Tropical Ecology* 13:97-113.
Loegering, J.P., J.D. Fraser, and L.L. Loegering
    1995. Ghost crab preys on a Piping Plover chick. *Wilson Bulletin* 10:768-769.
Luiselli, L., and G.C. Akani
    2002. An investigation into the composition, complexity and functioning of snake communities in the mangroves of south-eastern Nigeria. *African Journal of Ecology* 40:220-227.
Mathis, W.N.
    1989. A review of the beach flies of the Caribbean and Gulf of Mexico (Diptera: Canacidae). *Proceedings of the Biological Society of Washington* 102:590-608.
Mathis, W.N.
    1990. A revision of the shore-fly genus *Diphuia* Cresson (Diptera: Ephydridae). *Proceedings of the Entomological Society of Washington* 92:746-756.
Mathis, W.N.
    1991. Studies of Gymnomyzinae (Diptera: Ephydridae), I: A revision of the shore-fly subgenus *Pseudohecamede* Hendel (Genus *Allotrichoma* Becker). *Smithsonian Contributions to Zoology*, no. 522, 28p.

14

Mathis, W.N.
    1992. The first shore fly of the genus *Glenanthe* Haliday from the Australasian Region (Diptera: Ephydridae). *Proceedings of the Entomological Society of Washington* 94:78-82.
Mathis, W.N.
    1993. A revision of the shore-fly genera *Hostis* Cresson and *Paratissa* Coquillett. *Proceedings of the Entomological Society of Washington* 95:21-47.
Mazzotti, F.B., B. Bohnsack, M.P. McMahon, and J.R. Wilcox
    1986. Field and laboratory observations on the effects of high temperature and salinity on hatchling *Crocodylus acutus*. *Herpetologica* 42:191-196.
McKee, K.L.
    1995. Mangrove species distribution and propagule predation in Belize: an exception to the dominance-predation hypothesis. *Biotropica* 27:334-345.
McKenzie, N.L., and J.K. Rolfe
    1986. Structure of bat guilds in the Kimberley mangroves, Australia. *Journal of Animal Ecology* 55:401-420.
McKenzie, N.L., and A.N. Start
    1989. Structure of bat guilds in mangroves: environmental disturbances and determinism. *Texas Tech University Museum Special Publications,* 167-178.
Meier, A.J., R.E. Noble, and P.M. McKenzie
    1989. Observations on the nesting ecology of the White-cheeked Pintail. *Caribbean Journal of Science* 25:92-93.
Mitten, S., C.S. McKeon, and I.C. Feller
    2004. Winter and summer bird communities of Twin Cays, Belize. *Atoll Research Bulletin* 527:1-21.
Mumby, P.J., A.J. Edwards, E. Arias-Gonzalex, K.C. Lindeman, P.G. Blackwell, A. Gall, M.I. Gorczynska., A.R. Harborne, C.L. Pescod, H. Renken, C.C.C. Wabnitz, and G. Llewellyn
    2004. Mangroves enhance the biomass of coral reef fish communities in the Caribbean. *Nature* 427:533-536.
Olafsson, E., and S.G.M. Ndaro
    1997. Impact of the mangrove crabs *Uca annulipes* and *Dotilla fenestrata* on meiobenthos. *Marine Ecology Progress Series* 158:225-231.
Olmos, F., R. Silva-e-Silva, and A. Prado
    2001. Breeding season diet of Scarlet Ibises and Little Blue Herons in a Brazilian mangrove swamp. *Waterbirds* 24:50-57.
Pacala, S., and J. Roughgarden
    1984. Control of arthropod abundance by *Anolis* lizards on St. Eustatius (Neth. Antilles). *Oecologia* 64:160-162.
Palacios-Vargas, J.G., L.Q. Cutz, and C. Maldonado
    2000. Redescription of the male of *Coenaletes caribaeus* (Collembola: Coenaletidae) associated with hermit crabs (Decapoda: Coenobitidae). *Annals of the Entomological Society of America* 93:194-197.
Platt, S.G., and J.B. Thorbjarnarson
    2000. Nesting ecology of the American crocodile in the coastal zone of Belize. *Copeia* 3:869-873.

16

Warner, G.F.

1969. The occurrence and distribution of crabs in a Jamaican mangrove swamp. *Journal of Animal Ecology* 38:379-389.

PLATES

18

Plate 1. A. *Littoraria angulifera*; B. *Marmara* sp.; C. *Elaphidion mimeticum*; D. *Anolis sagrei*; E. *Phyllodactylus insularis*; F. *Boa constrictor*
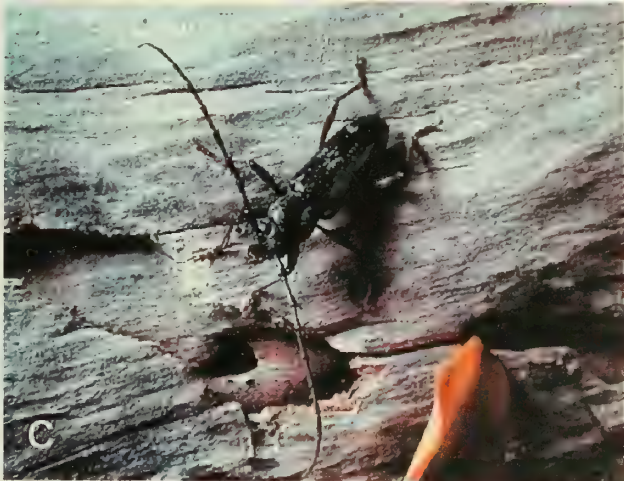
Plate 1

20

Plate 2. A. *Uca rapax*; B. *Ucides cordatus*; C. *Eurytium limosum*;
D. *Aratus pisonii*; E. *Armases ricordi*; F. *Goniopsis cruentata*

Plate 2

ATOLL RESEARCH BULLETIN

NO. 527

WINTER AND SUMMER BIRD COMMUNITIES OF TWIN CAYS, BELIZE

BY

STEPHEN MITTEN, C. SEABIRD McKEON, AND ILKA C. FELLER

WINTER AND SUMMER BIRD COMMUNITIES OF TWIN CAYS, BELIZE

BY

STEPHEN MITTEN[1], C. SEABIRD MᶜKEON[2], AND ILKA C. FELLER[2]

ABSTRACT

Avian species richness and abundance were studied in order to characterize the bird fauna of Twin Cays, Belize. Auditory and visual surveys were conducted for both migratory and resident species during winter and summer seasons. The Mangrove Yellow Warbler was the most common bird species on the islands in both seasons, with an estimated mean density of 13.4 birds/ha during the winter and 10.9 birds/ha in the summer. The importance of mangrove habitats to a diversity of migratory passerines and resident breeding birds along the Mesoamerican Barrier Reef is supported with 56 species of 20 families recorded from the islands.

INTRODUCTION

Few quantitative surveys of avian species have been conducted in mangrove forests in Central America. This may be due to the tangled maze of prop roots and boles that make moving through the mangrove extremely difficult and inhospitable. Mangroves are also perceived as not having sufficient bird species to warrant such a study. For example, current literature suggests that Central American mangroves are underutilized by migrant birds when compared to similar habitat outside of Central America (Petit et al., 1993). Nevertheless, mangroves have been found to be vital for some species' winter survival. Petit et al. (1995) found that in Panama, Northern Waterthrushes and Prothonotary Warblers were dependent on mangroves for their survival. Arendt (1992) reported that coastal mangroves on Caribbean islands were used by more than 50% of the migratory birds for at least a portion of their winter residence or as stopovers during migration. On some Caribbean islands, mangroves had greater densities of Northern Waterthrushes, Magnolia Warblers, American Redstarts and Yellow-bellied sapsuckers than any other habitat (Wunderle and Waide, 1993). Our primary research objectives were to document avian species structure (diversity, distribution, relative density), and trophic level complexity within the mangrove at Twin Cays during the winter and summer seasons, and to compare the avifauna between these two seasons. In this paper, we describe the avian species richness and relative abundance on Twin Cays, a small archipelago off the coast of Belize, during winter and summer 2004. Attempts to estimate density of all avian populations was not possible due to the small sample size for

[1] University of Missouri-St. Louis, International Center for Tropical Ecology, St. Louis, MO 63121.
[2] Smithsonian Environmental Research Center, Smithsonian Institution, 647 Contees Wharf Rd., Edgewater, MD 21037.

2

some species and bird movement between Twin Cays and the nearby cays. However, density measurements were estimated for the three most common winter species, Mangrove Yellow Warbler, Northern Waterthrush, and the Clapper Rail; and the two most common summer species, Mangrove Yellow Warbler and Green Heron.

STUDY AREA AND METHODS

We studied species richness, relative abundance, and trophic organization of migrant and resident birds in a mangrove habitat for 2 wk during January 1-14, 2004 and for 1 wk during July 8-15, 2004. The field research was undertaken at Twin Cays located about 20 km SE of Dangriga, Belize. Twin Cays is an archipelago of small peat-based mangrove islands encompassing approximately 72 ha of which 5 to 10 ha are shallow ponds whose sizes vary depending on the seasonal rainfall and inundation of salt water from the surrounding sea (Rodriguez and Feller, 2004). The vegetation characteristics of the study site, while superficially fitting the description of zonation patterns in mangrove forests elsewhere in the Caribbean (Davis, 1940; Thom, 1967; Ball, 1980), were highly mosaic. The vegetation on the two largest islands is dominated by a fringing forest of *Rhizophora mangle* (red mangrove), 4 – 6 m tall, along the water with stands of dwarf *R. mangle* trees, <1.5 m tall, *Avicennia germinans* (black mangrove) and *Laguncularia racemosa* (white mangrove) in mixed stands in the interior of the islands. In some areas, *Batis maritima* (saltwort) occurs in dense patches in the undergrowth. Other habitats include mud flats, moribund or dieback areas, and human-disturbed areas, including several clearcut stands with brush piles and two small garbage dumps. All of these habitats contribute significantly to the perceived avian "diversity" of Twin Cays. For more detailed descriptions of the vegetation of Twin Cays, see Woodroffe (1995) and Rodriguez and Feller (2004).

To inventory the birds in the mangrove on Twin Cays during the winter, we used auditory and visual encounter surveys of point counts, line transects, boat survey techniques, and opportunistic observations (Bibby et al., 2000). The same techniques, with the exception of line transects, were used during the summer session. All counts were conducted by Stephen Mitten who is familiar with the calls, songs, and plumage of the birds of Belize.

To assess numbers and kinds of water birds like Cormorants, Magnificent Frigatebirds and Brown Pelicans along the shoreline and overhead, a boat survey (following Bibby et al., 2000) was employed where a small motorboat 20-30 m out from shore, moved along the shoreline. All birds seen or heard between the boat and as far inland as possible were tallied until the shorelines of East Island and West Island were covered. Double counting of birds was minimized by maintaining a constant boat pace and observing where the birds landed so as not to count them again.

We also conducted 75 6-min, 100-m fixed-radius point counts in the winter and 62 6-min, 100-m fixed radius point counts in the summer to obtain distance sampling data (Buckland et al., 2001). Counts started at 7:00 AM and were completed by 11:30 AM. Points were located within 52 (100 m^2) plots that were randomly arrayed over East Island and West Island. During both field seasons, each plot was sampled at least once; some were sampled twice, but not in the same spot within the plot. Due to the

randomness of the point location, the habitat in which the count was taken was recorded. For example, some counts were in mudflats while others were in the fringe forest. Adjacent plots were not counted on the same day. All birds seen or heard within the 100-m^2 plots were counted. Distances were measured to all visible birds by a Bushnell laser range-finder accurate to ±1 m beyond 10 m out. All birds closer than 10 m were recorded as 5 m. Most detections were visual. Where detection was aural, we made the laser measurements to the most likely spot. Detections without a reliable distance measurement were discarded. No point counts were conducted during inclement or extremely windy weather. At the four-minute mark, "pishing" was undertaken for 30 s to raise any nearby birds. Species, number, and distance were recorded.

During the winter, we conducted only eight 150 m-line transects due to time constraints. Transects were kept to preexisting survey lines along ponds and mudflats, and forest edges. The habitats varied among the line transects. All birds seen and heard within 100 m on either side of the transect were counted, and distances were measured to all birds perpendicular to the line with the laser range-finder.

Effort was similar within census techniques, but varied between techniques and between seasons. For example, 9% more point counts were completed in the winter than in the summer, and no transects were carried out during the summer because of time constraint. Point counts received the majority of our field time. Opportunistic observations of birds seen outside of point counts, line transects, and boat surveys were noted, and individuals were counted. The results of these surveys within the mangrove also permitted estimation of species richness and relative abundance indices. Density measurements were obtained for the four most common species observed during point counts. Trophic level was determined from the literature.

Seasonal status of each species was based on Jones and Vallely (2001). In some cases, little information is available for the seasonal status of birds at Twin Cays. Thus, question marks in the appendices indicate a lack of knowledge. The seasonal status includes: (1) P=permanent resident (breeding either documented or assumed); (2) S= seasonal resident only (breeding documented or assumed); (3) V=visitor (nonmigratory that do not breed in area); (4) T= transient (migratory bird that neither breeds nor spends the winter in area); (5) W= winter resident (migratory bird that stays the winter).

We calculated the rarefaction curves for point count and transect census methods for the winter using EcoSim version 7.0, a Monte Carlo simulation technique (Gotelli and Entsminger, 2001). For point-count analyses, the mean number of detections of birds per point at each plot was calculated. The relative abundance (pcRA) value of a given species was expressed as the number of individuals observed per point count. Frequency (pcFR) was equal to the percentage of point counts the birds were observed considering the whole sample. The relative abundance (tRA) of a species encountered by the transect method was expressed as the number of individuals observed per 150 m-line transect, and the frequency (tFR) was determined as the number of transects in which a given species was encountered considering the total number of line transects. We used SPSS 10.0 one-way ANOVA followed by a Tukey *post hoc* tests to examine the relationship between the most numerous species observed by point counts.

Rough density estimates for the three most common winter-bird species and the two most common summer species observed by point counts were obtained using Distance 4.1 Release 2 software (Thomas et al., 2003). Distance 4.1 sampling key

function models (half-normal and hazard-rate with series expansion adjustments) were based on best fit estimators with minimum Akaike Information Criteria (AIC) and/or Delta AIC (AICc). Because of the small sample size, stratification of the data as to the type of habitat where the point count occurred was not implemented. Ideally, this would have been done. For example, Green Herons were more easily detected in the dwarf *R. mangle* stands than in the taller *R. mangle* fringe; thus, the encounter rate would be higher. However, for our purpose, it was presumed to be the same across all habitat strata. This, of course, is not true and causes some bias. Distance data were transformed into 11 intervals of 10 m each. Observational distance measurements were truncated in the field at 110 m and, in the case of the Mangrove Yellow Warbler, further truncated to 10% of the largest observation for analysis to exclude outliers. Nonclustered parameters were chosen with each model although there may have been some possible clustering with the Mangrove Yellow Warbler.

Bird species were identified to 11 feeding guilds based on diet and mode of feeding adapted from classification schemes by Verner (1984) and Thiollay (1994). These guilds are (1) F= species that feed on fish and some invertebrates; (2) P = pirate food from other species or scavenge; (3) F+REP+INV = species that feed on fish, small reptiles and invertebrates; (4) INV+VEG = species that feed primarily on small invertebrates and aquatic vegetative matter; (5) INV = species that feed primarily on small aquatic insects and invertebrates; (6) NEC = nectarivores; (7) R = raptors (carnivores); (8) FRU = frugivores (species that feeds on fruits and buds all year); (9) OMN = omnivores; (10) INS = insectivores; (11) INS+FRU = insectivores-frugivores (species that feed primarily on insects but will take fruit at certain times). Birds were also categorized according to seven habitat categories within Twin Cays. These categories were adapted and modified from schemes of Naka (2004). They are: (1) AIR =overhead; (2) AQU = aquatic; (3) AQUE = aquatic edge; (4) AQUF = aquatic fields (mud flats, wet sandy beaches and marshy fields); (5) C = canopy; (6) U = lower strata (understory and midstory); (7) A = found anywhere within the understory and canopy. Both feeding guilds and habitat categories were based on observations by Mitten during >120 h of behavioral observations in the field, and on the literature (Howell and Webb, 2001).

RESULTS

Species Richness and Composition

We recorded 56 avian species of 20 families on Twin Cays, Belize during the winter and summer of 2004 (Appendices 1 and 2; scientific names provided therein). Of these, 23 species or ~41% of the total, were seen exclusively during the winter while 12 species (21.4%) were found exclusively during the summer. Bird species found during both seasons constituted 38% of the total. A breakdown of the results from the winter and summer seasons follows.

1) <u>Winter</u>: Forty-four bird species of 20 families were recorded during the winter survey. Of these, three families comprised 45% of all species recorded (Ardeidae, Parulidae and Scolopacidae). The families Parulidae and Scolopacidae had the highest number of species (seven species each). With the exception of the Mangrove Yellow

Warbler, all species within these two families were winter migrants. By a small margin (54.5% to 45.5%, respectively), the number of migratory species (winter and transients) was greater than nonmigratory species, i.e. permanent residents, visitors that do not breed in the area, and seasonal species (breeding only). The family Ardeidae had the greatest number of permanent species represented with six. Most permanent residents were aquatic or semiaquatic species such as Brown Pelicans and members of the Family Ardeidae (see Appendix 2.). Based on male plumage, the Mangrove Yellow Warbler (*Dendroica petechi*) had both northern migrants (*Dendroica petechi petechi*) (~10 %) and resident populations (*Dendroica petechi erithachorides*) (~90%) represented on Twin Cays. More species were counted by point counts (24 species) than by the other methods, 20 by line transect, 14 by boat, and 12 species were incidentally observed outside of sampling protocol (see Appendix 1 and Fig.1).
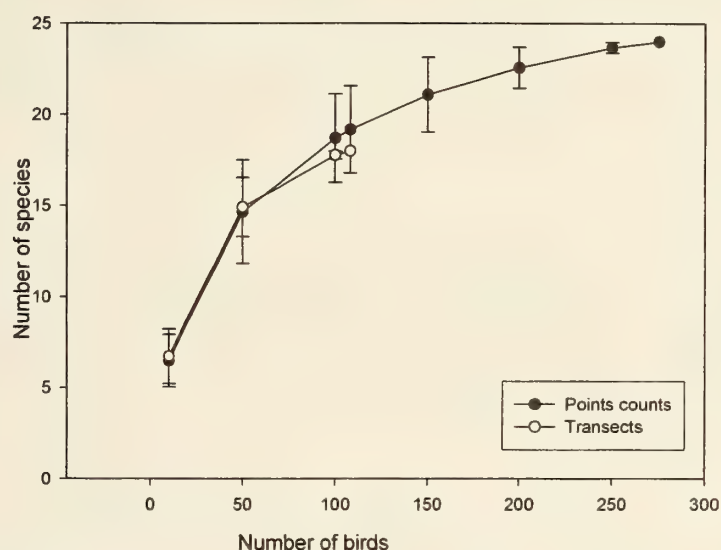
Figure 1. Species rarefaction curves in Twin Cays mangroves based on point counts and transects (Winter 2004).

Brown Pelicans, Magnificent Frigatebirds and Double-Crested Cormorants were excluded from point counts or line transects because they were seen but were either well outside the 100 m range or continually flying back and forth over the mangrove canopy. A nesting pair of ospreys was also excluded from line transect or point count data, so sampling method did not bias the data. Six species were recorded by only point counts, four by only line transects and excluding the four aforementioned species, one species was seen only by boat (Belted Kingfisher). Most species were seen by more than one method and 16 species were recorded by both point-counts and line-transect methods (Appendix 1). Accumulative species curves (i.e., rarefaction curves based on Monte Carlo simulations) revealed that the rate at which new bird species were being added had reached an asymptote with point counts but was still rising with line transects (Fig. 1). This is not surprising given the small number of line-transect samples (eight) and, with more effort, line transects may add number of species.

2) <u>Summer</u>: Thirty-three species of 17 families were recorded during the summer survey of Twin Cays (see Appendix 1). Two families comprised 33% of all species recorded (Ardeidae, and Parulidae; seven species and four species, respectively). All seven species of the family Ardeidae that were seen during the winter were observed during the summer, including the Little Blue Heron, which Valley and Jones (2001) list as a winter resident. Three of the four warblers were winter migrants that presumably never returned north. Permanent residents accounted for nearly 50%, and winter Neotropical migrants that never returned north made up ~18% of species observed. Seasonal residents (three are known for certain; White-Crowned Pigeon, Wilson's Plover and Brown-Crested Flycatcher) made up 9% of the total summer species composition. Hummingbird nests have been found previously on Twin Cays (Feller, personal observation), but it is not known which of the two summer hummingbird species breeds on the cays. They were not observed breeding in this survey, and thus were not included as seasonal breeders in data presented here. Ten species were counted during the boat survey, 19 by point counts, and 10 were incidentally seen outside of sampling protocol. Brown Pelicans, Magnificent Frigatebirds, and Double-Crested Cormorants were again excluded from the analysis but were nevertheless seen during point counts.

Species Abundance

Most species were encountered in low numbers in both seasons (Appendix 1). During the winter, four of the top five species that had high pcRA values were also the top four most frequently (high pcFR value) encountered species (i.e., Mangrove Yellow Warbler, Great-Tailed Grackle, Clapper Rail, and Northern Waterthrush; Plate 1). These same four species were also found in the top five highest tRA (Table 1, Appendix 1).

Table 1. Mean number of individuals per point count of the most common bird species at Twin Cays in winter and summer. Values are means ± 1 SD (number of birds seen in all point counts).

Bird species	Winter	Summer
Mangrove Yellow Warbler	1.7 ± 1.1 (67)	1.7 ± 0.9 (79)
Great-Tailed Grackle	1.2 ± 0.4 (29)	2.0 ± 2.0 (39)
Clapper Rail	1.3 ± 0.6 (25)	0
Northern Waterthrush	1.1 ± 0.3 (20)	0
Green Heron	0	1.6 ± 0.5 (23)

The only significant difference between these four species in mean number of individuals recorded per point count was between the Mangrove Yellow Warbler and the Northern Waterthrush ($F = 3.820$, $df = 100$, $P = 0.012$; Tukey *post hoc*, $P = 0.034$). The Mangrove Swallow had the second highest pcRA value but had one of the lowest relative frequency values (pcFR). This was due to having one large flock fly over during one point count. It was encountered on only two of the 75 point counts. The most frequently (high pcFR value) encountered species during the summer were the Mangrove Yellow Warbler,

Great-Tailed Grackle, and Green Heron, and they had the highest pcRA values. There was no significant difference among the Great-Tailed Grackle, Mangrove Yellow Warbler and Green Heron in mean number of individuals recorded per point count (ANOVA, $P > 0.05$). There was a notable increase in the pcFR for the Yucatan Vireo from winter to summer. The pcRA and pcFR values of the Green Heron increased between the winter and summer seasons. These values decreased in the Clapper Rail. The Mangrove Yellow Warbler was the single most abundant species within the mangrove and had the highest encounter rate no matter what season or sampling method used (around 25%).

Table 2. Density analyses of common bird species at Twin Cays, Belize: D = estimated density (birds/ha) ± 1 SE; 95% CI = log-based 95% confidence interval of birds/ha. Calculations of D and 95% CI are based on key function models for best fit using Distance 4.1 Release 2 software.

Species	Season	D	95% CI
Mangrove Yellow Warbler	Winter	13.4 ± 0.4	7.5, 24.1[a]
Mangrove Yellow Warbler	Summer	10.9 ± 5.6	4.2, 28.7[a]
Northern Waterthrush	Winter	4.8 ± 1.6	2.5, 9.0[b]
Clapper Rail	Winter	0.8 ± 0.3	0.4, 1.6[a]
Clapper Rail	Pooled	0.9 ± 0.2	0.5, 1.4[c]
Green Heron	Summer	1.1 ± 0.6	0.4, 2.9[a]

Key function models based on best fit:
[a] Hazard/Polynomial
[b] Half-normal/Cosine
[c] Hazard/Cosine

Density Measurements

Density measurements (number of individual/ha) were problematic given the small sample size. Nevertheless, approximate densities with the log-based 95% confidence intervals (CI) were calculated using the Distance 4.1 software for the three most common winter species (Mangrove Yellow Warbler, Clapper Rail, and Northern Waterthrush) and for the two most common summer species (Mangrove Yellow Warbler and Green Heron, Table 2). In addition, we pooled the data from the winter and summer Clapper Rail surveys to obtain an overall Clapper Rail density estimate. Sufficient data were not available to estimate the summer Clapper Rail population. The Great-Tailed Grackle had high pcRA and pcFR values, but there was considerable movement onto and off the cays from neighboring cays due to two active dump sites, which made a density estimate for this species unfeasible.

During the winter season, the Mangrove Yellow Warbler had the highest estimated "mean" density followed by the Northern Waterthrush and the Clapper Rail,

although low sample size limited the statistical power. The sizes of the bird populations were estimated by multiplying the mean density of birds/m^2 by total area (Rodriguez and Feller, 2004). If these densities are accurate, the total population of Mangrove Yellow Warblers, Northern Waterthrushes, and Clapper Rails on Twin Cays would be ~965, 343, and 56 individuals, respectively. In the case of the Mangrove Yellow Warbler and the Northern Waterthrush, substantial upward bias in point-count estimates of density may have occurred due to probable movement of birds towards the observer by "pishing". However, this practice allowed an increase in detectability. As Buckland et al. (2001) point out, bias occurs because probability of detection is a non-increasing function of distance from the point so that objects moving towards the observer are more likely to be detected when closer to the point, leading to overestimation of object density. Likewise, in the case of the Clapper Rail, density measurements were probably underestimated as birds moved away from the observer. The log-based 95% CI for the Mangrove Yellow Warbler, the Northern Waterthrush, and the Clapper Rail are given in Table 2. To compensate for these biases, the lower confidence interval for the Mangrove Yellow Warbler and the Northern Waterthrush and the higher confidence interval for the Clapper Rail were used to calculate more realistic population estimates of ~38, 181, and 113 individuals, respectively.

The summer density measurements for the Mangrove Yellow Warbler were almost 11 birds/ha, with 95% CI levels ranging from approximately 4 to 29 birds/ha (Table 2). Taking the lower confidence level for the same reasons as stated above, the Mangrove Yellow Warbler density estimate for Twin Cays during the summer stands at around 300 individuals. However, there was no significant difference between estimates obtained during the winter from those obtained during the summer. The estimated Green Heron summer density was just over 1 bird/ha, but ranging from near 0 to almost 3 birds/ha (Table 2). A crude estimate would put the number of Green Herons on Twin Cays as between 29 to 216 individuals with the actual number being closer to ~70 birds. Numbers were not sufficient to estimate the summer Clapper Rail population. Thus, we pooled the data from both winter and summer surveys to obtain an estimate of Clapper Rails. The pooled data resulted in a slightly higher estimate for density (i.e., from 0.8 to 0.9 birds/ha) and a tighter range for the 95% CI levels. The lower encounter rate for the Clapper Rail in the summer does not necessarily indicate a drop in the population, but more likely it indicates decrease in the detection function. Not only were summer point counts decreased from the winter season, the rails were much more secretive and less vocal in July than in January, contributing to the difficulty in obtaining sufficient data for analysis.

Trophic Organization

Survey data indicated that the aquatic field category was the predominant habitat for bird species on Twin Cays (see Appendix 2). It constituted 33% (summer) to 41% (winter) of the total bird species. Equal numbers of species were represented in most of the other habitats with five to nine species in each or 11 to 20% of the total. We found a small decline during the summer survey in the number of species using each habitat, but the relative percentage stayed roughly the same. The two exceptions were the canopy category, which increased from 15% to 27% of the total, and the understory, which

decreased by ~50% in number of species. This decrease was due to the significant reduction in understory Neotropical warbler species during the summer.

During the winter, the main avifauna guilds were groups that fed primarily on fish and small animals (12 species) and insectivores (11 species). These were followed by species that fed primarily on aquatic invertebrates (eight species), species that fed on aquatic vegetation and small invertebrates (four species), insectivore-frugivores (three species), omnivores (two species), and nectarivores, frugivores, raptors, and pirates having one species each. Clearly, primarily insectivorous species (combining insectivores including the vireos with species that fed on aquatic invertebrates) accounted for the vast number of species (47%) and fish eaters, including pirates and the laughing gull accounted for 32%. Frugivores and nectarivores accounted for <5%. These numbers stayed about the same during the summer with the exception of species feeding on insects and aquatic invertebrates, which decreased from 47% to ~39 % of the total. Again, this was due to the drop in Neotropical migrant shorebirds and warblers in the summer.

DISCUSSION

There are no previously published descriptions of avian species richness on Twin Cays. In this study, we found that the number of forest-restricted birds on the Cays was small. The majority of birds could be classified as either "wetland" birds or secondary growth species. The bird list therefore includes a number of species that are not restricted to mangrove forests but rather are either wetland species (e.g., Black-Bellied Whistling Duck, Lesser Scaup and Blue-Winged Teal), or inhabit the aquatic edge (Laughing Gull and Caspian Tern). In addition, distinctive vegetation types such as dwarf, scrub, and mixed woodland stands were also found within the islands (Rodriguez and Feller, 2004), and these habitats contributed significantly to the avian diversity of Twin Cays. The Twin Cays "bird list" at present constitutes 56 species: 44 species recorded during the winter and 33 species during the summer. Almost 38% of the bird species were recorded during both the winter and summer seasons.

The species accumulation curves from the point counts leveled off, which suggested that our sampling intensity was adequate for the seasons sampled. Spring and fall seasons are known to have greater numbers of migratory species as documented on neighboring Carrie Bow Cay (Ed Hunt, Carrie Bow Naturalist Log, Sept. – Oct. 1999; April – May 2001). Line transects with more effort may have produced a higher number of species. Blake and Loiselle (2001) documented that, at least within tropical forests, few species are obtained by both mist-netting and point counts. Some migrants are less territorial and less vocal and therefore harder to capture by point counts (Wang and Finch, 2002). However, this may not necessarily hold true for mangroves. While mist netting of birds for sampling may complement the visual and auditory searches by capturing more elusive birds, time constraints dictated that point counts and line transect were the best use of the time available.

Mangroves are a favorite habitat for a number of migrant species. (Arendt, 1992) Migrants and transients were 54% of the winter species detected. This is slightly higher than the 30% to 47% encountered in previous surveys of the West Indies (Wunderle and

10

Waide, 1993; Latta et. al., 2003). However, those studies did not concentrate on mangroves exclusively.

This study indicates that insectivores predominate during the winter, which is consistent with studies done elsewhere (Faaborg and Terborgh, 1980). Latta et al. (2003) predicted a shift to frugivores during the summer, but this does not hold true for mangroves. The small number of frugivores and nectarivores may be a function of lower food availability. During the summer, Green-Breasted Mangos and Rufous-Railed Hummingbirds were observed visiting the flowers of *A. germinans* and feeding on associated insects. The Cinnamon Hummingbird may be a seasonal bird here as well. The White-Crowned Pigeon, a frugivorous species, was recorded both in June and in January in very low numbers. According to Jones and Vallely (2001), this species resides primarily on the mainland during the winter.

The exact number of permanent residents on Twin Cays is not known. Possibly 25% of the recorded species could be permanent residents that live out their lives on the cays. Ardeids, in particular, appear to use the island for breeding. Two pairs of Great Egrets and a pair of Tricolored Herons were observed either preparing or sitting on nests. Green Herons and Yellow-Crowned Night Herons were the most frequently encountered, which suggested they were the most abundant herons. There was an increase in the Green Heron point-count relative abundance (pcRA) value and relative frequency value (pcFR) from the winter to the summer season. We think this represents a true increase in the Green Heron population because many of the birds encountered were juveniles. Jones and Valleley (2001) lists the little Blue Heron as a winter resident on the cays, but Little Blue Herons in juvenile plumage were seen during the summer on Twin Cays, which suggested year-round residency.

Pelicaniform birds, including Brown Pelicans, Double-Crested Cormorants, and Magnificent Frigatebirds, were very common around the fringe. Sixty-six Double-Crested Cormorants were counted one morning around Twin Cays. Flocks of Brown Pelican varied from day to day with up to 70 individuals on some days; on other days, only a few individuals were noted. Magnificent Frigatebirds nested on nearby cays, and their numbers fluctuated from day to day, as well.

A single Peregrine was observed during the winter presumably hunting the flocks of shorebirds that frequented the ponds within the interior of Twin Cays. A nesting pair of Osprey represented the only other raptors present, though Common Black Hawks were numerous in adjacent mainland mangrove forests.

Two rail species were documented. The Clapper Rail was common along the fringe, small creek waterways, and in the dense ponded dwarf stands. The Rufous-Necked Woodrail apparently resides on Twin Cays but in very low numbers. None, however, were found during the summer.

All together, 10 species of shorebirds were recorded on Twin Cays with eight species during the winter. Of these eight winter species, we observed two flocks of Least Sandpipers, consisting of more than 15 individuals, as well as solitary birds. In addition, there were at least three Black-Bellied Plovers and at least two pairs of Greater Yellowlegs. Ruddy Turnstones and Spotted Sandpipers were seen feeding around the dump. A Spotted Sandpiper was observed feeding on small *Uca* spp. Several flocks of Short-Billed Dowitchers, each with more than nine individuals, were also observed. A flock of five Short-Billed Dowitchers was observed during the summer along with two

Greater Yellowlegs. While absent or overlooked during the winter, two Semipalmated Plovers and 11 Wilson Plovers were observed in the summer. Of the 11 Wilson Plovers, at least three pairs were nesting, although only one nest with four eggs was discovered. It is of note that the breeding habitat of these birds is limited to the cleared, burned, and filled areas of the Cays.

Undoubtedly, the presence of tidal channels affects the distribution and habitat use by shorebirds at Twin Cays. Kelsey and Hassall (1989) have documented that wet moist soil harbors more invertebrates and therefore more food for water birds. Water depth plays a significant impact on the species use of habitats. Too much water or not enough water decreases available foraging habitat (Isola et al., 2000). Danufsky and Colwell's (2003) work suggested that habitat characteristics had a significant impact on species richness and densities for the winter shorebird community in California. They found that the amount of standing water correlated positively with the Wimbrel and negatively for the Dowitcher. As the hydrology of Twin Cays is altered with the cutting of survey lines and other anthropogenic development, it would be interesting to note changes in habitat use among migratory shorebirds; however, the low numbers of birds would require much time and effort to get substantial data.

A small number of Belted Kingfishers inhabit Twin Cays during the winter. The American Pygmy Kingfisher was not encountered, but has been noted from Twin Cays previously (Anne Chamberlain, Carrie Bow Naturalist Log, April, 2001), and has been recorded on other offshore islands (Jones and Vallely, 2001).

Insectivorous passerines composed a large percentage of the species recorded. Of the two vireo species, the Yucatan Vireo was encountered significantly more often than the Mangrove Vireo. There was a notable increase in the relative point count frequency value for the Yucatan Vireo from winter to summer. The Yucatan Vireo was observed behaving much like a sapsucker woodpecker, digging deep into tree branches and boles presumably to extract wood-boring insect larvae or other arthropods.

Only two species of flycatchers were recorded, which was surprising given the number of moths and other flying insects observed. The Great-Crested Flycatcher was observed during the winter and the Brown-Crested Flycatcher in the summer. There were at least three breeding pairs of the Brown-Crested Flycatchers, which was consistent with Jones and Vallely (2001) who reported that the Brown-Crested Flycatcher is migratory in Belize.

Three swallow species (Tree Swallow, Mangrove Swallow, and Barn Swallow) observed in the winter were not seen in the summer. However, we did record two martin species (Purple Martin and Gray-Breasted Martin) during the summer.

Nine species of warblers were documented in our surveys: seven during the winter and four during the summer. The Mangrove Yellow Warbler was by far the most numerous in both seasons. Three of the four summer warbler species were Neotropical migrants, two of which were not seen doing the winter (the Yellow-Throated Warbler and the Palm Warbler). The Palm Warbler was found feeding in the dense understory of *B. maritima*. During the winter, the Mangrove Yellow Warbler and Northern Waterthrush were the most numerous, followed by the American Redstart. The other warbler species appeared in low numbers. The high density of Mangrove Yellow Warblers (~7 - 24 birds/ha) and Northern Waterthrushes (~2 - 9 birds/ha) suggest that the mangrove is a rich habitat for insectivorous species. The Mangrove Yellow Warbler feeds primarily in

12

the mid to upper canopy while the Northern Waterthrush feeds on insects in the understory. One Mangrove Yellow Warbler was observed feeding on a small Mangrove Tree Crab, *Aratus pisonii*.

In conclusion, the mangrove forests of Twin Cays are vital to many bird species, particularly migratory passerines and wetland birds. High numbers of resident Clapper Rails, Green Herons, migrant Northern Waterthrushes, and both resident and migrant populations of Yellow Warblers use the islands during the winter and summer seasons. In all appearances, mangroves seem essential for some species' survival. The abundance of ground or understory bird species is primarily a function of habitat availability, food resource availability, and low predation pressure. Anthropogenic alteration of hydrology and forest structure, and the introduction of feral dogs assuredly will take their toll on birdlife. Changes are already visible on the Cays in the breeding presence of Wilson Plovers in clearcut and filled areas. As further changes are implemented, the suitability of the islands as habitat for the current assemblage of migratory and resident birds is likely to be reduced.

ACKNOWLEDGEMENTS

REFERENCES

Arendt, W.J.
    1992. Status of North American migrant landbirds in the Caribbean region: a
        summary. In *Ecology and Conservation of Neotropical Migrant Landbirds*,
        edited by J.M. Hagan, III and D.W. Johnston, 143-174. Smithsonian Institution
        Press, Washington, DC.

Ball, M.C.
    1980. Patterns of secondary succession in a mangrove forest in southern Florida.
        Oecologia (Berlin) 44:226-235.

Bibby, C.J., N.D. Burgess, D.A. Hill, and S.H. Mustoe
    2000. *Bird Census Techniques* (2nd edition). Academic Press, London.

Blake, J.G., and B.A. Loiselle
    2001. Bird assemblages in second-growth and old-growth forests, Costa Rica:
        Perspectives from mist nets and point counts. *The Auk* 118:304-326.

Buckland, S.T., D.R. Anderson, K.P. Burnham, J.L. Laake, D.L. Borchers, and L.
Thomas
    2001. *Introduction to Distance Sampling: Estimating Abundance of Biological
        Populations.* Oxford University Press, NY.

Danufsky, T., and M.A. Colwell
    2003. Winter shorebird communities and tidal flat characteristics at Humboldt Bay,
        CA. *The Condor* 105:117-129.

Davis, J.H.
    1940. The ecology and geologic role of mangroves in Florida, Papua Tortugas
        Laboratory 32:304:412. *Carnegie Institute, Washington Publication* Number
        517.

Faaborg, J., and J.W. Terborgh
    1980. Patterns of migration in the West Indies, In *Migrant Birds in the Neotropics:
        Ecology, Behavior, Distribution, and Conservation*, edited by A. Keast and
        E.S. Morton, 157-163. Smithsonian Institution Press, Washington, DC.

Gotelli, N.J., and G.L. Entsminger
    2001. EcoSim: Null models software for ecology. Version 7.0. Acquired Intelligence
        Inc. & Kesey-Bear. http://homepages.together.net/~gentsmin/ecosim.htm.

Howell, S.N.G., and S. Webb
    2001. *A Guide to the Birds of Mexico and Northern Central America.* Oxford
        University Press. NY.

Isola, C.R., M.A. Colwell, R.J. Safran, and O.W. Taft
    2000. Interspecific differences in habitat use by waterbirds foraging in managed
        wetlands of the northern San Joaquin Valley, California. *Waterbirds* 23:196-
        203.

Jones, H.L., and A.C. Vallely
    2001. *Annotated Checklist of the Birds of Belize.* Lynx Edicions. Barcelona, Spain.

Kelsey, M.G., and M. Hassall
    1985. Patch selection by Dunlin on a heterogeneous mudflat. *Ornis Scandinavica*
        20:250-254.

14

Latta, S.C., C.C. Rimmer, and K.P. McFarland
    2003. Winter bird communities in four habitats along an elevational gradient on Hispaniola. *The Condor* 105:179-197.

Naka, L.N.
    2004. Structure and organization of canopy bird assemblages in Central Amazonia. *The Auk* 121 (1):88-102.

Petit, D.R., J.F. Lynch, R.L. Hutto, J.G. Blake, and R.B. Waide
    1993. Management and conservation of migratory landbirds over-wintering in the Neotropics. In *Status and Management of Neotropical Migratory* Birds, edited by D.M. Finch and P.W. Stangel, 70-92. Gen. Tech. Rep. RM-229. USDA Forest Serv., Rocky Mt. Forest Range Exp. Sta., Fort Collins, CO.

Petit, D.R., J.F. Lynch, R.L. Hutto, J.G. Blake, and R.B. Waide
    1995. Habitat use and conservation in the Neotropics. In *Ecology and Management of Neotropical Migratory Birds: A Synthesis and Review of Critical Issues*, edited by T.E. Martin and D.M. Finch, 145-197. Oxford University Press, Inc. New York.

Rodriguez, W., and I.C. Feller
    2004. Mangrove landscape characterization and change in Twin Cays, Belize using aerial photography and IKONOS Satellite data. *Atoll Research Bulletin* 513:1-22.

Rützler, K., and I.C. Feller
    1996. Caribbean swamp communities. *Scientific American* 274(3):70-75.

Thiollay, J.M.
    1994. Structure, density and rarity in an Amazonian rainforest bird community. *Journal of Tropical Ecology* 10:449-481.

Thom, B.G.
    1967. Mangrove ecology and deltaic geomorphology: Tabasco, Mexico. *Journal of Ecology* 55:301-343.

Thomas, L., J.L. Laake, S. Strindberg, F.F.C. Marques, S.T. Buckland, D.L. Borchers, D.R. Anderson, K.P. Burnham, S.L. Hedley, J.H. Pollard, and J.R.B. Bishop
    2003. Distance 4.1. Release 2. Research Unit for Wildlife Population Assessment, University of St. Andrews, UK. http://www.ruwpa.standac.uk/distance/.

Verner, J.
    1984. The guild concept applied to management of bird populations. *Environmental Management* 8:1-14.

Wang, Y., and D.M. Finch
    2002. Consistency of mist netting and point counts in assessing landbird species richness and relative abundance during migration. *The Condor* 104:59-72.

Woodroffe, C.D.
    1995. Mangrove vegetation of Tobacco Range and nearby mangrove ranges, Central Belize Barrier Reef. *Atoll Research Bulletin,* 427:1-35.

Wunderle, J.M.., Jr., and R.B. Waide
    1993. Distribution of overwintering Neartic migrants in the Bahamas and Greater Antilles. *The Condor* 95:904-933.

APPENDIX 1

Common Name	Scientific Name	wpcFR	wpcRA	spcFR	spcRA	tFR	tRA	WB (N)	SB (N)	WI (N)	SI (N)
Brown Pelican*	Pelecanus occidentalis	*	*	*	*	*	*	11	12		
Double-Crested Cormorant*	Phalacrocrax auritus	*	*	*	*	*	*	66	36		
Magnificent Frigate*	Fregata magnificens	*	*	*	*	*	*	6	4		
Great Blue Heron	Ardea herodias	0.09	0.11	0.03	0.03	0.25	0.25	2	0		
Great Egret	Ardea alba	0.03	0.04	0	0	0.13	0.25	0	1		
Little Blue Heron	Egretta caerulea	0.03	0.03	0.05	0.05	0.38	0.5	1	1		
Tricolored Heron	Egretta tricolor	0.05	0.06	0.02	0.02	0.25	0.38	0	0		
Reddish Egret	Egretta rufescens			0.02	0.02			0	0		
Green Heron	Butorides virescens	0.12	0.12	0.27	0.37	0.38	0.5	0	6		
Yellow-Crowned Night Heron	Nyctanassa violacea	0.07	0.09	0.02	0.02	0.5	0.63	0	0		
Black-Bellied Whistling Duck	Dendrocyna autumnalis			0	0			0	0		1
Blue-Winged Teal	Anas discors	0	0			0.13	0.25	0	0		
Lesser Scaup	Aythya affinis	0	0			0	0	0	0	2	
Osprey*	Pandion haliaetus	*	*	0.02	0.02	*	*	2	0	3	
Peregrine	Falco peregrinus	0.01	0.01			0	0	0	0		
Rufous-Necked Woodrail	Aramides axillaris	0	0			0	0	0	0	1	
Clapper Rail	Rallus longirostris	0.27	0.33	0.13	0.16	0.75	1.88	6	2		
Black-Bellied Plover	Pluvialis squatarola	0.03	0.03			0.38	0.38	1	0		
Wilson's Plover	Charadrius wilsonia			0.03	0.08			0	0		
Semipalmated Plover	Charadrius semipalmatus			0	0			0	0		2
Greater Yellowlegs	Tringa melanoleuca	0.03	0.05	0	0	0.38	0.63	1	0		2
Whimbrel	Numenius phaeopus	0	0			0	0	0	0	1	
Marbled Godwit	Limosa fedoa	0	0			0	0	0	0	1	
Spotted Sandpiper	Actitis macularia	0	0			0	0	0	0	1	
Least Sandpiper	Calidris minutilla	0.01	0.2			0	0	0	0		
Ruddy Turnstone	Arenaria interpres	0	0			0	0	0	0	1	
Short-Billed Dowitcher	Limnodromus griseus	0.01	0.12	0.08	0.08	0.13	1.25	0	0		
Laughing Gull	Larus atricilla	0	0	0	0	0	0	0	0	1	1
Caspian Tern	Sterna caspia	0	0			0	0	0	0	1	
Royal Tern	Sterna maxima	0.01	0.03			0	0	5	0		2
Sandwich Tern	Sterna sandvicensis			0	0			0	0		1
White-Crowned Pigeon	Columba leucocephala	0	0	0.02	0.02	0	0	0	0	2	

Common Name	Scientific Name	tRA	tFR	spcRA	spcFr	wpcRA	wpcFr	SB (N)	WB (N)	SI (N)	WI (N)
Cinnamon Hummingbird	*Amazilla rutila*	0	0			0	0	0	0		1
Green-Breasted Mango	*Anthracothorax prevostii*			0	0			0	0	2	
Rufous-Tailed Hummingbird	*Amazilla tzacatl*			0.02	0.02			0	0		
Belted Kingfisher	*Ceryle alcyon*	0	0			0	0	4	0		
Great-Crested Flycatcher	*Myiarchus crinitus*	0.01	0.01			0	0	0	0		
Brown-Crested Flycatcher	*Myiarchus tyrannulus*			0.10	0.01			0	0		
Purple Martin	*Progne subis*			0.02	0.02			0	0		
Gray-Breasted Martin	*Progne chalybea*			0.03	0.13			0	0		
Tree Swallow	*Tachycineta bicolor*	0.01	0.11			0	0	0	0		
Mangrove Swallow	*Tachycineta albilinea*	0.03	0.63			0.13	0.63	0	0		
Barn Swallow	*Hirundo rustica*	0.01	0.01			0	0	0	0		
Grey Catbird	*Dumetella carolinensis*	0.03	0.03			0	0	0	0		
Mangrove Vireo	*Vireo pallens*	0.01	0.01	0	0	0	0	0	0		
Yucatan Vireo	*Vireo magister*	0.04	0.05	0.18	0.08	0	0	1	10	2	
Mangrove Yellow Warbler	*Dendroica petechia*	0.52	0.9	0.74	1.26	0.88	3.13	9	21		
Magnolia Warbler	*Dendroica magnolia*	0	0			0.13	0.13	0	0		
Yellow-Throated Warbler	*Dendroica dominica*			0.02	0.02			0	0		
Palm Warbler	*Dendroica palmarum*			0	0			0	0	1	
American Redstart	*Setophaga ruticillia*	0.04	0.04	0	0	0	0	0	0	1	
Ovenbird	*Seiurus aurocapillus*	0	0			0.13	0.13	0	0		
Northern Waterthrush	*Seiurus noveboracensis*	0.21	0.27			0.63	0.75	0	0		
Louisiana Waterthrush	*Seiurus motacilla*	0	0			0	0	0	0		1
Common Yellowthroat	*Geothlypis trichas*	0	0			0.13	0.13	0	0		
Great-Tailed Grackle	*Quiscalus mexicanus*	0.32	0.39	0.32	0.63	0.63	1.75	4	32		
TOTAL: 56 species		1.99	3.66	2.03	3.01		1.75	123	125		
		13 sp.	10 sp.	10 sp.	12 sp.		10 sp.				

*Excluded from point counts and line transects. WB (N) = winter boat count of individuals. SB (N) = summer boat count. wpcFr = winter point count frequency value for all birds observed, spcFr = summer point count frequency value for all birds observed. Point count frequency (pcFR) values are equal to the percentage of point counts the species was observed considering the total number of point counts. wpcRA = winter point count relative abundance. spcRA = summer point count relative abundance. Point count relative abundance (pcRA) values of a given species are expressed as the number of individuals observed per point count. Line transect relative abundance (tRA) value of a species encountered was expressed as the number of individuals observed per 150 m line transect. Line transect frequency (tFR) value was determined as the number of transects in which a given species was encountered considering the total number of 150 m line transects. WI (N) = Winter Incidentals (N), SI (N) = Summer Incidentals (N).

APPENDIX 2

Species #	Common Name	Seasonality	Habitat	Feeding guild
1	Brown Pelican	P	AQU	F
2	Double-Crested Cormorant	P or V?	AQU	F
3	Magnificent Frigatebird	P	AIR	P
4	Great Blue Heron	P or V?	AQUE	F+REP+INV
5	Great Egret	P or V?	AQUE	F+REP+INV
6	Little Blue Heron	W	AQUE	F+REP+INV
7	Tricolored Heron	P or V?	AQUE	F+REP+INV
8	Green Heron	P	AQUE	F+REP+INV
9	Yellow-Crowned Night Heron	P	AQUE	F+REP+INV
10	Blue-Winged Teal	T	AQU	INV+VEG
11	Lesser Scaup	T	AQU	INV+VEG
12	Osprey	P	AQUE	F
13	Peregrine	W or T?	AIR	R
14	Rufous-Necked Woodrail	P?	U	INV+VEG
15	Clapper Rail	P	U	INV+VEG
16	Black-Bellied Plover	W	AQUF	INV
17	Greater Yellowlegs	W or T?	AQUF	INV
18	Wimbrel	T	AQUF	INV
19	Marbled Godwit	W or T?	AQUF	INV
20	Spotted Sandpiper	W	AQUF	INV
21	Least Sandpiper	W or T?	AQUF	INV
22	Ruddy Turnstone	W	AQUF	INV
23	Short-Billed Dowitcher	W or T?	AQUF	INV
24	Laughing Gull	P or V?	AQU	OMN
25	Caspian Tern	W or T?	AQU	F
26	Royal Tern	V	AQU	F
27	White-Crowned Pigeon	S	C	FRU
28	Cinnamon Hummingbird	P?	C	NEC
29	Belted Kingfisher	W	AQUE	F
30	Great-Crested Flycatcher	T	C	INS
31	Tree Swallow	W	AIR	INS
32	Mangrove Swallow	P	AIR	INS
33	Barn Swallow	T	AIR	INS
34	Grey Catbird	W or T?	U	INS+FRU
35	Mangrove Vireo	P	C	INS+FRU
36	Yucatan Vireo	P	C	INS+FRU
37	Yellow Warbler	P and W	C	INS
38	Magnolia Warbler	W or T?	C	INS
39	American Redstart	W	U	INS
40	Ovenbird	W	U	INS
41	Northern Waterthrush	W	U	INS
42	Louisiana Waterthrush	W	U	INS
43	Common Yellowthroat	W	U	INS
44	Great-Tailed Grackle	P	A	OMN

Seasonality: P=permanent resident; B=seasonal resident; V=visitor (non-migratory; do not breed in area); T= transient (migratory; neither breeds nor spends the winter); W=winter resident (migratory; stays winter). Habitat: AIR=overhead; AQU=aquatic; AQUE=aquatic edge; AQUF=mudflats, wet sandy beaches, marshy fields; C= midstory, canopy; U=lower strata (understory to midstory); A=anywhere within mangrove. Feeding guild: F=fish, some invertebrates; P=pirate food or scavenge; F+REP+INV=fish, reptiles, invertebrates; INV+VEG=aquatic vegetation, invertebrates; INV=aquatic insects, invertebrates; NEC= nectarivores; R=raptors (carnivores); FRU=frugivores (fruits, buds); OMN=omnivores; INS=insectivores; INS+FRU=insectivores-frugivores (insects, fruit).

PLATE

Plate 1. A. Mangrove Yellow Warbler; B. Northern Waterthrush; C. Yucatan Vireo; D. Green Heron (Juvenile); E. Clapper Rail; F. Osprey

Plate 1

ATOLL RESEARCH BULLETIN

NO. 528

BENTHIC MICROBIAL MATS: IMPORTANT SOURCES OF FIXED
NITROGEN AND CARBON TO THE TWIN CAYS, BELIZE ECOSYSTEM

BY

SAMANTHA B. JOYE AND ROSALYNN Y. LEE

Figure 1. Site map of Twin Cays, Belize, noting the sampling locations and dates. Site abbreviations are: BF=Boa Flats; D=Dock; L=Lair; NWD=Northwest Dock; SCC=South Clear Cut; WP=West Pond; and, WS=Weather Station. See text for details.

BENTHIC MICROBIAL MATS: IMPORTANT SOURCES OF FIXED NITROGEN AND CARBON TO THE TWIN CAYS, BELIZE ECOSYSTEM

BY

SAMANTHA B. JOYE[1] AND ROSALYNN Y. LEE[1]

ABSTRACT

We collected surface microbial mats at sites on Twin Cays, Belize to determine rates of primary production and nitrogen transformations. A diverse array of cyanobacteria including filamentous, coccoidal and heterocystous cyanobacteria, as well as purple sulfur bacteria and heterotrophic bacteria, were important components of microbial mat communities. Sediment chlorophyll *a* concentrations illustrated a high photosynthetic biomass in surface sediments. Rates of primary carbon fixation, measured as gross oxygenic photosynthesis, and nitrogen fixation and denitrification, measured using specific metabolic inhibitors, were determined during day-and-night incubations. Primary production rates were similarly high across different mat types. Nitrogen fixation rates were substantial under *in situ* conditions and nighttime activity frequently exceeded daytime activity. *In situ* denitrification rates were very low in all incubations. In the presence of added nitrate, however, denitrification rates increased significantly during daytime and nighttime incubations so that they equaled or exceeded nitrogen fixation rates. Collectively, our data show that microbial mats are a significant source of fixed carbon and nitrogen to the Twin Cays ecosystem and suggest that mats may serve as an important component of the ecosystem's food web.

INTRODUCTION

Microbial mats are light-driven, laminated microbial ecosystems that are modern descendants of ancient microbial communities responsible for the initial oxygenation of the earth system (des Marias, 2003; Krumbein et al., 2003). Today, microbial mats may play a similarly important role in a variety of environments (Golubic, 1994) ranging from polar deserts (de los Rios et al., 2004) to hypersaline environments (Jørgensen et al., 1983; Campbell and Golubic, 1985; Paerl et al., 2003) to coral reefs (Wiebe et al., 1975), mangroves (Karsten et al., 1998) and intertidal marshes and mud flats (Bebout et al., 1987 and 1983; Skyring and Bauld, 1990; Joye and Paerl, 1994). Microbial mats are comprised of a diverse suite of autotrophic and heterotrophic microorganisms that carry out rapid rates of elemental cycling (Paerl et al., 2000). Despite incredible functional and phylogenetic diversity at the population level, microbial mats are highly structured communities with organization apparent at micrometer and centimeter scales through geochemical profiles (e.g., changes in oxygen or sulfide concentration over depth),

---

[1] Department of Marine Sciences, University of Georgia, Athens, GA, 30602-3636.

differing colors (e.g., reflecting dominant pigments of the microbes in each layer) or varying textures (e.g., from the binding, trapping, or accumulation of sediments or organic byproducts such as empty sheaths).

Microbial mats are often referred to as "self-sustaining" ecosystems (Des Marais, 2003) where primary production (oxygenic and anoxygenic photosynthesis and, to a lesser extent, chemosynthesis) and organic matter recycling (fermentation and terminal metabolism) processes are tightly coupled. Even though mats are characterized by tight internal coupling, export of microbial mat-derived material to the larger ecosystem has been demonstrated in many settings. For example, mats support secondary production of associated benthic invertebrates (Pulich and Scalan, 1987; Bouillon et al., 2004), which then serve as food items for fish and birds and mats may provide fixed nitrogen to associated higher plants (Bashan et al., 1998).

We determined rates of primary production (i.e., oxygenic photosynthesis) and nitrogen cycling (i.e., nitrogen fixation and denitrification) in microbial mats collected from several sites on Twin Cays, Belize. We determined rates during both daytime and nighttime incubations to evaluate the impact of oxygen production via photosynthesis on nitrogen cycling. We also identified the dominant cyanobacteria in microbial mat samples using microscopic techniques to help explain the observed patterns of nitrogen cycling.

PREVIOUS WORK

Among the bacteria, many cyanobacteria are unique in their ability both to use dinitrogen (N_2) as a primary nitrogen (N) source through N_2 fixation and to evolve oxygen (O_2) via oxygenic photosynthesis, making them important in carbon (C), oxygen (O) and nitrogen (N) cycles. Cyanobacteria are some of the most conspicuous components of microbial mats. Diverse types of cyanobacteria inhabit microbial mats, including filamentous nonheterocystous (e.g. *Microcoleous spp.* and *Lyngbya spp.*), coccoidal (e.g., *Chroococcus spp.* and *Aphanocapsa spp.*) and heterocystous (e.g., *Calothrix spp.* and *Nodularia spp.*) forms. In addition to playing important roles in C, N and O cycling, dense accumulation of filaments and excreted mucous help bind and stabilize sediments (Golubic et al., 2000), promoting establishment and eventual expansion of the mat.

Microbial mat communities are based ultimately on light availability because oxygenic and anoxygenic photosynthetic autotrophs produce the organic carbon that is later consumed by fermenting and heterotrophic bacteria. Oxygenic photosynthesis accounts for a large fraction (about 90% on average) of primary production in microbial mats and a large fraction of this activity is attributable to cyanobacteria (Kuhl et al., 2003). Nitrogen-fixing bacteria in mats provide the mat microbial community with an internal source of fixed N via N_2 fixation (Stal et al., 1984; Stal and Krumbein, 1985a; Stal and Heyer, 1987). The ability to provide primary sources of both fixed C and fixed N makes mats particularly important components of oligotrophic ecosystems.

One of the most interesting features of microbial mats is the simultaneous occurrence of processes that are metabolically incompatible, such as oxygenic

photosynthesis and anaerobic processes like N_2 fixation or sulfate reduction (Cohen and Rosenberg, 1989; Canfield and des Marias, 1991; Paerl et al., 1989 and 1994). The coexistence of aerobic and anaerobic processes in microbial mats results from tight coupling between production and consumption processes, redox heterogeneity and microzonation, and possibly novel metabolic adaptations (Paerl et al., 2000). Understanding how N cycling processes are regulated by O_2 concentrations in microbial mats is of particular interest because fixed N availability often limits primary production in marine environments (Howarth, 1991). In fact, N_2 fixation can be an extremely important N source in N-limited marine environments (Howarth et al., 1988a and 1988b).

Oxygen is a potent inhibitor of nitrogenase, the enzyme responsible for N_2 fixation (Bautista et al., 1985; Paerl et al., 1989; Fay, 1992; Stal, 1995). Maintaining high rates of primary production requires sufficient N supplies and N_2 fixation can alleviate N limitation. Consortial interactions between different microbes, such as tight coupling between O_2 production and consumption processes to maintain *in situ* O_2 concentrations at optimal (low) levels, may help optimize conditions for N_2 fixation in microbial mats (Steppe et al., 1996; Paerl et al., 2000). Alternatively, oxygenic photosynthesis and N_2 fixation may be separated temporally, e.g., high rates of oxygenic photosynthesis may occur during the day while N_2 fixation occurs at night when O_2 concentrations are lower (Bautista et al., 1985; Stal and Krumbein, 1987). Another possibility is that N_2 fixing microbes may spatially separate nitrogenase from potential O_2 toxicity by either isolating the enzyme within O_2-resistant heterocysts (Yates, 1977; Haselkorn, 1978) or by partitioning the cellular enzymatic machinery used in carbon fixation (O_2 evolution) from that of N_2 fixation (e.g., *Lyngbya aestuarii*, Paerl et al., 1991).

Oxygen concentration also influences other mat biogeochemical processes that are O_2-sensitive, such as denitrification, sulfate reduction and methanogenesis. Denitrification, or the reduction of nitrate to N_2, is mediated by denitrifying bacteria. Denitrifiers are facultative anaerobic heterotrophs, meaning that these microbes couple organic carbon oxidation to the respiration of either O_2 (when O_2 concentrations exceed about 20 μM) or nitrate (Joye, 2002). Denitrification is typically controlled by the availability of organic carbon, O_2 and nitrate (Joye, 2002). In many systems, nitrate availability limits denitrification rates but few studies have examined denitrification in microbial mats (Joye and Paerl, 1993 and 1994; Lee and Joye, in preparation).

MATERIALS AND METHODS

Study Site

This work was conducted at Twin Cays, Belize, a pair of oceanic mangrove islands that lie approximately 12 miles offshore (Feller et al., 2002). The islands are peat-based and receive no terrestrial (i.e., river) inputs of freshwater or sediment. The islands support distinct habitats, including mangrove forests, tidal creeks, and shallow ponds. Macroscopic forest vegetation is dominated by the red mangrove (*Rhizophora mangle*) but black (*Avicennia germinans*) and white (*Laguncularia racemosa*) mangroves are also present. Generally speaking, the mangrove forests can be divided into three zones based

on the height of the trees in that zone: fringe, transition and dwarf (Feller et al., 2002). The fringe zone, characterized by tall (about 5 m) trees, lies at the interface between the ocean or tidal creek and the edge of the mangrove forest. The transition zone, characterized by shorter trees (between 2-4 m), lies between the fringe and dwarf zones. The dwarf zone, characterized by short (about 1 to 1.5 m) trees, occupies a large fraction (about 37%, Rodriguez and Feller, this volume) of the island area. Surficial microbial mats are found throughout the different ecotones on Twin Cays but laminated microbial mats occurred mainly in the dwarf zone. The work described here was carried out in dwarf mangrove forests, which are ubiquitous in the interior of the islands.

Microbial mats were collected from seven dwarf mangrove sites, three on the east island (**BF**=Boa Flats, **L**=Lair and **WS**=Weather Station) and four from the west island (**D**=Dock, **NWD**=Northwest Dock, **SCC**=South Clear Cut and **WP**=West Pond) (Fig. 1). Two distinct areas were sampled at the WP site: one along the pond edge and one within the dwarf zone. The BF, WS and WP sites supported well-laminated microbial mats that grew atop thick (10's of cm) accumulations of organic debris (including old mat layers) along the edge of shallow ponds (Fig. 2a). The D and L sites supported localized accumulations of thin microbial mats that grew on top of a thin (<cm) organic layer that overlaid peat (Fig. 2a). At the NWD and WP dwarf sites, (noted WP-tab on Figs. 5 and 6), thin (<1 cm) laminated mats were found growing directly on top of peat. At the SCC site, thick (> cm) accumulations of mat grew on top of carbonate marl that was laid down when the region was clear cut.

Microscopy and Geochemical Characterization

The major cyanobacteria present in microbial mats were identified using epifluorescent microscopy. Individual layers of the microbial mats, delineated by changes in color, were carefully sampled using a sterile scalpel or dissecting pin. The sample was transferred to a microscope slide and examined under oil immersion. Multiple slides were examined for each depth horizon. Samples for chlorophyll a determination were collected in the field (n=3 to 5 for each mat type) and from samples used for microelectrode profiling (n=3 per core) by subcoring the mat with a cut-off 5 cc syringe. After collecting the subcore, excess organic material and/or peat were removed from the bottom of the core with a scalpel so that the sample contained only viable microbial mat. Each sample was transferred to a 15-ml centrifuge tube and 1-to-2 drops of a saturated magnesium carbonate solution were added to the mat to stabilize chlorophyll. Samples were stored frozen prior to analysis back at the University of Georgia laboratory. Chlorophyll a was determined by sonicating samples in a mixture of acetone, methanol, and deionized water at a ratio 45:45:10 to burst the cells. Next, pigments were extracted for 12-to-24 hours at 0°C. Following extraction, samples were centrifuged and chlorophyll concentrations were determined using a spectrophotometer (Lee and Joye, in preparation).

Samples for quantification of dissolved nutrient (dissolved inorganic nitrogen (DIN) = nitrate (NO_3^-) + ammonium (NH_4^+) and dissolved inorganic phosphate (DIP) = orthophosphate (PO_4^{3-})), concentrations and environmental data (hydrogen sulfide (H_2S) concentration, salinity and pH) were collected in the field from a depth of 5-to-10 cm

below the mat using a PVC piezometer and a hand-actuated vacuum pump. Samples were filtered through a 0.2 µm filter. Salinity was determined using a hand-held refractometer. pH was measured using a field electrometer calibrated with certified (National Bureau of Standards) buffers at pH 4 and 7. Other samples were either stored at 4°C (nutrients) or preserved (H_2S samples preserved by fixation with 500 µL of 20%-weight-per-volume zinc acetate solution) and then stored at 4°C. Nutrient and H_2S analyses were conducted using standard methods (Joye et al., 1996; Lee and Joye, in preparation).

Mats for use in experiments to determine N-cycling rates were collected the day before the experiment by cutting a 400-cm^2 section with a scalpel and sliding a flexible plastic sheet beneath the mat. Several (~3 to 4) sections were collected with care taken to maintain the integrity of the mat and placed into clean (10% HCl washed, deionized water-rinsed) plastic pans. Samples were transported to the laboratory and moistened with filtered (GF/F) ambient creek water to prevent desiccation. Incubations were performed using subsamples obtained by inserting minicorers (cut-off syringes with 1.15 cm^2 surface area) vertically into the large mat sections. From each core, the upper 0.5 cm was transferred to a 38-ml serum bottle for incubations as described below. Incubations were run at six-hour intervals during the day (about 0900 to 1500) or at night (2200 to 0400). At the same time as samples for N cycling rates were collected, four to five cores (5 cm diameter and 6 cm deep) were collected for determining rates of oxygenic photosynthesis. Water from adjacent tidal creeks was collected for use in the incubations described below.

Microbial Mat Primary Production

Depth profiles of O_2 concentrations and rates of oxygenic photosynthesis (via the light-dark shift method) were determined using O_2-specific microlectrodes (Revsbech et al., 1980; Revsbech et al., 1983a and 1983b; Joye et al., 1996). Microelectrode profiles were conducted in the laboratory using UNISENSE® microelectrodes, a UNISENSE® picoammeter, and a computer-controlled micromanipulator. In laboratory experiments, photosynthetically active radiation (PAR) flux was provided by a Fostec® full-spectrum light source. Oxygen concentrations and the rate of photosynthesis were determined at 100 µm intervals from about 1000 µm above the sediment water interface to the depth where oxygen concentration was zero. To evaluate heterogeneity in primary production rates, at least three profiles were obtained from each core and three-to-four cores were profiled from each site. PAR in the field and in the laboratory was measured using a LiCor model 2π quantum sensor coupled to a LiCor datalogger.

Microbial Mat Nitrogen Transformations

The combined acetylene (C_2H_2) reduction–acetylene block assay was used to estimate N_2 fixation and denitrification rates, respectively (Hardy et al., 1968; Joye and Paerl, 1994). We examined the effects of oxygenic photosynthesis on N cycling and coupling between photosynthetic CO_2 and N_2 fixation by comparing rates in different treatments during daytime and nighttime incubations. During daytime incubations, we

treatment allowed us to assess the effect of photosynthetic O_2 evolution on N transforming processes, i.e., light-supported N transformations in the absence of O_2 evolution. This treatment is noted "DCMU" on figures. Incubating samples in the dark (bottles wrapped in aluminum foil) eliminated activity coupled directly to light-generated (PSI or PSII) reductant and/or energy; this treatment provided estimates of *dark* N_2 fixation. To determine the impact of NO_3^- availability on rates of N_2 fixation and denitrification, we included a set of light-and-dark incubations amended with 1 mM nitrate and 2 mM glucose. These treatments provided *potential denitrification* rates and helped evaluate whether dissolved inorganic N concentrations regulate N_2 fixation. Rates in these treatments are referred to as *light NG* and *dark NG* later in the text and on the figures.

The details of the protocol have been described previously (Joye and Paerl, 1994). Briefly, samples were incubated for six hours during daytime or nighttime periods. DCMU-amended samples were run during the day only. Blanks (incubations of filtered creek water + C_2H_2 without any mat) were used to correct experimental treatments for trace ethylene (C_2H_4) contamination of C_2H_2. All treatments and blanks were run in triplicate. Ethylene production was quantified using flame ionization gas chromatography (Joye and Paerl, 1994). Production rates were transformed to N_2 fixation rates using an experimentally determined conversion factor (Lee and Joye, in preparation). Acetylene also blocks the transformation of N_2O to N_2 in denitrifying bacteria and denitrification rates were estimated by quantifying N_2O production rates during the incubation (Joye and Paerl, 1994). Nitrous oxide concentration was quantified using electron capture gas chromatography (Joye and Paerl, 1994; Lee and Joye, in preparation).

RESULTS

Microscopy and Geochemical Characterization

Twin Cays microbial mats are diverse in both overall morphology (Fig. 2A) and with respect to the cyanobacterial species present (Fig. 2B). The morphology of the mat varied as a function of the underlying substrate. Mats growing on top of peat had irregular (bumpy) surface topography (Fig. 2, A-3, A-4) while mats growing at the edges of ponds or atop organic layers had smoother surfaces. These different mats contained a variety of cyanobacteria. Mats growing directly on top of peat generally contained more heterocystous (e.g., *Nodularia spp.*) species whereas mats growing along the edges of ponds and those growing on organic layers overlaying peat were dominated by coccoidal (e.g., *Chroococcus spp.* and *Gloeocapsa spp.*) and filamentous, non-heterocystous species (e.g., *Microcoleus spp., Lyngbya spp., and Phormidium spp.*). Numerous heterotrophic bacteria and photosynthetic bacteria (e.g., *Chromatium, Thiocapsa*, and *Rhodopseudomonas*) were also observed in the microbial mats.

7
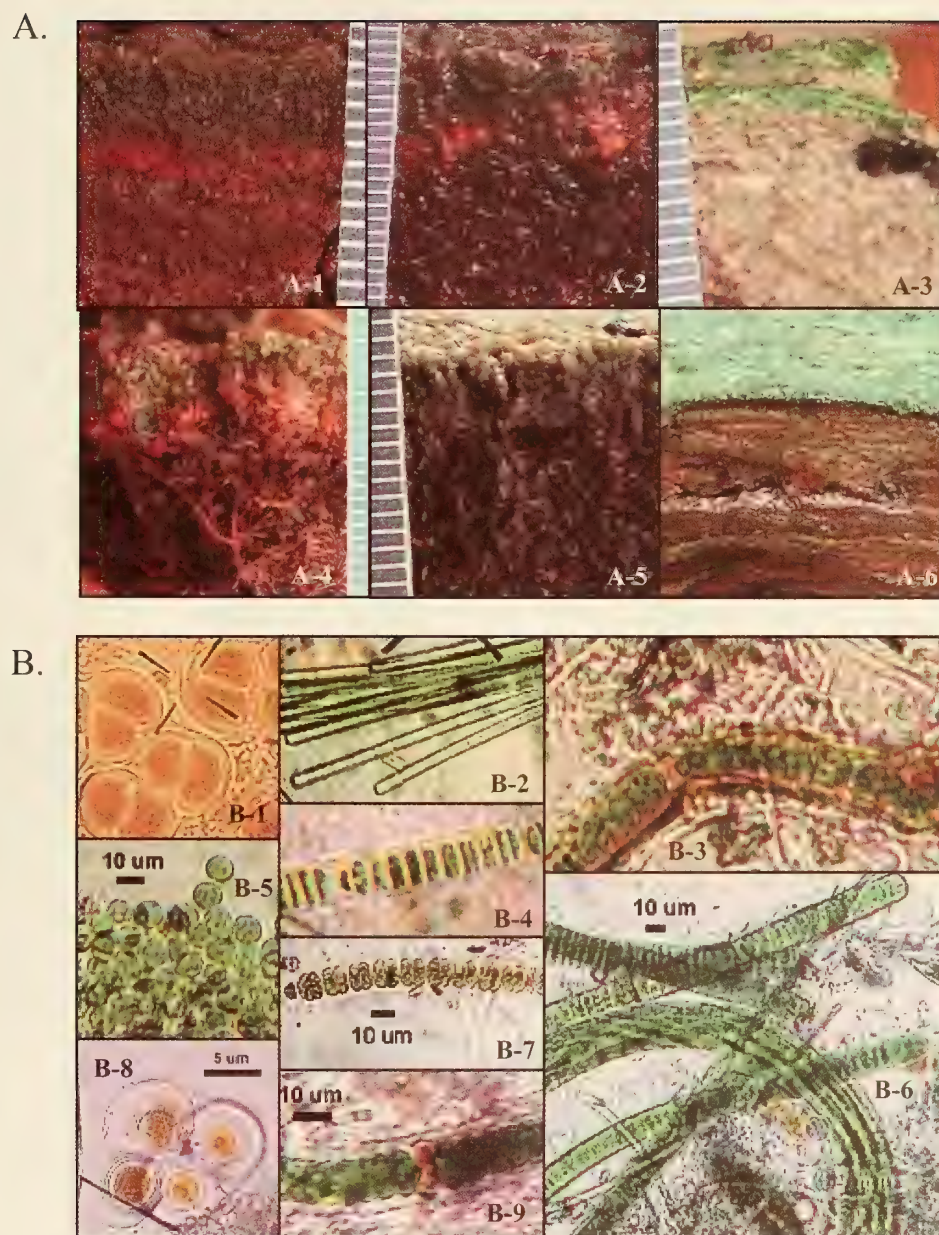
Figure 2. (A) Microbial mat morphologies observed on Twin Cays. Panels A-1 and A-2 show mats growing atop thin organic layers that overlay peat. Panels A-4 and A-5 show thin mats growing directly on top of peat. Panels A-3 and A-6 show mats from the SCC (3) and WP (6) sites. (B) Examples of the resident cyanobacteria in Twin Cays microbial mats: 1-Chroococcus sp.; 2-Microcoleus sp.; 3-Nodularia sp. (with opaque sheath); 4- Johannesbaptista sp.; 5- Aphanocapsa sp.; 6- Lyngbya sp. (thicker single filaments) and Microcoleus sp. (thin, multiple filaments within a sheath); 7-Johannesbaptista sp.; 8-Gloeocapsa sp.; and 9-Nodularia sp. (with clear sheath).

Average temperatures did not vary significantly between the three sampling dates. The maximum daily PAR flux was highest in June but day-to-day variation in PAR due to patchy cloud cover obscured significant differences between sampling dates. Variation in the concentration of chlorophyll *a* and pore water nutrients was noted (Table 1). Pore water at 10 cm was generally hypersaline. Chlorophyll *a* concentrations were highest in November 2000 but no significant differences were observed between the sites sampled at this time. In June 2001, chlorophyll *a* concentrations were significantly lower than those observed in November 2000. In March 2002, chlorophyll *a* concentrations were similar to those observed in June 2001 except at the WP site where high concentrations were observed. Pore water pH at 10 cm was usually between six and seven (data not shown). Pore water dissolved inorganic nitrogen (DIN = $NH_4^+ + NO_3^-$) concentrations varied substantially (~40 µM to >1.5 mM) beneath microbial mats. Dissolved inorganic phosphorus concentrations were very low (0.02 to 6 µM). The porewater DIN:DIP ratio varied but usually exceeded the Redfield Ratio of 15 (>100 to >1000). Pore water H_2S concentrations also varied, ranging from 0.19 to 3.5 mM.

Microbial Mat Primary Production

Pore water O_2 concentration was a function of PAR intensity and responded quickly to changes in the PAR availability (Fig. 3). Oxygen was rapidly consumed in sediments in the dark, limiting the oxygen penetration depth to 0.1 to 0.4 mm depth. As the PAR flux increased, oxygen concentrations gradually increased and the depth of oxygen penetration increased. A surface PAR flux greater than about 350 µE led to a subsurface peak in O_2 concentration (400 to 700 µM or >300% saturation) and an O_2 penetration depth between 1.1 and 2 mm (Fig. 3A). Integrating the gross oxygen production (GOP) rate over depth across increasing PAR availability showed that rates were similar at PAR fluxes between 100 and 2200 µE in the microbial mats from the edge of the Weather Station pond (values given represent the mean and error bars are standard deviation of the mean; n=3 profiles at each PAR flux). Even though the mean integrated GOP rates at ≥2000 µE were higher, the standard deviations were also higher so the rates were not significantly different from those observed at lower PAR fluxes.

GOP rates were similar across time and across sites. In June 2001, integrated GOP rates were comparable at the four sites examined (6 and 17 mmol O_2 m^{-2} hr^{-1} at 200 and 2000 µE, respectively) (Fig. 4A). Rates at the two light levels were only significantly different at the Dock site; however, when all data were pooled, rates at the higher PAR flux (2000 µE) significantly exceeded those observed at the lower PAR flux (200 µE).
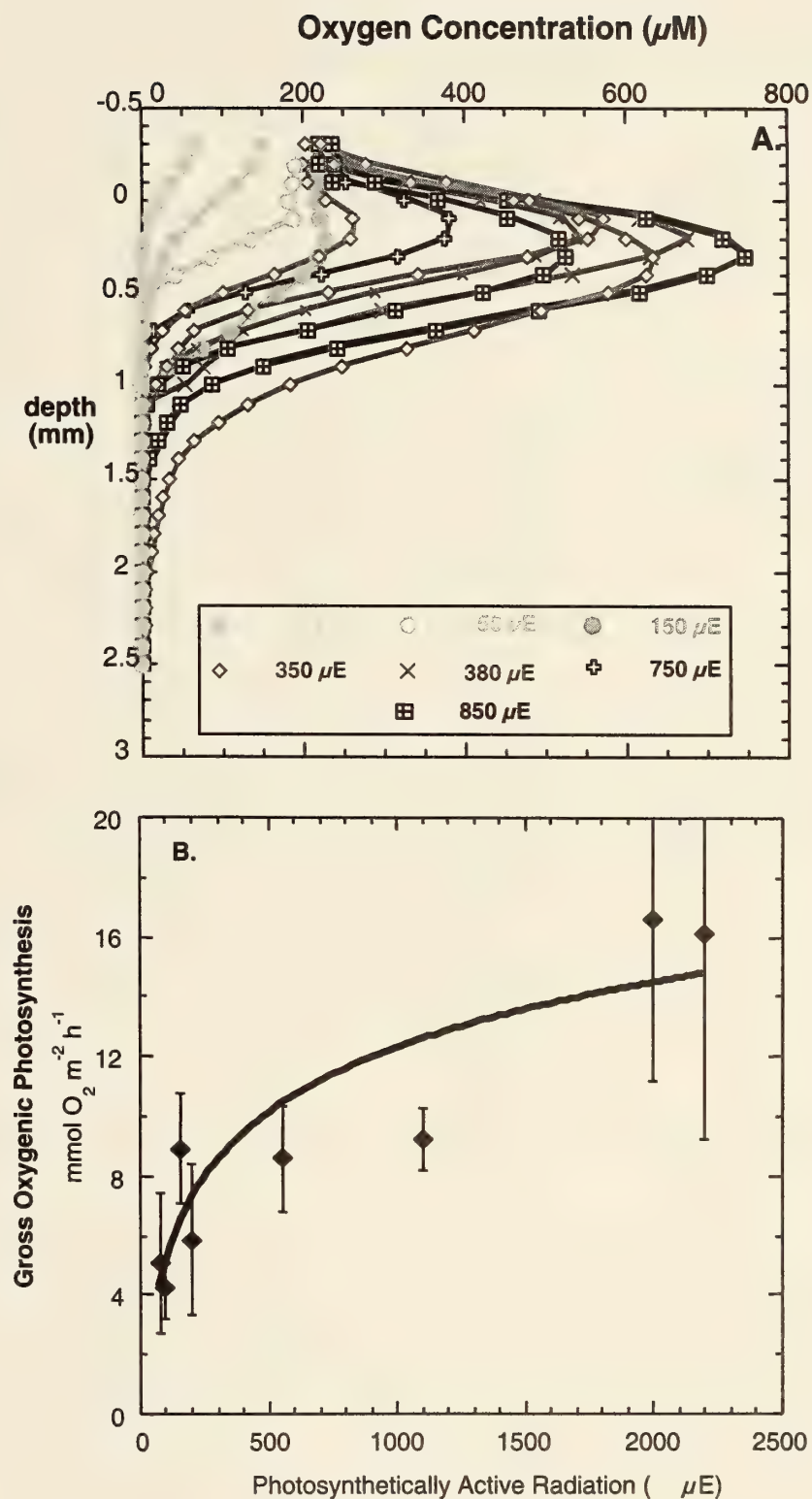
Figure 3. A. Pore water oxygen concentration over depth at different light levels (BF, Nov 2000). B. Gross oxygenic photosynthesis versus light availability. Values shown represent the mean and error bars are standard deviation of the mean of n=3 profiles at each PAR flux (WS, June 2001).

Table 1. Physical data and chlorophyll *a* and nutrient concentrations for the three sampling dates (average and (standard deviation) are shown).

Parameter:	Nov-00 Salinity[3]	Avg. Temp.[1] Chlorophyll *a*[4]	27.6 °C DIN[5]	Avg. PAR[2] DIP[5]	1800 μE H_2S[6]
Site: BF	36	157.5 (57)	288 to 1200	0.2 to 0.38	0.01
D	n.d.	n.d.	n.d.	n.d.	n.d.
L	n.d.	n.d.	n.d.	n.d.	n.d.
NWD	41 (3)	129 (80)	62 (49)	1 (0.1)	0.19 (0.1)
SCC	n.d.	n.d.	n.d.	n.d.	n.d.
WP	n.d.	n.d.	n.d.	n.d.	n.d.
WS	41.5 (5)	280.8 (127)	704 (1254)	2 (3)	0.32 (0.35)
Parameter:	Jun-01 Salinity[3]	Avg. Temp.[1] Chlorophyll *a*[4]	28.8 °C DIN[5]	Avg. PAR[2] DIP[5]	2400 μE H_2S[6]
Site: BF	49	48.9 (29)	274 to 310	0.2 to 1.5	0.4 to 0.5
D	48	50 (13)	313	0.37	0.44
L	51	84 (33)	86	0.27	0.31
NWD	40	28 (8)	59	0.17	0.56
SCC	n.d.	n.d.	n.d.	n.d.	n.d.
WP	n.d.	n.d.	n.d.	n.d.	n.d.
WS	n.d.	88.5 (11)	n.d.	n.d.	n.d.
Parameter:	Mar-02 Salinity[3]	Avg. Temp.[1] Chlorophyll *a*[4]	27.2 °C DIN[5]	Avg. PAR[2] DIP[5]	2000 μE H_2S[6]
Site: BF	40	66 (24)	1607	6.5	3.5
D	33.5	63 (29)	43.3	0.4	0.47
L	34	74 (34)	45.5	0.2	0.42
NWD	42	58 (29)	78	0.8	0.32
SCC	42	77 (9)	156	0.5	0.44
WP	42	244 (12)	1.2	0.02	n.d.
WS	50	101 (69)	121	3	1.1
[1]average temperature	[2]photosynthetically active radiation	[3]part per thousand	[4]mg m^{-2}	[5]μM [6]mM	n.d.=no data

Adjusting the GOP rates to account for differences in chlorophyll *a* concentration (i.e., dividing the GOP rate, mmol O_2 m^{-2} h^{-1}, by the sediment chlorophyll *a* concentration, mg Chl *a* m^{-2}) yields a biomass specific production rate in units of mmol O_2 (mg Chl *a*)$^{-1}$ h^{-1} and permits a more robust comparison of activity across sites (Fig. 4B). Chlorophyll *a* specific GOP rates at the Dock site were significantly different between PAR fluxes but at the other sites, as well as for the pooled data, rates were not significantly different between PAR fluxes. In March 2002, GOP rates were similar to those observed in June 2001 but chlorophyll *a*-specific GOP rates were 2-to-10 times higher.
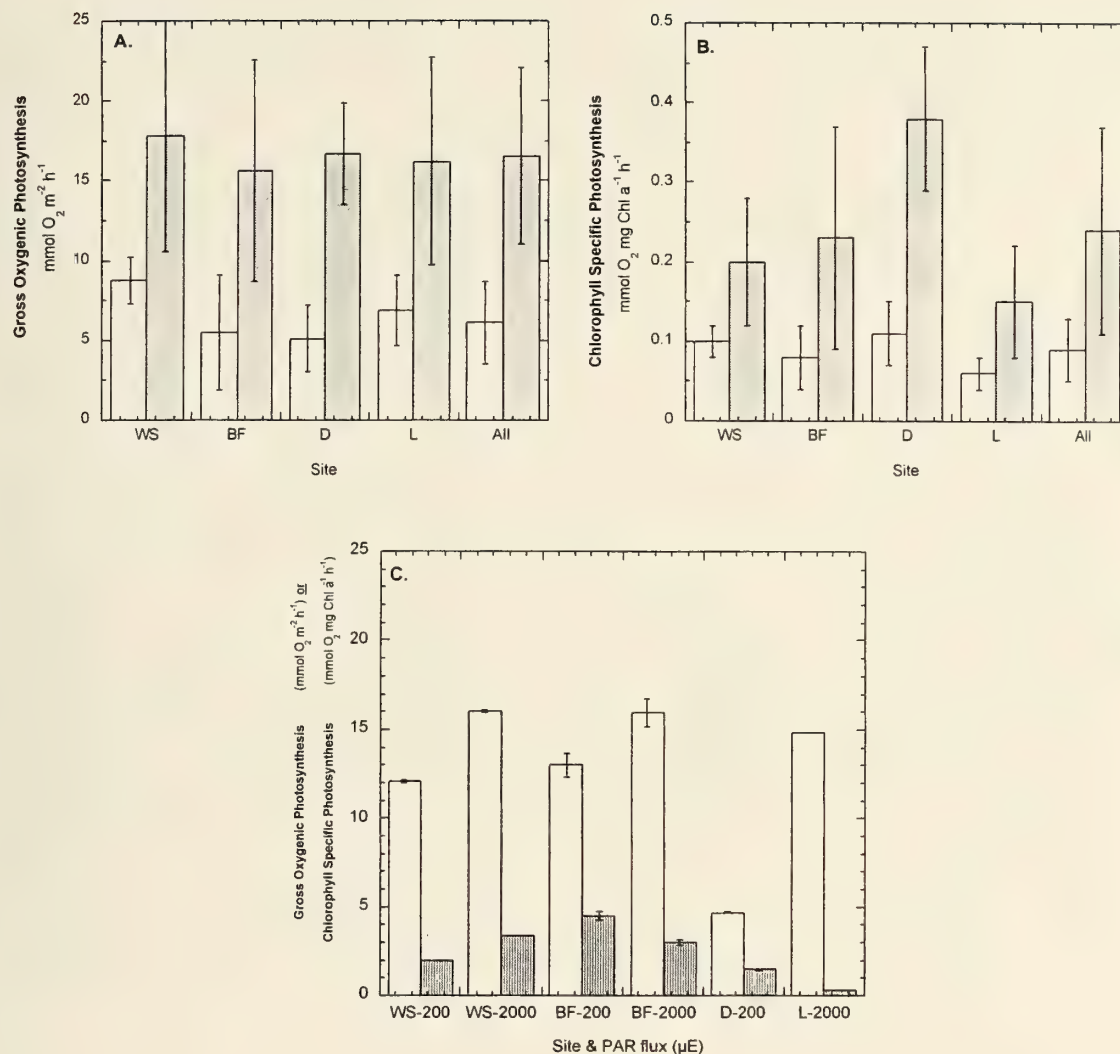
Figure 4. Rates of (A) GOP and (B) chlorophyll *a* specific GOP across sites in June 2001 (open=200 μE; hatched=2000 μE) and (C) GOP (open) and chlorophyll *a* specific GOP (speckled) across sites in March 2002 (only one PAR flux level available for D and L).

Microbial Mat Nitrogen Cycling

Nitrogen-fixation rates were variable but generally were higher in March compared to June (Fig. 5). Nighttime activity (15 to 20 μmol N m^{-2} h^{-1}) was generally equal to, or higher than, rates observed during daytime light (0 to 15 μmol N m^{-2} h^{-1}) or dark (2 to 20 μmol N m^{-2} h^{-1}) incubations. Samples amended with DCMU exhibited significantly higher N$_2$ fixation rates (30 to 500 μmol N m^{-2} h^{-1}) during the day relative to light samples (no DCMU), and DCMU-amended rates in March 2002 were significantly higher than rates observed in June 2001 (<100 to >250 μmol N m^{-2} h^{-1}). Addition of inorganic nitrogen such as NO$_3^-$ (in +NG treatments) reduced N$_2$ fixation rates during daytime and nighttime incubations, although differences were not always significant. *In situ* denitrification rates were low (<2 μmol N$_2$O m^{-2} h^{-1}) but potential denitrification

rates rivaled (20 to 200 μmol N m^{-2} h^{-1}), and in some cases exceeded, rates of N$_2$ fixation (Fig. 6). Denitrification rates were generally higher in dark-incubated treatments during daytime incubations. Daytime-dark rates and nighttime rates in +NG treatments were not significantly different.
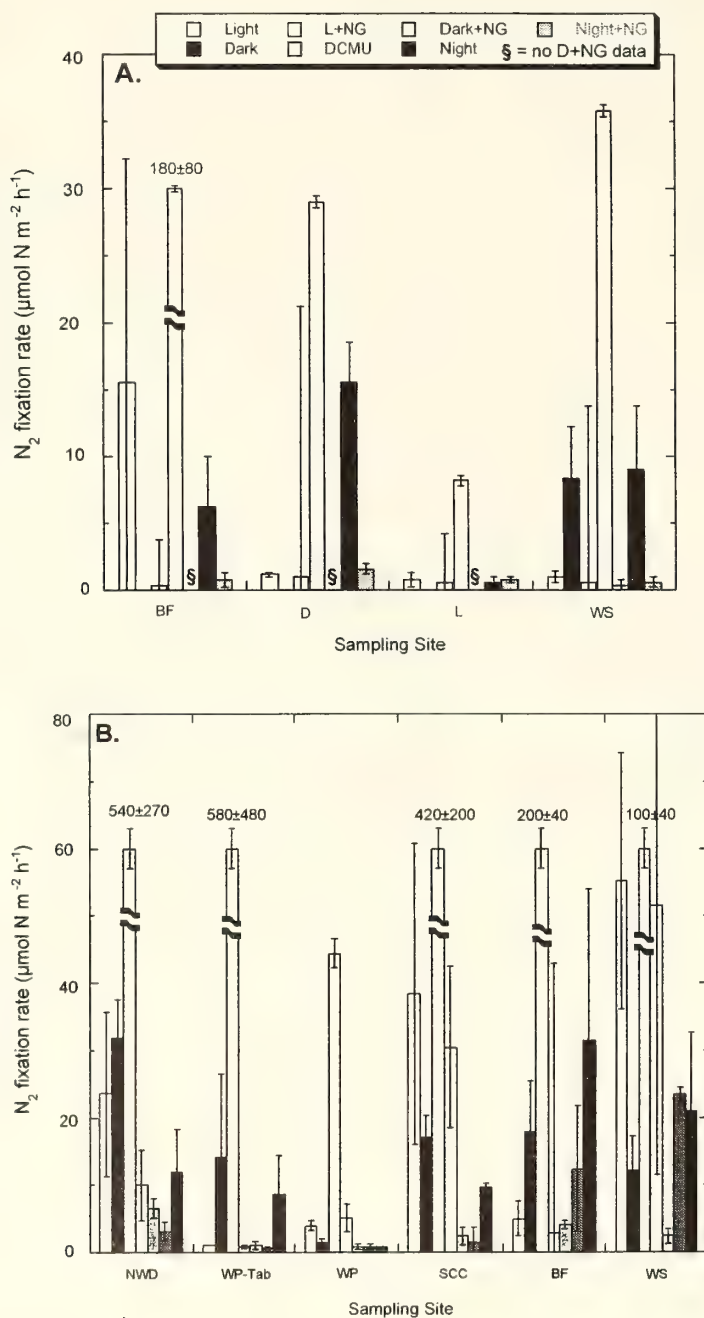
Figure 5. Day-night variability in N$_2$ fixation in microbial mats (**A**: 6/01, **B**: 3/02). Columns represent mean of n=3 samples; error bars=standard deviation of the mean. Maximum values and standard deviation for split columns are noted at the top of each column.

DISCUSSION

This is the first study to document the distribution of microbial mats and to evaluate their potential impact on biogeochemical cycling in oceanic mangrove ecosystems. Laminated microbial mats were abundant on surface sediments throughout the dwarf mangrove zone of Twin Cays. These microbial mats exhibited high rates of primary production, nitrogen fixation and potential denitrification suggesting they are important ecological and biogeochemical players in oceanic mangrove island systems.

A diverse suite of phototrophic microbes, including eukaryotes (diatoms) and prokaryotes (both cyanobacteria and photosynthetic bacteria), were observed in microbial mats. The photosynthetic bacteria observed in Twin Cays microbial mats (Fig. 2) are similar to those observed in other environments, including microbial mats from a hypersaline lagoon on San Salvador, Bahamas (Pinckney et al., 1995a and 1997), mats in evaporative salt ponds on Baja California Sur, Mexico (Lopez-Cortes, 1990; Bebout et al., 2002), saline lakes (Jørgensen et al., 1983) or mats occupying temperate intertidal flats (Joye and Paerl, 1994; Pinckney et al., 1995b). The dominant cyanobacteria in microbial mats varied between mats lying along the edges of ponds (e.g., WS, BF and WP) versus those in the dwarf zone occurring directly over the peat (e.g., NWD, WP-tab, L and D) versus those in an area where the mangrove had been removed via clear cutting (SCC). Mats along the pond edge were dominated by filamentous non-heterocystous cyanobacteria, including *Microcoleus* and *Lyngbya*. Coccoidal cyanobacteria and photosynthetic bacteria were also present. Mats overlying peat were unique in that they often contained heterocystous cyanobacteria, e.g., *Nodularia* (at NWD), in addition to coccoidal cyanobacteria, e.g., *Gloeocapsa* and *Chroococcus*, and some filamentous non-heterocystous forms (e.g., *Lyngbya*, at D, L, and WP-tab sites). Mats at the SCC site were dominated by *Spirulina*, a filamentous non-heterocystous cyanobacterium, and by *Aphanocapsa*, a coccoidal cyanobacterium. Photosynthetic bacteria, visible as a brown or pink layer beneath the cyanobacterial layer, were present in mats lying at the pond edge or on top of peat (see Fig. 2 A-1, A-2, A-4, and A-6). The dominant cyanobacteria in a particular mat sample helped explain, to some extent, the day-night patterns of N_2 fixation activity we observed (see below).

Microbial Mat Primary Production

The importance of oxygenic photosynthesis in Twin Cays microbial mats is evident from the depth distribution of dissolved O_2 in the pore water (Fig. 3A). In November 2000, dissolved O_2 penetrated only 0.1 to 0.5 mm into the microbial mat in the dark (0 μE). As the PAR flux increased, the shape-and-depth distribution of O_2 changed. The maximum pore water O_2 concentration increased from <100 μM at 0 μE to 800 μM O_2 at a PAR flux of 850 μE. The depth of the maximum O_2 concentration deepened from 0.2 to 0.7 mm and the O_2 penetration depth deepened by 1 mm, increasing from 0.5 mm in the dark to >1.5 mm at 850 μE. Though pore water O_2 concentrations increased with increasing PAR flux, the integrated rate of gross oxygenic photosynthesis (GOP) varied between 6 and 15 mmol O_2 m^{-2} h^{-1} across a range of PAR flux (Fig. 3B). We observed high variability between profiles because O_2 concentrations at a given depth often varied

by ±50%. This variability made it impossible to distinguish GOP at low PAR flux from that at high PAR flux. The relatively constant GOP rate at low (50 µE) and high (>1000 µE) PAR flux suggests that the phototrophic microbes in Twin Cays microbial mats are efficient (i.e., they photosaturate) at low PAR flux and that they are able to sustain high GOP activity even at high PAR fluxes. We did not observe any evidence of photoinhibition at PAR fluxes exceeding 2000 µE. Thus, Twin Cays microbial mats appear poised to photosynthesize at near maximum rates even at low PAR flux meaning that microbial mat light-driven primary production occurs during most of the daylight hours.

We did not quantify bacteriochlorophyll concentrations so the discussion of photosynthetic biomass below is based on chlorophyll a data. We stress, however, that photosynthetic bacteria are abundant in Twin Cays microbial mats and note that the importance of photosynthetic bacteria in benthic primary production is discussed elsewhere (Lee and Joye, in preparation). Photosynthetic biomass, as chl a, in Twin Cays microbial mats varied between about 30 and 300 mg chl a m^{-2} (Table 1) and biomass-specific primary production rates varied between 0.1 and 4 mmol O$_2$ (mg chl a)$^{-1}$ h^{-1} (Fig. 4). The chl a concentrations observed in Twin Cays microbial mats are comparable to those observed in other temperate and tropical sediments (e.g., Sournia et al., 1977; Potts, 1980; Joye et al., 1996; Pinckney and Paerl, 1997; Karsten et al., 1998; Camacho and de Wit, 2003).

Biomass-specific GOP rates in Twin Cays microbial mats in June 2001 were about an order of magnitude lower than those observed in tropical (Pinckney and Paerl, 1997) and temperate (Joye et al., 1996) microbial mats. In contrast, the biomass-specific GOP rates in Twin Cays microbial mats in March 2002 were comparable to those documented in tropical systems but were still about 3-times lower than those observed in high biomass temperate estuarine microbial mats. Seasonal variability in the biomass-specific GOP may reflect changes in microbial mat microbes' abilities to channel light-derived reducing equivalents into primary production. Lower tides which result in more severe exposure to UV in June may have stressed microbial mat microbes and led to lower photosynthetic efficiencies at that time compared with March which is in the 'wet' season (Lee and Joye, in preparation). UV-exposure has been shown to influence community structure and function in other microbial mats (Karsten et al., 1998; Sheridan, 2001) and future studies are planned to evaluate the impact of UV-exposure on the photophysiology of Twin Cays microbial mats.

Microbial mats are ubiquitous in dwarf mangrove zones along pond edges and along the bottom of shallow ponds. These habitats (dwarf red-and-black mangrove, open pond dwarf, floc zones and mixed dwarf stands; Rodriguez and Feller, this volume) account for about 44% of the land cover on Twin Cays (Rodriguez and Feller, this volume) covering about 332,854 m^2. We can derive a rough estimate of the annual primary production rate of microbial mats by assuming that half of this area (166,427 m^2) is covered by microbial mats (based on numerous surveys we have conducted in these areas, we consider this a conservative estimate) and assuming that, on an average day, a microbial mat receives ample PAR for 6 hours to support a GOP rate of 12 mmol O$_2$ m^{-2} h^{-1} (the average rate at low and high light, see Fig. 4). Using these values and assuming a photosynthetic quotient (O$_2$ evolved per CH$_2$O fixed) of one yields a value of 4.4 x 10^6

mol carbon or 5.3 x 10^7 g carbon fixed per year (gross) by microbial mats. Microbial mats are obviously an important source of labile organic carbon for the Twin Cays ecosystem.

Microbial Mat Nitrogen Transformations

This is the first study of microbial mats reporting contemporaneous data for biomass and rates of primary production, nitrogen fixation and denitrification. Nitrogen fixation rates in Twin Cays microbial mats were often comparable during the day and at night with rates ranging between 15-to-20 μmol N m^{-2} h^{-1} (Fig. 5). Nitrogen fixation activity measured in Twin Cays microbial mats is similar to that observed in other tropical and temperate microbial mats (Joye and Paerl, 1994; Pinckney and Paerl, 1997; Steppe et al., 2001). The day-versus-night patterns of N_2 fixation activity probably resulted from the various strategies used by the mat microbes to fix N_2. In mats dominated by heterocystous cyanobacteria (e.g., Fig. 5B, NWD), N_2 fixation activity during the day exceeded that at night. However, daytime activity was comparable in light and dark treatments suggesting that N_2 fixers had ample reductant to support activity in the dark. The source of this reductant appeared to be linked to photosynthesis because nighttime activity was much lower (one-half of daytime activity).

Oxygen production during photosynthesis was a potent regulator of N_2 fixation year round. Nitrogenase is inhibited by O_2 and the addition of DCMU, a photosystem-II (i.e., O_2 production) inhibitor, led to significant increases in nitrogen-fixation rates. At times, nitrogen-fixation rates in DCMU-amended treatments were an order of magnitude higher than those observed in light or dark treatments (Fig. 5B). As shown in Fig. 3, pore water O_2 concentrations increase rapidly as GOP rates increase. Similarly, blocking O_2 production with DCMU decreases pore water O_2 concentration at depths within the photic zone. The increase of N_2 fixation rates in DCMU treatments suggests that a substantial fraction of the nitrogenase present in these microbial mats is only active when freed from O_2 inhibition. This fraction of the population may be active during certain parts of the day (early morning or late afternoon) when ample light is available but pore water O_2 concentrations are below the threshold for inhibition of nitrogenase.

In mats dominated by filamentous nonheterocystous cyanobacteria (Fig. 6, A: WS and B: BF, WP-tab), daytime dark and DCMU-amended N_2 fixation rates exceeded light rates suggesting oxygen-regulation of N_2 fixation. However, in mats where both filamentous non-heterocystous cyanobacteria and coccoidal cyanobacteria were common, daytime light activity was comparable to that noted in dark treatments. Many cyanobacteria excrete copious amount of mucous which can relieve oxygen inhibition of nitrogenase by either stimulating heterotrophic bacterial oxygen consumption (i.e., respiration) or by serving as a diffusive shield against oxygen exposure. The existence of anoxic microzones may facilitate N_2 fixation in microbial mats (Tankere et al., 2002).
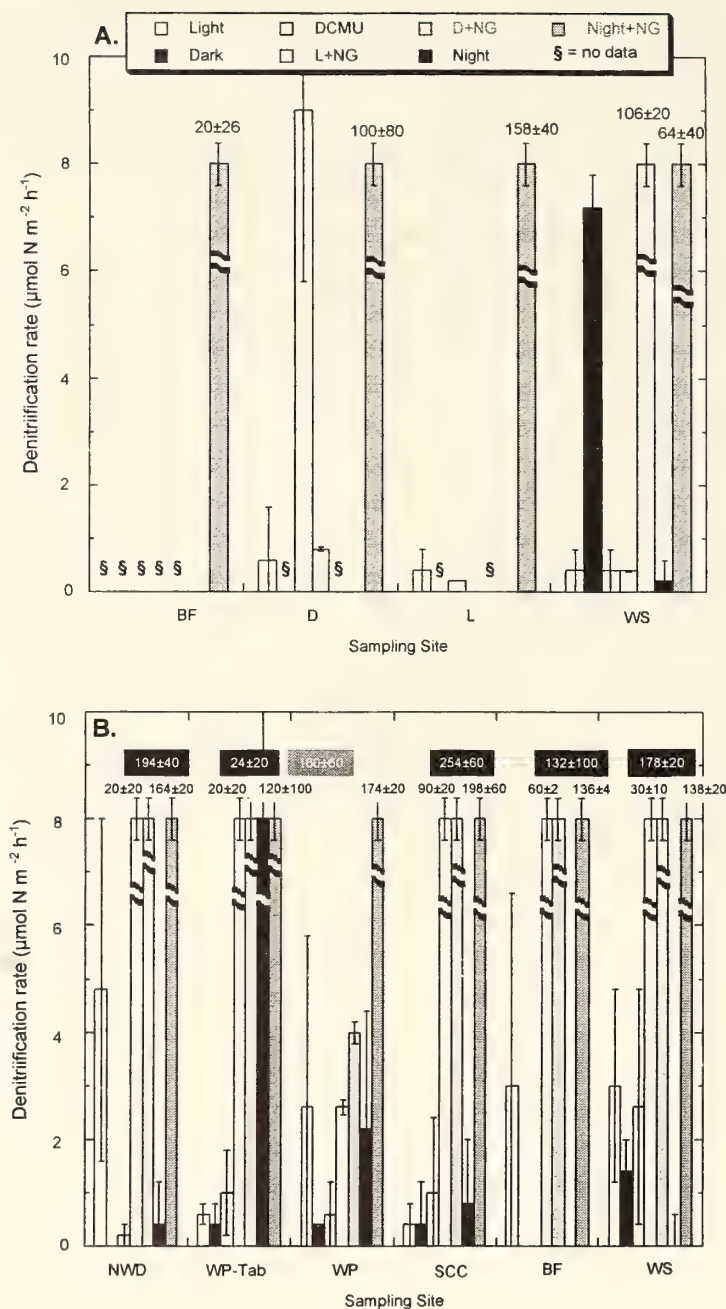
Figure 6. Day-night variability in microbial mat denitrification (**A**: 6/01, **B**: 3/02). Columns represent mean (n=3 samples) and error bars=standard deviation of the mean. Maximum rates and standard deviation for split columns are noted at the top of each column. Black or grey boxes refer to daytime-dark (black) or nighttime (gray) +NG treatments.

The variable pattern of N_2 fixation activity observed in Twin Cays microbial mats results from the different strategies used by N_2 fixing cyanobacteria to support the contemporaneous activity of the energy-demanding process of N_2 fixation with the energy-producing process of photosynthesis (Stal and Krumbein, 1985; Stal et al., 1994;

Steppe et al., 1996; Paerl et al., 2000). Part of the variability in N_2 fixation patterns likely results from the contribution of other microbes, e.g., sulfate-reducing bacteria or photosynthetic bacteria, to N_2 fixation. Cyanobacteria are certainly not the only microbes capable of N_2 fixation in microbial mats. A phylogenetically diverse suite of nitrogenase (*nif*H) genes has been observed in samples from similar microbial mats (Paerl et al., 1996 and 2000; Steppe et al., 1996; Omoregie et al., 2004). The experiments detailed here did not permit us to link directly a particular microbial group with N_2 fixation activity but ongoing and planned studies of *nif*H diversity and additional experiments aimed at determining the source of reductant for nitrogenase in these microbial mats may permit us to make such connections in the future.

Not surprisingly, the availability of inorganic nitrogen influenced N_2 fixation rates. The addition of inorganic nitrogen, such as nitrate (2 mM), in +NG treatments often resulted in substantial (80%) and instantaneous reduction in N_2 fixation rates (Fig. 5). However, in some cases, the addition of inorganic nitrogen had no effect on N_2 fixation (e.g., Fig. 5B, WS). Contrary to results of the +NG experiments, variability in pore-water nutrient and sulfide concentrations could not explain the patterns of N_2 fixation we observed. Nitrogen-fixation rates were not correlated with pore-water DIN concentrations (field DIN was usually < 500μM and was rarely >1000 μM, Table 1). Dissolved inorganic nitrogen concentrations (mainly ammonium; nitrate concentrations were usually <2 μM; data not shown) in the microbial mats were quite variable with the highest concentrations observed beneath mats along the pond edges (Table 1). Pore-water dissolved inorganic-phosphorus concentrations were extremely low (DIN:DIP ratios were always >16, suggestive of P limitation) and variability in DIP also did not correlate to N_2 fixation rates. Patterns in pore-water hydrogen-sulfide concentrations were also variable (highest beneath pond-edge mats) and did not help explain N_2 fixation rates. The available data suggest that the primary factors controlling N_2 fixation at these sites are pore-water oxygen concentrations and the availability of reductant (Lee and Joye, in preparation).

In situ denitrification rates in microbial mats were low (<4 μmol N m^{-2} h^{-1}). However, a tremendous potential for denitrification exists in these mats (up to 240 μmol N m^{-2} h^{-1}). Upon addition of nitrate and glucose, denitrification rates increased immediately and rates in light-and-dark treatments were sometimes comparable. At other times, rates in dark treatments exceeded those in light treatments, suggesting oxygen inhibition of denitrification. Nitrate availability clearly limited denitrification in these microbial mats and oxygen concentration was only a limiting factor when nitrate was available. Similar results were reported for a temperate microbial mat where denitrification rates were controlled by nitrate availability. Once nitrate became available, oxygen concentration also influenced (via inhibition) denitrification rates (Joye and Paerl, 1994).

Denitrification rates were generally higher in March 2002 compared to June 2001. Higher rates of denitrification during March may have resulted from wetter conditions which would generate anoxic conditions in the mat (as opposed to more aerobic conditions when the mat is dry and exposed during the day-time low-tide conditions that existed in June). Potential denitrification rates at night were remarkably similar between the different sites sampled in either June or March. Daytime *in situ* denitrification rates

were variable but the highest daytime denitrification activity was observed in mats with shallow O_2 penetration depths (Fig. 6B, WS, WP-E, BF). Shallow oxygen penetration depths may have permitted nitrate to flux through the aerobic zone into the anaerobic zone where it was accessible to denitrifying bacteria.

Overall, high rates of N_2 fixation and low denitrification rates under *in situ* conditions suggest that Twin Cays microbial mats are a net source of fixed N to the environment. Using the information presented above for primary production we can also extrapolate our N-fixation data to the Twin Cays system-scale. Based on the data presented in Fig. 5, a conservative average N_2 fixation is 10 μmol N m^{-2} h^{-1}. If we assume activity throughout the day-night cycle (the rate above averages day-and-night-time activity), then the average N input to the Twin Cays system is about 1.5×10^4 mol N or 2×10^5 g N per year. This is a conservative estimate that averages data from the different sites. Nonetheless, the magnitude of N_2 fixation activity underscores the importance of microbial mats as a significant N source for the Twin Cays ecosystem.

CONCLUSIONS

Microbial mat microorganisms are dynamic participants in biogeochemical cycling in Twin Cays sedimentary environments. Microbial mats are widespread occupying the sediment surface in dwarf mangrove zones, along pond edges and bottoms, and in clear-cut zones. A diverse assemblage of cyanobacteria was documented in Twin Cays microbial mats. In addition to cyanobacteria, eukaryotes (diatoms) and photosynthetic, chemosynthetic, and heterotrophic bacteria are also active in these microbial mats. Primary production and N_2 fixation by microbial mats represents a previously unrecognized source of fixed carbon and nitrogen to oceanic mangrove ecosystems. The absolute distribution of these mats on Twin Cays is unknown at present but remote sensing data suggests that up to 44% of the sediment could be occupied by microbial mats. Using conservative values for microbial mat distributions and activity, we estimate that these mats fix at least 5.3×10^7 g of carbon (gross) per year and 2×10^5 g N per year. Much of this labile organic carbon and nitrogen is probably transferred to other compartments in the Twin Cays food web. C-and-N cycling in microbial mats may also contribute to previously observed system-level patterns of nutrient limitation. Feller et al. (2002) reported that dwarf red mangrove trees on Twin Cays are phosphorus-limited. High rates of N_2 fixation by microbial mats within dwarf zones may enrich this zone with organic nitrogen, driving phosphorus limitation in the dwarf mangrove trees. Definitively linking microbial mats to the observed system-level patterns of nutrient limitation requires further study.

ACKNOWLEDGMENTS

REFERENCES

Bashan, Y., M.E. Puente, D.D. Myrold, and G. Toledo
   1998. *In vitro* transfer of fixed nitrogen from diazotrophic filamentous cyanobacteria to black mangrove seedlings. *FEMS Microbiology Ecology* 26:165-170.
Bautista, M.F., and H.W. Paerl
   1985. Diel nitrogen fixation in an intertidal marine cyanobacterial mat community. *Marine Chemistry* 16:369-377.
Bebout, B.M., S.P. Carpenter, D.J. Des Marais, M. Discipulo, T. Embaye, F. Garcia-Pichel, T.M. Hoehler, M. Hogan, L.L. Jahnke, R.M. Keller, S.R. Miller, L.E. Prufert-Bebout, C. Raleigh, M. Rothrock, and K. Turk
   2002. Long-term manipulations of intact microbial mat communities in a greenhouse collaboratory: simulating earth's present and past field environments. *Astrobiology* 2:383-402.
Bebout, B.M., M. Fitzpatrick, and H.W. Paerl
   1993. Identification of sources of energy for nitrogen fixation and physiological characterization of nitrogen-fixing members of a marine microbial mat community. *Applied and Environmental Microbiology* 59:1495-1503.
Bebout, B.M., H.W. Paerl, K.M. Crocker, and L.E. Prufert
   1986. Diel interactions of oxygenic photosynthesis and nitrogen fixation (acetylene reduction) in a marine microbial mat community. *Applied and Environmental Microbiology* 53: 2353-2362.
Bouillon, S., N. Koedam, W. Baeyens, B. Satyanarayana, and F. Dehairs
   2003. Selectivity of subtidal benthic invertebrate communities for local microalgal production in an estuarine mangrove ecosystem during the post monsoon period. *Journal of Sea Research* 51:133-144.
Camacho, A., and R. de Wit
   2004. Effect of nitrogen and phosphorus additions on a benthic microbial mat from a hypersaline lake. *Aquatic Microbial Ecology* 32:261-273.

20

Campbell, S.E., and S. Golubic
    1985. Benthic cyanophytes (cyanobacteria) of Solar Lake (Sanai). *Archives of Hydrobiology* 38/39:311-329.
Canfield, D.E., and D.J. Des Marias
    1991. Aerobic sulfate reduction in microbial mats. *Science* 251:1471-1473.
Cohen, Y., and E. Rosenberg (eds.)
    1989. *Microbial Mats: Physiological Ecology of Benthic Microbial Communities.* American Society for Microbiology, Washington, D. C.
Dank, S.M., E.H. Evans, and P.A. Whittaker
    1983. *Photosynthetic Systems: Structure, function and assembly.* John Wiley & Sons, Chichester.
de los Rios, A., C. Ascaso, J. Wierzchos, E. Fernandez-Valiente, and A. Quesada
    2004. Microstructural characterization of cyanobacterial mats from the McMurdo Ice Shelf, Antarctica. *Applied and Environmental Microbiology* 70:569-580.
des Marias, D.J.
    2003. Biogeochemistry of hypersaline microbial mats illustrates dynamics of modern microbial ecosystems and the early evolution of the biosphere. *The Biological Bulletin* 204:160-167.
Fay, P.
    1992. Oxygen relations of nitrogen fixation in cyanobacteria. *Microbiol. Rev.* 546: 340-373.
Feller, I. C., K.L. McKee, D.F. Whigham, and J.P. O'Neill
    2002. Nitrogen vs. phosphorous limitation across an ecotonal gradient in a mangrove forest. *Biogeochem.* 62:145-175.
Golubic, S.
    1994. The continuing importance of cyanobacteria. In: S. Bengston (Ed.) *Early Life on Earth* (pp. 334-340). Columbia University Press, New York.
Golubic, S., L. Seong-Joo, and K.M. Browne
    2000. Cyanobacteria: Architects of sedimentary structures. In: R. Riding and S.M. Awramik (Eds.) *Microbial Sediments* (pp. 57-67). Springer-Verlag, Berlin.
Hardy, R.W.F., R.D. Holsten, E.K. Jackson, and H. Burns
    1968. The acetylene-ethylene assay for nitrogen fixation: Laboratory and field evaluation. *Plant Physiology* 43:1165-1207.
Haselkorn, R.
    1978. Heterocysts. *Annual Review Plant Physiology* 29:319-344.
Hill, S.
    1988. How is nitrogenase regulated by oxygen? *FEMS Microbiology Reviews* 54:111-130.
Howarth, R.W.
    1991. Nutrient limitation of net primary production in marine ecosystems. *Annual Review of Ecology* 19:89-110.
Howarth, R.W., R. Marino, J. Lane, and J.J. Cole
    1988a. Nitrogen fixation in freshwater, estuarine and marine ecosystems: 1. Rates and importance. *Limnology and Oceanography* 33:669-687.

Howarth, R.W., R. Marino, and J.J. Cole
    1988b. Nitrogen fixation in freshwater, estuarine, and marine ecosystems: 2.
        Biogeochemical controls. *Limnology and Oceanography* 33:688-701.
Jørgensen, B.B., N.P. Revsbech, and Y. Cohen
    1983. Photosynthesis and structure of benthic microbial mats: Microelectrode and
        SEM studies of four cyanobacterial communities. *Limnology and Oceanography*
        28:1075-1093.
Joye, S.B., M.L. Mazzotta, and J.T. Hollibaugh
    1996. Community metabolism in microbial mats: The occurrence of biologically-
        mediated iron and manganese reduction. *Estuarine Coastal and Shelf Science*
        43:747-766.
Joye, S.B., and H.W. Paerl
    1993. Contemporaneous nitrogen fixation and denitrification in microbial mats: rapid
        response to runoff events. *Marine Ecology Progress Series* 94:267-274.
    1994. Nitrogen cycling in microbial mats: rates and patterns of denitrification and
        nitrogen fixation. *Marine Biology* 119:285-295.
Karsten, U., J. Maier, and F. Garcia-Pichel
    1998. Seasonality in UV-absorbing compounds of cyanobacterial mat communities
        from an intertidal mangrove flat. *Aquatic Microbial Ecology* 16:37-44.
Kuhl, M., T. Fenchel, and J. Kazmierczak
    2003. Growth, structure and calcification potential of an artificial cyanobacterial mat.
        In: W. E. Krumbein, D. Paterson, and G. Zavarzin (Eds.) *Fossil and recent
        biofilms, a natural history of life on planet Earth* (pp. 77-102). Kluwer
        Academic Publishers, Dordrecht, the Netherlands.
Krumbein, W.E., U. Brehm, G. Gerde, A.A. Gorbushina, G. Levit, and K. Palinska
    2003. Biofilm, Biodictyon, and Biomat - Biolaminites, Oolites, Stromatolites -
        Geophysiology, Global mechanisms and Parahistology. In: W.E. Krumbein, D.
        W. Paterson, and G.A. Zavarzin (Eds.) *Fossil and recent biofilms, a natural
        history of life on planet Earth* (pp. 1-28). Kluwer Academic Press Publishers,
        Dordrecht, the Netherlands.
Lee, R.Y., and S.B. Joye
    2004. Seasonal controls on nitrogen fixation and denitrification in oceanic mangrove
        sediments. *Marine Ecology Progress Series* (in prep.).
    2004. The relative importance of photo- versus chemo- autrotrophic primary
        production in mangrove microbial mats. *Aquatic Microbial Ecology* (in prep.).
Lopez-Cortes, A.
    1990. Microbial mats in tidal channels at San Carlos, Baja California Sur, Mexico.
        Geomicrobiology Journal 8:69-85.
Omoregie, E.O., L.L. Crumbliss, B.M. Bebout, and J.P. Zehr
    2004. Comparison of diazotrophic community structure in *Lyngbya sp.* and
        Microcoleus chthonoplastes dominated microbial mats from Guerrero Negro,
        Baja, Mexico. *FEMS Microbiology Ecology* 47:305-318.
Paerl, H.W., B.M. Bebout, and L.E. Prufert
    1989. Naturally occurring patterns of oxygenic photosynthesis and nitrogen fixation in
        a marine microbial mat: physiological and ecological ramifications. In: Y.

22

Cohen and E. Rosenberg (Eds.) *Microbial Mats: Physiological ecology of benthic microbial communities* (pp. 326-341). American Society for Microbiology Press, Washington, D. C.

Paerl, H.W., B.M. Bebout, C.A. Currin, M. Fitzpatrick, and J. Pinckney
  1994. Nitrogen fixation dynamics in microbial mats. In: L.J. Stal and P. Caumette (Eds.) *Microbial Mats: Structure, Development, and Environmental Significance* (pp. 325-337). Springer-Verlag, Berlin.

Paerl, H.W., M. Fitzpatrick, and B.M. Bebout
  1996. Seasonal nitrogen fixation dynamics in a marine microbial mat: Potential roles of cyanobacteria and microheterotrophs. *Limnology and Oceanography* 41:419-427.

Paerl, H.W., J. Pinckney, and T.F. Steppe
  2000. Cyanobacterial -bacterial mat consortia: examining the functional unit of microbial survival and growth in extreme environments. *Environmental Microbiology* 2:11-26.

Pinckney, J., and H.W. Paerl
  1997. Anoxygenic photosynthesis and nitrogen fixation by a microbial mat community in a Bahamian hypersaline lagoon. *Applied and Environmental Microbiology* 63:420-426.

Pinckney, J., H.W. Paerl, and B.M. Bebout
  1995a. Salinity control of benthic microbial mat community production in a Bahamian hypersaline lagoon. *Journal of Experimental Marine Biology and Ecology* 187:223-237.

Pinckney, J., H.W. Paerl, and M. Fitzpatrick
  1995b. Impacts of seasonality and nutrients on microbial mat community structure and function. *Marine Ecology Progress Series* 123:207-216.

Potts, M.
  1980. Blue-green algae (Cyanobacteria) in marine coastal environments of the Sanai peninsula; distribution, zonation, stratification and taxonomic diversity. *Phycologia* 19:60-73.

Pulich, W., and S. Rabalais
  1986. Primary production potential of blue-green algal mats on Southern Texas tidal flats. *The Southwestern Naturalist* 31:39-47.

Pulich, W., and R.S. Scalan
  1987. Organic carbon and nitrogen flow from marine cyanobacteria to semiaquatic insect food webs. *Contributions in Marine Science* 30:27-37.

Revsbech, N.P., and B.B. Jørgensen
  1983. Photosynthesis of benthic microflora with high spatial resolution by the oxygen microprofile method: capabilities and limitations of the method. *Limnology and Oceanography* 28:749-756.

Revsbech, N.P., J. Sørensen, T.H. Blackburn, and J.P. Lomholt
  1980. Distribution of oxygen in marine sediments measured with microelectrodes. *Limnology and Oceanography* 25:403-411.

Revsbech, N.P., B.B. Jørgensen, T.H. Blackburn, and Y. Cohen
   1983. Microelectrode studies of photosynthesis and O$_2$, H$_2$S and pH profiles of a microbial mat. *Limnology and Oceanography* 28:1062-1074.

Sheridan, R.
   2001. Role of ultraviolent radiation in maintaining the three-dimensional structure of a cyanobacterial mat community and facilitating nitrogen fixation. *Journal of Phycology* 37:731-737.

Skyring, G.W., and J. Bauld (Eds.)
   1990. *Microbial mats in Australian coastal environments*. Plenum Press, New York.

Sournia, A.
   1977. Notes on the primary production of coastal waters in the Gulf of Elat (Red Sea). *International Reviews for Hydrobiology* 62:813-819.

Stal, L.J.
   1995. Physiological ecology of cyanobacteria in microbial mats and other communities. *New Phytologist* 131:1-32.

Stal, L.J., and W.E. Krumbein
   1985a. Nitrogenase activity in the non-heterocystous cyanobacterium Oscillatoria sp. grown under alternating light-dark cycles. *Archives of Microbiology* 143:67-71.
   1985b. Oxygen protection of nitrogenase in the aerobically nitrogen-fixing, non-heterocystous cyanobacterium, Oscillatoria sp. *Archives of Microbiology* 143:72-76.
   1987. Temporal separation of nitrogen fixation and photosynthesis in the filamentous, non-heterocystous cyanobacterium, Oscillatoria sp. *Archives of Microbiology* 149:76-80.

Stal, L.J., and H. Heyer
   1986. Dark anaerobic nitrogen fixation (acetylene reduction) in the cyanobacterium *Oscillatoria* sp. *FEMS Microbiology Ecology* 45:227-232.

Stal, L.J., S. Grossberger, and W.E. Krumbein
   1984. Nitrogen fixation associated with the cyanobacterial mat of a marine laminated microbial ecosystem. *Marine Biology* 82:217-224.

Stal, L.J., H.W. Paerl, and B.M. Bebout
   1994. Heterocyst ous versus non-heterocystous cyanobacteria in microbial mats. In: L. J. Stal and P. Caumette (Eds.) *Microbial Mats: Structure, Development and Environmental Significance* (pp. 403-414). Springer-Verlag, Berlin.

Steppe, T.F., J.B. Olson, H.W. Paerl, R.W. Litaker, and J. Belnap
   1996. Consortial N$_2$ fixation: a strategy for meeting nitrogen requirements of marine and terrestrial cyanobacterial mats. *FEMS Microbiology Ecology* 21:149-16.

Steppe, T.F., J. Pinckney, J. Dyble, and H.W. Paerl
   2001. Diazotro phy in modern marine Bahamian stromatolites. *Microbial Ecology* 41:36-44.

Tankere, S.P.C., D.G. Bourne, F.L.L. Muller, and V. Torsvik
   2002. Microenvironments and microbial community structure in sediments. *Environmental Microbiology* 4:97-105.

Wiebe, W.J., R.E. Johannes, and K.L. Webb
   1975. Nitrogen fixation in a coral reef community. *Science* 188:257-259.

24

Yates, M.G.
    1977. Physiological aspects of nitrogen fixation. In: W. Newton, J.R. Postgate, and C. Rodriguez-Barruero (Eds.) *Recent Developments in Nitrogen Fixation* (pp. 219-270). Academic Press, New York.

Zehr, J.P., B.D. Jenkins, S.M. Short, and G.F. Steward
    2003. Nitrogenase gene diversity and microbial community structure: a cross-system comparison. *Environmental Microbiology* 5:539-554.

TROPHIC INTERACTIONS WITHIN THE PLANKTONIC FOOD WEB IN
MANGROVE CHANNELS OF TWIN CAYS, BELIZE, CENTRAL AMERICA

BY

EDWARD J. BUSKEY, CAMMIE J. HYATT, AND CHRISTA L. SPEEKMANN
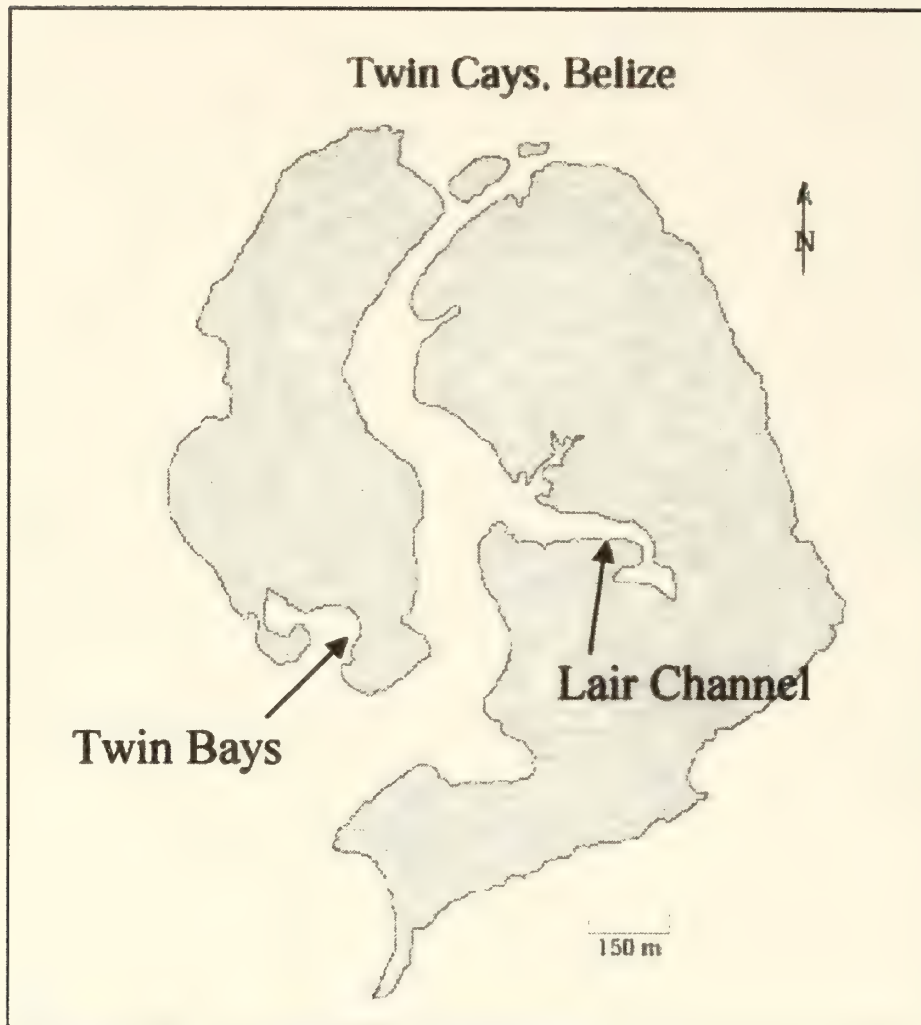
Figure 1. Index map showing study sites, Twin Cays, Belize

TROPHIC INTERACTIONS WITHIN THE PLANKTONIC FOOD WEB IN MANGROVE CHANNELS OF TWIN CAYS, BELIZE, CENTRAL AMERICA

BY

EDWARD J. BUSKEY , CAMMIE J. HYATT, AND CHRISTA L. SPEEKMANN

ABSTRACT

The tidal channels of mangrove islands such as Twin Cays, Belize support a productive and diverse microplankton assemblage. In turn, this microplankton community supports large populations of copepods that form dense aggregations in the prop-root environment along the margins of these channels. The growth rate of the phytoplankton community and the grazing rate of the heterotrophic microzooplankton community were measured using the seawater dilution method. In separate experiments, the grazing rate of the swarm-forming copepod *Dioithona oculata* on natural microplankton assemblages was measured. Chlorophyll concentrations in the natural plankton assemblages used in these experiments ranged from 1-to-11 µg Chl a L^{-1}. Dinoflagellate populations typically ranged from 17-to-50 cells ml^{-1}, with heterotrophic dinoflagellates generally exceeding autotrophic forms in abundance. Ciliates were the second most abundant form of heterotrophic microzooplankton with populations ranging from 1-15 cells ml^{-1}. Results of the dilution experiments indicate that, during the study period, microzooplankton grazed between ca. 60-90% of potential phytoplankton production and phytoplankton growth exceeded microzooplankton grazing in all experiments. Grazing studies with *D. oculata* indicated that copepod ingestion rates were highest on ciliates and autotrophic dinoflagellates and that copepod populations are capable of grazing about 10% of the protozoan population each day.

INTRODUCTION

Microzooplankton populations, composed mainly of heterotrophic protozoa and small larval metazoa, such as copepod nauplii, form an important trophic link between phytoplankton and larger zooplankton, such as copepods (Sherr et al., 1986; Stoecker and Capuzzo, 1990). Heterotrophic protists feed efficiently on pico- and nanoplankton size classes that are too small for many copepods, and they repackage this organic matter into sizes that are more easily consumed by copepods (Sherr and Sherr, 1992). Copepods in turn may play an important role in structuring the planktonic community; by grazing on microplankton-sized particles such as ciliates and dinoflagellates they may indirectly

enhance the growth of small-sized phytoplankton that are grazed by protozoa (Sherr and Sherr, 1994; Buskey et al., 2003). This study examines the trophic interactions between phytoplankton, heterotrophic microplankton and copepods within the mangrove channels of Twin Cays, Belize.

The dominant copepod species found in the mangrove channels is *Dioithona oculata* (Ferrari et al., 2003). Adult and late copepodites of this species form dense swarms in light shafts penetrating the mangrove canopy during the day. These swarms disperse at dusk and reform at dawn (Ambler et al., 1991). Copepod densities average 30 copepods ml^{-1} in swarms (Buskey et al., 1996) but are reduced to only a few copepods L^{-1} when the copepods are dispersed at night (Ferrari et al., 2003). These extraordinary densities within swarms suggest that competition for food must be intense. Gut pigment analysis demonstrated that swarming copepods had less chlorophyll in their guts compared with dispersed copepods at night (Buskey et al., 1996), but this difference could also be due to a diel pattern of feeding behavior which has been demonstrated in other copepod species (e.g. Mackas and Bohrer, 1976).

In addition, copepods are well known to be omnivorous and gut-pigment analysis reveals nothing about feeding on heterotrophic organisms that do not contain chlorophyll. The adaptive value of this swarming behavior is thought to include protection from predation, reduced dispersion and enhanced mating opportunities (Hamner and Carlton, 1979). These benefits are thought to compensate for the energetic costs of swarming behavior in terms of reduced feeding within swarms (due to intense competition) and high metabolic costs of increased swimming speeds to maintain swarms within light shafts in spite of tidal currents (Buskey et al., 1996; Buskey, 1998). These currents may also renew the food supply to a swarm, reducing food competition. *D. oculata* also have strong diel patterns in their reproductive behavior and physiology. Females carry their eggs externally in two clusters and these eggs usually hatch at night between midnight and 6 AM (Ambler et al., 1999) while the copepods are dispersed from their swarms. If adult *D. oculata* fed on their own nauplii they would be exposed to high predation pressure if eggs hatched within swarms during the day.

In this study we measured phytoplankton growth and microzooplankton grazing using the seawater dilution method (Landry and Hassett, 1982) and we measured the feeding rates of adult *Dioithona oculata* on natural microplankton assemblages using 24-hour incubations similar to the method used by Gifford and Dagg (1988). A second set of experiments using 12-hour day-or-night incubations was performed to determine if there was a natural diel pattern in the feeding rate of *D. oculata*. Finally, in order to determine if *D. oculata* prey on their own young, we ran feeding experiments with adult *D. oculata* and newly released nauplii.

3

METHODS

Field experiments were carried out at Twin Cays, a pair of mangrove-covered islands ca. 2 km NW of the Smithsonian Institution's field station on Carrie Bow Cay in Belize, Central America. Microzooplankton-grazing experiments were carried out during April, 2002. Seawater for microzooplankton grazing studies was collected in the Lair Channel of Twin Cays (Fig. 1) by gently submerging a 20 L carboy with a-200 μm mesh screen over the opening to exclude larger zooplankton. The dilution method (Landry and Hassett, 1982; Landry et al., 1995) was used to assess the growth of phytoplankton populations and the grazing impact of microzooplankton. This method uses serial dilution of natural microplankton assemblages to decouple growth of phytoplankton and grazing of microplankton. The method is based on the assumption that microzooplankton grazing declines linearly with increased dilution of natural microplankton assemblages with filtered seawater (decreasing grazers' encounter rate with food) but that phytoplankton growth is unaffected by this dilution.

Filtered seawater for dilution experiments was produced by gravity filtering seawater from the Lair Channel successively through 3.0 and 0.2 μm porosity Gelman capsule filters. Mixtures of filtered seawater and whole seawater containing the natural microplankton community were then prepared consisting of 100%, 50%, 25% or 10% whole seawater. Triplicate 500 ml polycarbonate bottles were filled with each mixture. Small amounts of phytoplankton nutrients (equivalent to F/200, Guillard and Ryther, 1962) were added to each bottle to compensate for a possible reduction in regenerated nutrients caused by the dilution of grazers. Three additional bottles containing whole seawater were incubated without added nutrients to assess the effects of nutrient addition. Initial samples (in triplicate) for enumeration of microzooplankton and for chlorophyll *a* analysis were collected from each dilution mixture. The bottles were incubated under ambient temperature and light conditions by hanging them in a mesh bag suspended in partial shade beneath the pier on Carrie Bow Cay. The alternate periods of shade and sunlight along the edge of the pier simulate the light environment within the mangrove channels and the gentle rocking motion of the waves beneath the pier served to help keep the bottles mixed. At the end of the incubation period, final samples were taken from each bottle for microzooplankton enumeration and chlorophyll analysis.

The apparent growth rate of phytoplankton in individual bottles (based on changes in chl *a*) was calculated using the exponential growth model (Landry and Hassett, 1982):

$$P_t = P_0 \exp[k-g]t$$

where P_0 and P_t are chl *a* concentrations at the beginning and end of the experiment, respectively; k and g are instantaneous coefficients of phytoplankton growth and grazing mortality, respectively; t is time (d). Coefficients k and g were determined from linear regression of apparent growth rate of phytoplankton ($1/t \ln[p_t/p_0]$) on the fraction of diluted seawater. The negative slope of the line is the daily grazing coefficient (g) and the

4

y-intercept is the phytoplankton growth rate (k) with added nutrients. Phytoplankton growth in control bottles with no added nutrients was calculated as $(1/t \ln [p_t/p_0] + g)$.

Copepods for grazing experiments were collected during early morning or late afternoon from swarms at one of two locations along the shoreline of Twin Cays: either from Twin Bays or the Lair Channel (Fig. 1). Swarms of *Dioithona oculata* typically form in shafts of sunlight within the shade along the fringes of red-mangrove shorelines (Ambler et al., 1991). Copepods were collected with a 153-µm mesh sieve attached to a small transparent plastic bag. While snorkeling, the sieve was gently pulled through the swarm to capture copepods and then the top of the plastic bag was sealed and returned to a nearby small boat. The copepods were gently rinsed into a large plastic bucket containing seawater. A plastic carboy of whole seawater, excluding mesozooplankton, was collected at the same site by placing a piece of 200 µm mesh over the opening to the carboy and gently submerging it.

At the beginning of a grazing experiment, the carboys of whole seawater were gently mixed and poured into five 500-ml polycarbonate bottles. The polycarbonate bottles were gently mixed again, a sample of 100 ml was removed from each bottle for chlorophyll analysis, and a 60 ml sample was removed for microzooplankton enumeration. A target number of approximately 25 copepods was added to each of three experimental bottles for each experiment. The actual number of copepods was counted at the end of experiments. Two bottles did not receive copepods and were used as controls. All bottles were then topped with whole seawater and sealed with parafilm before tightening the screw caps. The bottles were then placed in a mesh bag and hung in partial shade along the edge of the pier at Carrie Bow Cay to incubate. An initial set of four experiments was run with 24-hour incubations during April 2000 to determine the feeding rates of *Dioithona oculata* on various groups of microplankton. A second series of experiments were performed during March 2001 with 12-hour incubations during daylight (6AM - 6PM) or nighttime hours (6PM - 6AM) to determine if there were diel variations in feeding behavior. Following the period of incubation, the bottles were gently inverted several times to ensure that the contents were well-mixed, and then 100 ml samples were removed for chlorophyll analysis and 60 ml samples were removed for preservation with Lugol's Iodine and later microzooplankton enumeration.

Initial examination of microzooplankton samples was performed on Carrie Bow Cay to learn which species of dinoflagellate were heterotrophic and which were autotrophic because formaldehyde or glutaraldehyde-preserved samples (which would retain chlorophyll autofluorescence) could not be transported easily back to Texas for analysis. Samples from the whole seawater (20 ml) were fixed with three drops of 37% formalin and gently filtered onto a 0.4-µm polycarbonate filter with a 0.4 µm-Metricel filter as a backing to ensure a more even distribution of cells on the sample filter. These samples were examined using a Zeiss epifluorescent microscope with a blue excitation filter to determine the composition of autotrophic and heterotrophic dinoflagellates in our samples. Sketches and measurements were made of the predominant autotrophic and

heterotrophic dinoflagellates for later reference in counting the Lugol's Iodine-preserved samples.

Samples for total chlorophyll *a* analysis were filtered through 0.4 µm porosity polycarbonate membrane filters; size-fractionated chlorophyll *a* samples were first filtered through a 10 µm-porosity polycarbonate membrane filter and then again through a 0.4 µm-porosity filter. Filters were placed in 10 ml of 95% acetone in a glass scintillation vial, refrigerated in the dark (bottles covered with aluminum foil), and chlorophyll was allowed to extract for 12 hours. Chlorophyll concentration was measured using a Turner Designs TD-700 fluorometer using the nonacidification method of Welschmeyer (1994). Field calibration of the fluorometer was confirmed using a solid fluorescence standard (Turner Designs 7000-994). During the dilution experiments performed in April 2002, a Turner Designs Aquafluor portable fluorometer was used for chlorophyll *a* measurements.

Samples for microzooplankton analysis were preserved in 5% (v/v) Lugol's Iodine. Dinoflagellates were typically more numerous than ciliates in our samples so samples of 10 ml were settled to enumerate dinoflagellates and 40 ml samples were settled in an Utermohl chamber to enumerate ciliates. Notes on the sizes and shapes of autotrophic and heterotrophic species examined in the field were used to differentiate autotrophic and heterotrophic species in the Lugol's Iodine-preserved samples. The dimensions of each cell were measured under the microscope at (300X) magnification for later estimation of cell biovolumes using the equations recommended by Hillebrand et al. (1999). These biovolumes were converted to carbon estimates using the conversion factor for ciliates of Putt and Stoecker (1989) and that of Menden-Deuer and Lessard (2000) for heterotrophic dinoflagellates. Filtration and ingestion rates of the copepods were calculated based on the equations of Frost (1972).

A second set of experiments was run to determine if *Dioithona oculata* would prey on their own nauplii. Adults collected from swarms in the late afternoon were placed in a small 20 L aquarium overnight. The next morning water from this aquarium was gently passed through a nested pair of sieves. The top sieve (153 µm mesh) retained the adult *D. oculata* while allowing the newly hatched nauplii to pass through and be retained on the second sieve (20 µm mesh). The nauplii captured on the second sieve were gently rinsed into a plastic petri dish. These were then used in the predation experiments. Known numbers of nauplii were counted out and added to each of four 70 ml-clear-plastic tissue culture flasks. Six adult copepods were added to three of the four flasks while a fourth flask served as a control without added copepods. These flasks were then incubated for ca. 24 hours on a shaded table in a small water bath with ambient-temperature seawater flowing through (28 - 29 °C). After incubation, Lugol's Iodine was added to the contents of the tissue culture flask to kill and stain the copepods and the number of nauplii and copepods counted.

RESULTS

The microplankton assemblage near Twin Cays during the first set of experiments in April 2000 was characterized by moderately high chlorophyll a concentrations (6.4 - 11 µg L^{-1}), abundant dinoflagellates (ca. 5-18 x 10^3 L^{-1} for autotrophs, 7-34 x 10^3 L^{-1} for heterotrophs), numerous aloricate ciliates (ca. 1-3 x 10$^3$ L^{-1}) and a small number of tintinnids (<1 L^{-1}) (Fig. 2). During the second set of experiments in March 2001, the microplankton assemblages at the same locations were characterized by lower chlorophyll a concentrations (3.4 - 6.5 µg L^{-1}), lower dinoflagellate concentrations (ca. 2-4 x 10^3 L^{-1} for autotrophs, 5-10 x 10^3 L^{-1} for heterotrophs), fewer ciliates (0.2 - 1.6 x 10^3 L^{-1}) and a similar concentration of tintinnids (<1 L^{-1}). Although we did not examine the distribution of chlorophyll a into different size fractions during the first set of experiments in April 2000, during the second set of experiments in 2001 chlorophyll a was fairly evenly distributed between the <10-µm size fraction (1-3 µg L^{-1}) and the >10-µm size fraction (2-3.5 µg L^{-1}). During the microzooplankton grazing experiments in April 2002, the waters of Lair Channel were characterized by lower chlorophyll a concentrations (1.1–2.3 µg L^{-1}), abundant dinoflagellates (ca. 4-17 x 10^3 L^{-1} for autotrophs, 6-34 x 10^3 L^{-1} for heterotrophs), numerous aloricate ciliates (ca. 2-14 x 10^3 L^{-1}) and a small number of tintinnids (<1 L^{-1}).
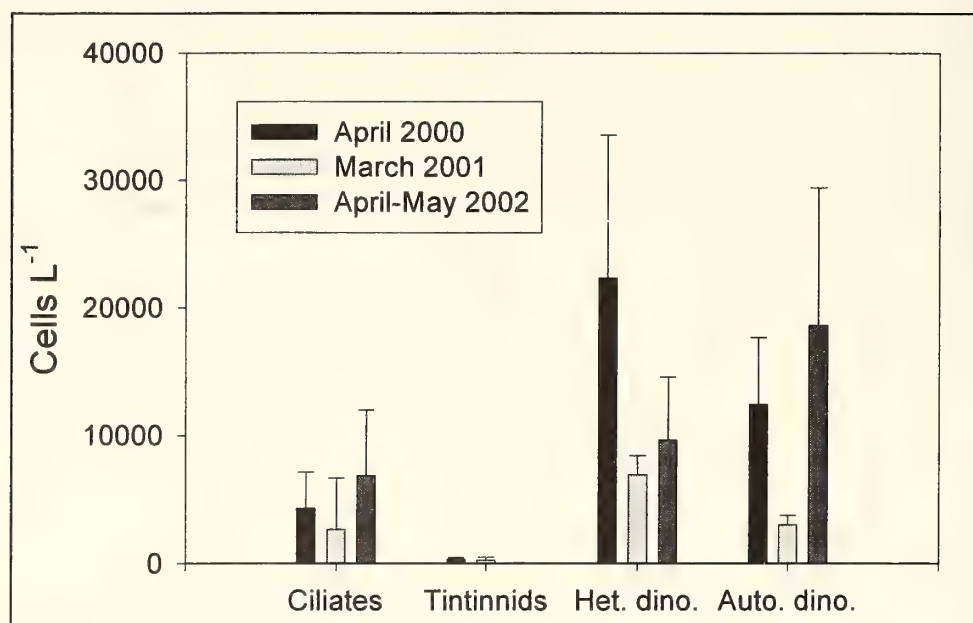
Figure 2. Mean abundances ($\pm$1 SD) of broad categories of microplankton in the mangrove channels of Twin Cays during each of the three years of this study, including ciliates, tintinnids, heterotrophic dinoflagellates (het. dino.) and autotrophic dinoflagellates (aut. dino.). Means from four experiments in April 2000, eight experiments in March 2001 and four experiments in April-May 2002.

The microplankton assemblage in the water column of the mangrove channels near Twin Cays was dominated by a fairly small number of genera. The autotrophic dinoflagellates belonged mainly to species of *Peridinium*, *Gymnodinium* or *Prorocentrum* in samples taken in all three years (2000-2002). Other common phytoflagellates in samples from both years included cryptomonads and euglenoids. Among the heterotrophic dinoflagellates, the dominant genera included *Protoperidinium*, *Gyrodinium*, *Gymnodinium* and *Cochlodinium* during both sampling years. Ciliate populations were strongly dominated by *Mesodinium* with smaller numbers of *Laboea* in both years. During 2001 and 2002, *Strombidium* were also abundant. Although generally rare compared with the aloricate ciliates, the most abundant tintinnids were species of *Eutintinnus* and *Tintinnopsis*. The size-frequency distributions for broad taxonomic categories of microplankton indicate that most ciliates had cell volumes comparable to equivalent spherical diameters (ESD) between 10-20 μm (Fig. 3). Autotrophic dinoflagellates were present in a wide range of sizes from ca. 6-40 μm ESD, whereas a large proportion of heterotrophic dinoflagellates were <20 μm ESD.

The results of the four dilution experiments are revealed through plots of apparent phytoplankton growth as a function of dilution factor (Fig. 4). The slope of the regression line represents the grazing coefficient (g) and the y-intercept of the regression line represents the growth rate of the phytoplankton with added nutrients when released from grazing (k). The grazing coefficients measured in these experiments ranged from 0.32-0.95 (d^{-1}) and phytoplankton growth coefficients ranged from 0.94-1.55 (d^{-1}) with added nutrients and from 0.66-1.27 (d^{-1}) in control bottles without added nutrients (Table 1). The enhanced growth of the 100% seawater dilution with f/200 nutrient additions compared with similar incubations without nutrient addition indicates that the phytoplankton populations in the mangrove channels were nutrient-limited. The phytoplankton growth coefficients for control bottles without added nutrients correspond to a range of 0.95-1.83 doublings per day, indicating very rapid phytoplankton growth (Table 1). Growth of broad categories of microplankton within the control bottles was lower with specific growth rates (mean ± SD) of ciliates, autotrophic dinoflagellates and heterotrophic dinoflagellates calculated at 0.22 ± 0.31, 0.35 ± 0.25 and 0.15 ± 0.11 d^{-1}, respectively. The calculated estimates of potential phytoplankton production, actual phytoplankton production and grazing indicate that microzooplankton grazing removed between 57-91% of potential phytoplankton production per day (Table 1).
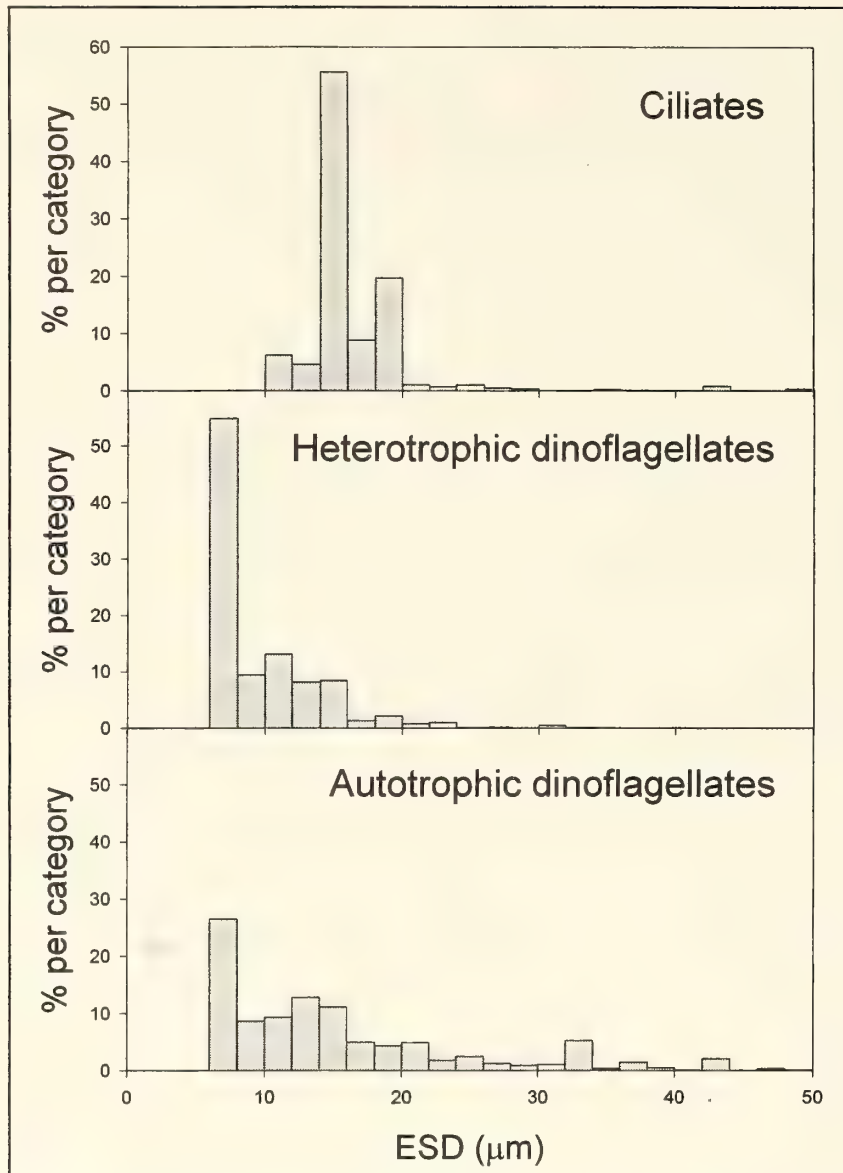
Figure 3. Size-frequency distribution of ciliates, heterotrophic dinoflagellates and autotrophic dinoflagellates based on pooled data from control samples of grazing experiments performed during April 2000. Cell volumes were calculated and then converted to equivalent spherical diameters (ESD).
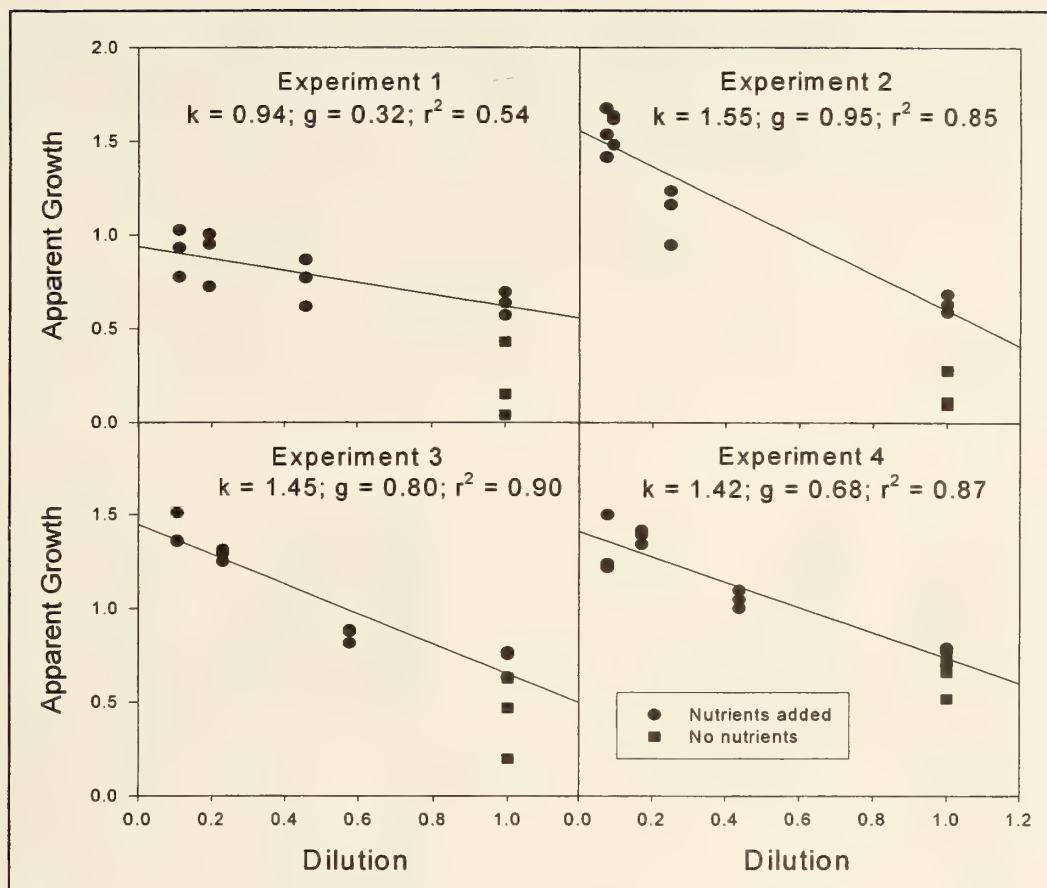
Figure 4. Results of dilution experiments. Apparent growth is plotted against the actual dilution factor based on chlorophyll *a* measurements. The phytoplankton growth coefficient (k) is the y-intercept of the regression line and the grazing coefficient (g) is the negative slope of the regression line. Squares represent the apparent growth of undiluted seawater without added nutrients. All experiments performed with samples collected from Lair Channel.

Table 1. Grazing by microzooplankton on phytoplankton based on dilution experiments. k: phytoplankton growth in bottles with nutrients added; μ: phytoplankton growth in control bottles without nutrients added; phytoplankton doublings = μ /ln2; g = grazing coefficient; potential phytoplankton production = $(P_0e^{\mu}) - P_0$; actual phytoplankton production = $(P_0e^{(\mu-g)}) - P_0$; % potential production removed = (potential production – actual production)/potential production.

Date (2002)	k (d^{-1})	μ (d^{-1})	Phyto doublings(d^{-1})	g (d^{-1})	% potential phyto prod. removed (d^{-1})
25 April	0.94	0.66	0.95	0.32	57
28 April	1.55	1.11	1.60	0.95	91
30 April	1.45	1.25	1.80	0.80	77
5 May	1.42	1.27	1.83	0.68	69

10

Clearance rates of *Dioithona oculata* estimated from 24-hr incubation experiments indicated generally higher clearance rates on ciliates compared with those for dinoflagellates (autotrophs or heterotrophs) or total phytoplankton, estimated with bulk chlorophyll *a* measurements (Fig. 5).
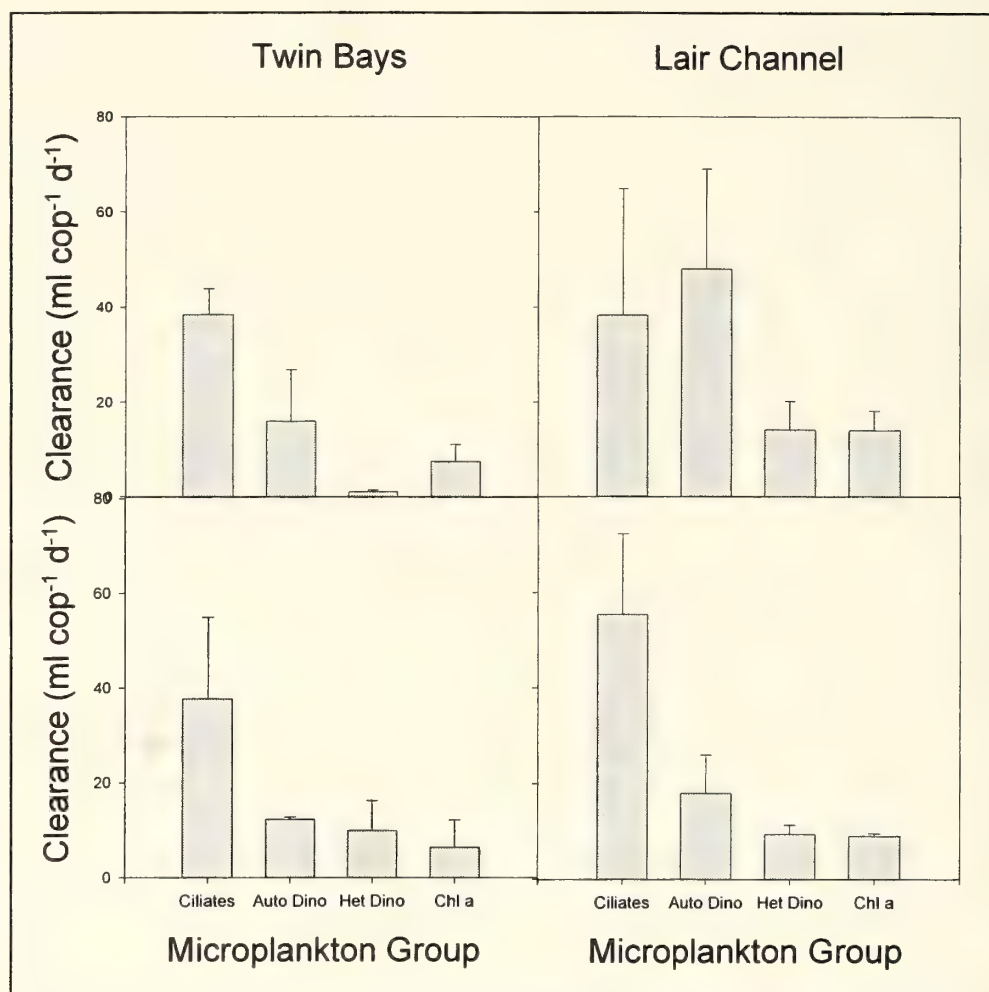
Figure 5. Daily clearance rates of *Dioithona oculata* on ciliates, autotrophic dinoflagellates (Auto Dino), heterotrophic dinoflagellates (Het Dino) and total phytoplankton measured as chlorophyll *a* (Chl a) during 24-hour incubation experiments carried out during two days with samples collected in Twin Bays and during two days with samples collected in Lair Channel during April 2000.

Mean clearance rates on ciliates ranged from about 38-55 ml copepod^{-1} d^{-1} with clearance rates on autotrophic and heterotrophic dinoflagellates ranging from 12-48 and from 1-14 ml copepod^{-1} d^{-1}, respectively. Clearance rates from 12-hour incubations were performed to determine if there were diel patterns of grazing in *D. oculata* independent of any effects caused by swarming behavior. These 12-hour clearance rates were

doubled to produce daily clearance rates comparable with the 24-hour incubation experiments. There was no consistent pattern of clearance rates being higher during day or night (Fig. 6) suggesting that there is no innate pattern of diel variations in feeding. Clearance rates calculated in these 12-hour incubations were somewhat higher than those found in the 24-hour incubations.
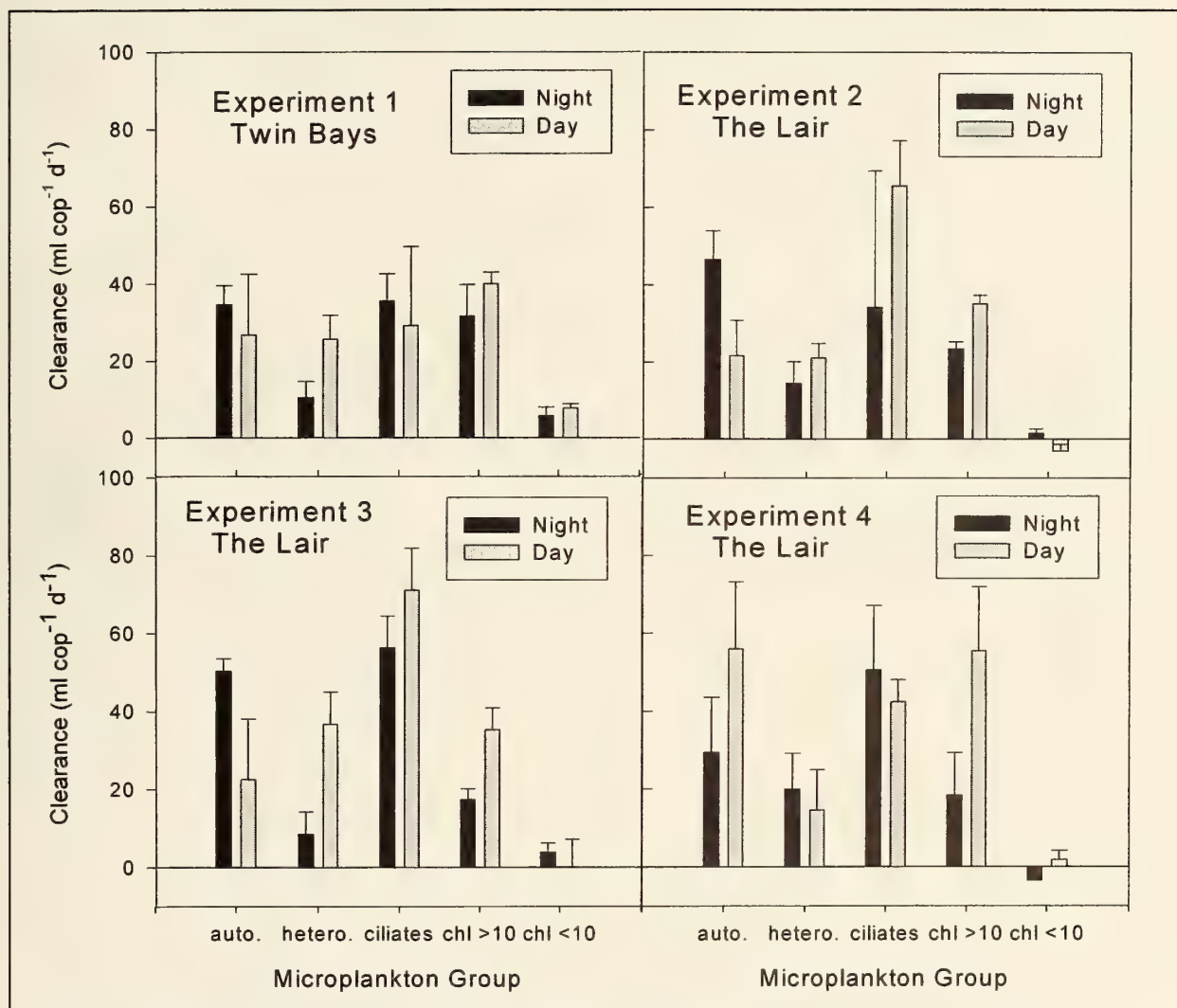
Figure 6. Clearance rates of *Dioithona oculata* on autotrophic dinoflagellates (auto.), heterotrophic dinoflagellates (hetero.) and size-fractionated total phytoplankton measured as chlorophyll *a* greater than 10 μm (chl >10) and as chlorophyll *a* less than 10 μm (chl < 10) during 12-hour incubation experiments carried out during either daylight or nighttime conditions in April 2001.

This may be due, in part, to the generally lower abundances of protozoan populations during the 12-hour incubations in 2001 (Fig. 2). When clearance rates for ciliates, autotrophic dinoflagellates and heterotrophic dinoflagellates are plotted as a function of

12

their initial abundance in samples, there is a general trend of lower clearance rates at higher cell abundances (Fig. 7). Clearance rates for ciliates during the 12-hour incubations ranged from 30-71 ml copepod^{-1} d^{-1}, and clearance rates on autotrophic and heterotrophic dinoflagellates ranged from 22-56 and 10-37 ml copepod^{-1} d^{-1}, respectively. The overall mean clearance rates for both sets of experiments were 47, 32 and 16 ml copepod^{-1} d^{-1} for ciliates, autotrophic dinoflagellates and heterotrophic dinoflagellates, respectively. Growth rates of microplankton within control bottles during both sets of grazing experiments were uniformly low for all broad categories with mean specific growth rates of 0.02, 0.04 and 0.01 d^{-1} for ciliates, autotrophic dinoflagellates and heterotrophic dinoflagellates, respectively.
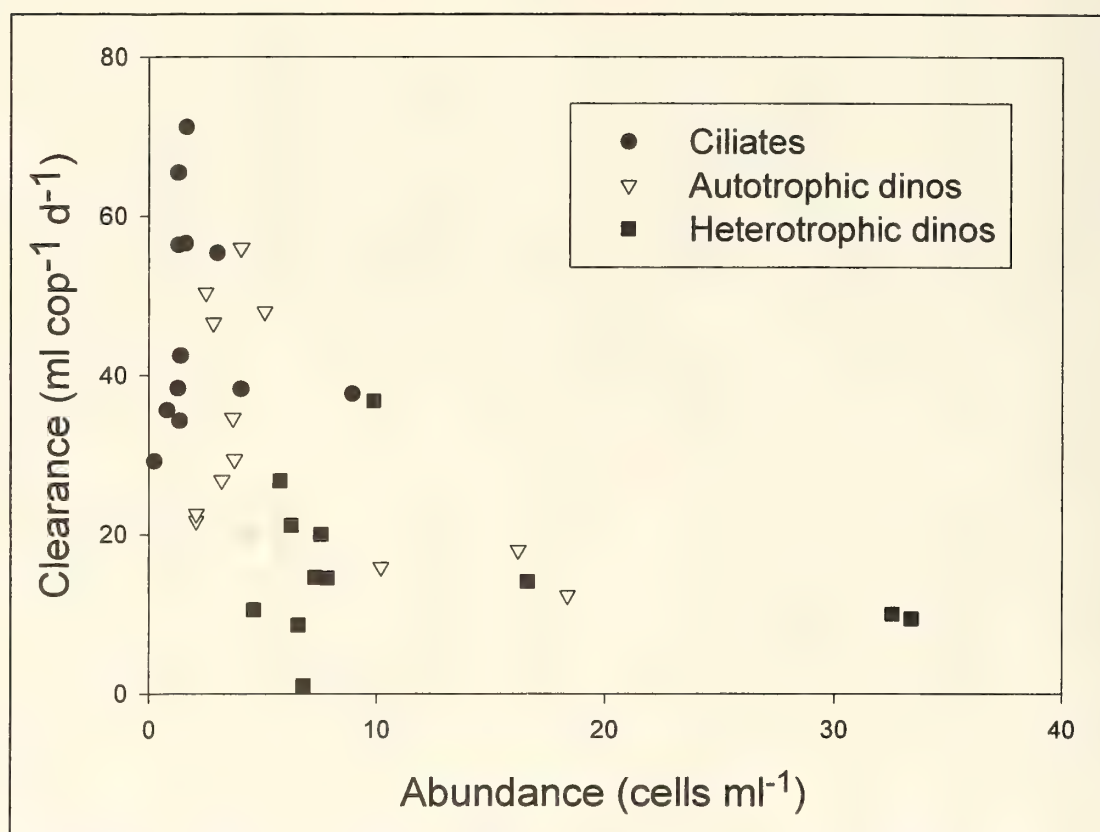
Figure 7. Relationship between clearance rate of *Dioithona oculata* on ciliates, autotrophic and heterotrophic dinoflagellates to their initial abundance in 12- and 24-hour incubation experiments.

Ingestion rates estimated from clearance rates, abundances and cell volumes during 24- hour incubation experiments ranged from mean values of 46-114 ng C copepod^{-1} d^{-1} for ciliates, 50-136 ng C copepod^{-1} d^{-1} for autotrophic dinoflagellates and 1-38 ng C copepod^{-1} d^{-1} for heterotrophic dinoflagellates (Fig. 8). Although *Dioithona oculata* has generally higher clearance rates on ciliates (Fig. 5), autotrophic

dinoflagellates may comprise a similar or slightly larger proportion of their ingested food. Heterotrophic dinoflagellates, while often very abundant in these experiments, had both lower clearance rates and were of generally smaller size and, therefore, account for a smaller fraction of the estimated carbon ingested by *D. oculata*. Combined ingestion on these three food categories was ca. 200 ng C copepod^{-1} d^{-1} for each of the four experiments.
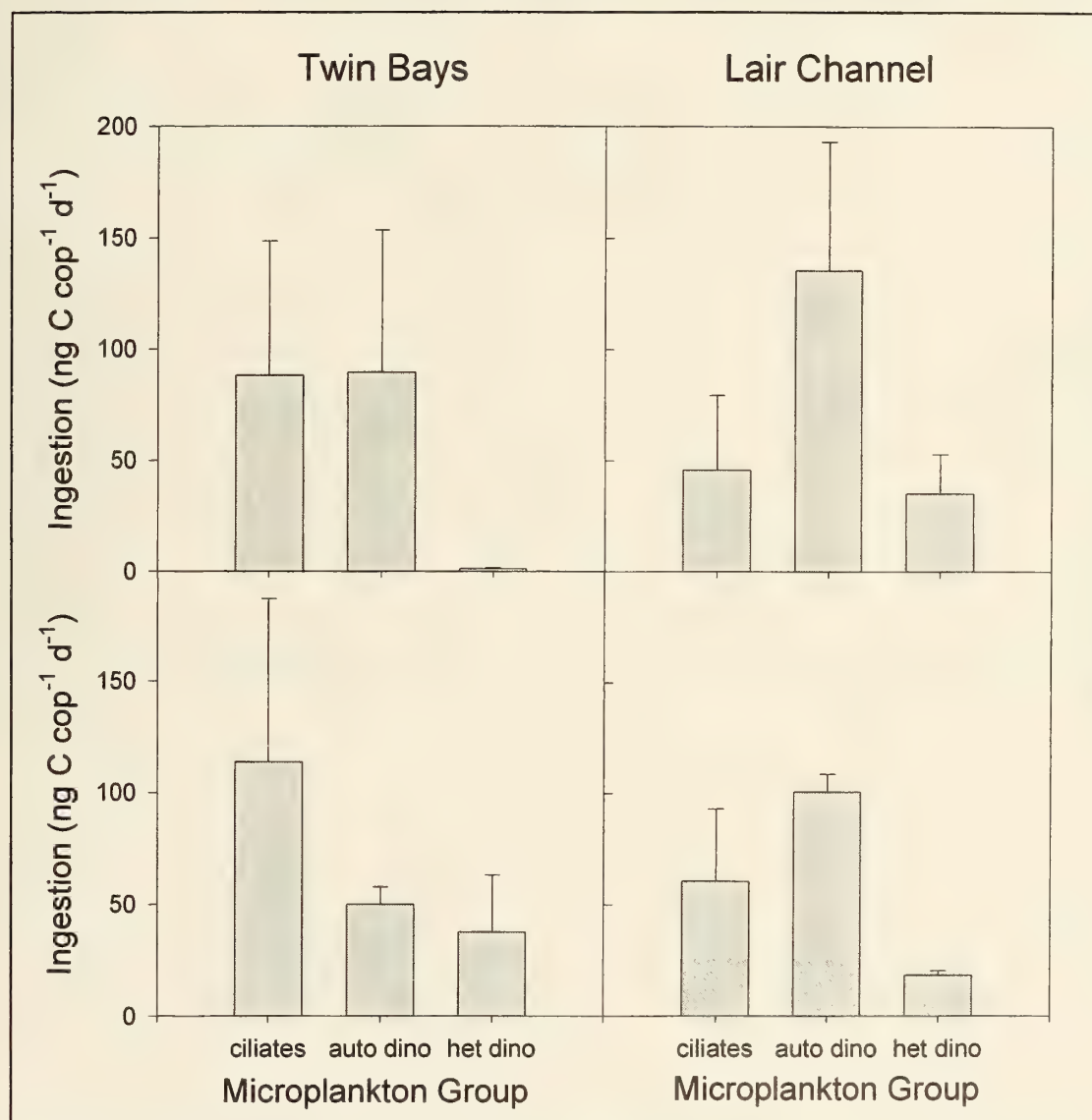
Figure 8. Estimates of carbon ingested by *Dioithona oculata* as ciliates, autotrophic dinoflagellates (auto dino) and heterotrophic dinoflagellates (het dino) for 24-hour incubation experiments carried out during two days with samples collected in Twin Bays and during two days with samples collected in Lair Channel.

14

Predation rates of *Dioithona oculata* on its own nauplii increased linearly over the range of prey densities tested (0.2–8 nauplii ml^{-1}, Fig. 9). Maximum predation rates were ca. 2.5 nauplii copepod^{-1} h^{-1}. However, there is no evidence of saturation in the functional response relationship so higher predation rates may be achieved at higher densities of nauplii. By comparing the size distributions of the natural prey distributions before and after feeding by copepods during the 24-hour incubation experiments, it is apparent that copepods feed preferentially on larger prey (>14 μm equivalent spherical diameter, Fig.10).
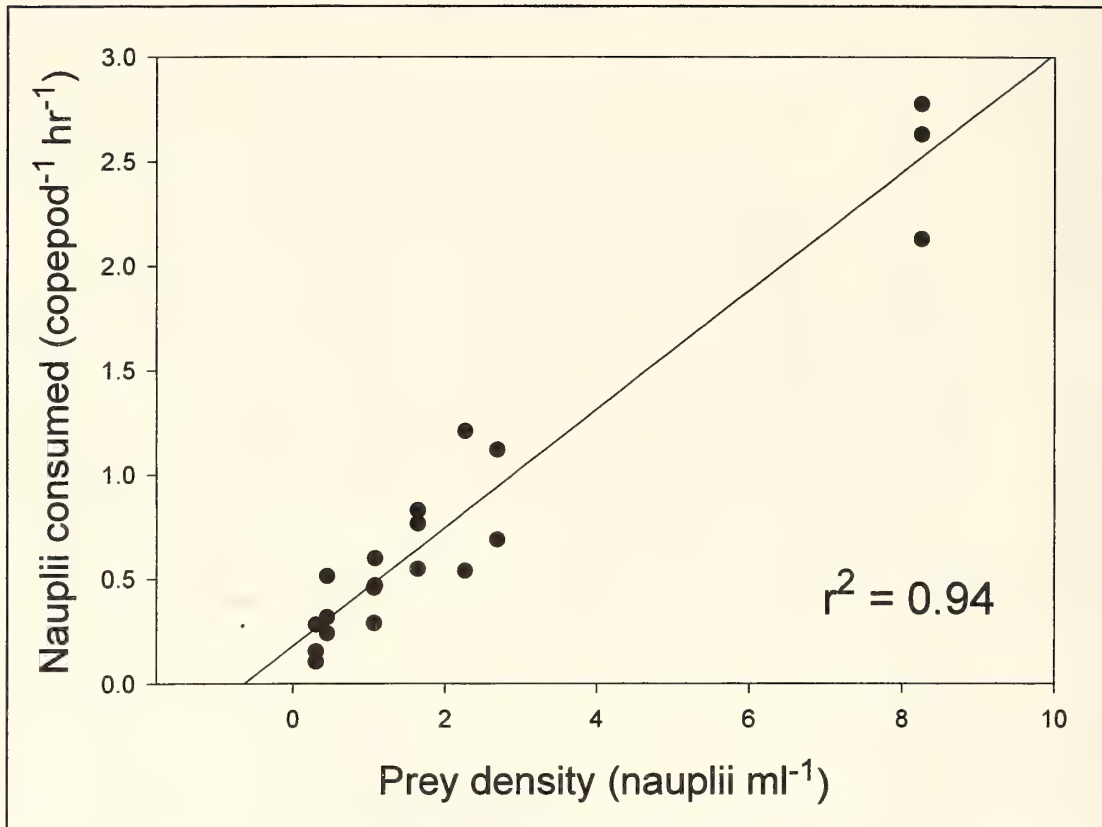
Figure 9. Ingestion rates of adult *Dioithona oculata* on newly hatched nauplii of *D. oculata* as a function of prey density.
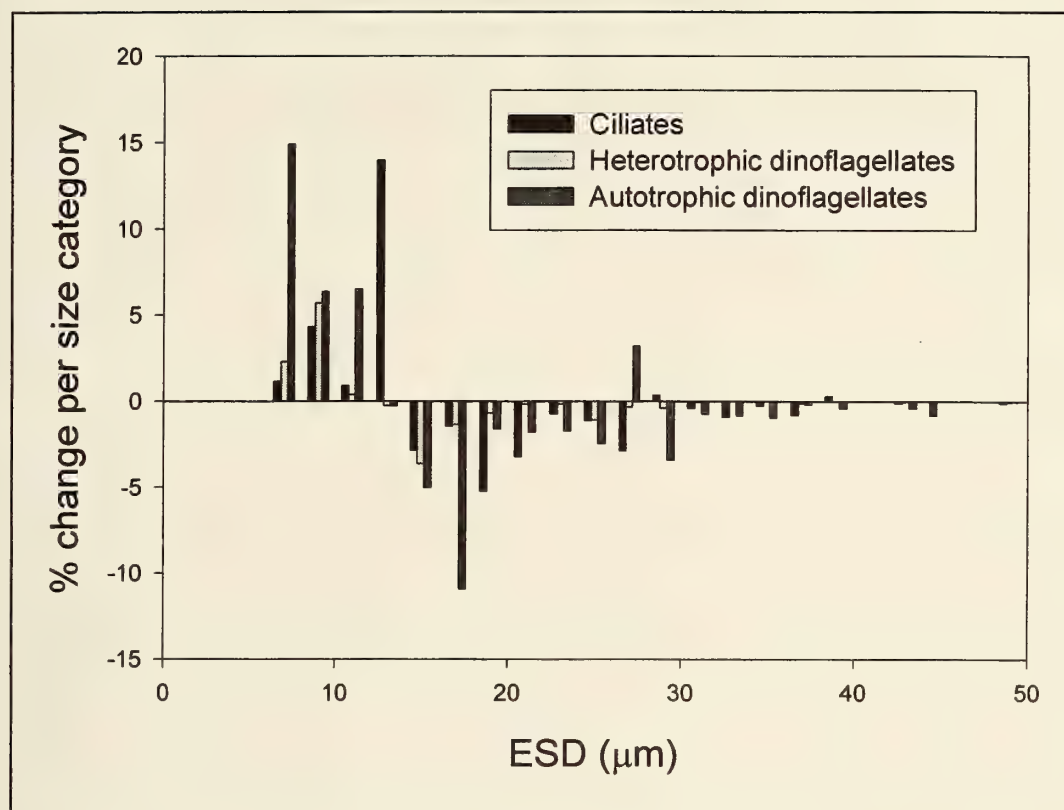
Figure 10. Percentage change in size categories of microplankton between the beginning and end of 24-hour feeding experiments with *Dioithona oculata*. Decreases in size categories indicate that copepods were removing cells through grazing.

DISCUSSION

Microzooplankton are abundant in the mangrove channels of Twin Cays and they are an important trophic link between the pico- and nanoplankton-sized organisms they feed on and the larger zooplankton that in turn feed on them. For broad categories of protists, heterotrophic dinoflagellates were most abundant, followed by aloricate ciliates and tintinnids (Fig. 2). Abundances of heterotrophic dinoflagellates and ciliates in this study (mean ca. 2-20 ml $^{-1}$) are comparable to those found in other studies of coastal regions in tropical and subtropical environments (e.g. Lynn et al., 1991; Buskey, 1993; Strom and Strom, 1996). Few studies have been published on the microzooplankton of mangrove channels but similar densities of ciliates are found in undisturbed mangrove creeks of North Queensland, Australia (McKinnon et al., 2002). Likewise, the low densities of tintinnids found in this study (mean < 1 ml^{-1}) are comparable to the densities reported for mangrove waters on the southeast coast of India (Godhantaraman, 2001).

It is now generally accepted that microzooplankton are the major grazers of phytoplankton throughout much of the world's oceans (Strom, 2002 and references therein). Based on our studies, the microzooplankton community in the mangrove channels of Twin Cays is a major consumer of phytoplankton, consuming ca. 60-90% of the potential phytoplankton production per day (Table 1). In this study, apparent growth of phytoplankton exceeded grazing by the microzooplankton community in all four experiments. In contrast, in a study of mangrove creeks with higher phytoplankton biomass than in the mangrove channels of Twin Cays (McKinnon et al., 2002), phytoplankton growth and grazing were found to be roughly in balance although microzooplankton grazing usually exceeded phytoplankton primary production. The grazing rates we measured with the dilution method are more comparable to measurements made in other less eutrophic coastal environments (e.g. Tamigneaux et al., 1997).

The phytoplankton community of the mangrove channels of Twin Cays appears to be highly productive. Phytoplankton growth rates estimated from changes in chlorophyll *a* concentrations in our dilution incubations indicate rapid phytoplankton growth equivalent to a range of 0.9 to 1.8 doublings per day (Table 1). Low concentration nutrient additions to dilution incubations (equivalent to f/200) caused a pronounced increase in phytoplankton growth rates (Fig. 4, Table 1) indicating that phytoplankton populations of Lair Channel were nutrient-limited. Nutrient additions are recommended for studies in oligotrophic waters where dilution may reduce the availability of recycled nutrients to phytoplankton (Landry et al., 1995). Nutrient additions are typically omitted in studies of more eutrophic coastal waters where it is assumed or known that nutrients are not limited (e.g. Gallegos, 1989; McKinnon et al., 2002). Given the evidence for nutrient limitation of phytoplankton growth in the mangrove channels of Twin Cays, nutrient additions are appropriate for these dilution experiments.

The dominant copepod in the mangrove channels of Twin Cays, *Dioithona oculata*, forms dense swarms along the fringes of the mangrove channels. Given the extremely high densities of copepods within these swarms (over 30 copepods ml^{-1}, Buskey et al., 1996) it seems likely that competition for food within swarms is intense. Previous attempts to compare the feeding behavior of swarming copepods during the day to that of dispersed copepods at night using the gut pigment method (Dagg, 1983) indicated that copepods within swarms generally had lower levels of chlorophyll *a* in their guts than dispersed copepods at night (Buskey et al., 1996). This difference could be due to higher competition for food in the dense swarms during the day compared with that of dispersed copepods at night, or due to a diel feeding behavior of the copepods where feeding is most intense at night (Mackas and Bohrer, 1976; Stearns, 1986). In addition there are a number of potential problems with interpretation of feeding rates using the gut pigment method (e.g. Baars and Helling, 1985; Conover et al., 1986) although this method can still be used to indicate relative feeding rates on phytoplankton

if it is assumed that gut passage rates and pigment destruction rates are the same under the conditions being compared. However, the gut pigment method only provides information for feeding rates on chlorophyll-containing cells (phytoplankton) and yields no information on the feeding rate on heterotrophic organisms. Many copepods are omnivorous in their diets and consume both phytoplankton and heterotrophic microzooplankton (Gifford and Dagg, 1988; Tiselius, 1989).

The results of our grazing experiments clearly indicate that *Dioithona oculata* is omnivorous and feeds on a mixture of microplankton including copepod nauplii (Fig. 9), ciliates and both autotrophic and heterotrophic dinoflagellates (Fig. 5). Relatively few detailed studies have been made of the feeding behavior of marine cyclopoid copepods compared with that of calanoid copepod species. The most common and best-studied marine cyclopoid copepods belong to the closely related genus *Oithona*. However, the overall behavior pattern of most *Oithona* species is in sharp contrast to the behavior of *D. oculata*. Species of *Oithona*, such as *O. nana*, *O. plumifera* and *O. similis,* have behavioral patterns that are characterized by long periods of inactivity during which they sink slowly (Hwang and Turner, 1995; Svensen and Kiorboe, 2000; Paffenhofer and Mazzocchi, 2002). In contrast, *D. oculata* swims nearly continually with a jerky motion and is capable of swimming at sustained speeds of up to 2 cm s^{-1} (Buskey et al., 1996). Marine cyclopoid copepods, such as *Oithona* species, are thought to feed only on moving prey and to detect their prey primarily through mechanoreception (Svensen and Kiorboe, 2000; Paffenhofer and Mazzocchi, 2002). It seems unlikely that *D. oculata* could feed in this manner because of the difficulty in detecting prey based on mechanoreception within a dense swarm of actively swimming copepods. It is unknown how *D. oculata* detect and capture their food but it is clear that they are feeding within these dense swarms based on both gut pigment analysis (Buskey et al., 1996) and the ability to collect fecal pellets from copepods captured from swarms (unpublished observations).

There have been relatively few studies of feeding by marine cyclopoid copepods on natural plankton assemblages. *Oithona similis* has been shown to feed primarily on ciliates and dinoflagellates greater than 10 μm diameter (Nakamura and Turner, 1997) and to have average carbon ingestion rates similar to those we found for *Dioithona oculata*. However, the average clearance rates measured for this copepod in the rich coastal waters of Massachusetts were much lower than those we found for *D. oculata* in the mangrove channels of Belize. In a study of unidentified *Oithona* species in the Antarctic (Lonsdale et al., 2000), clearance rates on protozoa were more similar to those found this study. It does appear that *D. oculata* feed preferentially on larger-sized food particles which would be consistent with a more raptorial-feeding mode than a filter-feeding mode. Based on the change in size frequency distribution of microplankton before and after grazing by *D. oculata* (Fig.10), it is apparent that these copepods only have a grazing impact on particles larger than 14 μm equivalent spherical diameter. Higher clearance rates on ciliates and autotrophic dinoflagellates compared with

18

heterotrophic dinoflagellates also indicate a preference for classes of microplankton which include more large cells.

Although metazoan microzooplankton were not abundant enough in our incubation experiments to determine feeding rates of adult *D. oculata* on natural assemblages, it is clear that these copepods are capable of feeding on active metazoan prey such as copepod nauplii (Fig. 9). These rare, large prey could be important components of their diet in nature. Other marine cyclopoid copepods have been shown to feed readily on copepod nauplii (Lampitt, 1978; Nakamura and Turner, 1997) including cannibalistic feeding on their own nauplii (Uchima and Hirano, 1986). *D. oculata* females have evolved a diel periodicity in the timing of egg production and egg hatching; egg clusters are produced during the day while the copepods swarm but the eggs hatch from these clusters (attached to the female body) when the copepods are dispersed at night (Ambler et al., 1999). This is highly useful as an antipredator adaptation for both adult females and newly hatched nauplii. Females bearing egg clusters are more visually conspicuous and are preyed on preferentially by planktivorous fish when not in swarms (Collumb, 2000); nauplii would be heavily preyed upon if they were hatched within high-density swarms during the day (Fig. 9).

Feeding of *Dioithona oculata* swarms will have an important impact on microplankton populations in mangrove channels. Average clearance rate of these copepods on ciliates is 40 ml per copepod per day. Thus a population density of 25 copepods per liter can potentially consume all ciliates in the water column. However, the average density within swarms of *D. oculata* can exceed 30 copepods per ml (Buskey et al., 1996); at this density a swarm potentially can clear the water of ciliates in less than two minutes. However, average current speeds in prop-root environments are ca. 1 cm s^{-1} (Buskey, unpublished data) so since swarms hold position within light shafts the food to swarms is continually renewed. Average densities of copepods throughout the entire mangrove channel, based on population density estimates taken at night when copepods are dispersed from swarms, are ca. three copepods per liter (Ferrari et al., 2003). At this density, *D. oculata* could consume ca. 10% of the ciliate population per day. However, given the slow growth rates measured for heterotrophic protozoa in our experiments, copepods could still play an important role in structuring the microzooplankton community, especially given their apparent size-selective feeding behavior (Fig. 10).

ACKNOWLEDGMENTS

REFERENCES

Ambler, J.W., F.D. Ferrari, and J.A. Fornshell
  1991. Population structure and swarm formation of the cyclopoid copepod *Dioithona oculata* near mangrove cays. *Journal of Plankton Research* 13:1257-1272.

Ambler, J.W., F.D. Ferrari, J.A. Fornshell, and E.J. Buskey
  1999. Diel cycles of molting, mating, egg production and hatching in the swarm-forming copepod *Dioithona oculata*. *Plankton Biology and Ecology* 46:120-127.

Baars, M.A., and G.R. Helling
  1985. Methodological problems in the measurement of phytoplankton ingestion by gut fluorescence. *Hydrobiological Bulletin* 19:81-88.

Buskey, E.J.
  1993. Annual pattern of micro- and mesozooplankton abundance and biomass in a subtropical estuary. *Journal of Plankton Research* 15:907-924.

Buskey, E.J.
  1998. Energetic costs of swarming behavior for the copepod *Dioithona oculata*. *Marine Biology* 130:417-423.

Buskey, E.J., H. DeYoe, F. Yochem, and T. Villareal
  2003. Effects of mesozooplankton removal on trophic structure during a bloom of the Texas "brown tide": a mesocosm study. *Journal of Plankton Research* 25:215-228.

Buskey, E.J., J.O. Peterson, and J.W. Ambler
  1996. The swarming behavior of the copepod *Dioithona oculata*: in situ and laboratory studies. *Limnology and Oceanography* 43:513-521.

Collumb, C.J.
  2000. Mesozooplankton population dynamics: factors affecting reproduction and predation. PhD. Dissertation. The University of Texas at Austin. 167pp.

Conover, R.J., R.Durvasula, S. Roy, and R. Wang
  1986. Probable loss of chlorophyll-derived pigments during passage through the gut of zooplankton, and some consequences. *Limnology and Oceanography* 31:878-887.

Dagg, M.J.
  1983. A method for the determination of copepod feeding rates during short time intervals. *Marine Biology* 75:63-67.

Ferrari, F.D., J.A. Fornshell, L. Ong, and J.W. Ambler
  2003. Diel distribution of copepods across a channel of an over-wash mangrove island. *Hydrobiologia* 499:147-159.

Frost, B.W.
  1972. Effects of size and concentration of food particles on the feeding behavior of the

20

marine planktonic copepod Calanus pacificus. *Limnology and Oceanography* 17:805-917.

Gallegos, C.L.
  1989. Microzooplankton grazing on phytoplankton in the Rhodes River, Maryland: non-linear feeding kinetics. *Marine Ecology Progress Series* 57:23-33.

Gifford, D., and M.J. Dagg
  1988. Feeding of the estuarine copepod Acartia tonsa Dana: carnivory vs. herbivory in natural microplankton assemblages. *Bulletin of Marine Science* 43:458-468.

Godhantaraman, N.
  2001. Seasonal variations in taxonomic composition, abundance and food-web relationship of microzooplankton in estuarine and mangrove waters, Parangipettai region, southeast coast of India. *Indian Journal of Marine Science* 30:155-160.

Guillard, R.R.L., and R.H. Ryther
  1962. Studies of marine planktonic diatoms I. *Cyclotella nana* Hudstedt and *Detonula convervacea* (Cleve) Gran. *Canadian Journal of Microbiology* 8:229-239.

Hamner, W., and J. Carlton
  1979. Copepod swarms: attributes and role in coral ecosystems. *Limnology and Oceanography* 24:1-14.

Hillebrand, H., C.D. Durselen, D. Kirschtel, U. Pollingher, and T. Zohary
  1999. Biovolume calculations for pelagic and benthic microalgae. *Journal of Phycology* 35:103-124.

Hwang, J.S., and J.T. Turner
  1995. Behavior of cyclopoid, harpacticoid and calanoid copepods from coastal waters of Taiwan. *P.S.Z.N.I.: Marine Ecology* 16:207-216.

Lampitt, R.S.
  1978. Carnivorous feeding by a small marine copepod. *Limnology and Oceanography* 23:1228-1231.

Landry, M.R., and R.P. Hassett
  1982. Estimating the grazing impact of marine micro-zooplankton. *Marine Biology* 67:283-288.

Landry, M.R., J. Kirshtein, and J. Constantinou
  1995. A refined dilution technique for measuring the community grazing impact of microzooplankton, with experimental tests in the central equatorial Pacific. *Marine Ecology Progress Series* 120:53-63.

Lonsdale, D.J, D.A. Caron, M.R. Dennett, and R. Schaffner
  2000. Predation by *Oithona* spp. on protozooplankton in the Ross Sea, Antarctica. *Deep-Sea Research II* 47:3273-3283.

Lynn, D.H., J.C. Roff, and R.R. Hopcroft
  1991. Annual abundance and biomass of aloricate ciliates in tropical neritic waters of Kingston, Jamaica. *Marine Biology* 110:437-448.

Mackas, D.L., and R.N. Bohrer
    1976. Fluorescence analysis of zooplankton gut contents and an investigation of diel feeding patterns. *Journal of Experimental Marine Biology and Ecology* 25:77-85.
McKinnon, A.D., L.A. Trott, M. Cappo, D.K. Miller, S. Duggan, P. Speare, and A. Davidson
    2002. The trophic fate of shrimp farm effluent in mangrove creeks of North Queensland, Australia. *Estuarine, Coastal and Shelf Science* 55:655-671.
Menden-Deuer, S., and E.J. Lessard
    2000. Carbon to volume relationships for dinoflagellates, diatoms and other protist plankton. *Limnology and Oceanography* 45:569-579.
Nakamura, Y., and J.T. Turner
    1997. Predation and respiration by the small cyclopoid copepod Oithona similes: how important is feeding on ciliates and heterotrophic flagellates? *Journal of Plankton Research* 19:1275-1288.
Paffenhofer, G.A., and M.G. Mazzocchi
    2002. On some aspects of the behavior of *Oithona plumifera* (copepoda: Cyclopoida). *Journal of Plankton Research* 24:129-135.
Putt, M., and Stoecker, D.K.
    1989. An experimentally determined carbon: volume ratio for marine oligotrichous ciliates from estuarine and coastal waters. *Limnology and Oceanography* 34:1097-1103.
Sherr, E.B., and B.F. Sherr
    1992. Trophic roles of pelagic protests: phagotrophic flagellates as herbivores. *Archiv fur Hydrobiologie. Beiheft Ergebnisse Limnologie* 37:165-172.
Sherr, E.B., and B.F. Sherr
    1994. Bactivory and herbivory: key roles of phagotrophic protists in pelagic food webs. *Microbial Ecology* 28:223-235.
Sherr, E.B., B.F. Sherr, and G.A. Paffenhofer
    1986. Phagotrophic protozoa as food for metazoans: a "missing link" in marine pelagic food webs. *Marine Microbial Food Webs* 1:61-80.
Stearns, D.E.
    1986. Copepod grazing behavior in simulated natural light and its relation to nocturnal feeding. *Marine Ecology Progress Series* 30:65-76.
Stoecker, D.K., and J.M. Capuzzo
    1990. Predation on protozoa: its importance to zooplankton. *Journal of Plankton Research* 12: 891-908.
Strom, S.L.
    2002. Novel interactions between phytoplankton and microzooplankton: their influence on the coupling between growth and grazing rates in the sea. *Hydrobiologia* 480:41-54.

Strom, S.L., and M.W. Strom

    1996. Microplankton growth, grazing and community structure in the northern Gulf of Mexico. *Marine Ecology Progress Series* 130:229-240.

Svensen, C., and T. Kiorboe

    2000. Remote prey detection in *Oithona similis*: hydromechanical versus chemical cues. *Journal of Plankton Research* 22:1155-1166.

Tamigneaux, E., M. Mingelbier, and L. Legendre

    1997. Grazing by protests and seasonal changes in the size structure of protozooplankton and phytoplankton in a temperate nearshore environment (western Gulf of St. Lawrence, Canada). *Marine Ecology Progress Series* 146:231-247.

Tiselius, P.

    1989. Contribution of aloricate ciliates to the diet of *Acartia clausi* and *Centropages hamatus* in coastal waters. *Marine Ecology Progress Series* 56:49-56.

Uchima, M., and R. Hirano

    1986. Predation and cannibalism in neritic copepods. *Bulletin of the Plankton Society of Japan* 33:147-149.

Welschmeyer, N.A.

    1994. Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and phaeopigments. *Limnology and Oceanography* 39:1985-1992.

ART IN THE SWAMP: USING FIELD ILLUSTRATION TO PREPARE
DRAWINGS OF MANGROVE COMMUNITIES AT TWIN CAYS,
BELIZE, CENTRAL AMERICA

BY

MARY PARRISH AND MOLLY KELLY RYAN
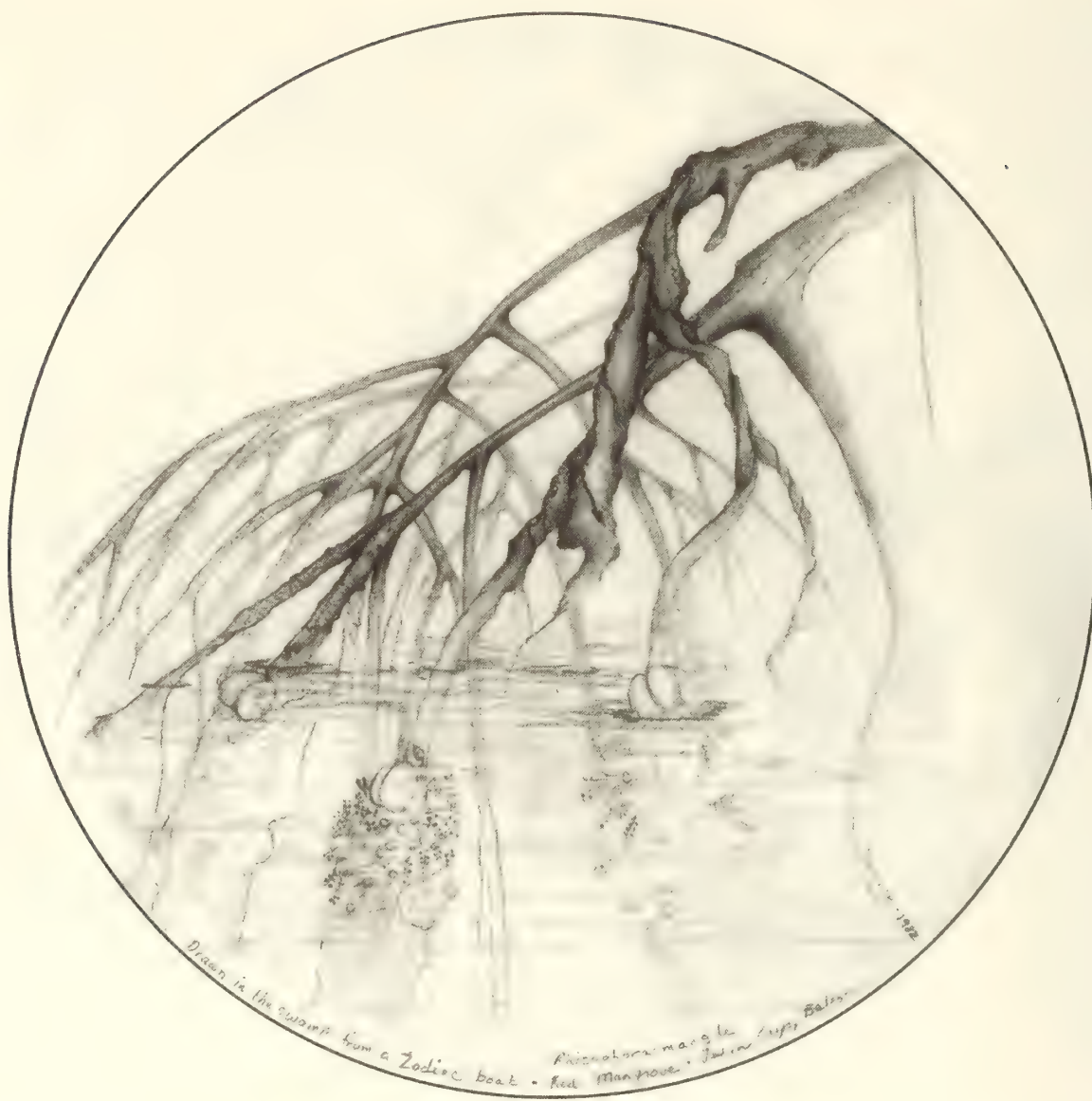
Figure 1. Field sketch of red mangrove stilt roots drawn while afloat in a boat.
Illustration by Mary Parrish, 1982.

ART IN THE SWAMP: USING FIELD ILLUSTRATION TO PREPARE DRAWINGS OF MANGROVE COMMUNITIES AT TWIN CAYS, BELIZE, CENTRAL AMERICA

BY

MARY PARRISH[1] and MOLLY KELLY RYAN[2]

ABSTRACT

In 1981, the Smithsonian West Atlantic Mangrove Program (SWAMP) began sending scientific illustrators into the field with scientists to study the mangrove environment and prepare drawings of the ecological communities of Twin Cays. Rather than working only from preserved specimens and photographs in a museum setting, artists observed and sketched terrestrial, intertidal and subtidal habitats in situ and collected live specimens that were drawn and photographed at the field station on Carrie Bow Cay, Belize. The project was called *Art in the SWAMP*.

INTRODUCTION

Scientific illustrators generally prepare drawings in a studio setting using preserved specimens, verbal description and photographs as reference material. Traditionally, the organisms are carefully positioned on a white or black background with lighting from the upper left so that taxonomic characters are presented in a clear and predictable way to the scholarly reader.

Klaus Ruetzler, sponge biologist and director of the Caribbean Coral Reefs Ecosystems Program, instead asked artists to produce a series of illustrations based on field observation that were not prepared for taxonomic description, but were designed to show how plants and animals appeared in life and interacted in the terrestrial, intertidal and subtidal communities of the mangrove ecosystem. He invited several artists to work alongside scientists at the Carrie Bow Cay field station to observe and sketch the environment in situ. Among these, Smithsonian artists Candy Feller, Molly Ryan and Mary Parrish have participated in this project, with Candy Feller as lead artist for many years.

[1] Department of Paleobiology, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560.

[2] Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560.

PREVIOUS WORK

Historically, artists have often been an important part of scientific expeditions: Captain James Cook employed the artist William Hodges, and later John Webber, to document his voyages around the world that began in 1768 (Thomas, 2003); David Starr Jordan asked Bistrow Adams to accompany him to the Pribylof Islands to draw fur seals from nature in 1896 (Jordan, 1896); William Beebe invited artists Isabel Cooper, Helen Tee-Van and Don Dickerman to join his *Arcturus* expedition to illustrate his discoveries from New York to the Galapagos Islands in 1925 (Beebe, 1926); and the ornithological illustrator, Louis Agassiz Fuertes, participated in many field expeditions including those organized by the American Museum of Natural History and the Field Museum. Many other examples exist in the history of science.

Natural history artists such as John James Audubon and Roger Tory Peterson, and fine artists such as Leonardo da Vinci and Albrecht Dürer insisted on the importance of working from nature. In the late 1800s, sketching and painting directly from nature became central to the work of Realist painters such as John Constable, and later those of the Barbizon, Impressionist and Post-Impressionist schools that included fine artists such as Vincent van Gogh, Claude Monet, and Paul Gauguin. These artists rebelled against the classical academic tradition of painting in the studio and moved outdoors to paint "en plein air" (in the open air). A school of Plein Air painters is still active today.

Photography has replaced some of the work of the field artist, but illustration is still used to synthesize complex subject matter, such as ecological communities, or to clarify structure in specimens that the camera cannot record adequately. In addition, illustration portrays nature in a way that is aesthetically unique.

Excellent previously published pen-and-ink stipple drawings of communities that were helpful to this project include Sarah Landry's illustrations for *Sociobiology: The New Synthesis* by Edward O. Wilson (Wilson, 1975), and John Sibbick's illustrations for *Reef Evolution* by Rachael Wood (Wood, 1999). Sarah Landry worked for *Art in the SWAMP* during one field season as well.

METHODS AND RESULTS

Photographs were used when a literal depiction of a scene was needed, but for many projects, illustration worked better than photographs to present the information required by the scientists. Three-dimensional cutaways, composite drawings of idealized communities made from a series of observations that took place over a period of time, clarification of material through the elimination of distracting information such as mud, sand or debris, enhancement of details that would otherwise be too subtle to communicate, and use of block diagrams were some of the techniques illustrators used to help present data that a camera could not (Figs. 1 - 3). Illustration was also used to depict specimens whose shape, color patterns and structure were difficult to preserve, such as sponges, tunicates and other soft-bodied invertebrates, when depth of field extended beyond the camera's capability, or parallax would cause distortion in a photograph.
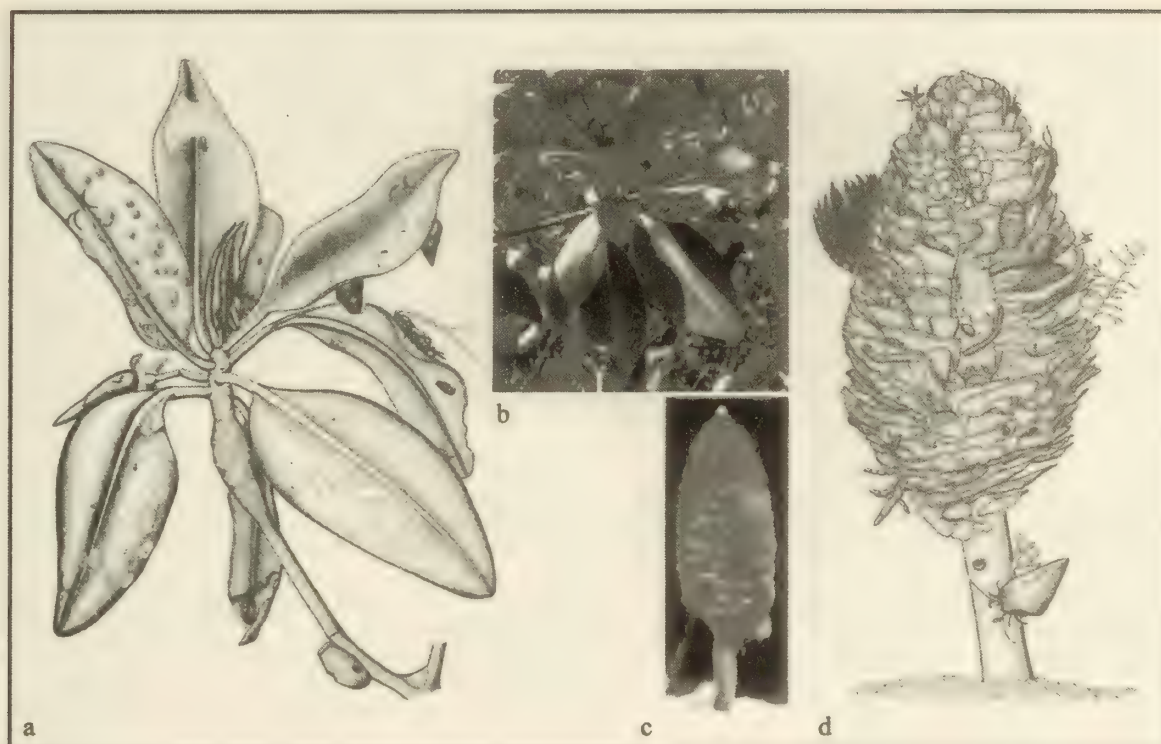
Figure 2. Stipple drawings of idealized communities shown with photographs of the same subject matter: a) Red mangrove, *Rhizophora mangle*, leaf cluster with insects and snails. b) Marine alga, *Rhipocephalus phoenix*, with microcommunity. Clarity of subject matter is the hallmark of a good pen-and-ink drawing. Illustrations by Candy Feller. Photo in 2a by Mary Parrish. Photo in 2b by Candy Feller.

The Field Sketch

Field observation and sketching are very important for all artists who want to portray nature in their work. Nature is infinitely complex, subtle, exciting and unpredictable. What artists imagine in their studios or copy from a photograph or other artists' work is useful but will never be as valuable as what is observed firsthand in the field. Perspective, light, shadow, color, composition, and other artistic considerations can be seen more clearly in the field, and biological information such as the interactions between plant, animal and environment, and mannerisms and habits of species are understood best by direct observation. Immersion in nature impacts illustrators and, in turn, the illustration - both scientifically and aesthetically. While discomforts in the field can hamper work in some respects, the benefits outway the difficulties.

A field sketch is direct, usually gestural (Fig. 3a), and can capture a scene with a freshness that is often lost when drawing from a photograph or preserved specimens. The field sketch can later be used as a compositional base for an illustration where details are more carefully worked out and the drawing is more refined (Fig. 3b).

Occasionally, field sketches were prepared for aesthetic rather than scientific reasons (Fig. 1). Drawings of this type can help individuals, who might otherwise be indifferent or hostile towards towards an ecosystem, gain an appreciation of the ecosystem while at the same time bring pleasure to those who are fond of it already.

Figure 3. a) Field sketch of red mangrove roots and seedlings with two anoles. b) Surface features of the illustration sketched in the field; cutaway block diagram showing peat substratum was sketched in the Carrie Bow Cay laboratory from a sample collected by Karen McKee. Sketches by Mary Parrish.

Scientific Collaboration

While many illustrations were prepared independently by Candy Feller, such as those in Figure 2, artists Mary Parrish and Molly Ryan often worked alongside scientists acting as field assistant, dive buddy, and photographer, as well as artist. For example, Molly Ryan worked with Klaus Ruetzler to prepare a community illustration of the sponge *Tedania ignis* (Fig. 4). Drawings were often developed during the research process and recorded in a sketch as discoveries were made. Collaboration in the field, the laboratory, and during informal discussions throughout the day and evening were beneficial to the illustration process.

Often one or more host specimens were collected and returned to the Carrie Bow Cay laboratory in order to observe and extract live organisms hidden inside and to prepare a composite drawing. Examples of composite illustrations can be seen in the mangrove branch (Fig. 2a), marine alga (Fig. 2b), and sponge (Fig. 4b). Artists first drew the host organism. After noting posture and position, associated plants and animals were removed, sketched, and photographed. Finally, all the individual elements were combined into a single drawing. Specimens, such as sponges, were carefully cut in order to draw their internal structures. Artifacts left by any inhabitants, such as spaces and burrows, were also recorded. Artists prepared drawings with cutaway views and inset enlargements in order to show the subsurface details when necessary (Figs. 3b, 4a).

Each organism was identified by a scientist and the subsequent drawing was checked for accuracy. Specimens were preserved and labeled to use as voucher and reference material upon return to the museum. Photographs were also used for reference when completing the final illustration in the studio.
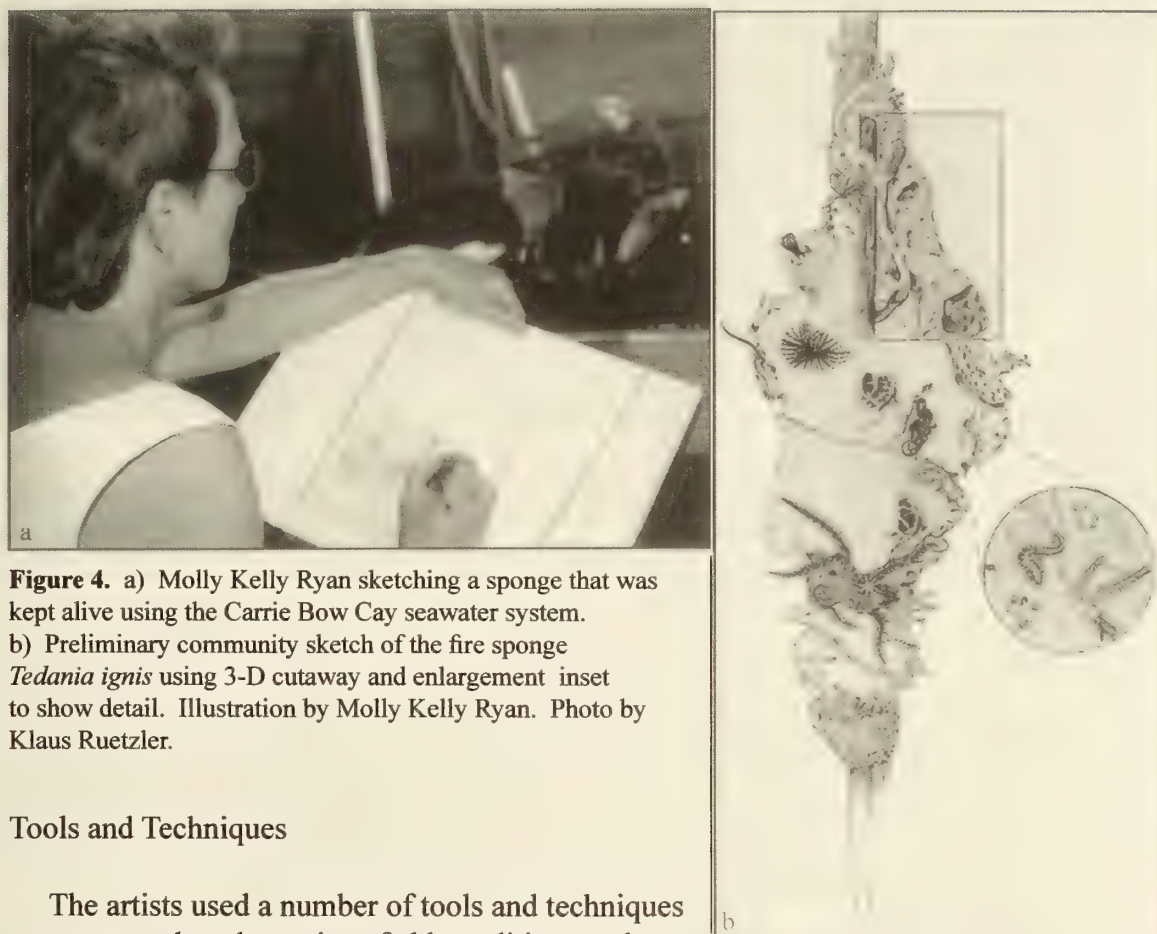
Figure 4. a) Molly Kelly Ryan sketching a sponge that was kept alive using the Carrie Bow Cay seawater system. b) Preliminary community sketch of the fire sponge *Tedania ignis* using 3-D cutaway and enlargement inset to show detail. Illustration by Molly Kelly Ryan. Photo by Klaus Ruetzler.

Tools and Techniques

The artists used a number of tools and techniques to accommodate the various field conditions and habitats found at Twin Cays. The supply list included tracing or other drawing paper, drafting film (which is stable even when wet), watercolor paper, large metal clips, rubber bands, tape, retractable pencils, leads, pencil sharpener, knife, kneaded eraser, tape, plastic bags, drawing board, sunglasses, bug repellent, collecting bags, field notebook, rain gear, and camera. Optional supplies were additional media such as watercolor or acrylic paint, charcoal, ink, or other materials.

Large clips, rubber bands and tape were used to hold paper steady in windy conditions. Watercolor paper often comes in a pad that is glued on all four sides and was useful on windy days. When a sketch on this paper was finished, the adhesive edges of the paper were slit with a knife and the drawing lifted off the pad. Retractable mechanical pencils were used in the field because the leads were protected while not in use. Sun is very reflective on white paper, so artists worked in the shade or wore sunglasses. Plastic bags were used to protect supplies from rain and seawater. Many of these supplies seem self-evident, but if they are forgotten in a remote area problems arise.

Illustration tools varied according to the habitats of specific subject matter and the size and scale of the community to be drawn. Microcommunities were collected and drawn live in the Carrie Bow Cay lab using microscope and camera lucida (Fig.5a). Large scale communities, such as mudflats and overviews of the mangrove swamp (Fig. 5b), and occasionally even underwater scenes were drawn in situ (Fig. 6).

6

Figure 5. a) Candy Feller drawing live microorganisms with a camera lucida. b) Mary Parrish sketching the mudflat community at Aanderaa Flats. Photos by Chip Clark.

Traditional drawing materials were generally used to make illustrations above water, but Candy Feller and Mary Parrish also tried methods of drawing underwater. Candy Feller designed an underwater drawing board and experimented with a number of papers and drawing media (Fig. 6). Mike Carpenter, Carrie Bow Cay station manager, designed an underwater drawing table constructed with PVC pipe (Fig. 6b). A wooden graphite pencil on Denril drafting film that was attached to a plexiglass board turned out to be the simplest and the best method for drawing underwater.

Figure 6. a) Candy Feller using her prototype drawing board. b) Mary Parrish using an underwater drawing table constructed by Mike Carpenter. c) Mary Parrish drawing subtidal mangrove roots at Twin Cays using Candy Feller's underwater drawing board. d) Underwater sketch of the reef NE Carrie Bow Cay and Twin Cays. Photo 6a by Klaus Ruetzler. Photo 6b by Mike Carpenter. Photo 6c by Chip Clark. Field sketch 6d by Mary Parrish.
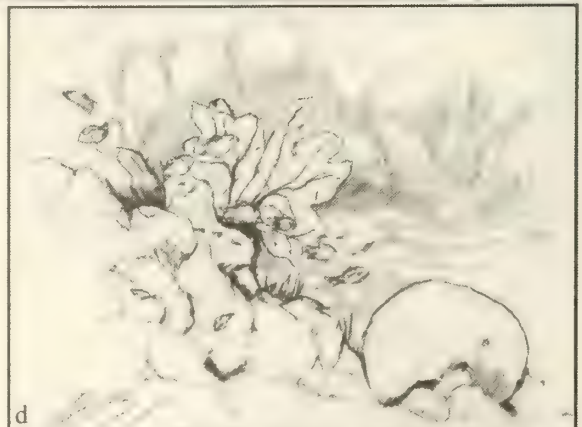
Composition

   Preparing a community illustration that is scientifically correct and still appears
natural is difficult and composition is one of the most challenging parts of developing the
illustration (Fig. 7).   Nature usually does not present itself in the manner required by the
scientist for the illustration or a photograph would have been used in the first place.
Idealized communities, sometimes called "family portraits", contain many more
animals and activities than are seen normally at any given moment and views that show
underground, underwater and above ground views simultaneously are two examples this
problem.
   Often a scientist wants to show a great deal of information in the space of a single
drawing that may be reduced to a very small size, such as a 3.25 inch column width,
when published.   A drawing can easily become cluttered if the composition is poor and
confusing, and aspects such as light source, scale and perspective are not well designed.
For these reasons, a significant amount of planning takes place and preliminary sketches
are developed and exchanged between artist and scientist prior to preparing a final
rendering. The more organisms that are needed in the illustration, the more difficult the
composition will be and the longer it will take to finish the drawing.

Figure 7. Stipple drawing of idealized mangrove mudflat community showing proper composition and
stipple technique. Illustration Molly Kelly Ryan.

8

The focal area of an image should generally be offset from the center and located towards one of the corners. The artist should lead the eye around the drawing using lights, darks, line, and form to move the eye in a comfortable way that is not too jarring. Foreground, middle ground and background should be established through the use of tone and scale. Line weight that is heaviest is used for foreground information. This will give the effect of aerial perspective because the stronger elements will appear to move forward in space. Contrast in lights, darks, and texture will add visual interest to a composition. Block diagrams (such as the one seen in Fig. 3b) can also help organize the composition of a complex illustration. Proper aerial and linear perspective, scale, correct shading and water reflections, along with interesting subject matter and careful rendering of the drawing, will all help create a successful illustration.

Final Rendering

Guidelines were established to assure aesthetic cohesiveness among illustrations prepared by several artists. Line weight, size of illustration, percent reduction of drawing for publication, technique, drawing substrate, method of showing enlargements, plate arrangement, use of borders and use of scales were all standardized. Drawings were planned for 1/3 reduction (to 67%). Artists rendered the illustrations in pen-and-ink stipple technique using 4x0 technical pens on Denril drafting film to prepare the final art. Care was taken to make sure stipples were round, not ragged. Stipples were spaced far enough apart so as not to touch one another and so that they did not block up when reduced. Fresh ink was used to ensure that the ink was very black. As ink ages it becomes gray because the carbon separates from the alchol and cannot be remixed. A good reference for preparing scientific illustrations is *The Guild Handbook of Natural Science Illustration*, edited by Elaine R.S. Hodges (2003).

CONCLUSIONS

Science provides the data needed to understand and conserve the mangrove ecosystem and to relate it to the needs of the larger world. Scientific illustration synthesizes this information visually for dissemination to scholarly and lay communities. A substantial portfolio of sketches, finished stipple drawings, and photographs has been prepared for use in scientific and popular publications, exhibits, and websites.

The mangrove is scientifically and aesthetically exciting. Having experienced Twin Cays my (MP) main response to the mangrove is not scientific curiosity alone. I probably speak for my other colleagues too when I say that I am awed by the beauty of the mangrove. While I sit quietly to draw, the usual mangrove characters appear: brown anoles; mangrove crabs and warblers; termite nest; seed pods just beginning to grow in the mangrove branches; subtidal roots covered with sponges, tunicates and ophiuroids; fish swimming among the roots. These and many other communites are available for studyut it is the aesthetic inspiration of the mangrove that I value most (Fig. 8).

Figure 8. A mangrove warbler catches a moth at Twin Cays. Field Sketch by Mary Parrish.

ACKNOWLEDGMENTS

REFERENCES

Beebe, William
    1926. *The Arcturus Adventure.* G.P. Putnam's Sons, New York and London.

Hodges, Elaine R.S., ed.
    2003. *The Guild Handbook of Scientific Illustration*, 2nd edition. John Wiley & Sons, Inc.

Jordan, David Starr
    1896. *Report of Fur-Seal Invstigations, 1896-1897, Part 1.* Government Printing Office, Washington, D.C.

Thomas, Nicholas
    2003. *Cook: the Extraordinary Voyages of Captain James Cook.* Walker & Company, New York.

Wilson, Edward O.
    1975. *Sociobiology: The New Synthesis.* Harvard University Press. Cambridge.

Wood, Rachael.
    1999. *Reef Evolution.* Oxford University Press. Oxford.

ATOLL RESEARCH BULLETIN

Nos. 509–530