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Editor: Dr. Dean F. Martin Co-Editor: Mrs. Barbara B. Martin
Institute for Environmental Studies, Department of Chemistry, University of South Florida,
4202 East Fowler Avenue, Tampa, Florida 33620-5250
Phone: (813) 974-2374; e-mail: dmartin@chuma1.cas.usf.edu
Business Manager: Dr. Richard L. Turner
Department of Biological Sciences, Florida Institute of Technology,
150 West University Boulevard, Melbourne, Florida 32901-6975
Phone: (321) 674-8196, e-mail: rturner@fit.edu
http://www.floridaacademyofsciences.org

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DEAN F. MARTIN, *Editor* BARBARA B. MARTIN, *Co-Editor*

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Environmental and Chemical Sciences

DIAZINON AND CHLORPYRIFOS TOXICITY TO THE FRESHWATER ASIATIC CLAM, *CORBICULA FLUMINEA* MULLER, AND THE ESTUARINE HOOKED MUSSEL, *ISCHADIUM RECURVUM* RAFINESQUE.

JON M. HEMMING*[(1)] AND WILLIAM T. WALLER[(2)]

[(1)]United States Fish and Wildlife Service, Panama City Field Office,
1601 Balboa Avenue, Panama City, FL 32405
[(2)]Institute of Applied Science, University of North Texas, P.O. Box 310559, Denton, TX 76203

ABSTRACT: *Organophosphate resistance of the Asiatic clam* Corbicula fluminea *and hooked mussel* Ischadium recurvum *were preliminarily assessed with diazinon and chlorpyrifos exposures. The diazinon 96-hour LC_{50} for* I. recurvum *was estimated to be 1,354 μg/L with a 95% upper confidence limit (UCL) of 1,501 μg/L and 95% lower confidence limit (LCL) of 1,041 μg/L. Chlorpyrifos appeared more toxic to* I. recurvum *than diazinon with an estimated 96-hour LC_{50} of 960 μg/L (LCL of 890, UCL 1,040 μg/L).* C. fluminea *was more tolerant with an estimated diazinon 96-hour LC_{50} of 4,067 μg/L (LCL 2,847, UCL 5,814 μg/L). The protectiveness of bivalve isolation through valve closure was examined in behaviorally regulated exposures that demonstrated noteworthy changes in tolerance of* C. fluminea *exposed to 15,251 μg/L diazinon. An exposure duration thought to preclude bivalve self-isolation also indicated high organophosphate resistance with a 21-day diazinon LC_{50} estimation for* C. fluminea *of 548 μg/L (LCL 455 μg/L, UCL 658 μg/L).*

Key Words: Diazinon, chlorpyrifos, bivalve, *Ischadium recurvum*, *Corbicula fluminea*

THE focus of this research was to evaluate the toxicity of organophosphorus pesticides to lamellabranch bivalves. Preliminary results led to the examination of behavioral regulation in bivalves and extended exposure. The resulting data provided for subsequent testing of the sensitivity of these bivalves for biological monitoring purposes.

Bivalves investigated for use as biological indicators of water quality were the freshwater Asiatic clam *Corbicula fluminea* Muller, 1774 and estuarine, hooked mussel *Ischadium recurvum* Rafinesque, 1820 (Doherty, 1990; Graney et al., 1984).

Both species are in the subclass Lamellibrachia and widely distributed throughout the United States. *I. recurvum* is an indigenous species, but *C. fluminea* is an invasive bivalve having been introduced into U.S. waters in the early 1900's (McMahon, 1982). Both bivalve species are hermaphroditic (McMahon and Williams, 1982), able to produce dispersal filaments (McMahon, 1982), and known primarily as highly invasive fouling agents (Doherty, 1990; Graney et al., 1984; Chanley, 1970). The numerous similarities between these species allowed for comparative study of relative tolerance to organophosphate pesticides.

Bivalves of the subclass Lamellibranchia are filter feeders that posses a greatly enlarged, specialized gill. The gill provides a large exposed surface area for feeding and respiration. Bivalves are often isosmotic in marine environments and only slightly hyperosmotic in fresh waters. These adaptations minimize water loss across the osmotically permeable gill area. However, the relatively large area of permeable tissues utilized for respiration and feeding facilitates rapid uptake and storage of lipophilic pesticides and metals.

Kramer and co-workers (1989) noted that the concentration of pollutants in molluscs can serve as an indicator for the level of pollution in the environment. Bivalves accumulate some toxicants, making them suitable for the characterization of specific ecosystems. An equilibrium concentration can be obtained after only several weeks of exposure (Kramer et al., 1989). Stirling and Okumus (1994) stated that it is well known that high concentrations of contaminants can play an important role as stressors to mussels. Mussels can respond to stress by valve closure, inhibition of byssal thread production, decreased respiration and filtration rate, and as a consequence, poor growth (Stirling and Okumus, 1994).

Organophosphorus insecticides have become an environmental concern. Their widespread use in agriculture and domestic lawn and garden applications have led to the contamination of aquatic environments via runoff and wastewater treatment plant discharge. Two organophosphorus pesticides are diazinon [0,0-diethyl 0-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate] and chlorpyrifos [0,0-diethyl 0-(3,5,6-trichloro-2 pyridinyl) phosphorothioate]. Diazinon is sold under several trade names including Spectracide, Sarolex, and Diazitol, among others. Although the sale of chlorpyrifos has recently been restricted, annual production of diazinon is almost 4 million kg in the United States (Robertson and Mazzella, 1989).

Organophosphorus pesticides act at nerve endings primarily by phosphorylation of the acetylcholinesterase enzyme (AChE; Gysin and Margot, 1958). Inhibition of AChE retards normal control of nerve impulse. Organophosphates are efficiently absorbed via inhalation, ingestion and diffusion through permeable membranes including the epidermis. Breakdown of the pesticide within an organism occurs predominately through hydrolysis. However, if the breakdown is slow, as is the case for some organophosphorus pesticides, the toxicants may be stored in body fat tissues (Morgan, 1989; Eckert et al., 1988).

The experiments reported here were designed to evaluate the toxicity of organophosphorus pesticides to a freshwater and an estuarine bivalve species. Results were utilized in detemining the usefulness of these species for water quality monitoring in these environments.

METHODS—*96-Hour LC_{50} values*—Utilizing existing LC_{50} data for bivalve molluscs, multiple range finding tests were performed on adult, field collected organisms from areas of high population density. Forty *I. recurvum* (four replicates of ten) were exposed to each concentration in a dilution series of diazinon or chlorpyrifos aqueous solutions and a filtered sea water control. Twenty-four *C. fluminea* (four replicates of six) were exposed to each concentration in a dilution series of a diazinon aqueous solution with reconstituted hard water control. Diazinon was obtained from the commercial product Spectracide: Lawn and Garden Insect Control, which is 25% active ingredient (diazinon) and 75% inert ingredients. Chlorpyrifos was purchased in the form of ORTHO: Dursban Lawn and Insect Spray containing 5.3% chlorpyrifos and 94.7% inert ingredients. Stock solutions were prepared daily by pipetting the pesticide product into 2 L of the respective control medium. Serial dilutions were made daily from 1 L of the stock for a total of seven concentrations in *I. recurvum* testing and five diazinon exposure concentrations for *C. fluminea* testing.

Static exposures were performed in covered 500-mL Carolina dishes. The 300 ml exposure solutions were replaced daily. Mortality was observed as failure of valve closure upon prodding. Mortality was recorded daily upon static renewal and dead clams were removed. Organophosphate concentrations were quantified via GC analysis. Probit analysis was performed on the data if there were at least two partial kills and a monotonically increasing response. If those assumptions were not met, the Trimmed Spearman-Karber method was used to analyze the data.

Behaviorally controlled exposures—The response of *C. fluminea* to diazinon exposure was further examined under behavior-regulated exposures. Ninety-six clams were used in this assay. Twenty-four clams were exposed in four replicates of six clams to the highest concentration (15,251 µg/L) used for the 96-hour LC_{50} estimation. A second set of twenty four mussels were exposed simultaneously to the same concentration. Individuals in the second group of mussels were bound after the initial 24 hours of exposure to eliminate periodic "re-sampling" of the exposure solution. Mussels were bound with cross-sections of 0.5 inch tygon tubing forced over the shell perpendicular to the hinge. The same procedure was followed for 48 clams exposed to reconstituted hard water. All replicate solutions were replaced daily. Mortality was recorded daily upon static renewal and dead clams were removed for unbound clams. Bound clams were not examined for mortality during the controlled behavior portion of the exposure.

After 96 hours of exposure, all replicates in each concentration were placed in clean reconstituted hard water. At this time all bound clams were unbound by removing the tygon cross-sections. The clams were left in the reconstituted hard water rinse for 48 hours. At 24-hour periods, the clams were re-examined for mortality. Mortality was compared between bound and unbound clams in each of the two exposure concentrations to determine the influence voluntary "re-sampling" had on diazinon toxicity.

Tests of 21-day exposures—The extreme tolerance of *C. fluminea* to diazinon, even when the behavior was not restricted, prompted an additional test to examine the LC_{50} of *C. fluminea* over 21 days of exposure. The exposures were carried out and the results analyzed in the same manner as was the 96-hour LC_{50} test (static renewal in covered 500-mL Carolina dishes).

Diazinon and control solutions were prepared and replaced daily. Six diazinon concentrations were used for exposure. The highest concentration (3,022 µg/L) was doubled in volume and used as a stock solution for serial dilutions to achieve the five lower concentrations. Mortality was recorded daily and dead clams were removed before static renewals. The experiment exposed 18 clams (3 replicates of 6 clams) to six diazinon concentrations and a reconstituted hard water control.

RESULTS—Ischadium recurvum *diazinon LC_{50} determination*—The diazinon 96-hour LC_{50} for the hooked mussel was estimated to be 1,354 µg/L with a 95% Upper Confidence Limit (UCL) of 1,501 µg/L and 95%(LCL) of 1,041 µg/L. The highest concentration in this analysis caused 100% mortality. However, one higher concentration that did not cause complete mortality was omitted from the analysis due to the inability of both statistical programs to compute median toxicity.

TABLE 1. Aqueous diazinon concentrations (μg/L, ppb), number of mussels exposed to each concentration, cumulative mortality and percent mortality at 96-hours from a 96-hour LC_{50} estimation assay for *Ischadium recurvum*.

Concentration	No. exposed	No. dead at 96-h	% Mortality at 96-h
Sea-water control	40	3	7.5
1,456	40	26	65
1,715	40	31	77.5
2,300	40	39	97.5
3,800	40	40	100
5,480	40	38	95
7,040	40	40	100
8,950	40	40	100

Subsequently, the data showed a monotonic response and were homogeneous. Exposure concentrations and mussel mortality are provided in Table 1.

Ischadium recurvum *chlorpyrifos LC_{50} determination*—The data from the exposure of *I. recurvum* to chlorpyrifos (Table 2) were analyzed with the Trimmed Spearman-Karber analysis with a 20% trim. The chlorpyrifos 96-hour LC_{50} for the hooked mussel was estimated to be 960 μg/L with a 95% UCL of 1,040 μg/L and a 95% LCL of 890 μg/L. The upper 95% confidence in the chlorpyrifos LC_{50} estimate was approximately equal to the lower 95% confidence about the diazinon LC_{50} estimate (1,041 μg/L), which may suggest that the estimated LC_{50} values are different.

Corbicula fluminea *diazinon LC_{50} determination*—The 96-hour diazinon LC_{50} for *C. fluminea* was estimated to be 4,067 μg/L with a lower 95% confidence of 2,847 μg/L and an upper 95% confidence of 5,814 μg/L diazinon. The ability of clams to isolate themselves from their immediate environment may, in part, explain the high diazinon resistance and the non-monotonic response observed as the concentrations increased by large amounts. Exposure concentrations and mussel mortality are provided in Table 3.

Behaviorally-controlled exposures—Behaviorally-regulated exposures (clams manually closed with tygon tubing) demonstrated noteworthy changes in the tolerance of *C. fluminea* exposed diazinon. After the initial 24-hour exposure, four mortalities occurred for each group of twenty-four clams in the high diazinon concentration. No mortality was observed for the total forty-eight clams in reconstituted hard water. Unbound clams in diazinon exposures continued to suffer mortality throughout the diazinon exposure. Twenty-one unbound clams died during the 96-hour exposure to diazinon. However, no deaths occurred in the bound clams up to the 48-hour post exposure period. No mortality of bound or unbound control clams occurred in reconstituted hard water during the 96-hour exposure or in the 48-hour post exposure period.

TABLE 2. Aqueous chlorpyrifos concentrations (μg/L, ppb), number of mussels exposed to each concentration, and cumulative mortality and percent mortality at 96-hours from a 96-hour LC_{50} estimation assay for *Ischadium recurvum.*

Concentration	No. exposed	No. dead at 96-h	% Mortality at 96-h
Sea-water control	40	3	7.5
772	40	5	12.5
1,134	40	33	82.5
2,550	40	38	95
4,170	40	38	95
7,728	40	39	97.5
11,528	40	40	100

Test of 21-day exposure—The 21-day diazinon LC_{50} estimation for *C. fluminea* was based on a Trimmed Spearman-Karber analysis. The 21-day diazinon LC_{50} for *C. fluminea* was estimated to be to 548 μg/L with a 95% lower confidence of 455 μg/L and a 95% upper confidence of 658 μg/L diazinon.

DISCUSSION—Ischadium recurvum *LC_{50} determination*—Acutely toxic organophosphate concentrations are high for the estuarine *I. recurvum.* This is not uncommon for many bivalve species. The U.S. Environmental Protection Agency's Aquatic Toxicity Information Retrieval database (AQUIRE) reports 96-hour EC_{50} values for *Crassostrea virginica* (American or virgin oyster) as high as 1,000 μg/L diazinon and 10,200 μg/L chlorpyrifos. The freshwater bivalve tested, *Corbicula fluminea*, has an estimated 96-hour LC_{50} value for diazinon of 4,067 μg/L with a 95% UCL of 5,814 μg/L and 95% LCL of 2,847 μg/L. High organophosphate tolerances have also been reported for more estuarine species. The estimated 96-hour LC_{50} for juvenile *Cyprinodon variegatus* (Sheepshead minnow) exposed to diazinon is 1,400 μg/L (Goodman et al., 1979) and juvenile *Opsanus beta* (Gulf toadfish) exposed to chlorpyrifos has an estimated 96-hour LC_{50} concentration of 520 μg/L (Hansen et al., 1986). Despite the resistant nature of the freshwater clam *C. fluminea*, freshwater organisms are often much less resistant to organophosphates. *Lepomis machrochirus* (Bluegills) and *Salmo gairdneri* (Rainbow trout) have estimated 24-hour LC_{50} values of 52 μg/L and 380 μg/L diazinon, respectively (Cope, 1965). Some invertebrates are even more sensitive (*Ceriodaphnia dubia* 48-hour LC_{50}, 0.5 μg/L diazinon and *Hyalella azteca* 0.29 μg/L chlorpyrifos; AQUIRE, 1997).

The unidentified pesticide carriers ("inert" organic solvents) in which these active ingredients (diazinon and chlorpyrifos) are sold may have affected the results of the toxicity tests. If these carriers were toxic to the mussels or affected the toxicity of the active ingredients, the results may reflect the activity of these anonymous participants to some unknown extent.

The heterogeneity of the variances (caused by a non-monotonic response) was likely attributed to the unique abilities of mussels to isolate themselves from a toxic exposure to varying degrees, while still in the presence of that toxicant. Exposure is a function of the magnitude, duration and frequency with which an organism

TABLE 3. Aqueous diazinon concentrations (μg/L, ppb), number of clams exposed to each concentration, and cumulative mortality and percent mortality at 96-hours from a 96-hour LC_{50} estimation assay for *Corbicula fluminea*.

Concentration	No. exposed	No. dead at 96-h	% Mortality at 96-h
Hard water control	24	0	0
920	24	0	0
1,875	24	4	17
3,786	24	15	63
7,607	24	15	63
15,251	24	19	79

interacts with a biologically available toxicant. Bivalves have some control over the frequency and duration of such an interaction, providing the initial toxic insult is not acute. Mortality occurs under these conditions when bivalves cannot completely isolate themselves, periodically sample the environment, or have survived levels of toxic insult sufficient to cause mortality prior to isolation. The latter situation can be complicated by the fact that the isolated environment often already includes the toxicant, which is the trigger to isolate. Bivalves need be exposed to a toxic substance for a certain duration within a given frequency to have an acute reaction to it.

Corbicula fluminea *diazinon LC_{50} determination*—The estimated 96-hour diazinon LC_{50} for *C. fluminea* was high even when compared to other bivalve species such as *I. recurvum*. The ability of mussels to isolate themselves from their immediate environment may, in part, explain the high resistance and the non-monotonic response as the exposure concentrations increased by large amounts. Additionally, the high diazinon concentrations *C. fluminea* succumbed to are unlikely to be present in the environment unless extreme circumstances exist.

The ability of *C. fluminea* to incur such high levels of diazinon insult may have been partially explained by the ability to isolate themselves from the unsuitable environment through valve closure. The results of the toxicity test prompted an examination of the ability of *C. fluminea* to protect themselves from adverse exposure. This was examined in a regulated behavior study in which clams were forced closed for 72 hours after the initial 24 hours of exposure to diazinon.

Behaviorally-controlled exposures—When comparing manually-closed clam survival to those which were able to open at will, less mortality occurred in the behaviorally-regulated clams. In fact, no mortality occurred after clams were closed manually even though they were exposed to high diazinon concentrations for 24 hours prior to forced closure. Both bound exposures suggested that the clams could cope anaerobically for the 72-hour period during which they were manually closed. Isani and co-workers (1989) exposed the bivalve *Scapharca inaequivalvis* to sea water flushed with nitrogen to promote anaerobiosis for up to 96 hours without inducing mortality, but that the duration depends on the species. The combined results indicated that the clams were capable of isolating themselves from the unsuitable environment by means of voluntary valve closure.

Some clams which were not bound were observed actively siphoning the exposure solution. This was assumed based on the occurrence of gaped valves with siphons protruding in an open and active manner. The high mortality which did occur in unbound clams in diazinon solutions may have been the result of periodic "re-sampling" of the environment over the exposure period.

Tests of 21-day exposure—The estimated diazinon LC_{50} for *C. fluminea* over this extended time period may represent the toxicity of diazinon to the clams better than that determined for the 96-hour exposure, because it likely precludes their ability to isolate themselves from their environment and operate anaerobically. The diazinon concentration (458 μg/L) that *C. fluminea* could withstand was still high in comparison to many aquatic species. For example, 96-hour diazinon LC_{50} values have been reported at 136–500 μl/L for *Lepomis macrochirus* and 100–1,000 μg/L for *Salvelinus fontinalis*. Invertebrates such as *Pteronarcys californica* (96-hour $LC_{50} = 25$ μg/L) and *Hyalella azteca* (96-hour $LC_{50} = 6.5$ μg/L) are often even less tolerant of diazinon exposure (AQUIRE, 1997). However, this high level of exposure is not likely to be present in the environment in a manner that would persist for three weeks unless conditions were extreme.

CONCLUSIONS—The toxicity of organophosphorus pesticides to *I. recurvum* and *C. fluminea* was found to be relatively low. In the case of *C. fluminea*, toxicity was low even when compared to other resistant bivalves. The ability of the bivalves to isolate themselves from a toxic insult provided variable results. The toxicity of diazinon to *C. fluminea* was dependent on valve closure and the same could be assumed for *I. recurvum*. The dramatically increased survival for clams that were not provided the opportunity to periodically re-sample their toxic environment may suggest that toxicity for bivalves is a more complicated matter than for other organisms. However, the inability of bivalves to flee such insults may only be somewhat compensated for by their ability to isolate themselves within the unfavorable environment itself.

The tolerance of *C. fluminea* to diazinon is remarkably high even when such isolations are less of a factor as could be seen by the 21-day exposure. Although it is unknown how long *C. fluminea* can voluntarily isolate itself via valve closure, the behaviorally regulated test suggests that such voluntary isolations do not occur for extended periods. The very low susceptibility of *C. fluminea* to organophosphates is likely to be a function of something other than behavior and permeability alone.

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RARITY AND CONSERVATION OF FLORIDA SCRUB PLANTS

Jaclyn M. Hall[(1)] and Thomas W. Gillespie[(2)]

[(1)]Department of Geography, University of Florida, P.O. Box 117315, Gainesville, FL 32611-7315
[(2)]Department of Geography, University of California Los Angeles, Los Angeles, CA 90095-1524

Abstract: *Florida scrub is a globally imperiled ecosystem with high endemism. This study examined the distribution of 38 plant species associated with the Florida scrub habitat in 20 remaining fragments in Pinellas, a county that has experienced a 99% reduction in scrub area since 1900. In particular, we identify if natural history (dispersal type, life cycle strategy, life-form, habitat specificity) and biogeographic (range type) characteristics associated with selective extinction can be used to predict rare species by incidence in remaining fragments. There were few associations or significant differences among natural history characteristics and rarity in Florida scrub. However, anemochory was negatively associated with species richness suggesting that fragments with low species richness contain a high proportion of wind-dispersed species. The biogeographic characteristic of range type (Florida endemic vs. non-endemic) was the best predictor of species incidence at a local scale, and regional scale distributions of species in Florida were associated with local scale distributions in remaining fragments. A conservation assessment of species and remaining fragments is discussed relating to protection status of all sites and focusing on sites with high diversity of scrub species, scrub obligates, and endangered species.*

Key Words: Florida scrub, fragmentation, natural history characteristics, rarity

Florida scrub refers to several phases of a xeric community characterized by sclerophyllus evergreen plants with patches of open, bare, infertile sand and little herbaceous development (Myers, 1990). These scrub communities, generally restricted to upland sites in peninsular and coastal Florida, are fire-maintained and fire-dependent ecosystems with an episodic fire frequency every 10–100 years (Abrahamson et al., 1984; Hawkes and Menges, 1996). Florida scrub has been extremely fragmented due to urban and rural development and there is an increasing interest in identifying and protecting remaining fragments (Myers and Ewel, 1990; Stout, 2001).

In general, most fragmented systems exhibit an imperfect but significantly nested pattern in which the species composition of the patches with lower species richness is a nested subset of patches with higher species richness (Patterson, 1987). For mammals and birds, a number of natural history characteristics associated with selective extinction, such as population densities, dispersal ability, reproductive success, and historical effects have been hypothesized to explain nested patterns (Patterson, 1987; Bolger et al., 1991; Culter, 1991; Bird and Boecklen, 1998). However, there is little empirical research on plant natural history characteristics and vulnerability to local extinction in habitat fragments, although there is no shortage of theories for why certain plant species should become locally extinct in fragmented systems like Florida scrub (Howe, 1984; Heywood et al., 1994; Davies et al., 2000).

The extinction of large mammals and birds in successively smaller fragments of Florida scrub has been well documented, while the secondary extinction of vertebrate-dispersed plants, although often proposed, is poorly documented (Howe, 1984; McCoy and Mushinsky, 1994; da Silva and Tabarelli, 2000). Fire frequency has decreased in remaining fragments of Florida scrub, especially in urban areas, and this can result in the invasion of hardwood hammock vegetation (Myers, 1990; Hawkes and Menges, 1996). This may result in the selective extinction of certain plant life cycle strategies or life-forms (Menges, 2000). Annual plant species, that generally prefer open areas, may be at a competitive disadvantage compared to perennial plants in remaining scrub fragments (Menges and Kohfeldt, 1995). Herbs have been identified as more extinction-prone than woody plants as a result of fragmentation and fire suppression (Quintana-Ascencio and Menges, 1996). Habitat specificity may also be associated with selective extinction in fragmented systems. In particular, species restricted to one habitat type may be more susceptible to local extinction than species that occur in two or more habitat types (Rabinowitz, 1986; Gaston, 1994). Finally, the regional scale biogeography of species may be used to identify extinction- prone species at a local spatial scale. Species with small ranges may be more extinction-prone than species with large ranges (Terborgh and Winter, 1980; Rabinowitz, 1986). Although this pattern is not a mechanistic explanation of selective extinction, a number of studies have recently noted that regional scale patterns can predict 75% of local scale patterns (Gaston, 2000).

Florida scrub is a globally imperiled ecosystem with high endemism and many plant species are restricted to only a few fragments (Stout et al., 1988; McCoy and Mushinsky, 1994; Ricketts et al., 1999). Scrub communities within the gulf coastal region of Florida have been poorly studied and there are no published studies or inventories on plant communities for a number of counties in Florida, including Pinellas County, which is the most densely populated and urbanized county in Florida (Brewer and Suchan, 2001; Stout, 2001). Pinellas, historically, was covered with more than 1800 ha of Florida scrub but urban development has resulted in a 99.9% reduction in scrub's original extent in this county (Hall, 2002). A systematic conservation assessment of remaining fragments and species could provide insight into conservation priorities for Pinellas County and the State of Florida (Marguales and Pressey, 2000).

This research on Florida scrub has two primary objectives. First, we will identify if natural history and biogeographic characteristics can be used to identify rare species by incidence in remaining fragments of Florida scrub. Second, we will undertake a conservation assessment of Florida scrub species and remaining fragments in Pinellas County.

Study Area—This study was undertaken in Pinellas County, Florida, located on the central West Coast of Florida. Pinellas County is a large peninsula that historically was covered with flatwoods, sandhills, and Florida scrub (Myers and Ewel, 1990). Today, natural scrub vegetation is restricted to private lands and seven city and county parks. This research was undertaken in 20 of the largest and best remaining fragments of Florida scrub in Pinellas County. These fragments ranged is size from 78 ha to 0.5 ha with half of the fragments being less than 5 ha.

METHODS—A list of Florida scrub species was created to assess scrub species richness in remaining fragments in Pinellas County. The scrub species list included native vascular plants that are either endemic or near endemic to Florida scrub according to Wunderlin (1998). Extensive searches were undertaken at all sites to determine the presence/absence of scrub species. Fieldwork was undertaken from January 2000 to November 2001. All sites were visited a minimum of five times throughout the years to ensure the identification of fertile specimens of all scrub species (Hall, 2002).

Natural history and biogeographic data were collected for all scrub species. Species were classified as autochory (self dispersal), anemochory (wind dispersal), or zoochory (animal dispersal) (van der Pijl, 1982). The life cycle strategies of all species were classified as annual or perennial and the life-forms of all species were classified as herbaceous or woody (Bell and Taylor, 1982; Nelson, 1996; Taylor, 1998; Wunderlin, 1998). Habitat specificity identified species as obligate to Florida scrub or facultative species that can occur in other xeric upland communities. All species were classified as obligate or facultative based on field observations, voucher specimens in the University of South Florida herbarium, and habitat descriptions in Wunderlin (1998). Species range type was classified as endemic to the State of Florida or non-endemic. Data on species regional distributions in Florida was calculated as the number of counties ($N = 67$) for which a species has been recorded (Wunderlin, 2000). Vulnerable species were identified from a state list of threatened and endangered plants (FNAI, 2000).

Data analysis—A nestedness score was calculated following methods used by Atmar and Patterson (1993). Nestedness scores range from 0, which represents a perfectly nested system, to 100, which represents a random subset. A Spearman's rank correlation was used to identify if there is a significant association between species richness at each site and the proportion of different dispersal types, life cycle strategies, life-forms, habitat specificity, and range type at each site. Mann-Whitney U tests were used to identify significant differences between natural history characteristics of species and incidence of species at the 20 study sites. This identifies significant differences between incidence of species and dispersal types, life cycle strategies, life-forms, habitat specificity, and range type. A Spearman's rank correlation was also used to identify an association between the regional distribution of species in Florida and local distribution of species by incidence in Pinellas County.

Conservation priorities for scrub species were assessed over two spatial scales. Local or county conservation priorities were based on species incidence in Pinellas County. Regional or state conservation priorities were based on the number of counties in which the species has been recorded. Fragment conservation priorities were based on the number of threatened and endangered plants at each site.

RESULTS—*Natural history of Florida scrub species*—There were 38 species identified as associated with Florida scrub in Pinellas County, Florida (Appendix 1). Scrub species richness by site ranged from 30 to 10 species. No species occurred in all 20 sites, although four species were recorded in 19 sites. Dispersal types in scrub plants were dominated by autochory (22 sp.), followed by anemochory (11 sp.), and zoochory (5 sp.). Most species were perennial (23 sp.) as compared to annual (15 sp.) and a majority of species were herbaceous (30 sp.) as compared to woody (8 sp.). Based on habitat specificity, 18 species were classified as obligate or restricted to Florida scrub and 20 species can occur in other xeric pine communities. There were 13 species endemic to the state of Florida. Two species were listed as threatened by the State of Florida (*Lechea cernua* and *Garberia heterophylla*), and four species were listed as endangered (*Chrysopsis floridana*, *Lechea divaricata*, *Bigelowia nuttalii*, and *Asclepias curtissii*).

TABLE 1. Associations between species richness and the proportion of individual natural history characteristics by site.

Natural history	Spearman's rank correlation
Dispersal type	
Autochory	0.205
Anemochory	−0.530*
Zoochory	0.282
Life cycle type	
Annual	−0.042
Perennial	0.04
Life-form	
Herbaceous	0.263
Woody	−0.263
Habitat specificity	
Obligate	0.292
Facultative	−0.29
Range type	
Florida endemic	0.509*
Non-endemic	−0.509*

* Significance levels = $P < 0.05$.

Natural history characteristics and rarity—The nestedness score for all species and sites was 23.24 suggesting a relatively nested pattern. The proportions of natural history and biogeographic characteristics by site were compared with species richness at each site (Table 1). There was a significant negative correlation between anemochory and species richness and a positive correlation between species endemic to Florida and species richness. A comparison of natural history and biogeographic characteristics and incidence in scrub fragments found no significant difference for most categories (Table 2). There was a significant difference between incidence of Florida endemics and non-endemics. When species incidence in Pinellas was compared to species distribution in Florida, there was a significant positive correlation (Spearman's rank correlation coefficient = 0.557, $P < 0.0001$).

Conservation priority of species and sites—Rare species were ranked by incidence within Pinellas County and Florida (Table 3). Three species were recorded in only one site, three species were recorded in only two sites, and three species were recorded in only three sites. These species should receive a high conservation priority within the county. When species were ranked by incidence in Florida, only two species were recorded in less than 10 counties and deserve a high conservation priority at the State level. Big Scrub, Boyd Hill South, Boyd Hill North and Lansbrook fragments deserve a high conservation priority within Pinellas based on endangered and threatened species richness (Table 4). Sites with greater species richness did not necessarily have the most vulnerable species.

TABLE 2. Comparison of species incidence in 20 fragments of Florida scrub and natural history characteristics.

Natural history characteristics	Mann-Whitney U test significance levels
Autochory vs. anemochory	0.693
Autochory vs. zoochory	0.485
Anemochory vs. zoochory	0.913
Annual vs. perennial	0.416
Herbaceous vs. woody	0.847
Obligate vs. facultative	0.136
Florida endemic vs. non-endemic	0.019

DISCUSSION—*Predicting rarity*—Florida scientists have long been interested in predicting species richness and rarity in habitat fragments (Gaston, 1994). Species richness in habitat fragments is relatively easy to predict because the species-area relationship is one of the most significant and well-documented patterns in ecology. Indeed, species richness of Florida scrub plants in Pinellas County was significantly correlated with the size of the scrub fragment (Spearman's rank correlation coefficient = 0.760, $P < 0.0001$) (Hall, 2002). However, predicting species composition or which species will become locally extinct and hence rare has been problematic.

Obviously, the best method for examining rarity in plants is extensive field surveys, but this had not been done in Pinellas County and has not been done for a number of counties in Florida (Stout, 2001). Population viability analysis is one method for predicting local extinction and rarity. However, this method requires extensive long-term studies, generally over five years, of individual species and their detailed life history characteristics such as plant dormancy, seed dormancy, periodic recruitment, clonal growth, and models of stochastic growth rates, metapopulation dynamics, and disturbance cycles (see Menges, 2000 for review). Another method for possibly predicting rarity could be through identifying natural history and biogeographic characteristics associated with local extinction (Davies et al., 2000; Gillespie, 2001). There are a number of distributional databases and regional floras

TABLE 3. Conservation priority of rare plants based on distribution in Pinellas Country and Florida.

Species	Incidence in Pinellas	Species	Incidence in Florida	Incidence in Pinellas
Paronychia rugelii	1	*Bigelowia nuttalii*	2	1
Bigelowia nuttalii	1	*Chrysopsis floridana*	4	3
Asclepias curtissii	1	*Dalea pinnata*	13	2
Polygonella robusta	2	*Stipulicida setacea*	13	16
Bulbostylis warei	2	*Polygonella ciliata*	14	4
Dalea pinnata	2	*Lechea divaricata*	16	6
Polygonella gracilis	3	*Paronychia rugellii*	17	1
Palafoxia feayi	3	*Lechea cernua*	17	10
Chrysopsis floridana	3	*Aristida gyrans*	17	14

TABLE 4. Conservation priority of remaining Florida scrub sites in Pinellas County based on species listed as endangered and threatened in Florida and species richness at each site.

Species	Boyd Hill South	Big Scrub	Boyd Hill North	Lansbrook	Weedon Island	Chautauqua East	Dunedin Cemetery	Alderman
Bigelowia nuttalii	X							
Asclepias curtissii		X						
Chrysopsis floridana	X		X					
Lechea divaricata	X	X	X	X	X			
Garberia heterophylla		X				X	X	X
Lechea cernua	X	X	X	X	X	X	X	X
Total	4	4	3	3	2	2	2	2
Species richness	29	30	24	24	15	21	14	23

that contain comparative natural history characteristics that may be used to predict rare species in areas not yet surveyed.

However, natural history characteristics of scrub plants were of limited utility for predicting rarity. Pinellas County has lost many animal vectors such as bears, Florida scrub jays, and a number of native rodents (Hall, 2002). However, there was no statistical evidence that dispersal mechanisms of scrub species makes a plant more vulnerable to local extinction, even though four species-poor sites contained no animal-dispersed species. The only significant association between species richness and dispersal type in our study was a negative association between richness and the proportion of wind-dispersed plants. This suggests that the proportion of wind-dispersed plants is greater in fragments with lower species richness. There were no associations or significant differences between life cycle type, life-form, or habitat specificity.

Biogeographic patterns were better at predicting rarity than natural history characteristics for scrub species in Pinellas County, Florida. A simple categorical classification of species as endemic to Florida or non-endemic, which is similar to the classification of natural history characteristics, was the best predictor of rare species. Furthermore, incidence of species in Florida is associated with the distribution of species in fragments within Pinellas County. This pattern suggests that the distribution of species at a regional scale may be used to predict the distribution of species at a local scale. A similar pattern has been noted for other taxa where species with large ranges at a regional scale also have the largest ranges at a local spatial scale (Gregory and Blackburn, 1998; Gaston, 2000). There are a growing number of regional databases that map the distribution of plant species that could be used to test this hypothesis as it relates to predicting patterns of rarity within fragmented systems. This suggests that regional scale data on distribution can be used as a first order assessment of distribution of species at a local scale for areas where little inventory data exists.

Most research on fragmented systems have focused on fauna and noted that nestedness is closely associated with habitat area. Small mammals on mountaintops

(3.8), land birds on islands in Baja California (7.2), and land birds on Queen Charlotte Islands, Canada (15.9), all have lower nestedness scores than our results (23.24) (Atmar and Patterson, 1993). It may be the case that plants in fragmented systems are not as nested as animals because small fragment size may be less detrimental to a plant population than an animal population. However, more research is needed on nestedness in plant communities to determine if this is a general biogeographic pattern.

CONCLUSION—*Conservation priority for species and sites*—At a local scale, the nine species that are distributed in three or fewer fragments (Table 3) deserve the highest conservation priority. It should be noted that only three of these plants are listed as threatened or endangered by the state (FNAI, 2000). It may be the case that all plants with low incidence in Pinellas should be monitored at the state level because they appear to be susceptible to the habitat fragmentation that is occurring throughout Florida (Myers and Ewel, 1990; Stout, 2001). At a regional scale, *Bigelowia nuttalii* and *Chrysopsis floridana* deserve the highest conservation priority because they are restricted to fewer than five counties. Both species have been identified as endangered by the state (FNAI, 2000).

The seven scrub fragments in Table 4 deserve the highest priority for conservation. Three of these fragments (Boyd Hill South, Boyd Hill North, Weedon Island) are protected parks while the other four fragments have no protection. Big Scrub contained four threatened and endangered species and had the highest species richness. This site is not protected, and, in our opinion, deserves the highest priority for conservation in Pinellas County. Although the state's more coastal scrub sites contain fewer restricted range species than better-studied scrubs of Florida's central ridge system, we believe every county should make protecting endangered scrub habitats a high priority.

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APPENDIX 1. Scrub species in Pinellas County, Florida.

Family	Scientific name	Incidence
APOCYNACEAE	*Asclepias curtissii**	1
ASTERACEAE	*Pityopsis graminifolia*	19
ASTERACEAE	*Palafoxia integrafolia*	16
ASTERACEAE	*Palafoxia feayi*	3
ASTERACEAE	*Garberia heterophylla**	7
ASTERACEAE	*Chrysopsis floridana**	3
ASTERACEAE	*Carphephorus corymbosus*	12
ASTERACEAE	*Bigelowia nuttalii**	1
ASTERACEAE	*Balduina angustifolia**	12
CARYOPHYLLACEAE	*Stipulicida setacea var. lacerata*	16
CARYOPHYLLACEAE	*Paronychia rugelii*	1
CARYOPHYLLACEAE	*Paronychia americana**	8
CISTACEAE	*Lechea torreyi*	15
CISTACEAE	*Lechea divaricata*	6
CISTACEAE	*Lechea deckertii*	8
CISTACEAE	*Lechea cernua**	10
CISTACEAE	*Helianthemum nashii**	4
CISTACEAE	*Helianthemum corymbosum*	19
CLUSIACEAE	*Hypericum reductum**	4
COMMELINACEAE	*Callisia ornata**	7
CYPERACEAE	*Rhynchospora megalocarpa**	10
CYPERACEAE	*Bulbostylis warei**	2
CYPERACEAE	*Bulbostylis ciliatafolia*	19
EMPETRACEAE	*Ceratiola ericoides**	7
ERICACEAE	*Lyonia fruticosa*	9
ERICACEAE	*Lyonia ferruginea**	15
FABACEAE	*Galactia regularis*	19
FABACEAE	*Dalea pinnata var. adenopoda*	2
OLEACEAE	*Osmanthus megacarpus**	5
PINACEAE	*Pinus clausa**	18
POACEAE	*Aristida gyrans**	14
POLYGONACEAE	*Polygonella robusta*	2
POLYGONACEAE	*Polygonella polygama*	12
POLYGONACEAE	*Polygonella gracilis*	3
POLYGONACEAE	*Polygonella ciliata*	4
SCROPHULARIACEAE	*Gratoila hispida*	11
SCROPHULARIACEAE	*Seymeria pectinata*	10
SELAGINELLACEAE	*Selaginella arenicola**	12

* = Obligate to Florida scrub.

SHORT-TERM EFFECTS OF NUTRIENT ADDITION ON GROWTH AND BIOMASS OF *THALASSIA TESTUDINUM* IN BISCAYNE BAY, FL

E. A. Irlandi[1*], B. A. Orlando[2], and W. P. Cropper, Jr.[3]

University of Miami, Center for Marine and Environmental Analyses,
4600 Rickenbacker Causeway, Miami, FL 33149

Abstract: *Landuse in watersheds has a large impact on the quality of water and sediment in adjacent bays and estuaries. Changes in pore water nutrients and sediment composition due to anthropogenic impacts to the surrounding watershed may potentially influence the growth and biomass of rooted, estuarine vegetation. This study examined spatial patterns in growth and biomass of the seagrass* Thalassia testudinum *during the early spring growing season in an estuary with a highly disturbed watershed (Biscayne Bay, FL). In addition, the plants' response to nutrient addition via fertilizer spikes as a proxy to increased pore water nutrient levels was evaluated. Spatial differences in biomass occurred among the four sites with those on the western side of the bay that receive nutrient inputs from terrestrial run off having greater biomass per* m^2 *than the two sites on the eastern side of the bay. The addition of nutrients via fertilizer spikes did not influence biomass, but the production of new plant tissue (*mm^2*/day) as a measure of growth was increased by an average of 45% with the addition of fertilizer spikes at all sites. The results indicate that the structure (biomass) of these subtropical* T. testudinum *meadows in an estuary with a highly disturbed watershed did not change with short-term nutrient addition, but the function of the meadows was altered through enhanced production.*

Key Words: *Thalassia testudinum*, seagrass, growth, nutrients, Biscayne Bay, FL

Landuse practices in watersheds can have a large impact on the quality of the water and the sediment in adjacent bays and estuaries. Fertilizer applications to farms and homes, waste-water treatment, and septic systems contribute to increased nutrient loading that can fuel blooms of phytoplankton and/or macroalgae (e.g., Lapointe and Clark, 1992; Valiela et al., 1992; Duarte, 1995; McClelland et al., 1997; Valiela et al., 1997; Hauxwell et al., 1998, 2000; McGlathery, 2001). These blooms can reduce the penetration of light to the seafloor and contribute to the decline of seagrass. In addition to affecting seagrass beds via light reduction, death and decay of phytoplankton and macroalgae may contribute to increased nutrient loads and organic matter to sediments with resulting changes in pore water chemistry (Zimmerman and Montgomery, 1984; Sassi et al., 1988; Williams, 1990). Nutrient enrichment of the sediment can also occur as sediment particles and organic matter from land are carried by runoff and deposited to the sea floor (Trefry et al., 1992).

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[1] Current address: Department of Marine and Environmental Systems, Florida Institute of Technology, Melbourne, FL 32901. [2] Current address: South Florida Water Management District, West Palm Beach, Fl 33406. [3] Current address: School of Forest Resources and Conservation, University of Florida, Gainesville, FL 32611-0410.
* Corresponding author: irlandi@fit.edu

Seagrasses are rooted in the sediment and draw at least a portion of their nutrients from the pore water (Patriquin, 1972; Short and McRoy, 1984; Short, 1987; Fourqurean et al., 1992b; Stapel et al., 1996). Addition of nutrients and changes in sediment composition will affect pore water chemistry and may ultimately influence the growth and biomass of seagrasses. Experimental additions of nutrients to the pore water of seagrass meadows have produced a range of responses. In some instances growth and/or biomass have been enhanced while in others they have not, depending on the degree of nutrient limitation in a particular system and the species concerned (Orth, 1977; Bulthuis and Woelkerlin, 1981; Roberts et al., 1984; Dennison et al., 1987; Williams, 1987; Short et al., 1990; Erftemeijer et al., 1994; Fourqurean et al., 1995). Long-term nutrient additions have also been shown to influence species composition of seagrass meadows in South Florida. *Halodule wrightii* out-competes *Thalassia testudinum* in tropical systems with an increase in phosphorus in the sediment pore water associated with the addition of bird feces from rookeries and perches (Powell et al., 1989; 1991; Fourqurean et al., 1995).

Biscayne Bay is a subtropical estuary on the southeast coast of Florida, U.S.A (Fig. 1). The watershed for the bay is highly disturbed with intense urbanization along the northern portions and extensive agricultural development in the southern region. Flood-control canals drain this highly altered watershed resulting in significant modifications to the natural timing and quality of fresh water discharges to the bay. Instead of slow release sheet flow, much of the freshwater and terrestrially derived nutrients entering the bay are now in the form of point-source release from canals. In an effort to improve the health of the Everglades system located west of Biscayne Bay, the Army Corps of Engineers is in the process of making changes to the existing canal and drainage system (U.S. Army Corps of Engineers, 1999). The goal of the "re-plumbing" effort is to increase fresh water surface flow to the Everglades and Florida Bay. To achieve this, existing surface water flow currently discharged via canals to Biscayne Bay will be diverted to the west.

In an effort to evaluate the status of *Thalassia testudinum*, the major species of seagrass in Biscayne Bay, prior to the proposed changes in surface-water flow, field surveys were conducted to assess plant biomass, morphometrics, and production at several locations (Irlandi et al., 2001). *Thalassia testudinum* from sites on the western side of the bay that are heavily influenced by freshwater discharge from canals exhibited narrower leaf widths in all seasons and years sampled, and lowered production only during years of prolonged reductions in salinity associated with high rain fall amounts during the typically dry season. In the present study we tested if *T. testudinum* in Biscayne Bay, a subtropical estuary, is currently limited by pore-water nutrients and if the effect of nutrient addition to the sediment would be similar among sites of varying location. We accomplished this by adding tree fertilizer spikes to the sediment and measuring changes in growth and biomass of the seagrass at multiple sites.

METHODS—*Study sites*—Four sites were chosen to test the effects of nutrient addition on seagrass growth and biomass: Barnes Sound, Chicken Key, Sands Key and Broad Creek (Fig. 1). Barnes Sound

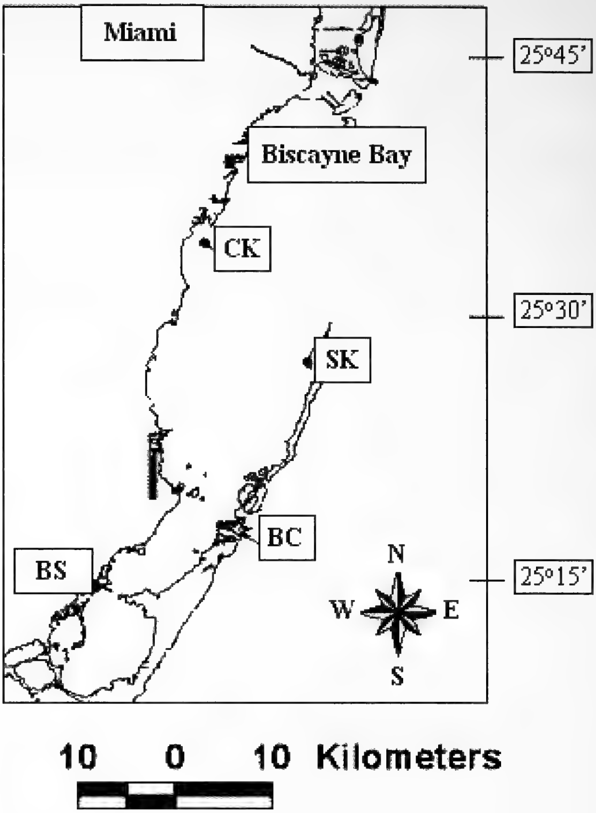

FIG. 1. Location of study sites in Biscayne Bay, Florida. CK = Chicken Key, SK = Sands Key, BC = Broad Creek, and BS = Barnes Sound.

and Chicken Key are located on the western side of the bay and are influenced by terrestrial sheet flow runoff from urban and agricultural areas. We chose sites that were not immediately adjacent to canal discharge points as the seagrass in these areas experience rapid and extreme fluctuations in salinity that contribute to reduced shoot density and biomass compared to sites on the western margin that are not immediately adjacent to canals (Irlandi et al., 2001). Sands Key and Broad Creek are located on the eastern side of the bay distant from point-source discharges from canals and with limited influence of terrestrially derived nutrients from adjacent undeveloped barrier islands.

Experimental set up—A 25-m long by 4-m wide grid was haphazardly established at each site with the long side of the grid running parallel to shore. Thirty evenly spaced (six across at 5-m spacing by five down at 1-m spacing) points were marked with wooden dowels (ca. 5-mm diameter and 1-m long) within each grid. Placement of fertilizer addition treatments was then determined using a randomized blocked design. Three points along each row (or block) of the grid were randomly assigned nutrient addition treatments to produce three treatment and three control points in each of the five rows of the grid.

Growth and biomass of seagrass—In February 1998, we inserted Jobes® tree fertilizer spikes (8% ammoniacal nitrogen, 18% P_2O_5, and 18% K_2O) into the sediment at the nutrient addition points. We initiated the experiment during the winter/spring dry season to limit potential interactions with salinity reductions that occur on the western side of the bay in association with run off and canal discharge during

TABLE 1. Results from 3-way randomized block ANOVA on the maximum daily growth rates measured as mm^2 tissue produced per day for *Thalassia testudinum* at four sites (random) in Biscayne Bay with and without the addition of tree fertilizer spikes (nutrients- fixed).

Source	df	MS	F	P
Site	3	194.46	3.59	0.037
Treatment	1	1616.35	152.39	0.0011
Block (site)	16	54.13	0.86	0.62
Site * treatment	3	10.61	0.16	0.92
Treatment * block	16	67.12	1.065	0.40
Error	80	63.03		

the rainy season. After approximately 5 weeks (sufficient time to allow nutrients to diffuse into the pore water—Williams, 1987; Erftemeijer et al., 1994) we marked individual shoots adjacent to each dowel for growth using standard leaf marking techniques (Dennison, 1990; Short and Duarte, 2001). The marked shoots were recovered 1-week later and the area of new leaf production was determined by multiplying the length of new growth by the width of the leaf. Since growth rates of seagrass leaves vary with age, we standardized all measurements to daily leaf area production of the youngest leaf on the shoot. We also assessed above-ground plant biomass for nutrient-enriched and control plots at each site by cutting the shoots at the sediment surface from a 400-cm^2 area (20 $\times$ 20 cm quadrat) adjacent to each wooden dowel ca. 8 weeks after the nutrient spikes were placed in the field. We chose to wait 8 weeks so that sampling of biomass would coincide with spring growth.

Separate 3-factor randomized block ANOVAs were used to analyze differences in the dependent variables, growth as a measure of leaf area produced by the youngest shoot and biomass, among blocks (random and represented by rows of the grid) nested within sites (random), among sites, and between nutrient conditions (fixed). Homoscedasticity of error variances was confirmed prior to analysis of leaf area produced (Cochran's test). Heteroscedastic error variances in the biomass data, however, required transformation of the data prior to analysis. Tukey's post hoc comparisons were employed to determine which sites differed from each other when statistical differences among sites were detected.

RESULTS—*Growth and biomass*—There was a significant difference in maximum daily growth rates measured as mm^2 of tissue produced per day among sites and between high and low nutrient conditions (Table 1). New tissue production was ca. 25%–35% higher at Broad Creek (24.1 mm^2/day $\pm$ 1.8 SE), than at Sands Key (19.7 $\pm$ 1.6), Chicken Key (20.9 $\pm$ 1.4), and Barnes Sound (18.0 $\pm$ 1.6) (Fig. 2a). Growth was also on average ca. 45% greater with the addition of nutrients than from control plots (24.4 $\pm$ 1.2 vs. 17.1 $\pm$ 0.9 mm^2/day, respectively) (Fig. 2b).

Estimates of total biomass varied among sites, but were not influenced by the addition of nutrients to the sediment (Table 2). Biomass estimates ranged from a high of 95.9 g/m^2 ($\pm$ 18.1 SE) to a low of 29.3 g/m^2 ($\pm$ 5.5 SE) and were higher at the sites on the western side of the bay (Barnes Sound and Chicken Key) than at the sites on the eastern side of the bay (Sands Key and Broad Creek) (Fig. 3).

DISCUSSION—Spatial patterns in growth and biomass, and the plants' growth response to nutrient addition suggest that *Thalassia testudinum* was nutrient limited at the locations studied in Biscayne Bay. Biomass was greater at the two sites on the western side of the bay that receive nutrient inputs from terrestrial run off (Tables 1 and 2; Figs. 2 and 3), and growth was increased with the addition of fertilizers at all

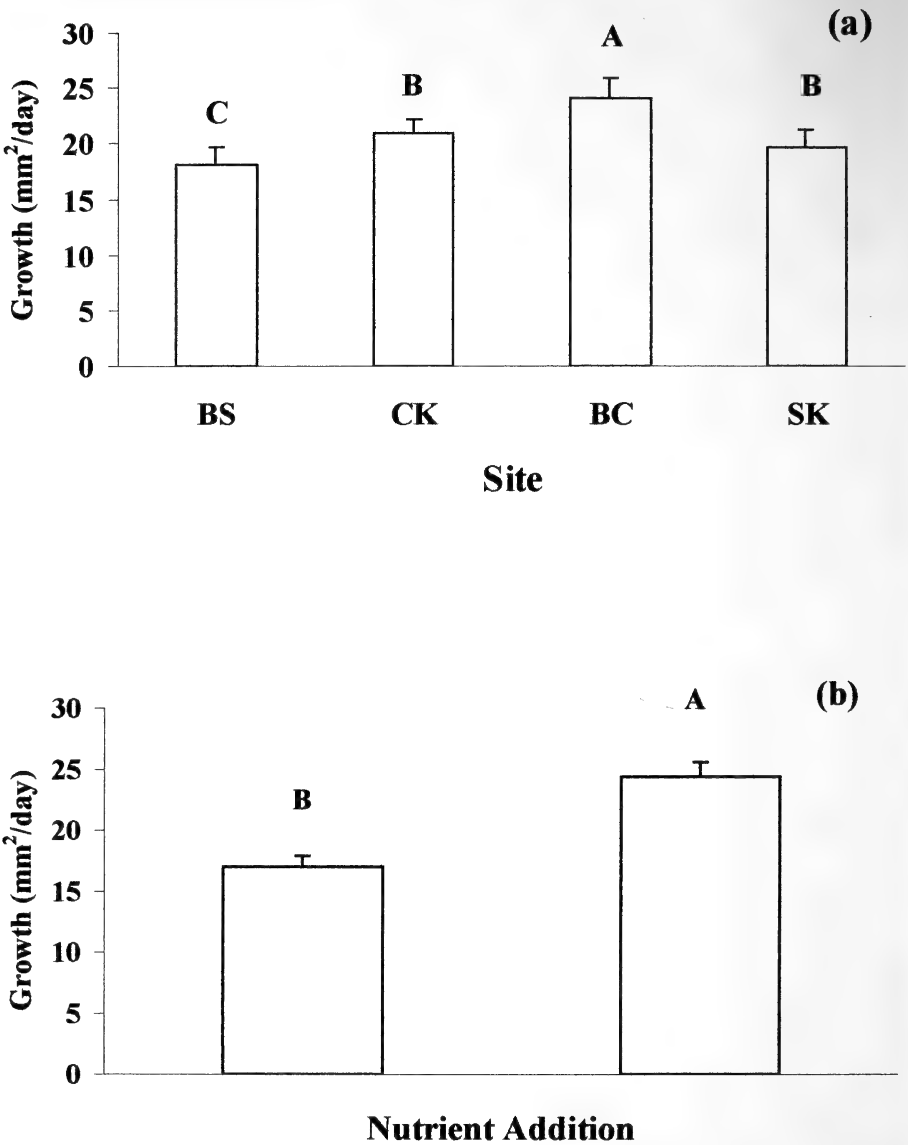

FIG. 2. The average daily growth (mm^2/day) for the youngest leaves on each shoot for each of the four study sites (a), and with and without the addition of nutrient spikes (b). ANOVA results are provided in Table 1. In this and all subsequent figures like letters denote sites that did not differ significantly in Tukey post hoc comparisons, and error bars represent ± one standard error.

sites (Table 1; Fig 2). Whether nitrogen or phosphorus is the limiting nutrient is unclear, however, and the type of limiting nutrient may vary spatially in the bay. In carbonate-dominated systems typical of tropical regions, phosphorus is typically the limiting nutrient due to adsorption of the phosphorus by carbonate sediments

TABLE 2. Results from 3-way randomized block ANOVA on biomass of *Thalassia testudinum* from 20 cm × 20 cm quadrats at four sites (random) in Biscayne Bay with and without the addition of tree fertilizer spikes (nutrients- fixed).

Source	df	MS	F	P
Site	3	23.19	69.70	0.00
Nutrients	1	0.021	1.46	0.31
Block (site)	16	0.33	0.74	0.74
Site * nutrient	3	0.014	0.025	0.99
Nutrients * block	16	0.59	1.31	0.21
Error	80			

making it unavailable for uptake by seagrass (Short, 1987). Studies in Florida Bay and the Bahamas have suggested that a positive relationship between phosphorus in the pore water and seagrass biomass is indicative of phosphorus-limited growth (Powell et al., 1989; 1991; Short et al., 1990; Fourqurean et al., 1992a). The carbonate composition of the sediments in Biscayne Bay varies spatially with marine carbonates dominating the sediments on the eastern side of the bay and terrigenous sediments and mangrove peats dominating the sediments along the mainland (Wanless, 1969; Wanless et al., 1995).

There are many biogeochemical processes that influence the concentration of nutrients in the pore water and in the sediment, and measures of pore-water or water-column nutrients do not necessarily represent nutrient availability as it does not take into account turnover times of nutrient pools (Howarth, 1988). Analysis of the nutrient content of tissue samples can provide an integrated assessment of long-term nutrient availability and elucidate patterns in nutrient limitation (Gerloff and Krombholz, 1966; Atkinson and Smith, 1983; Duarte, 1990; Fourqurean et al., 1992b; Fourqurean and Zieman, 2002). Plant tissues collected for another study in January 1998 from Chicken Key, Sands Key, and Broad Creek exhibited comparable N:P ratios for seagrasses at the three sites (40:1, 30:1, and 41:1, respectively) (Irlandi and Orlando, unpublished data). Other sites on the western side of the bay directly adjacent to canal discharge points, however, had ratios of ca. 60:1 demonstrating increased incorporation of nitrogen into tissues at sites that potentially receive nutrient inputs from canal discharge. These elevated N:P ratios would suggest that seagrasses in the bay are generally nitrogen limited and not phosphorus limited, but a more detailed investigation would be required to confirm this.

Our results from this and other investigations in Biscayne Bay demonstrate that growth and biomass of *Thalassia testudinum* in Biscayne Bay are variable in space and time and are influenced by many factors including salinity (Irlandi et al., 2001) and nutrients (this study). While there were spatial differences in biomass among sites, the addition of nutrients did not result in increased biomass, but did increase growth rates. This indicates that the structure of the seagrass meadows (e.g., biomass) did not change with nutrient additions over the time frame of our study, but the function of the meadows was altered through enhanced production. Continued nutrient enrichment of the pore water over longer time frames, however, may produce measurable differences in seagrass biomass.

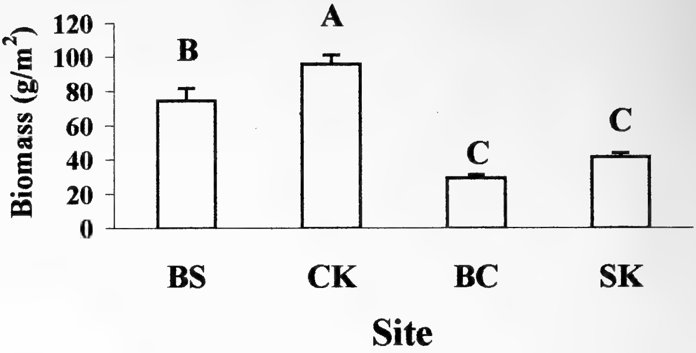

FIG. 3. The average above-ground biomass (g/m^2) of *Thalassia testudinum* for each of the four study sites. ANOVA results are presented in Table 2.

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RECENT OCCURRENCE OF THE SMALLTOOTH SAWFISH, *PRISTIS PECTINATA* (ELASMOBRANCHIOMORPHI: PRISTIDAE), IN FLORIDA BAY AND THE FLORIDA KEYS, WITH COMMENTS ON SAWFISH ECOLOGY

Gregg R. Poulakis[(1)1] and Jason C. Seitz[(2)]

[(1)]Florida Marine Research Institute, Florida Fish and Wildlife Conservation Commission, Charlotte Harbor Field Laboratory, 1481 Market Circle, Unit 1, Port Charlotte, Florida 33953-3815 and Department of Biological Sciences, Florida Institute of Technology, 150 West University Boulevard, Melbourne, Florida 32901-6975
[(2)]Collier County Environmental Services Department, 2800 North Horseshoe Drive, Naples, Florida 34104

Abstract: *Encounters with the smalltooth sawfish,* Pristis pectinata, *in Florida Bay and the Florida Keys were documented by soliciting information from anyone who might have encountered them in these areas. Each person who had information was asked the same series of questions to determine the date and location of the encounter, estimated total length (ETL), and habitat characteristics. A total of 1,632 sawfish encounters occurring between 1990 and 2002 were reported to us (89% occurred between 1998 and 2002). Sawfish were encountered during the day and night. Most sawfish were probably immature when encountered. The smallest sawfish (≤1 m ETL) were found in ≤2.4 m of water and virtually all larger immature sawfish (1.1–2.9 m ETL) were found in ≤10 m of water. The largest sawfish (≥3 m ETL) were found in shallow water (≤10 m) and deeper water (to 122 m). This study is the first to document the regular occurrence of smalltooth sawfish in water deeper than 10 m. A wide variety of habitat types were reported and included mud, sand, seagrass, limestone hard bottom, rock, coral reef, and sponge bottom. Most sawfish encounters in Florida occurred between March and August, though numerous encounters were reported throughout the year. Most encounters consisted of a single sawfish being observed or caught on hook and line, but groups of 2–20 similar-sized individuals were also reported. These data support our previous observation that the United States sawfish population is larger than previously estimated, and that sawfish are relatively common in south Florida.*

Key Words: Endangered species, Everglades, Florida Bay, Florida Keys, largetooth sawfish, nursery, *Pristis pectinata*, *Pristis perotteti*, *Pristis pristis*, ray, smalltooth sawfish

Sawfishes belong to a small group of batoid elasmobranchs that are cosmopolitan in coastal tropical and subtropical waters, including estuaries and freshwater (Bigelow and Schroeder, 1953; Last and Stevens, 1994). As the name implies, these fishes possess an elongated, blade-like snout (rostrum) that has lateral, tooth-like denticles (rostral teeth) set in sockets. The rostrum, often referred to as the "saw", is used during feeding and for defense (Breder, 1952; Compagno and Last,

[1] Correspondence: poulakis@comcast.net; www.floridasawfish.com

1999). Despite their worldwide distribution and distinct morphology, little is known about the biology of this group of fishes.

Although worldwide sawfish systematics remain unsettled (Compagno and Cook, 1995), two species of sawfishes (*Pristis pectinata* Latham, 1794 and *Pristis perotteti* Müller & Henle, 1841) have been historically recognized in the western Atlantic (*P. perotteti* has recently been referred to by some authors as *P. pristis*). Both western Atlantic species are found in similar habitats (Bigelow and Schroeder, 1953; McEachran and Fechhelm, 1998), but the largetooth sawfish, *P. perotteti*, typically ranges south and west of Florida (Baughman, 1943; Thorson, 1974). The vast majority of published records of sawfish encountered in Florida refer to the smalltooth sawfish, *P. pectinata*, with hundreds having been historically reported from both coasts of the state (e.g., Henshall, 1895; Evermann and Bean, 1898). The largetooth sawfish has only been reported three times in the state, the last time being prior to 1960 (Seitz and Poulakis, 2002). Thus, we assume that the extant sawfish in Florida are *P. pectinata*.

In Florida, smalltooth sawfish were regularly reported in early ichthyofaunal surveys (e.g., Henshall, 1891; Evermann and Bean, 1898), but for a variety of reasons the species became scarce in the state. Because of their unique morphology and large size, sawfish have been particularly susceptible to fishing nets (Evermann and Bean, 1898). Although sawfish were not targeted commercially, removing them from nets often involved killing the animals for logistical and safety reasons (Henshall, 1895). Fishing mortality, combined with K-selected life history, caused sawfish populations to decline in the United States during the nineteenth and twentieth centuries (Snelson and Williams, 1981; Hoenig and Gruber, 1990). Because of the population decline, conservation efforts directed toward western Atlantic sawfishes have been initiated from various organizations beginning in 1992, such as the World Conservation Union, the State of Florida, and the American Fisheries Society (IUCN, 1996; Florida Fish and Wildlife Conservation Commission, 1999; Musick et al., 2000). The smalltooth sawfish was listed as endangered in the United States under the Endangered Species Act on April 1, 2003 (United States National Marine Fisheries Service, 2003).

Seitz and Poulakis (2002) used an interview method to document the occurrence of smalltooth sawfish in Florida. This research demonstrated the existence of a reproductive population of sawfish in the United States, allowed us to take the first steps toward understanding sawfish ecology in Florida, and helped us realize that sawfish were being captured to the south of our original study area. The survey has been successful (despite obvious limitations) because it has bridged the gap that exists between scientists and laymen. Because of the success of this initial data collection effort, we expanded our encounter database and our methodology has been adopted by other sawfish researchers. Incidental captures of sawfish have been occasionally reported in the literature from Florida Bay and the Florida Keys (e.g., Tabb and Manning, 1961; Starck, 1968; Sogard et al., 1989), but to date, no studies have focused on sawfish in these areas of the state. The goals of this study were to: (1) document the location of recent (1990–2002) encounters with smalltooth sawfish in Florida Bay and the Keys and (2) further define aspects of sawfish ecology in Florida.

STUDY AREA—A wide range of habitats exist in the south Florida study area. Shallow, seagrass-covered, carbonate mudbanks are a common feature in Florida Bay, a large (1500 km^2) lagoonal estuary situated between the Florida Keys and the mainland (Sogard et al., 1989). The mudbanks are typically flat on top and slope into various relatively shallow basins. In general, mudbanks toward the eastern side of Florida Bay are long and narrow, and banks on the western side are wider and larger. Florida Bay supports macroalgal, mud bottom, and hard bottom communities. It has extensive mangrove and saltmarsh shorelines, and constitutes the southernmost portion of Everglades National Park (Fernald and Purdum, 1998). Since the late 1800s, the Everglades ecosystem, which naturally drained into Florida Bay, has been ditched and drained for farming and flood control. Today, about 80% of the water that historically passed through the Everglades and emptied into Florida Bay is discharged to east and west coast estuaries (Fernald and Purdum, 1998).

In contrast to Florida Bay, the Florida Keys and their associated coral reef tract have physiographic characteristics and species compositions similar to the tropical reefs of the Bahamas and the Caribbean (Fernald and Purdum, 1998). The reef tract is comprised of bank reefs (primary reef systems; 7 to 15 km seaward of the larger Keys), patch reefs (smaller, isolated reefs typically found between shore and bank reefs), and Dry Tortugas reefs (more staghorn coral, ca. 139 km west of Key West). In general, bank reefs consist of elongate limestone spurs (covered with macroalgae and living corals) and grooves (valleys of sand and rubble between the spurs). Patch reefs are typically found associated with seagrass beds in water ca. 2 to 9 m deep.

MATERIALS AND METHODS—Because of their unusual appearance and relatively large size, sawfish are easily recognizable and tend to represent a memorable experience for those who encounter them. Encounters with sawfish in Florida Bay and the Keys were documented by soliciting information from anyone who would possibly encounter these fish (e.g., recreational and commercial fishermen, fishing guides; Seitz and Poulakis, 2002). We also ran newspaper articles, appeared on local television fishing shows, and circulated posters that asked for anyone with any information on these fish since 1990 to contact us (by telephone, mail, e-mail, or website). Posters were distributed beginning in January 1999, and covered the area from Charlotte Harbor to Key West, Florida by April 2001. The posters were displayed where anglers and boaters would likely encounter them (e.g., bait and tackle shops, boat ramps). Each person that had information was asked the same series of questions about their encounter(s). The survey included determination of the date and location of the encounter, estimated total length (ETL), and habitat characteristics (e.g., water depth, habitat type). In cases where a person reported a size range for a fish, we used the midpoint of the range. Photographic or video documentation of encounters was obtained when available. All encounters were plotted on charts as exact points or in general areas (depending on the detail of the available information) and summarized using a geographic information system (GIS; Florida Fish and Wildlife Conservation Commission, 2000). In most cases, the exact location of the encounter(s) could be determined during the interviews. Although size at maturity is currently unknown for *P. pectinata*, we assume that this species matures at about 3 m total length or larger, based on size at maturity data for *P. perotteti*, and because *P. pectinata* reaches a larger maximum length (Thorson, 1976; Last and Stevens, 1994). Encounters reported in Seitz and Poulakis (2002) that occurred in northern Florida Bay are included for continuity purposes.

RESULTS—A total of 414 interviews were conducted that documented 1,632 sawfish encounters in Florida Bay and the Keys (i.e., south Florida) between 1990 and 2002 (89% occurred between 1998 and 2002; Figs. 1–2). Of these encounters, 88% were observations or hook and line captures and 9% were captured on longlines. Other methods reported that resulted in sawfish encounters included commercial and experimental gill nets, shrimp trawls, cast nets, seines, and lobster pot line entanglement, as well as sightings from helicopters, ultralite airplanes, and divers. Virtually all of the sawfish that were captured were bycatch of fishermen targeting sharks, tarpon (*Megalops atlanticus*), snook (*Centropomus undecimalis*), or red drum (*Sciaenops ocellatus*). Sawfish were encountered during the day and

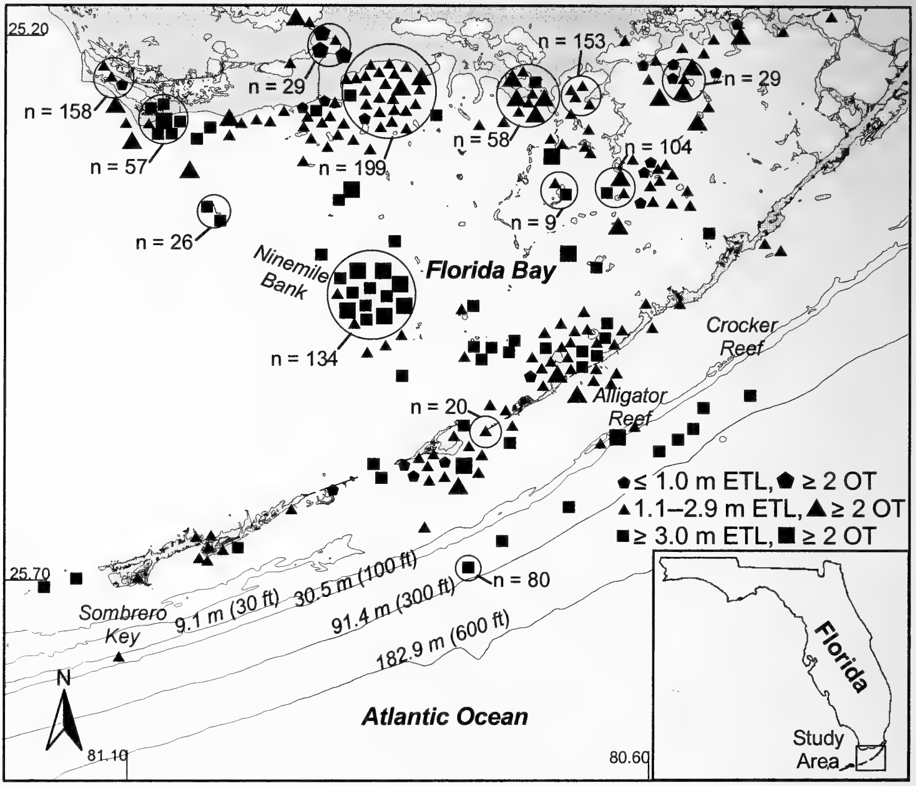

FIG. 1. Map of Florida Bay and the upper Florida Keys where at least 1,348 sawfish were encountered between 1990 and 2002. Each small symbol indicates an encounter with a single sawfish and each large symbol indicates ≥2 sawfish observed together (OT) in an estimated total length (ETL) size class. Sixty-six sawfish were reported from this section of the study area without specific location information.

night. It is important to note that several biases affect these data (e.g., more charter boat trips during the winter tourist season, most recreational fishing occurs during the day, longlines typically soak at night).

Size—Most sawfish encountered in south Florida probably had not reached sexual maturity when they were encountered (ca. 68% <3 m ETL; mean = 2.4 m ETL; Fig. 3). The same observation was made when all encounter information from Florida was examined (mean = 2.1 m ETL; Fig. 4). A total of 52 sawfish from south Florida were very small (≤1 m ETL), potentially young-of-the-year, 1,050 were larger (1.1–2.9 m ETL), but probably still sexually immature, and 519 were very large (≥3 m ETL), and probably sexually mature. Eleven sawfish were reported without length estimates.

Water depth—Of the sawfish encounters in south Florida where water depth was estimated (n = 642), 67% (regardless of size) came from water ≤10 m deep (Figs.

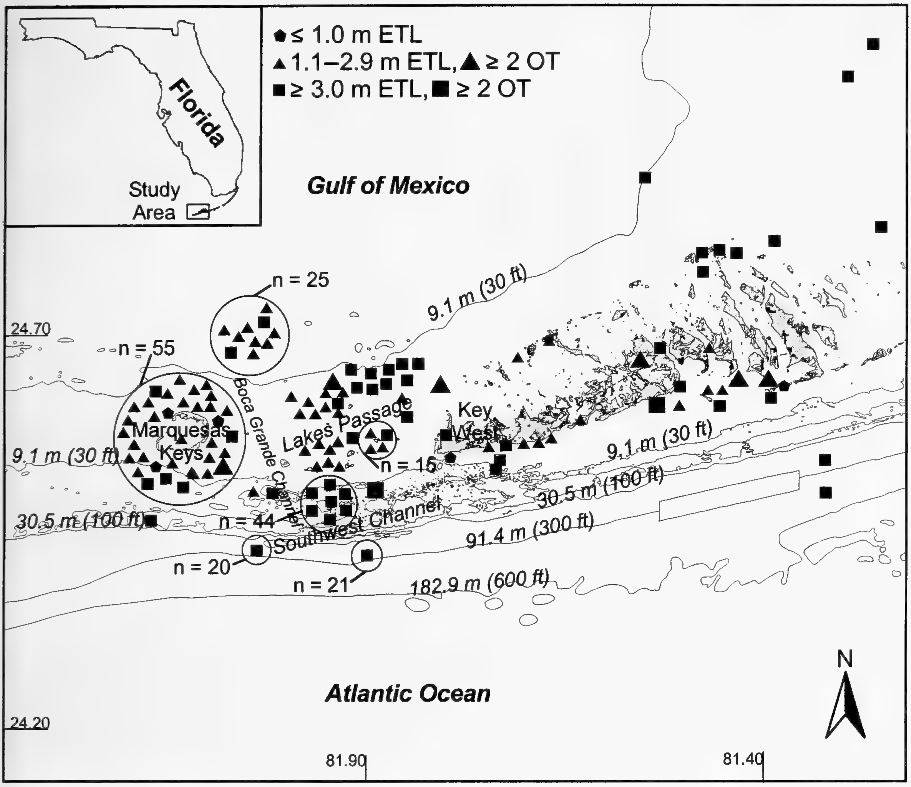

FIG. 2. Map of the lower Florida Keys where at least 260 sawfish were encountered between 1990 and 2002. An additional 24 encounters came from waters further west off the Dry Tortugas (not shown). Each symbol indicates an encounter with a single sawfish and each large symbol indicates ≥2 sawfish observed together (OT) in an estimated total length (ETL) size class.

1–2). Of the 33% that came from water >10 m deep, 70% were found on the bottom in >70 m of water. The smallest sawfish (≤1 m ETL) were found in ≤2.4 m of water. Virtually all (98%) of the larger immature sawfish (1.1–2.9 m ETL) were found in ≤10 m of water (maximum = 62 m). The largest sawfish (≥3 m ETL) were found both in shallow water (37% in ≤10 m of water) and deeper water (46% in >70 m of water; maximum = 122 m).

Habitat—The primary habitat types where sawfish were encountered in south Florida were: mud (61%), sand (11%), seagrass (10%), limestone hard bottom (7%), rock (4%), coral reef (4%), and sponge bottom (2%). Sawfish were also reported associated with sea fans, an artificial reef, a culvert pipe, a freshwater spring, and an oil rig.

Seasonality—Month or season was reported for many sawfish encounters in Florida. Of all encounters in the state since 1990 where the month of the encounter was known (n = 439), 78% occurred between March and August. However,

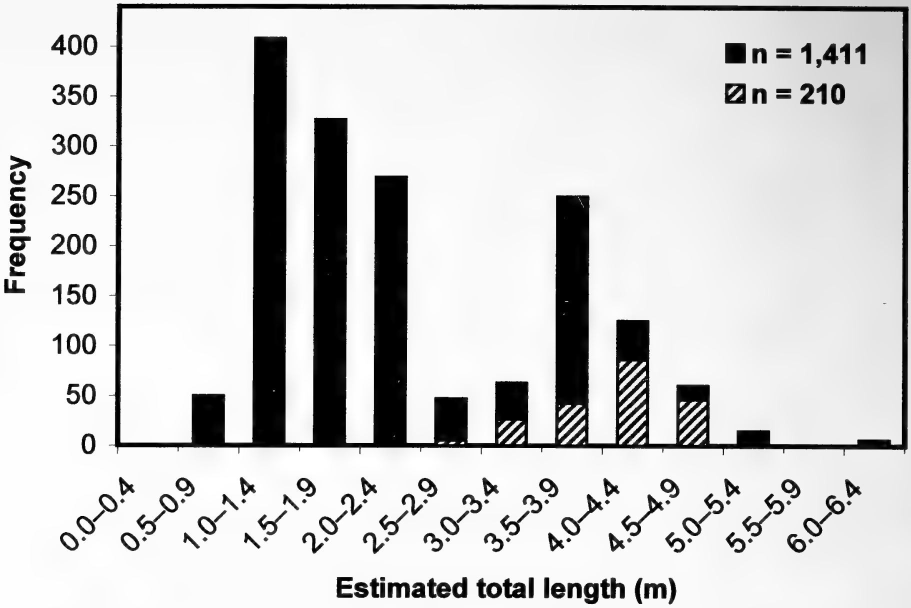

FIG. 3. Estimated total lengths (ETL) of all sawfish reported with length data from Florida Bay and the Florida Keys between 1990 and 2002 (n = 1,621; mean = 2.4 m ETL; range = 0.3–6.1 m ETL). Hatched areas are sawfish caught in water deeper than 10 m (maximum = 122 m) off the Keys and dark areas correspond to sawfish from all other shallow areas (≤10 m) depicted in Figs. 1 and 2 (including the Dry Tortugas). There is one sawfish in the smallest size class. Eleven sawfish were reported without length estimates.

numerous encounters were reported in all months. The smallest sawfish (≤1 m ETL) were encountered mostly during April and May. Larger immature sawfish (1.1–2.9 m ETL) were encountered mostly between May and August. The largest sawfish (≥3 m ETL) were encountered mostly between March and June. Of the encounters where only a season could be confidently reported (n = 924), "spring" (36%) and "winter" (33%) were most commonly reported, followed by "summer" (19%) and "fall" (12%).

Groups—Most sawfish encounters in south Florida were reported as single fish being observed or caught on hook and line, but there were numerous reports of several sawfish observed together. Groups of 2–20 similar-sized individuals (0.7–5.2 m ETL) were reported. Cape Sable (East Cape), northern Florida Bay (e.g., Snake Bight, Terrapin Bay, Madeira Bay), and Ninemile Bank were commonly reported localities where several sawfish were observed together (Fig. 1).

DISCUSSION—Because of the paucity of published data on smalltooth sawfish, the first step in learning about sawfish ecology in the United States is to determine their present range. Records of smalltooth sawfish in the United States have been documented by various sources (Adams and Wilson, 1995; Seitz and Poulakis,

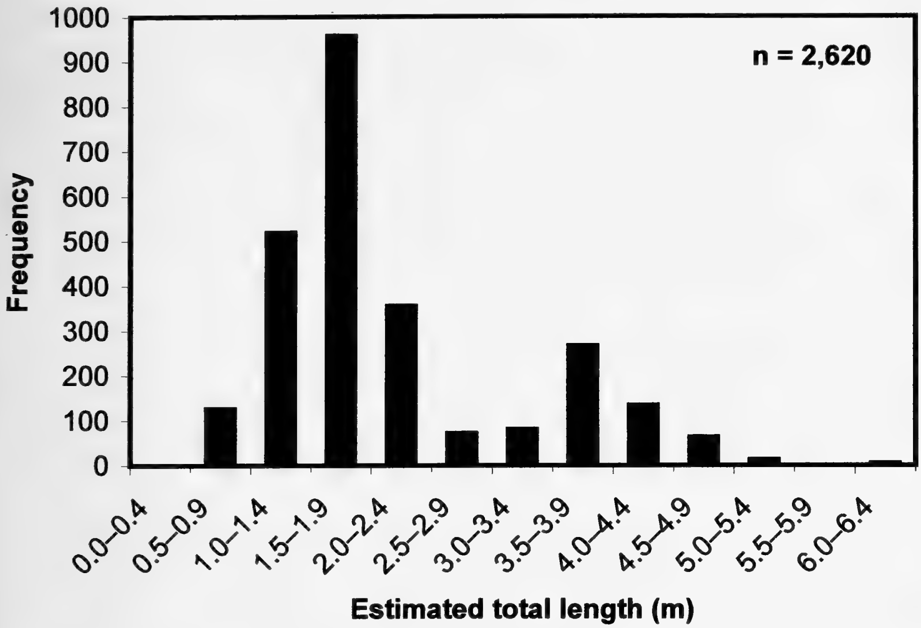

FIG. 4. Estimated total lengths (ETL) of all sawfish reported with length data from anywhere in Florida between 1990 and 2002 (mean = 2.1 m ETL; range = 0.3–6.4 m ETL). There are two sawfish in the smallest size class, there is one sawfish in the 5.5–5.9 m ETL size class, and there are seven sawfish in the largest size class. Sixty-three sawfish were reported without length estimates.

2002). Seitz and Poulakis (2002) demonstrated that a reproductive sawfish population still exists in southwest Florida, because neonates and juveniles are regularly encountered there. This paper demonstrates that sawfish are relatively common in southernmost Florida and that they use a variety of habitats not previously identified, including coral reefs and coastal waters deeper than 10 m.

Sawfishes are considered inhabitants of "shallow" (e.g., Bigelow and Schroeder, 1953; Thorson, 1982a), "shallow coastal" (e.g., van der Elst, 1981; Camhi et al., 1998), "estuarine" (e.g., Thorson, 1982a; McEachran and Fechhelm, 1998), and "freshwater" (e.g., Schwartz, 1984; Camhi et al., 1998) habitats, typically in water $\leq$10 m deep (Bigelow and Schroeder, 1953; Schwartz, 1984). This study demonstrates that smalltooth sawfish ($\geq$3 m ETL) regularly occur in water deeper than 10 m. To date, we know of at least 210 sawfish that have been recently encountered in water >10 m deep off the Florida Keys by longliners, shrimp trawlers, recreational anglers, and SCUBA divers (n = 148 >70 m). From our data, it is clear that smalltooth sawfish are regularly found in water up to 122 m.

A general description of habitat use by smalltooth sawfish can be inferred based on our data. Sawfish are present throughout the year in Florida. The smallest sawfish ($\leq$1 m ETL) are found most often in shallow (<2.4 m), protected, mangrove areas during April and May. Immature sawfish (1.1–2.9 m ETL) are found most often in shallow water ($\leq$10 m) between May and August, primarily in northern Florida Bay.

The largest sawfish ($\geq$3 m ETL) are found most often in shallow water ($\leq$10 m) between March and May, and in deeper water (to 122 m) throughout the year.

Sawfishes have been declining in numbers throughout the world because they occur in heavily fished coastal waters where they become entangled in nets, and because they have a limited reproductive potential (Thorson, 1982b; Hoenig and Gruber, 1990). Although both western Atlantic species are protected from harvest in Florida, much remains to be learned about the ecology of the smalltooth sawfish, and more research is needed if this species is going to survive in the United States.

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DEVELOPMENTAL PATTERNS AND GROWTH CURVES FOR OVULATE AND SEED CONES OF *PINUS CLAUSA* (CHAPM. EX ENGELM.) VASEY EX SARGO. AND *PINUS ELLIOTTII*, ENGELM. (PINACEAE)

Ronald F. Mente[(1)] and Sheila D. Brack-Hanes[(2)]*

[(1)]939 45th Street North, St. Petersburg, Florida 33713
[(2)]Collegium of Natural Sciences, Eckerd College, St. Petersburg, Florida 33733

Abstract: Pinus clausa *(Chapm. ex Engelm.) Vasey ex sargo. and* Pinus elliottii, *Engelm. ovulate/ seed cones have distinctive developmental patterns and growth curves. This is determined by using a morphological key to identify seven cone stages based on umbo and apophysis color and appearance from the time of pollination to maturity.* Pinus elliottii *has its earliest and both of its latest stages with length growth significantly greater than width growth and one middle stage where the opposite is true. The length-width ratio, therefore, for* P. elliottii, *approximates an inverted bell-shaped growth curve, whereas cones of* Pinus clausa, *a sympatric species, has a length to width growth pattern that increases with each stage without marked spurts of growth. Therefore, its developmental pattern and growth curve approximates a linear curve. Both trees, therefore, can be recognized by growth patterns that may be considered universal for each species. Features of umbo, apophysis, cone development and growth are useful and reliable taxonomic characters that should accompany the suite of features used to identify each* Pinus *species. In addition, these features provide another taxonomic benefit, the recognition of pine hybrids in the field. This could be very useful economic information now with apparent global climatic changes. Some hybrids may exhibit growth vigor, whereas the parent species may be less adaptable.*

Key Words: Pinaceae, *pinus*, seed cones, developmental patterns

Histological, morphological and developmental studies have contributed valuable information about taxonomy and reproductive strategies in conifers, but even more significant are the implications of plant growth in response to global climatic changes that are occurring now. Some previous developmental studies of conifers include those of embryos and ovulate/seed cones (Buchholz, 1918; Chamberlain, 1935; Konar and Ramchandani, 1958; Gifford and Foster, 1989; Mente and Brack-Hanes, 1990; Takaso and Tomlinson, 1989,1990,1991). In 1935, Chamberlain reported that gymnosperm embryos exhibit differentiation and growth in length as illustrated for *Pinus* by Buchholz (1918). More recently, Mente and Brack-Hanes (1990) demonstrated differential growth rates for ovuliferous scale features (umbo and apophysis) in *P. elliottii* cones. These studies have also contributed to our knowledge of reproductive strategies. Such strategies are thought to be changing in response to global warming and this could result in changes for the

* Author for correspondence.

economics of agriculture and forestry. Pines, as an economically valued crop, are represented in the literature with abundant details of their life cycle, reproductive strategies and development of tree structural components. For instance, traditional field studies indicate that pine pollen and seed cone initiation occur once a year (Ferguson, 1904; Buchholz, 1918; Haupt, 1941; Mergen and Koerting, 1957). On the other hand, reproductive cycles for *Pinus* species (includes pollen and seed cone initiation and growth to maturity) have been reported to extend over a period of two to three years (Konar and Ramchandani, 1958; Gifford and Foster, 1989).

There is significant interest in the observation of morphological characteristics, developmental patterns and rates of growth (expressed as growth curves) in pines and other living organisms because we now recognize a rapid, world-wide loss of species and their environments and perhaps even changes in what may be called universal growth patterns. In this further study of pines, the ovulate/seed cone developmental patterns and growth curves for two pine species in the Diploxylon Section, Insignes Group, the sand pine *P. clausa* (Engelm.) Vasey and the slash pine *P. elliottii* Engelm., will be investigated. *Pinus clausa* grows in coastal and interior "acid sand-ridges and dunes" of central and southwest Florida to south Alabama (Small, 1933; Long and Lakela, 1976). *Pinus elliottii* grows in flatwoods and coastal plains of central and north Florida, west to Louisiana and north to South Carolina (Small, 1933; Long and Lakela, 1976). These species will be examined employing quantitative analyses of growth stage measurements.

Materials and Methods—*Study sites—P. clausa* (Engelm.) Vasey ovulate/seed cones (168) were collected from a population of trees at Boyd Hill Nature Park in St. Petersburg, Florida (7–10 ft. above sea level). *Pinus elliottii* is represented by 299 ovulate/seed cones from trees on the campus of Eckerd College (3 ft. above sea level).

Collection times—The cone collections of *P. clausa* were made from May, 1989 through October, 1992 and provided ovulate and seed cones of all growth stages (Table 1). *Pinus elliottii* cones were collected from April, 1988 to October, 1992. Not all of the cone stages were present concurrently on the trees during the study periods.

Sampling methods—Paired length and width measurements were made as observed and/or collected (Mente and Brack-Hanes, 1990) and the cones grouped into the six growth stages (Stages 1–6) immediately after pollination (Stage 1a) according to the key in Table 1 and as illustrated by *P. elliottii* (Figs. 1–7). Developmental stages are defined by umbo and apophysis color and morphology (Figs. 1–7). Length to width ratios were calculated with matched pair data and plotted in Fig. 8 to show growth curves.

Stages of cone growth—Pinus clausa and *P. elliottii* have seven growth stages as defined by color and/or the most advanced external morphological changes that can be observed in the umbo (distal tip of the ovuliferous scale) (U) and apophysis (proximal, inflated portion of the ovuliferous scale) (A) (Table 1, Figs. 1–7). Stage 1a (Fig. 1) is the very small rosy/green open ovulet cone at pollination. Stage 1 (Fig. 2) is represented by umbo development in the rosy/green closed cone just after pollination and Stage 2 (Fig. 3) is umbo senescence (umbo appears brown). Stage 3 (Fig. 4) has brown, mature umbos with green, incipient apophysis growth. At this stage, apophyses appear as swollen green areas proximal to the umbos, whereas Stage 4 (Fig. 5) is represented by a green cone, *i.e.*, there are green apophyses cone-wide with small brown, dorsal umbos. Stage 5 (Fig. 6) shows apophysis senescence (brown-streaked areas at the proximal base of umbos) and Stage 6 (Fig. 7) is the completely mature, *i.e.*, brown, woody cone ready for seed dispersal, either open or closed.

TABLE 1. Key to determine growth stages (S) in *Pinus* ovulate and seed cones[a] based on umbo and apophysis color and morphology as determined in this study of *P. clausa* and *P. elliottii* and illustrated by *P. elliottii* in Figures 1–7.

Description	Stage
Cones open (umbos thin or hair-like)	S1a
Cones closed (green umbos inflated)	S1
Umbos brown or turning brown, no apophyses	S2
Umbos brown, green apophyses apparent & developing	S3
Green apophyses developed cone-wide	S4
Green apophyses with brown near umbo	S5
Brown apophyses, mature, woody cone	S6

Stage	Identification by ovulate-seed cone morphology
1a	Open cone, rosy/green (time of pollination)
1	Closed cone, rosy/green, umbo development
2	Brown cone, umbo senescence/mature
3	Brown cone, turning green with developing apophyses
4	Green cone, fully developed apophyses
5	Green cone, turning brown, apophysis in senescence
6	Brown (woody) cone, mature apophysis

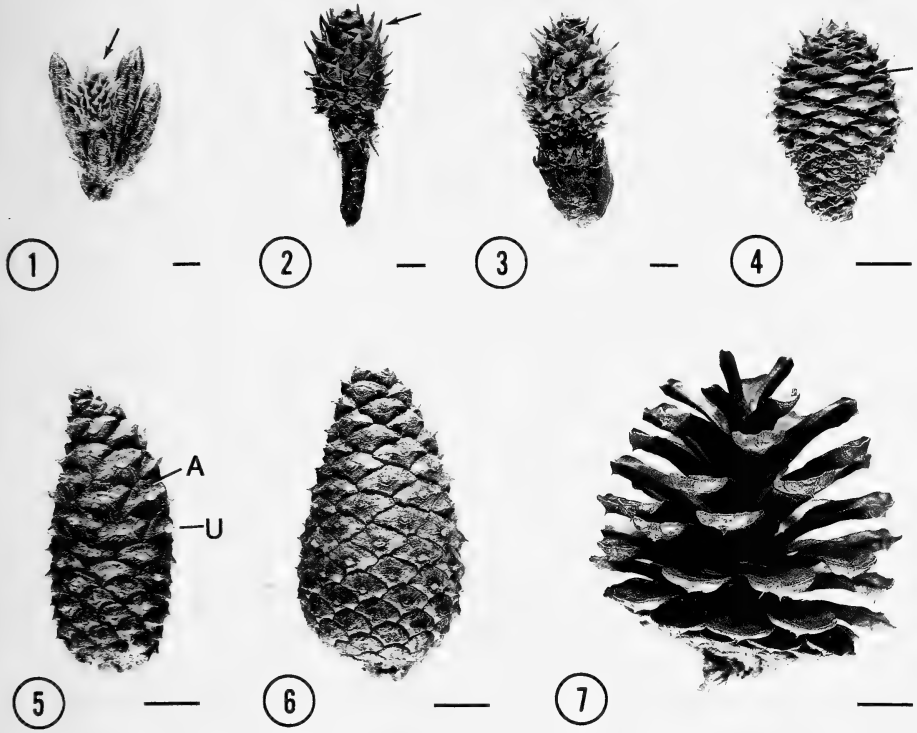
[a] Fresh specimens, usual morphology.

RESULTS—*Pinus clausa*—*Pinus clausa* ovulate/seed cone growth in length always exceeds growth in width for all stages (Table 2, Fig. 8). Therefore, the cone length to width ratio continuously and gradually increases from Stage 1 through Stage 6, to generate an approximate linear growth curve. At maturity, *P. clausa* has small, sessile seed cones.

Pinus elliottii—*Pinus elliottii* ovulate/seed cone growth in width is greater than that of length in Stage 4 as indicated by the L/W ratios in Table 2 and Figure 8, and it is greater in length to width for Stages 1,5 and 6. Stage 6 shows a nearly flat/very slight decrease (0.02) in the L/W ratio. This may be due to sample size (237 for Stage 5 versus 17 for Stage 6). Therefore, the L/W ratios yield a curve that approximates an inverted bell-shape. At maturity, *P. elliottii* cones are large and pedunculate.

General—All growth stages of the two species show similar development of umbos and apophyses. In addition, Stages 1a and 1 of both are distinguished by a pedunculate, green cone having a bloom on scales that show only a green, hair-like, umbo tip. By Stage 3, however, the peduncle of *P. clausa* becomes slightly obscured by an overgrowth of ovuliferous scales. Cones of *P. elliottii* are pedunculate in all growth stages.

DISCUSSION—*Pinus*—This seed cone developmental study supports the conclusions of Mente and Brack-Hanes (1990) concerning the reliability of seed cone growth patterns for taxonomic purposes. The taxonomy of *Pinus* is challenging



FIGS. 1–7. Stages (S) of *Pinus elliottii* ovulet and seed cones. 1. S1a, open cone (arrow) among young shoots Bar = 1.00 cm. 2. S1, closed cone with inflated young, green umbos having hair-like tip (arrow) Bar = 1.00 cm. 3. S2, brown, mature umbo. Bar = 1.00 cm. 4. S3, green apophysis (arrow) beginning to be apparent on growing ovuliferous scales. Bar = 0.6 cm. 5. S4, inflated green apophysis (A) and mature umbo (U) on scales. Bar = 0.90 cm. 6. S5, apophyses beginning to mature (turn brown) on scales. Bar = 1.00 cm. 7. S6, all apophyses mature and brown on open woody, mature cone. Bar = 0.85 cm.

and additional features certainly could be useful. Cone growth curves and comparative differential growth rates can be applied to the identification of species or hybrids, even though there may be several *Pinus* species with similar growth curves. Certainly, there are numerous accounts of varieties and even some nursery hybrids, but a paucity of reports of naturally occurring hybrids (Righter and Duffield,1951; Duffield, 1952; Perry, 1991). This indicates the apparent extreme difficulty of field identification. When many species/varieties are identified in the field, but few hybrids (Martinez, 1948; Perry, 1991), the most probable rationale is that the hybrids are unrecognized. Therefore, the use of conservative reproductive structures such as seed cones would be invaluable. Cone growth curve analysis, growth rates, and incremental growth, when established, are therefore suggested as other features to use with the suite of traditional and reliable morphological characteristics that now define a species or distinguish a hybrid.

The recognition of naturally occurring hybrids is worthwhile because they may be demonstrating adaptive responses to changing climatic conditions that are now

TABLE 2. Length (L) to width (W) ratios for developmental stages (S) of *P. clausa* (*P.c.*) and *P. elliottii* (*P.e.*) cones. Numbers of cones measured (N) have means ($\bar{x}$) averaged for each stage with Standard Deviation (s.d.) and Standard Error (s.e.). All measurements are in mm.

	S	N	$\bar{x}$ L	$\bar{x}$ W	L/W	s.d.	s.e.
P.c.	1	65	12.0	9.0	1.35	0.09	0.01
	2	4	20.0	13.3	1.53	0.05	0.03
	3	7	25.4	15.9	1.63	0.11	0.04
	4	17	34.5	18.6	1.82	0.14	0.03
	5	17	42.5	22.0	1.94	0.09	0.02
	6	58	53.8	26.0	2.12	0.11	0.01
P.e.	1	4	14.0	7.5	1.87	0.11	0.05
	2	13	19.8	14.1	1.40	0.07	0.02
	3	18	21.4	16.0	1.34	0.12	0.03
	4	10	26.8	22.3	1.20	0.16	0.05
	5	237	56.9	26.9	2.09	0.12	0.01
	6	17	83.0	40.0	2.07	0.04	0.01

detrimental to the parent species. Some of these hybrids may be not only fertile, but have features of immediate importance. They may be useful in arid or otherwise harsh environments as providers of additional sources of wood, wood products or edible seeds. Furthermore, hybrids may improve crop production by giving resistance to microbial and insect predators. Moreover, the advantages pointed out for the recognition and use of natural hybrids apply not only to pines, but to other economically valuable crops, as well.

This developmental study suggests that more seed cones of pine species from other geographic areas and climatic zones need to be observed to determine details of incremental growth, overall developmental patterns and growth curves. Of particular interest for a comparative study, would be observations of pine species such as *P. strobus* L. and *P. lambertiana* Dougl., that grow in regions with shorter growing seasons.

SUMMARY—The recognized loss of countless numbers of species and irreversible damage to our environments has caused us to redirect our focus to common, but yet-to-be scientifically established knowledge about recognized species. Therefore, more scientific inquiry is being applied to tacit knowledge about known species of plants and animals to discover growth patterns that are different from traditional assumptions. For instance, Lampl and co-workers (1992) described "discontinuous, aperiodic saltatory spurts" of growth in human development, a description that, now, may also be applied to some pine ovulate/seed cones. Distinguishing a growth pattern is dependent on differential growth in length and width. Moreover, it is apparent that external morphology implies concurrent internal differentiation at each stage. This, in fact, points out the need for more histological studies of early seed cone stages of *Pinus* species similar to the work of Takaso and Tomlinson (1991) for taxodiaceous species.

The study by Lampl and co-workers (1992) and this one of growth patterns in pines support the implication of universal growth patterns for all higher plant and

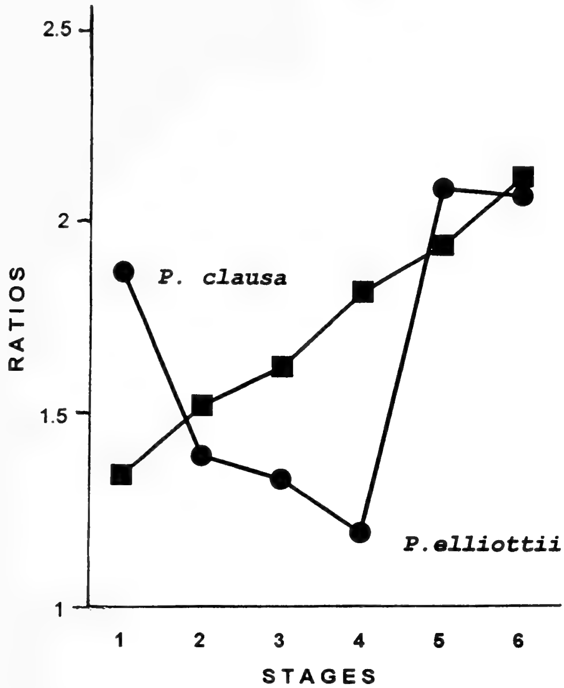


FIG. 8. Ratios of cone Length/Width from Table 2 for *P. clausa* and *P. elliottii* for growth stages as defined in Table 1.

animal species. Consequently, there is a need for more base-line studies such as this one of *Pinus*, to expand on this hypothesis.

Although taxonomic data, growth and reproductive strategies have significance in themselves, even more important are considerations for the future of agriculture and forestry economics. Therefore, the authors of this paper believe that an understanding of the developmental patterns and dynamics of incremental growth in species may have application in monitoring the effects of climatic change on them.

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LUMPY JAW IN WHITE-TAILED DEER SUBJECTED TO A SEVERE FLOOD IN THE FLORIDA EVERGLADES

KRISTI MACDONALD[1] AND RONALD F. LABISKY

Department of Wildlife Ecology and Conservation, University of Florida, Gainesville, Florida 32611

ABSTRACT: *White-tailed deer* (Odocoileus virginianus seminolus) *inhabiting the wet prairie and tree island habitats of the Florida Everglades were subjected to a severe flood in the fall and winter of 1994–95. Water levels in the study area peaked at 1.3 m in January 1995, more than double the 0.6-m level that restricts deer mobility, and forced surviving deer to crowd onto the slightly elevated (1 m) tree islands. Of 51 radio-collared deer (≥1 year in age) being monitored in Big Cypress National Preserve and Everglades National Park at the onset of flooding in November 1994, 25 (49%) had died by 31 March 1995. Two of the dead deer, a 3-yr-old female and a 2-yr-old male, had symptoms typical of actinomycosis or lumpy jaw, which is manifested clinically by severe osteomyelitis of the mandibles. The disease likely was caused by malnutrition and gum injuries incurred from consuming woody forage on the tree islands to which the deer were confined. These apparent cases of lumpy jaw are the first reported from white-tailed deer in Florida.*

Key Words: White-tailed deer, *Odocoileus virginianus seminolus*, Everglades, Florida, flood, disease, lumpy jaw, actinomycosis

THE development of actinomycotic lesions generally is attributed to introduction of resident oral bacteria *Actinomyces* sp. into a deep injury in the gums. It is believed that gum injuries, especially of the perialveolar area, are inflicted when animals consume coarse, spiky vegetation (Emily and Frahm, 1991; Butler, 1986). Following introduction into the tissue, the bacteria may invade the soft tissue or bone. "Lumpy jaw" refers to infection of the mandible, maxilla, palatine, or turbinate bones, which results in necrosis of the bone, progressive growth and replacement by a granulomatous mass, and tooth loss (Howe, 1981; Wobeser, 2001). In captive macropods, a group in which this disease is very common, outbreaks usually occur during cool and wet conditions when animals are stressed due to overcrowding (Butler and Burton, 1980). Animals with advanced stages of lumpy jaw may be unable to masticate due to severe pain and swelling (Butler, 1986; Howe, 1981).

Lumpy jaw occurs in wild and domestic ruminants on a worldwide scale (Howe, 1981). It has been documented in wild ungulates of North America, including white-tailed deer (*Odocoileus virginianus*), mule deer (*O. hemionus*) (Honess and Winter, 1956); Columbian black-tailed deer (*O. hemionus columbianus*) (Cowan, 1946); barren-ground caribou (*Rangifer tarandus groenlandicus*)

[1] Current address: Department of Ecology, Evolution and Natural Resources, Rutgers University, New Brunswick, New Jersey 08901.

(Miller et al., 1975); moose (*Alces alces*) (Ritcey and Edwards, 1958); and bighorn sheep (*Ovis canadensis*) (Hoefs and Bunch, 2001). This paper describes the first documentation of lumpy jaw in white-tailed deer (*Odocoileus virginianus seminolus*) in Florida. The two cases occurred during an unprecedented flood in the Everglades in 1994–95, which resulted in deer being largely confined to tree islands (MacDonald, 1997).

Study Area—The 200-km^2 study area (25 °38′ N, 80 ° 00′ W) included the southern region of Big Cypress National Preserve and the upper Shark River Slough area of Everglades National Park in extreme southwestern Florida. The climate is classified as sub-tropical with mean monthly temperatures ranging from 14°C in January to 28°C in August (Duever et al., 1986). The region is typified by a distinct dry winter season (November-April) and wet summer season (May-October). Mean annual rainfall is 136 cm, two-thirds of which falls during the summer. The habitat consists mainly of contiguous wet prairie (87%), which is typified by a hydroperiod of 50–150 days, interspersed with hardwood tree islands (Duever et al., 1986; Miller, 1993). These tree islands are elevated approximately 1 m above the surrounding prairie and suffer little or no seasonal flooding under normal weather conditions (Craighead, 1984). Forage is generally of low nutritional quality due to the poor soils (Harlow and Jones, 1965), and possibly the late successional stage of the vegetation (Belden et al., 1988).

Normal surface water levels in the study area range between about 0.4 m above in the summer wet season and –0.4 m below in the winter dry season. The flood of 1994–95 was the result of unusually heavy rainfall (>100 cm) during August-October 1994, and heavy rains (>20 cm) stemming from Tropical Storm Gordon on 14–15 November 1994. Surface water levels peaked at 1.3 m in January 1995, more than double the 0.6 m level that restricts deer movements (Loveless, 1959), and did not begin to subside until April 1995.

Methods—Twenty-five (49%) of 51 radio-collared adult deer being monitored (by aerial telemetry) at the initiation of the 5-mo flood had died by 31 March 1995, a finding consistent with the high rates of mortality of deer reported during previous floods in the Everglades (Loveless, 1959; Lampton, 1982; Langenau et al., 1984). The 49% mortality was more than double the annual mortality rate of adult deer in the same study area during preflood years (Labisky et al., 1995). Carcasses of the 25 dead deer were recovered at the earliest possible time following detection of a transmitter in mortality mode. Necropsies were conducted on site to determine cause of death when possible, and skulls and mandibles were collected for later examination. Abnormal lesions were found in the mandibles of 2 deer. These lesions, examined by D. J. Forrester (Department of Pathobiology, University of Florida, Gainesville, Florida), were typical of those resulting from actinomycosis. However, diagnostic confirmation of the disease by histopathological or microbiological techniques was precluded because of the severe tissue decomposition of carcasses at time of recovery.

Results and Discussion—The first case of apparent lumpy jaw was detected in a 3-yr-old female deer whose home range had encompassed a large area of wet prairie. At the onset of flooding in November, this deer moved 1 km southward into an area of clustered small tree islands, where she remained until 24 January 1995, when the transmitter went into mortality mode. The carcass, which was retrieved on 31 January 1995, revealed that the deer likely died from malnutrition as evidenced by the obvious definition of her spine and lack of a fat deposit at the base of her tail. Examination of skull and mandible, after cleaning, revealed the presence of lumpy jaw (Fig. 1, top). The destruction of the bone was more severe in the left mandible than in the right. Molar 1 on the left mandible had been lost and many small sinuses were present. Both sides of the left mandible exhibited extreme deterioration of the succuli, and food impaction could be distinguished on the right side.

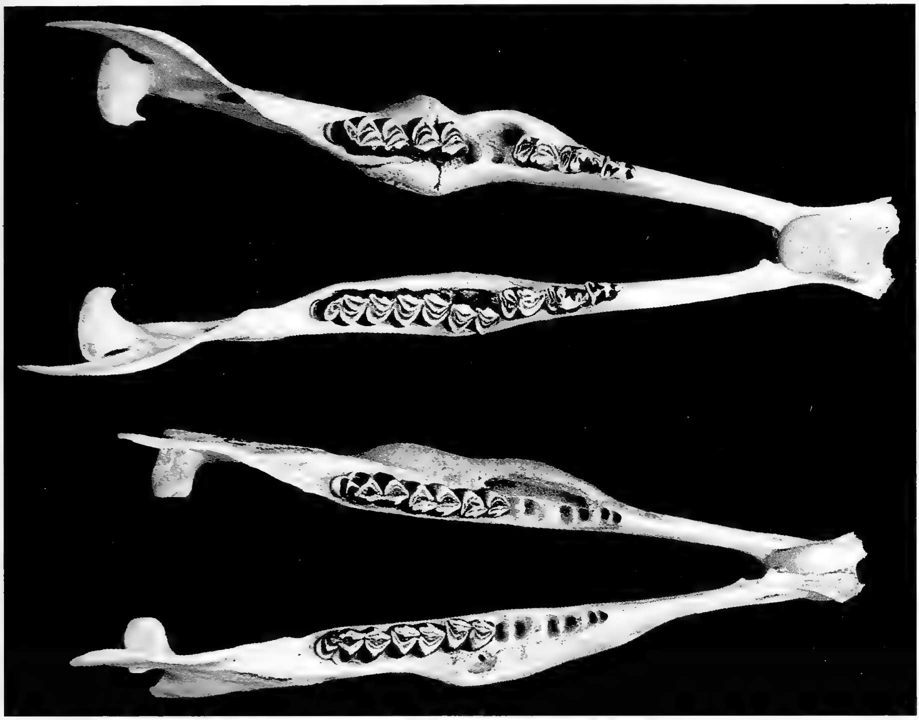

FIG. 1. Lower mandibles of white-tailed deer afflicted with actinomycosis or lumpy jaw: 3-yr-old female (top), 2-yr-old male (bottom).

The second case of apparent lumpy jaw occurred in a 2-yr-old male whose home range principally encompassed wet prairie with small, scattered tree islands. As water levels rose in mid-November, this deer left his pre-flood home range and traveled 3 km northeasterly to a large tree island. This deer remained within the tree island until his death on 13 March 1995. His carcass, recovered on 15 March, was entangled in *Smilax* vines in meter-deep water. Aerial radio-telemetry indicated that this deer became entangled between 1 March and 3 March. However, he survived entanglement for at least 10 days before dying of probable malnutrition and stress, which was strongly evidenced by the color (light pink) and consistency (gelatin-like) of his bone marrow. Subsequent examination of the jawbones indicated that the animal had severe osteomyelitis of both mandibles (Fig. 1, bottom). The granulomatous bone in the left mandible had a large drainage sinus. Lower premolars 1, 2 and 3 on both mandibles had been lost. It is likely that the affliction with lumpy jaw restricted the mastication of food, and was indirectly responsible for the death of this deer.

In conclusion, 2 (8%) of the 25 deer that died during the flood likely were afflicted with lumpy jaw. In contrast, none of 57 radio-collared adult deer recovered on the same study site during pre-flood years showed any sign of maxillary or mandibular abnormalities. Thus, environmental conditions likely contributed directly to the probable occurrence of the disease. As water levels began to rise in the Everglades, deer moved onto the slightly elevated tree islands when water depths restricted their mobility on the wet prairie (MacDonald, 1997). Because deer of this region include a large amount of herbaceous vegetation from the wet prairies in their diet (Loveless, 1959; Harlow, 1961; Labisky et al. 2003), it may be that when forced to feed exclusively on tree island plant species during the flood, they suffered deleterious effects. Because the vegetation available to them on tree islands was more woody or fibrous than their traditional forage, gum injury could have been common, predisposing the deer to suffer malnutrition and eventual death.

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GEOLOGY AND PALEONTOLOGY OF A CALOOSAHATCHEE FORMATION DEPOSIT NEAR LEHIGH, FLORIDA

Thomas M. Missimer and Amy E. Tobias

CDM Missimer, 9311 College Parkway, Suite 1, Fort Myers, FL 33919

Abstract: *A 1.5 m deep channel incised into the Tamiami Formation near Lehigh (Sec. 10, T. 44 S., R. 27 E.) in Lee County, Florida contained a Blancan-age (Pliocene) vertebrate and molluscan assemblage. At the base of the deposit vertebrates and a diverse assemblage of mollusks were present. The middle of the deposit contained numerous species of mollusks and corals. Here the predominant molluscan species were* Turritella perattenuata, Pyrazisinus campanulatus, Cerithium litharium, Conus adversarius tryoni, Chione elevata, *and* Carditamera arata. *Corals found included* Solenastrea hyades, Trachyphyllia *sp., poritids,* Stylophora affinis, *and* Montastrea annularis. *Near the top of the deposit, a bed occurred containing predominantly the gastropod* Pyzazisinus scalatus. *The top of the deposit was capped by a thin freshwater limestone containing the mollusk* Planorbella disstoni. *The Blancan vertebrate assemblage is believed to be between 2.5 to 2 Ma in age and correlates well to the basal Caloosahatchee Formation of Captiva Island, which had a maximum estimated age of about 2.14 Ma.*

Key Words: Blancan, Caloosahatchee Formation, Lehigh, vertebrates, Tamiami Formation, Fort Thompson Formation

Geological work on the Caloosahatchee Formation began in the 1880s, when Angelo Heilprin made a series of exploratory trips up the Caloosahatchee River to Lake Okeechobee (Heilprin, 1886). Heilprin provided a general description of the riverbanks where the Caloosahatchee Formation crops out and where he collected numerous mollusks for study. Later, Dall (1890–1903), published more detailed geological and paleontological studies of the Pliocene and Pleistocene sediments exposed on the banks of the Caloosahatchee River. In the 1930s and 1940s, Wendall C. Mansfield, Druid Wilson, Helen I. Tucker, and Axel Olsson made studies of the Caloosahatchee and Fort Thompson formations in southwestern Florida at a number of locations. These studies were published in Tucker and Wilson (1932, 1933) and Olsson and Harbison (1953). In the 1950s and 1960s, extensive work was conducted on the Caloosahatchee and Fort Thompson formations by DuBar (1958, 1962). Recently, the general stratigraphy of the Neogene in northwestern Lee County and southwestern Charlotte County was summarized by Missimer (1998, 2001).

Each year hundreds of excavation (pits) are made in Florida. Usually, shallow stratigraphic sections are exposed for a short time. With consideration that the flat topography of Florida does not produce many outcrops of subsurface rocks, it is quite important to document the geology and paleontology of these temporary exposures. The stratigraphic section described within this paper was partially

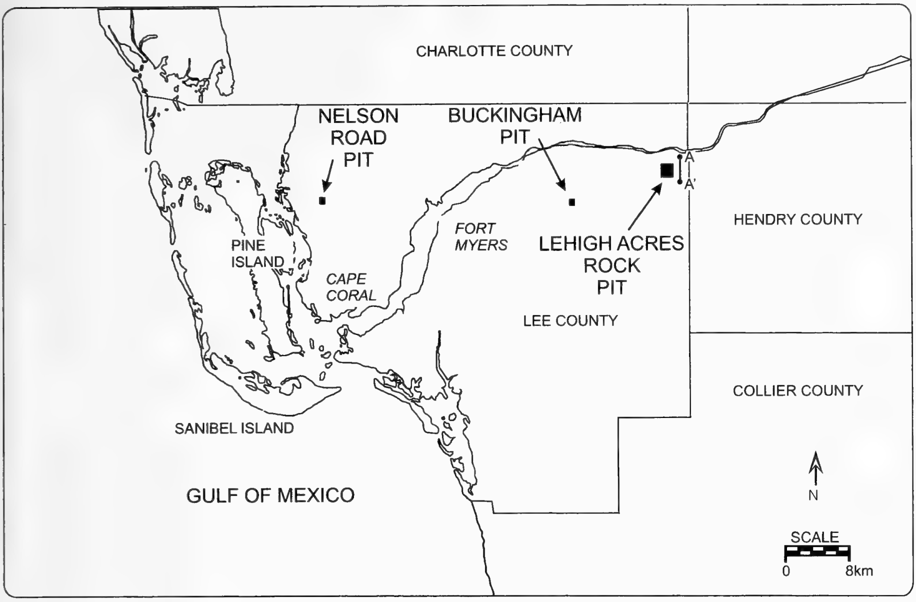

FIG. 1. Map of Lee County, Florida showing the locations of the Lehigh Acres, Buckingham, and Nelson Road pits and a geologic section (A-A′).

exposed and excavated from late 1979 to about mid 1984. The mining pit was located in Lee County, Florida near the town of Lehigh (NE 1/4, Section 10, Township 44S, Range 27E) (Fig. 1 and 2). Collection of invertebrate and vertebrate fossils from the Caloosahatchee Formation section was made primarily from spoils with some dives made into the pit to assess the geometry of the channel, the stratigraphy, and the predominant fossil types in each bed. Although many geologists and paleontologists have visited the exposures within the dewatered pits, few have published their findings.

METHODS—Over a 5-year period from 1979 to 1984, direct observations were made as a pit was excavated across a Caloosahatchee Formation deposit. Measurements were made and the orientation of the deposit was determined in the field using aerial photographs and a field compass. Periodic dives were made into the mining pit to observe the deposit and to collect some fossil samples. Invertebrate and vertebrate fossils were collected from the spoils as the deposit was mined.

Stratigraphy of the Lehigh Acres pit—Excavation penetrated between 6 to 9 m (25 to 30 feet) below land surface. Sediments were excavated from the lower part of the Tamiami Formation to the top of the Fort Thompson Formation (Fig. 3).

The deepest stratigraphic unit penetrated was the Buckingham Limestone Member of the Tamiami Formation (terminology of Missimer, 1992). Mansfield (1939) originally named this unit the Buckingham Marl and described it from a pit located near Buckingham in Lee County (Fig. 1) and from several locations exposed along the banks of the Caloosahatchee River. This unit is a light gray to white lime mud containing calcitic fossils, quartz sand, rock fragments, and phosphorite nodules. The composition is highly variable. There is a disconformity lying on top of the Buckingham Limestone, separating it from the overlying limestone. Based on geologic logs of wells drilled within 2 km (1 mile) of the Lehigh Acres

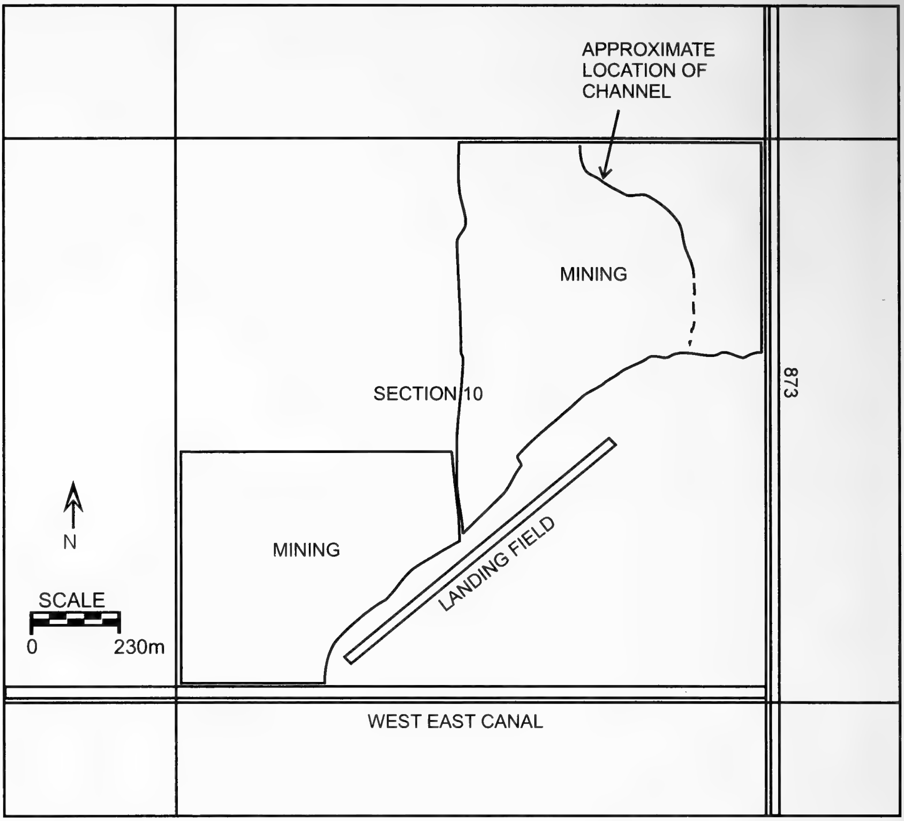

FIG. 2. Map showing the mining areas in the Lehigh Acres Pit and the location of the channel containing the Caloosahatchee Formation deposit.

Pit, the thickness of the unit is about 6 m (20 feet) (Fig. 3). Only the upper 1 to 2 m (3 to 6 feet) of the unit were penetrated by the excavation.

A light gray to light brown, well indurated limestone lies above the Buckingham Limestone. This unit is highly fossiliferous, but mostly molds and casts of the diverse fossil assemblage are preserved. However, some calcitic oysters and pectens are present. This unit is unnamed, but is the probable equivalent of the Pinecrest or Golden Gate Member of the Tamiami Formation (Missimer, 1992). The unit is between 2 and 3 m (6 and 10 feet) thick at the site. The limestone is the uppermost stratigraphic unit within the Tamiami Formation at this location.

A shallow channel is incised into the limestone at the top of the Tamiami Formation at the mining pit (Fig. 4). This channel has a maximum depth of 1 to 2 m (3 to 6 feet). It is infilled within a highly fossiliferous limestone (Fig. 5). Vertebrate fossils occur in abundance at the bottom of the feature and numerous species of aragonitic and calcitic mollusks are preserved. There are also many preserved corals. The sediments contained in this thin deposit occur within the Caloosahatchee Formation. The top of the formation is a disconformity.

A thin, commonly laminated, fossiliferous limestone lies atop the Caloosahatchee and Tamiami formations in the pit area. This unit is light brown to brown in color and ranges from 0 to 0.5 m (0 to 1.5 feet) in thickness. The unit is hard and contains variable amounts of quartz sand. At some locations it is laminated, particularly in the upper 2 to 5 cm (1 to 2 inches). The limestone contains a low diversity of

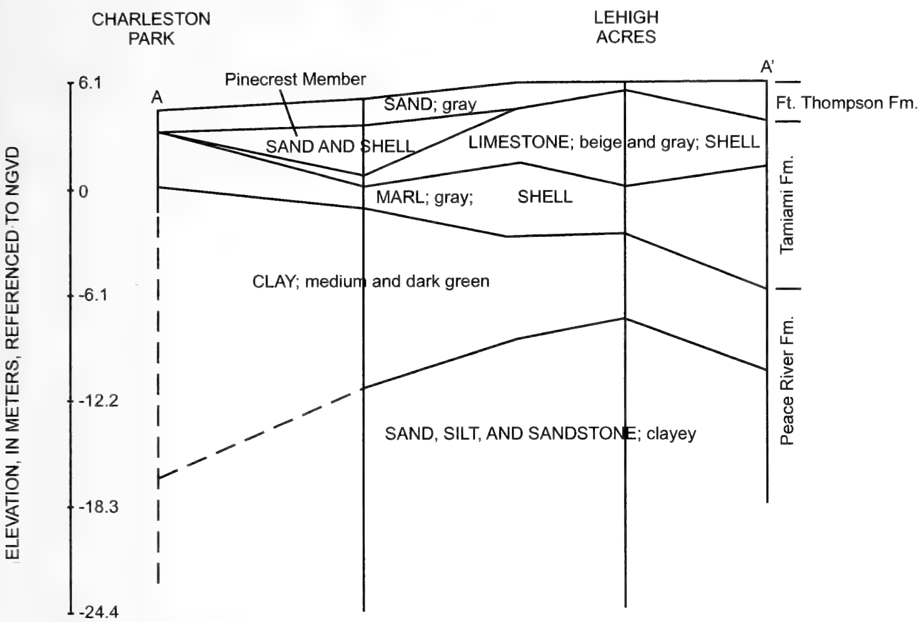

FIG. 3. Geologic section A-A′ showing stratigraphic units within the Peace River, Tamiami, and Fort Thompson formations.

fossil mollusks with the bivalve *Chione elevata* (Say) predominating. This unit lies within the Fort Thompson Formation.

An unlithified quartz sand unit overlies most of the site. This unit is 0 to 0.5 m (0 to 1.5 feet) in thickness. It is light gray in color and the grain size of the sand ranges from medium to fine. The quartz sand unit lies either in the Fort Thompson Formation or within a marine terrace deposit, such as the Pamlico Sand.

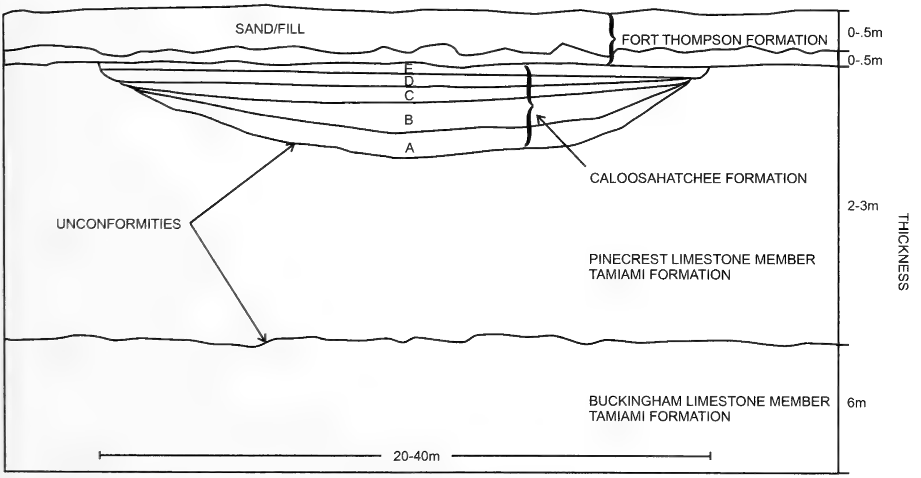

FIG. 4. Geometry of the channel-fill deposit at the Lehigh Acres Pit.

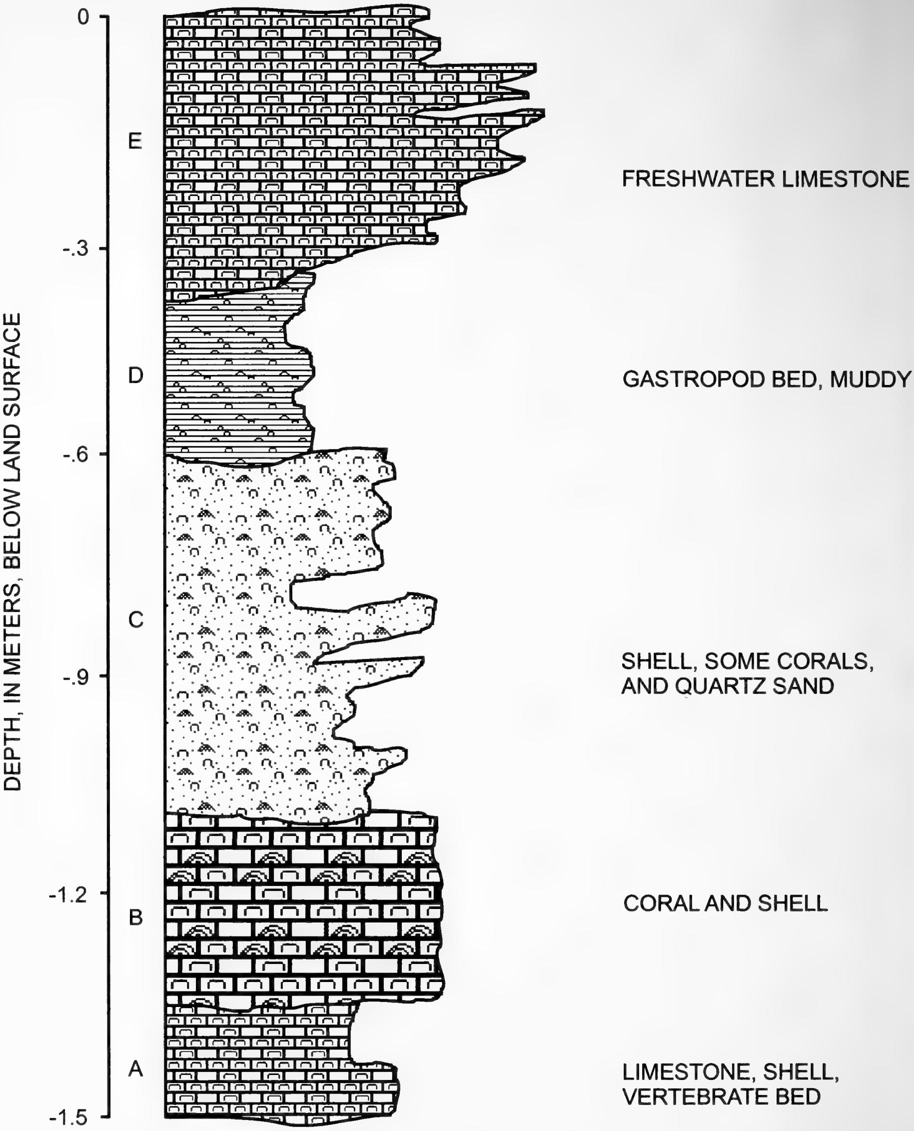

FIG. 5. Stratigraphic section of the Caloosahatchee Formation within the channel.

RESULTS AND DISCUSSION—*Site description*—The incised channel was intersected by many mining cuts that were oriented essentially east-west. The channel was oriented northwest-southeast to north-south (Fig. 2). It was a maximum of 2 m (6 feet) in depth in the centerline. Based on several snorkel dives, the width of the channel was 10 to 15 m (30 to 50 feet). The overall geometry and orientation of the channel is similar to some of the smaller, modern streams that are tributaries to the Caloosahatchee River, such as Hickey Creek or Bedman Creek.

Based on the dives, the Caloosahatchee Formation deposit within the channel can be divided in the mid-channel into five units (Fig. 4 and 5). The lithology of the beds varied from indurated, hard fossiliferous limestone to quartz sand and shell to a soft, marly limestone (Fig. 5). The lowest bed (A) is a light gray limestone containing a variety of invertebrate and vertebrate fossils. Bed B is a coralline boundstone in some locations and a marly limestone in other locations. Bed C is a highly variable bed of quartz sand and shell with some cementation. Bed D is a light to medium gray muddy bed containing predominantly gastropods. Bed E is a hard, indurated, freshwater limestone. The top of bed E is a disconformity. The deposit appears to be a single shoaling-upward sequence or parasequence. The overall stratigraphy is similar to the lower part of the Caloosahatchee Formation deposit mapped at the Nelson Road Pit in northwestern Lee County (Missimer, 2001). Because of the difficulty in observing the unit underwater and the variation within the deposit, it is not possible to describe the stratigraphy of the sediments in detail. Observations of the geometry of the channel were made and some samples were collected from the beds at various locations to define the predominant fossil types.

Invertebrate paleontology—Invertebrate fossils were collected predominantly from spoil as the channel sediments were excavated. Some samples were collected directly from the channel deposit when it could be reached by diving. A list of invertebrates collected from both the beds and spoils is given in Appendix A. The invertebrates are not assigned to each bed because the individual bed sampling was limited. Selected mollusks are shown in Fig. 6 and corals in Fig. 7. A total of 51 species of bivalves, 73 species of gastropods, and 7 species of corals were identified from the deposit. The micro-mollusks were not studied in detail.

Within each bed, there were considerable differences in diversity of mollusks. Bivalves and gastropods were common throughout the lower three beds (A,B,C) with the predominant bivalves being: *Chione elevata* (Say), *Anadara tranversa* (Say), *Carditamera arata* (Conrad), *Argopecten gibbus* (Linnaeus), *Anodontia alba* Link, and *Transennella conradina* Dall. The most significant gastropod species (characteristic of the Caloosahatchee Formation) from the interval were: *Turbo rhectogrammicus* Dall, *Conus adversarius tryoni* Heilprin, *Siphocypraea problematica* Heilprin, and *Hystrivasum horridum* (Heilprin) (Portell, 2001). Gastropods predominate the invertebrate assemblage in bed D. The peritidal gastropod *Cerithium litharium* Dall was very common with *Pyrazisinus scalatus* (Heilprin) and *Pyrazisinus campanulatus* (Heilprin). There was little diversity of invertebrates in bed E, which is a freshwater limestone and contains the freshwater gastropod *Planorbella disstoni* (Dall).

Direct observation of corals occurred only within beds B and C with most corals occurring in bed B. Commonly occurring corals were: *Trachyphyllia bilobata* (Duncan), *Dichocoenia caloosahatcheensis* Weisbord, *Solenastrea hyades* Dana, and *Diploria strigosa* Dana.

Vertebrate paleontology—The lowermost part of the channel deposit (bed A) contained a rich deposit of vertebrate fossils. No vertebrate fossils were observed in the overlying beds. Identification and analysis of the vertebrate assemblage

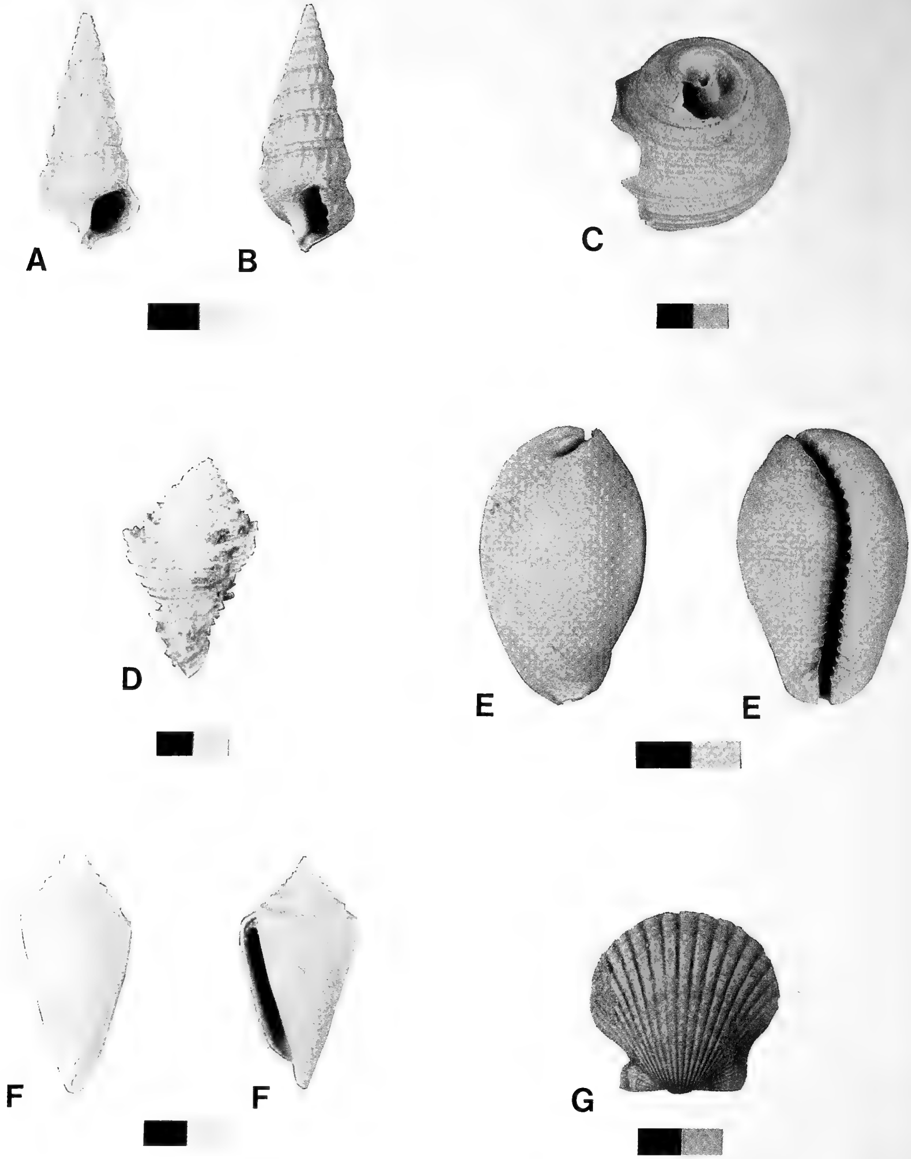

FIG. 6. Selected mollusks. The bar scale under each mollusk is 2 cm. A. *Pyrazisinus scalatus* (Heilprin, 1886), B. *Pyrazisinus campanulatus* (Heilprin, 1886), C. *Turbo rhectogrammicus* Dall, 1892, D. *Hystrivasum horridum* (Heilprin, 1886), E. *Siphocypraea problematica* Heilprin, 1886, F. *Conus adversarius tryoni* Heilprin, 1886, G. *Argopecten gibbus* (Linnaeus, 1758).

was made by Gary Morgan (Morgan and Hulbert, 1995). A complete list of the vertebrates is given in Appendix B. Selected vertebrates are shown in Fig. 8. Within the vertebrate assemblage, 24 species were identified with four unknowns. The distribution of vertebrate taxa included: fourteen land mammals, two marine

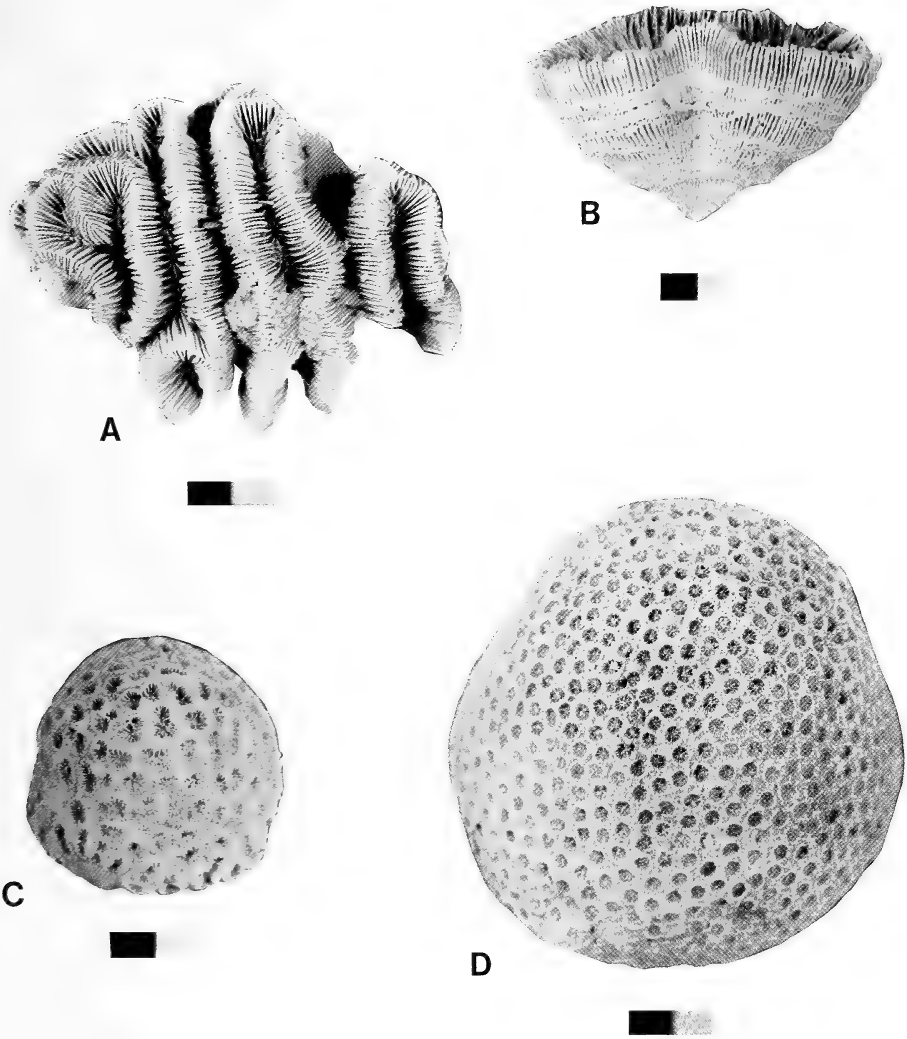

FIG. 7. Selected corals. The bar scale is 2 cm. A. *Diploria strigosa* (Dana, 1848), B. *Trachyphyllia* sp. cf. *T. bilobata* (Duncan, 1863), C. *Dichocoenia caloosahatcheensis* Weisbord, 1974, D. *Solenastrea hyades* (Dana, 1846).

mammals, five fish (four sharks), one alligator, one snake, and one freshwater turtle.

The most significant vertebrates found in the deposit are a Blancan assemblage. This group of vertebrates included: *Trachemys platymarginata* (freshwater turtle), *Glossotherium chapadmalense* (small ground sloth), *Nannippus peninsulatus* (small, three-toed horse), and *Platygonus bicalcaratus* (taper).

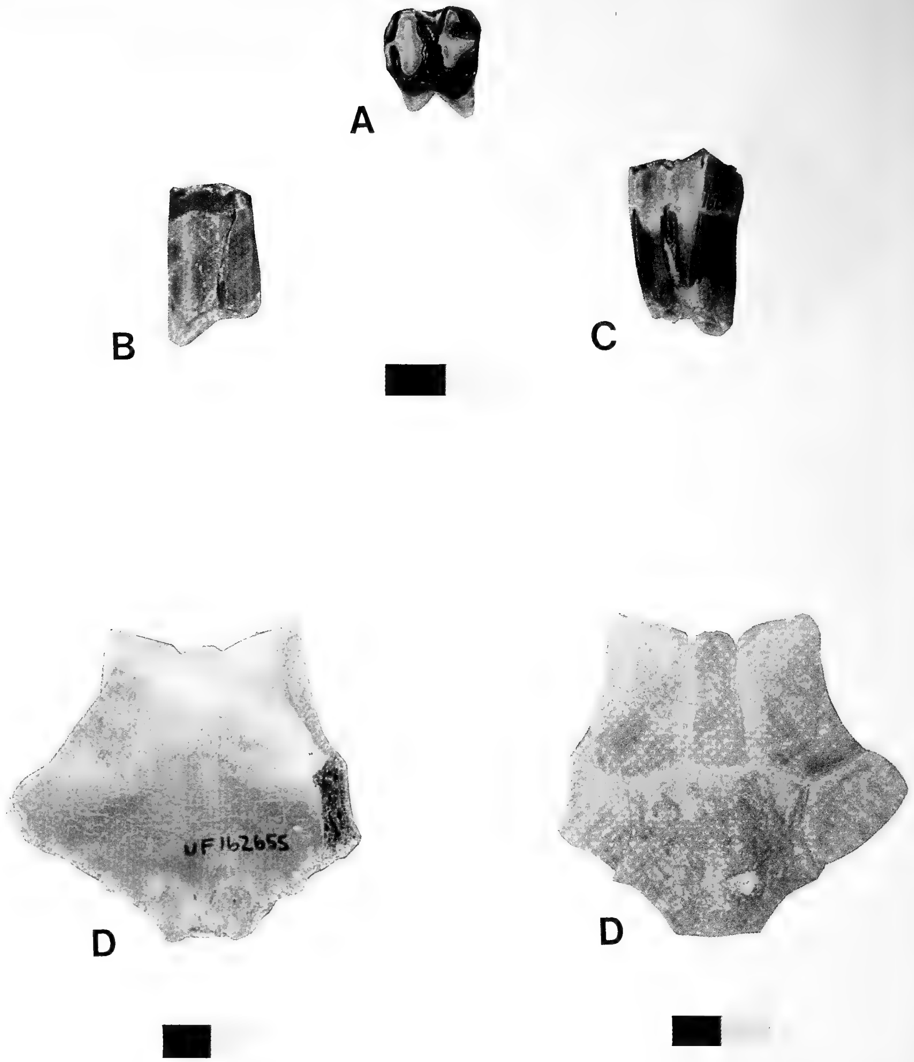

FIG. 8. Selected vertebrates. The bar scale is 2 cm. The catalogue numbers are from the Florida Museum of Natural History (University of Florida [UF]). A. *Platygonus bicalcaratus* (UF 162691), B. *Glossotherium chapadmalense* (UF 162664), C. *Nannippus peninsulatus* (UF 162667), D. *Trachemys platymarginata* (UF 162655).

Implications on age of the Caloosahatchee Formation—The age of the Caloosahatchee Formation has been debated for many years (Missimer, 1993). Dall and Harris (1892) suggested that the formation is Pliocene based on the ratio of extinct to living mollusks. DuBar (1958, 1974) suggested the entire Caloosahatchee Formation was Pleistocene based on the presence of a fossil horse skull, *Equus leidyi* (Hay). Brooks (1968) and Conklin (1968) placed the Pliocene-Pleistocene boundary in the middle of the formation based on the reassignment of some specific lithologic members into the overlying Fort Thompson Formation and others into the Caloosahatchee Formation. Perkins (1977) used the mapping of discontinuity surfaces to separate the Pleistocene sediments of southern Florida and his lowermost Pleistocene (Q1) surface occurs within the upper Caloosahatchee Formation. Bender (1973) used the uranium/helium dating method to determine the age of some corals collected from the Caloosahatchee Formation. These ages were 1.97 and 1.88 Ma (corrected to the time scale of Berggren and co-workers [1995]). Missimer (1997) used magnetostratigraphy and a single strontium date to suggest that the age of the Caloosahatchee Formation in a core from Captiva Island (W-16242) is 2.14 or 1.77 to 0.6 Ma. The age at the base of the formation in the core could not be determined because of the inability to obtain samples for magnetostratigraphic analysis.

A Blancan vertebrate assemblage was found in the lowermost part of the channel deposit. Blancan vertebrates are believed to have occurred in the age range from 2.5 to 2 Ma. (Lundelius et al., 1987; Morgan and Hulbert, 1995). The exact correlation of the Blancan vertebrate age range to the most current Global Magnetic Time Scale (GMTS) is not certain. If the Blancan vertebrate time scale is correlated to chron C2r, then the age range using the GMTS of Berggren and co-workers (1995) would be 2.58 to 1.95 Ma. The Blancan vertebrate assemblage in the basal bed suggests that the lower part of the Caloosahatchee Formation is Pliocene in age at this location. The occurrence of the Blancan assemblage in the lower part of the formation suggests that the age of the lower Caloosahatchee Formation is most likely 2.14 Ma based on the lowermost possible age suggested by Missimer (1997). None of the invertebrate fossils found in the deposit yielded age-diagnostic data.

Conclusions—A lower Caloosahatchee Formation deposit was studied at a rock pit located near Lehigh in Lee County, Florida. The deposit lies disconformably on the Tamiami Formation within an incised channel. The 1.5 m (5-foot) thick deposit contained an open-shelf limestone at the base, a coralline limestone in the middle and a peritidal limestone topped by a freshwater limestone at the top. The deposit is a single shoaling-upward sequence or parasequence and is very similar to a lowermost Caloosahatchee Formation deposit at the Nelson Road Pit in northwestern Lee County as described by Missimer (2001).

A diverse assemblage of mollusks and corals occurs within the deposit with 51 species of bivalves, 73 species of gastropods, and 7 coral species identified. A significant assemblage of vertebrate fossils was found primarily in the lowermost bed of the deposit. The vertebrate assemblage is Blancan-age based on the occurrence of *Trachemys platymarginata*, *Glossotherium chapadmalense*, *Nannippus peninsulatus*, and *Platygonus bicalcaratus*.

Based on the Blancan vertebrate assemblage and the stratigraphic position of the deposit, the lower Caloosahatchee Formation at this location is late Pliocene.

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Appendix A. A complete list of bivalves, gastropods, corals, and barnacles found at the Lehigh Acres Pit in Lehigh, Florida.

Bivalves

Anadara sp. A
Anadara sp. B
Anadara rustica (Tuomey and Holmes, 1857)
Anadara transversa (Say, 1822)
Glycymeris arata (Conrad, 1841)
Glycymeris arata floridana (Olsson and Harbison, 1953)
Isognomon sp.

PECTINIDAE

Lindapecten sp. cf. *L. muscosus* (Wood, 1828)
Lindapecten muscosus (Wood, 1828)
Argopecten anteamplicostatus (Mansfield, 1936)
Argopecten gibbus (Linnaeus, 1758)
Plicatula marginata Say, 1824
Spondylus rotundatus Heilprin, 1886
Anomia simplex Orbigny, 1842
Lucina pensylvanica (Linnaeus, 1758)
Parvilucina multilineata (Tuomey and Holmes, 1857)
Parvilucina trisulcata (Conrad, 1841)
Codakia orbicularis (Linnaeus, 1758)
Lucinisca nassula (Conrad 1846)
Lucina radians (Conrad, 1841)
Anodontia alba Link, 1807
Diplodonta punctata (Say, 1822)
Chama gardnerae Olsson and Harbison, 1953
Pseudochama radians (Lamarck, 1819)
Carditamera arata (Conrad, 1832)
Pleuromeris tridentata (Say, 1826)

Trachycardium isocardia (Linnaeus, 1758)
Trigoniocardia columba (Heilprin, 1886)
Laevicardium mortoni (Conrad, 1831)
Dinocardium robustum (Lightfoot, 1786)
Spisula solidissima similis (Say, 1822)
Tellina sp.
Tellina aequistriata Say, 1824
Tellina alternata tayloriana Sowerby, 1867
Tellina similis Sowerby, 1806
Semele sp.
Semele bellastriata (Conrad, 1837)
Tagelus divisus (Spengler, 1794)
Mercenaria campechiensis (Gmelin, 1791)
Chione elevata (Say, 1822)
Anomalocardia collosa Olsson and Harbison, 1953
Transennella conradina Dall, 1883
Pitar simpsoni (Dall, 1889)
Macrocallista nimbosa (Lightfoot, 1786)
Macrocallista maculata (Linnaeus, 1758)
Dosinia elegans (Conrad, 1843)
Gemma magna floridana Olsson and Harbison, 1953
Parastarte triquetra (Conrad, 1846)
Sphenia sp.
Caryocorbula contracta Say, 1822

Gastropods

Turbo floridensis Olsson and Harbison, 1953
Turbo rhectogrammicus Dall, 1892
Tricolia sp.
Neritina sphaerica Olsson and Harbison, 1953
Rissoina sp.
Cochliolepis holmesi (Dall, 1889)
Caecum sp. cf. *C. plicatum* Carpenter, 1858
Caecum cinctum Olsson and Harbison, 1953
Caecum coronellum Dall, 1892
Caecum nitidum Stimpson, 1851
Turritella apicalis Heilprin, 1886
Turritella perattenuata Heilprin, 1886
Turritella wagneriana Olsson and Harbison, 1953
Vermicularia recta Olsson and Harbison, 1953
Lemintina decussata (Gmelin, 1791)
Modulus carchedonius (Lamarck, 1822)
Cerithidea scalariformis (Say, 1825)
Pyrazisinus campanulatus (Heilprin, 1886)
Pyrazisinus scalatus (Heilprin, 1886)
Cerithium sp.
Cerithium sp. cf. *C. litharium* Dall, 1892
Cerithium sp. cf. *C. triticium* Olsson and Harbison, 1953
Cerithium heilprini Dall, 1892
Cerithium litharium Dall, 1892
Cerithium preatratum Olsson and Harbison, 1953
Cerithium triticium Olsson and Harbison, 1953
Rhinoclavis caloosaensis (Dall, 1892)
Crepidula convexa Say, 1822

Strombus sp. cf. *S. alatus* Gmelin, 1791
Trivia pediculus (Linnaeus, 1758)
Siphocypraea problematica Heilprin, 1886
Neverita duplicata (Say, 1822)
Ficus communis Roding, 1798
Hanetia mengeana (Dall, 1890)
Calotrophon ostrearum (Conrad, 1846)
Columbella sp. cf. *C. rusticoides* Heilprin, 1886
Anachis sp.
Melongena corona (Gmelin, 1791)
Busycon contrarium (Conrad, 1840)
Busycon echinatum (Dall, 1890)
Busycotypus spiratus (Lamarck, 1816)
Nassarius vibex (Say, 1822)
Latirus infundibulum (Gmelin, 1791)
Fasciolaria apicina Dall, 1890
Fasciolaria tulipa (Linnaeus, 1758)
Fusinus caloosaensis florida Olsson and Harbison, 1953
Oliva sayana Ravenel, 1834
Olivella sp.
Olivella dodona Olsson and Harbison, 1953
Olivella fargoi Olsson and Harbison, 1953
Mitra lineolata Heilprin, 1886
Turbinella regina Heilprin, 1886
Hystrivasum horridum (Heilprin, 1886)
Scaphella floridana (Heilprin, 1886)
Marginella onchidella Dall, 1890
Marginella pardalis Dall, 1890
Granulina ovuliformis (Orbigny, 1841)
Conus adversarius tryoni Heilprin, 1886
Conus spuroides Olsson and Harbison, 1953
Conus stearnsii Conrad, 1869
Conus waccamawensis B. Smith, 1930
Terebra dislocata (Say, 1822)
Clathrodrillia solida (C.B. Adam, 1830)
Crassispira perspirata (Dall, 1889)
Cymatosyrinx lunata (Lea, 1843)
Pyramidella crenulata (Holmes, 1859)
Acteocina candei (Orbigny, 1842)
Bulla striata Bruguiere, 1789
Microtralia occidentalis (Pfeiffer, 1854)

Freshwater Gastropods

Viviparus georgianus (Lea, 1834)
Planorbella disstoni (Dall, 1890)
Stenophysa meigsii (Dall, 1890)

Corals

PORITIDAE

Trachyphyllia sp. cf. *T. bilobata* (Duncan, 1863)
Diploria strigosa (Dana, 1848)
Oculina sp.
Montastrea annularis (Ellis and Solander, 1786)
Dichocoenia caloosahatcheensis Weisbord, 1974

Stylophora affinis Duncan, 1863
Solenastrea hyades (Dana, 1846)

Barnacles

BALANIDAE

APPENDIX B. A complete list of vertebrates found at the Lehigh Acres Pit in Lehigh, Florida.

Negaprion brevirostris
Carcharhinus sp.
Carcharhinus sp. cf. *C. obscurus*
Galeocerdo cuvier

DIODONTIDAE
COLUBRIDAE

Trachemys platymarginata
Alligator mississippiensis

cf. DUGONGIDAE
SIRENIDAE

Glossotherium chapadmalense
Eremotherium sp.
Nannippus peninsulatus
Equus sp.
Hemiauchenia sp.
Hemiauchenia blancoensis
Hemiauchenia macrocephala
Odocoileus sp.
Odocoileus virginianus
Platygonus bicalcaratus
Tapirus sp.

GOMPHOTHERIIDAE
FELIDAE

Mammut sp.
4 unknowns

PEST MANAGEMENT PRIORITIES AMONG FLORIDA'S ORGANIC VEGETABLE GROWERS

Hugh A. Smith[(1)], Everett R. Mitchell[(2)*], and John L. Capinera[(1)]

[(1)]Department of Entomology and Nematology, University of Florida, Gainesville, FL
[(2)]Agricultural Research Service, US Department of Agriculture, Gainesville, FL

Abstract: *Certified organic farmers from throughout the state of Florida provided information on cropping cycles, pests, and pest management practices. Florida's mixed organic vegetable farms are characterized by a broad range of pest problems due to the high degree of crop diversity on the farms, and the varied growing conditions found in the state, from temperate to subtropical. Because of the diversity of crops grown, no one pest predominates. With few exceptions, the pest problems encountered by organic growers are not different from those affecting conventional farmers. Organic growers tend to tolerate higher levels of pest damage, perhaps because of the higher market value of organic produce. Many organic growers exhibit a commitment to crop diversity that is based on farming philosophy as well as economics, and a willingness to experiment with new crops and new market niches. The complexity and flexibility of Florida's mixed organic vegetable farms enables growers to respond to pest pressure in many instances by reducing or eliminating a highly susceptible crop. Growers' responses revealed the need for increased ability to identify both pest and beneficial arthropod species. Several growers requested assistance in increasing natural on-farm biological control. Challenges to carrying out on-farm research under diverse cropping systems are discussed.*

Key Words: Organic farming, sustainable agriculture, integrated pest management, polyculture

Organic crop production is one of the most rapidly developing sectors of U.S. agriculture (Greene, 2001). Acreage of certified organic crops more than doubled in the U.S. in the 1990s (ERS, 2001). A significant portion of organic farms are small farms (approx. 2 ha) that produce a variety of vegetables. One third of the small farms producing mixed vegetables in the U.S. are certified organic (ERS, 2001).

Swisher and co-workers (1994) profiled Florida's commercial organic vegetable growers in 1994, when there were approximately 21 certified organic vegetable growers farming 492 ha (1215 A) in the state. In 1997, approximately 412 ha (1017 A) of certified organic vegetables were grown in Florida (ERS, 2001). In both 1994 and 1997, slightly over four percent of the organically grown vegetables in Florida were produced on farms 2.02 ha or less.

Mixed organic vegetable farms in Florida tend to be distinguished from conventional farms by the diversity of crops, the permissible tools and practices, the business approach, and the overall scale of the operation (Swisher et al., 1994; Swisher and Monaghan, 1995). Mixed organic vegetable growers in Florida often react to pest damage by reducing the amount of a crop grown or by switching crops.

* Deceased.

They tend to be more entrepreneurial than conventional growers, experimenting with new crops and new markets (Swisher et al., 1994).

Given these fundamental differences, the research and extension programs developed for conventional agriculture at many land grant and federal institutions may not adequately address the production problems of organic producers. In order to determine research priorities for mixed organic cropping systems, a survey about insect pests and pest management problems in Florida was sent to a group of certified organic vegetable growers. An additional purpose of the survey was to identify types of educational and training materials related to insect pest management that would be most useful to managers of organic farming systems.

METHODS—In early spring of 2000, informal interviews were held with six certified organic vegetable growers in north central Florida concerning their pest problems and pest management practices. These interviews were used to design a formal questionnaire aimed at gathering basic information to prioritize research efforts by federal and university personnel addressing the pest management needs of Florida's mixed organic vegetable growers.

The survey was divided into four groups of questions. The first set of questions focused on background information, such as the year of initial certification, the number of hectares farmed organically as opposed to conventionally, the amount of land owned or rented, years of experience in conventional and organic agriculture, and information on people employed on the farm other than the primary grower. These parameters can affect the decisions growers make concerning pest management and other aspects of crop production.

Growers were asked in the second section of the survey to list the crops they grew during different seasons, the approximate acreage devoted to each crop, the pests that affected each crop, and measures applied to manage the pest.

Growers were presented with a list of specific arthropod management tools in the third section. They were asked to list the pests and crops to which they had applied the method, and to comment on its apparent effectiveness. The list included *Bacillus thuringiensis* (Bt), neem, rotenone, pyrethrum, insecticidal soap, insecticidal oil, mail-order parasitoids and predators, resistant crop varieties, trap crops, and pheromone-based techniques. Growers were also given the opportunity to describe other methods, such as home-made pest management remedies.

In the final section, growers were asked how they diagnose and assess the impact of arthropod pest problems. Growers were asked if they employed some form of scouting program, if they had ever been unsure if an insect on their crop was beneficial or harmful, and how they determined if a product was acceptable for use on a certified organic farm. In addition, growers were asked if insect pests had either caused them to reduce the area devoted to a certain crop, or prevented them from growing a high value crop. Growers were asked to list their primary insect pests.

In the final question of the survey, growers were asked to rank five areas of production and to indicate which areas presented the greatest problems throughout the course of the year. The five areas listed were marketing, nutrient management, insects, weeds, and disease. The rankings were analyzed using the Kruskal Wallis procedure (PROC NPAR1WAY; SAS, 1996).

In early November 2000, the survey was mailed to about 40 mixed vegetable growers who had been certified by Florida Organic Growers and Consumers, an agency that certifies about 90% of the organic vegetable growers in Florida. The survey was mailed with a stamped, self-addressed envelope for its return, and included information on how to contact the survey team should questions concerning the form arise. An attempt was made to contact all recipients by telephone the week the survey was mailed. Recipients who had not returned their questionnaire by early December were telephoned again, and for a final time in January. During these subsequent telephone calls, growers were given the option to provide the information over the phone, or to be interviewed in person on their farm.

RESULTS—*Background information*—A total of 25 growers from throughout the state of Florida responded to the survey. Three respondents had farms in the Florida

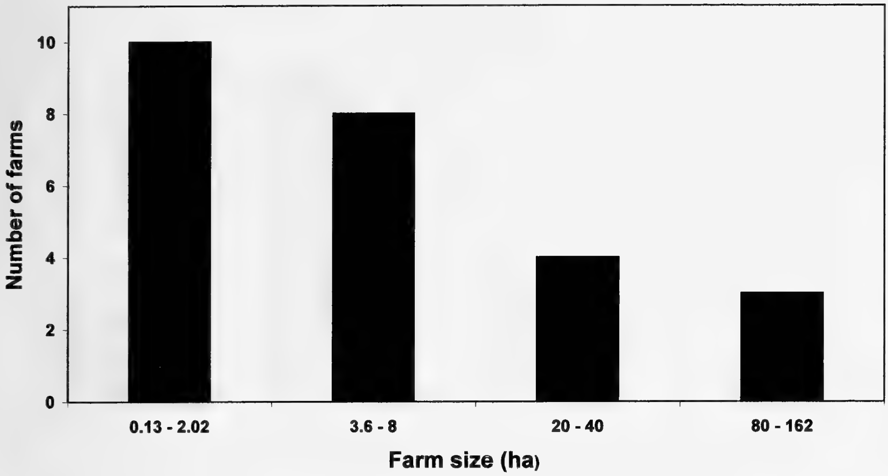

FIG. 1. Size distribution of farms in survey.

panhandle, eight were from north central Florida, nine were in the central portion of the state, and five farms were located in the southern end of the peninsula between Immokalee and Homestead. Fourteen growers returned the survey by mail, six were interviewed on the telephone, and five were interviewed in person.

The farms ranged in size from 0.13 ha (0.33 A) to 162 ha (400 A) (Fig. 1). Almost half (10) of the farms were 2.02 ha (5 A) or less in size. Seven of the farms included in the survey were certified organic between 1979 and 1989 (Fig. 2). The remaining 18 farms were certified between 1990 and 2000. Twenty farms were solely organic, whereas five of the farms were part of a larger operation that contained conventionally-farmed land, with the organic component comprising from 6 to 85% of the farm.

Seventeen growers owned all the land they farmed. Eight farms contained 30–96% rented land. Growers had from as little as one to as many as 25 years of organic farming experience, with 40% claiming five or fewer years of experience (Fig. 3). Eight growers had no experience in conventional farming, whereas seven growers had 20 or more years of experience with conventional farming (Fig. 4).

More than half the farms had only one person working full-time. Eleven farms had between two and seven full-time personnel, and one farm reported 11 full-time employees. Eleven farms regularly hired seasonal workers. The number of seasonal workers hired ranged from two to 50. The number of weeks each year that they were hired ranged from four to 50. Most of the larger farms employed seasonal or part-time workers, and many of the smaller farms did as well.

Data were not gathered in a manner suitable for statistical analysis, but there was no obvious relation between farm size and other parameters such as years of experience or number of employees. Smaller farms that produced crops such as watermelon (*Citrullus lanatus* [Thunb.] Matson & Nakai) which require extra labor at harvest sometimes required more employees than larger farms producing crops that are more easily harvested.

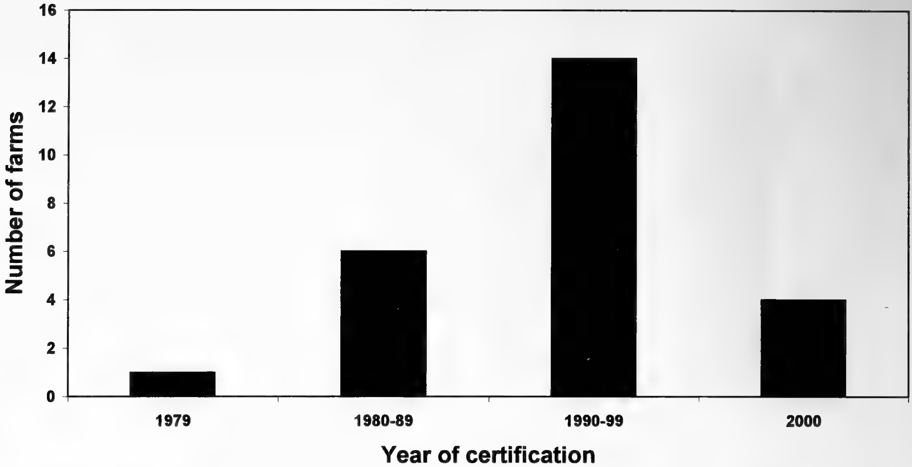

FIG. 2. Original date of organic certification for farms in survey.

Crops—The majority of farms (20) in the study grew crops from about six common horticultural plant families (x = 6.3, STD = 2.25). Plant families tended to be represented by a few or more different genera. Numerous cultivars of the same crop, such as kales or lettuces, were often grown on one farm. Most farms were highly diverse, with cropping systems that contained several or dozens of cultivars grown through the course of the year. However, a few farms with simpler systems were also represented. One farm grew only tomatoes, another only lettuces, and two focused on herbs or herbs and mushrooms. Six farms had a significant tree crop component. Tree crops consisted of pecans and persimmons in the Panhandle, and

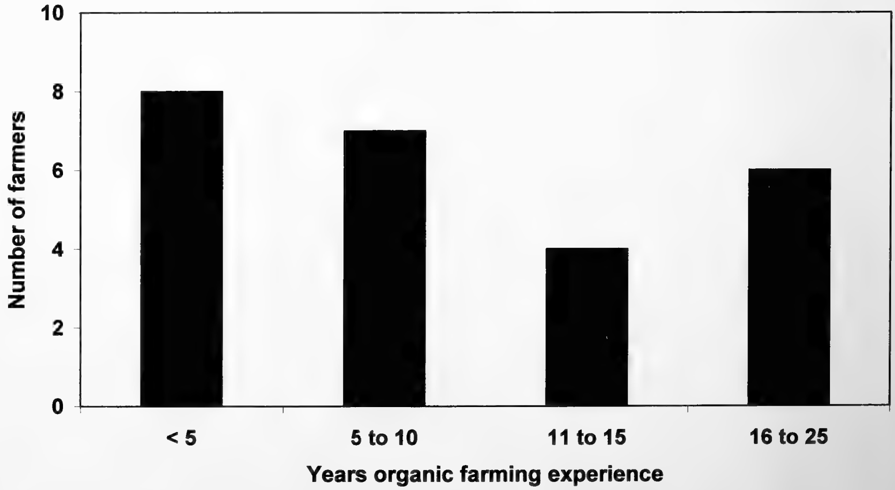

FIG. 3. Number of years farmers in survey have farmed organically.

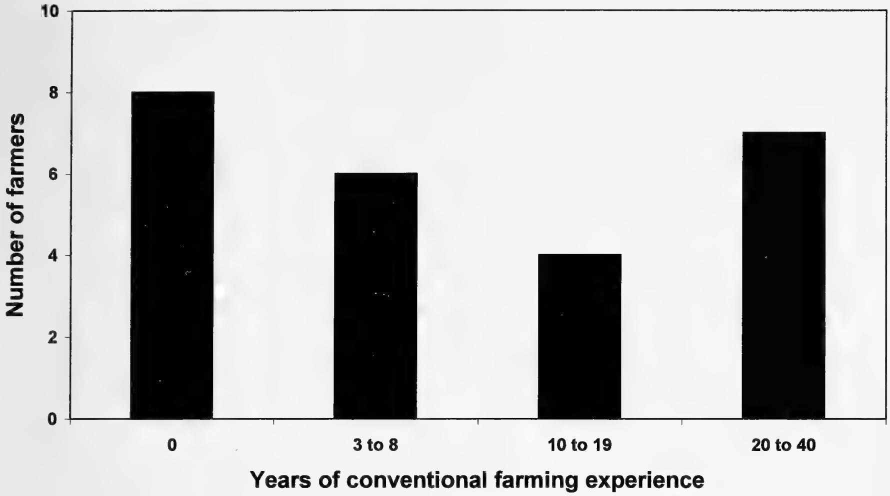

FIG. 4. Number of years farmers in survey have farmed conventionally.

a variety of avocados and tropical fruits throughout southern portions of the state. Pests of tree crops were not included in the study.

The primary crop groups grown during fall and winter consisted of lettuces (*Lactuca sativa* L.), Brassicas, tomatoes (*Lycopersicon esculentum* Mill.), peppers (*Capsicum annuum* L.), beans (*Phaseolus vulgaris* L.), squashes (*Cucurbita* spp.), spinach (*Spinacia oleracea* L.), beets (*Beta vulgaris* L.), chards (*B. vulgaris* L.), and carrots (*Daucus carota* L.) (Table 1). Lesser crops grown during fall and winter included garlic (*Allium sativum* L.) and onions (*Allium cepa* L.) (Amaryllidaceae), cut flowers, edible flowers such as violas (Violaceae), strawberries (Rosaceae), and other berries. Farms growing herbs and mushrooms did so year-round.

Major crops grown during the spring were squashes and melons, tomatoes, peppers, eggplant (*Solanum melongena* L.), and potatoes (*Solanum tuberosum* L.), sweet corn (*Zea mays* L.), and legumes (Table 2). Common bean and cow pea (*Vigna unguiculata* [L.] Walp.) comprised the majority of legumes, although one farm had extensive peanut (*Arachis hypogaea* L.) acreage.

Only 20% of the farms produced crops during the summer. Some of these farms, and many that ceased production, grew cover crops during the summer. Sorghum (*Sorghum vulgare* Pers.), millet (*Panicum miliaceum* L.), and iron and clay cowpea cultivars were the cover crop species most frequently mentioned. Many of the cucurbits, solanaceous and leguminous crops grown during the spring were also grown during the summer, as were okra (*Abelmoschus esculentum* [L.] Moench.) and sweetpotato (*Ipomoea batatas* [L.] Lam.).

Primary pests—Lepidoptera were the primary pests mentioned for most crop groups (Table 3). In addition to the crop families listed in Table 3, Lepidoptera complexes were among the most frequently listed pests for leguminous crops and lettuces. Aphids were significant pests of all crop groups, and were the most

TABLE 1. Major crops grown during fall and winter.

Crop family	Cultivars	Percent of farms
Compositae	Lettuces	64
Cruciferae	Brassicas	60
Solanaceae	Tomatoes, peppers	52
Fabaceae	Beans, peas	36
Cucurbitaceae	Squashes	32
Chenopodiaceae	Spinach, beets, chards	32
Umbelliferae	Carrots	24

common pest of leguminous crops, affecting 46% of bean and pea growers. Few pests overall were reported for members of the Chenopodiaceae.

Pest control methods—Most growers (88%) used some formulation of *Bacillus thuringiensis* (Bt) on a regular basis to manage a wide range of Lepidoptera larvae and the Colorado potato beetle (*Leptinotarsa decemlineata* [Say]) (Table 4). Growers uniformly reported Bt as effective. Insecticidal soaps were used by 64% of growers, primarily against aphids and whiteflies. Only half the growers using insecticidal soaps reported that they were consistently effective. Twenty-four percent of the growers used an azadirachtin product (neem) against a variety of arthropods. Some growers mentioned that neem seemed to work better as a preventative than as a cure, and others mentioned that it was too expensive. Few found it consistently effective. Only three growers reported using rotenone regularly, indicating that it was effective against stink bugs (Pentatomidae) and certain Coleoptera. Four growers used pyrethrum alone or combined with rotenone, and reported that results were variable.

Mail-order ladybird beetles, lacewings, predatory mites and mantids were released on-farm by 36% of the growers, about half of whom were satisfied with the results. Thirty-two percent of the growers released mail-order parasitoids of whiteflies (*Bemisia tabaci* Gennadius and *Bemisia argentifolii* Bellows & Perring), Lepidoptera eggs, or leafminers (*Liriomyza* spp.). Less than half these growers felt that the parasitoid introductions were effective. Twenty percent of the growers had tested some form of trap cropping, although none expressed confidence in its efficacy. Only a few growers used insecticidal oils. Growers were not asked about the use of diatomaceous earth, but this control material was mentioned as being effective by several growers.

TABLE 2. Major crops grown during spring.

Crop family	Cultivars	Percent of farms
Cucurbitaceae	Squashes, watermelons	52
Solanaceae	Tomatoes, peppers, eggplants, potatoes	48
Fabaceae	Common bean, southern pea	16
Gramineae	Sweetcorn	16

TABLE 3. Insect pests of major crops.

	Percent of growers affected
Solanaceous (19 growers)	
Tomato hornworm[1]	42
Armyworms, cutworms	38
Colorado potato beetle	37
Stink bugs	26
Aphids	26
Whiteflies	21
Others[2]	22
Cucurbits (16 growers)	
Lepidoptera[3]	50
Whiteflies	44
Aphids	25
Stink bug	19
Squash bug	19
Others[4]	3
Crucifers (15 growers)	
Lepidoptera[5]	44
Aphids	22
Others[6]	11

[1] *Manduca sexta* L.
[2] Thrips, pepper weevil, fire ants.
[3] Squash vine borer (*Melittia cucurbitae* [Harris]), pickleworm (*Diaphania nitidalis* Stoll), melonworm (*D. hyalinata* L.).
[4] Cucumber beetles, fire ants, leaf-footed bugs (Coreidae).
[5] Primarily cabbage looper (*Trichoplusia ni* [Hübner]).
[6] Yellow-margined leaf beetle, flea beetles (Chrysomelidae: *Epitrix* spp.).

Almost half (12) the growers described using alternative or home-made pest management methods. Most of these (7) involved interplanting crops to encourage beneficial insects or to repel/distract pest species. Five growers described home-made soap or detergent sprays. Two growers prepared botanical sprays, and on one farm brightly colored dishes filled with soapy water were used to trap a variety of pests.

Most growers (92%) scouted for insect pests, and 68% scouted daily, although apparently in an informal fashion. Almost 90% referred to the Organic Material Review Institute or Florida Organic Growers to determine if a given product was acceptable for use on a certified organic farm. Certified organic growers may only

TABLE 4. Pest management methods used by organic farmers.

Method	% Using	% Reporting as effective	Target
Bacillus thuringiensis	88	100	Lepidoptera, Colorado potato beetle
Insecticidal soap	64	50	Aphids, whiteflies
Mail-order predators	36	50	various
Mail-order parasitoids	32	>50	Leafminers, Lepidoptera eggs, whiteflies
Azadirachtin	24	few	various
Rotenone	4	100	Stink bugs, beetles
Pyrethrum	3	–	various

use products indicated by the Organic Materials Review Institute, which publishes a list of acceptable materials every year.

Almost three-quarters (72%) of the growers stated that they had been at one time unsure if an arthropod on their crop was beneficial or harmful. Less than half (44%) the growers regularly sought professional assistance (University of Florida, County Extension, or Division of Plant Industry) in identifying unknown pests on their farm. Twenty-four percent stated that they would attempt to identify the pest from reference books or the internet. The remainder did not respond to the question, or in a few instances indicated that they would ask other farmers.

Pest impact—There was no difference in level of ranking for marketing, fertilizer management, insect, disease, or weed management among the 23 growers who responded to this question (CHISQ = 3.04; DF = 4; P = 0.55). Marketing tended to be ranked lowest ($x \pm SE = 3.5 \pm 0.34$), meaning that it posed the fewest problems. Insect and disease management followed (2.9 ± 0.23 and 2.9 ± 0.27 respectively). Nutrient management and weed management tended to be ranked highest (2.7 ± 0.34 and 2.7 ± 0.29).

While only 16% of the growers reported that insect pests had prevented them from growing high value crops, 72% responded that insect pests had forced them to reduce production of certain crops. The details provided by growers on the threatened crops and the pests affecting them are indicative of the difficulties inherent in developing pest management programs targeted at mixed organic vegetable growers.

Fifteen growers together reported that eleven distinct insect pests reduced yield of seven different crops. Squash and brassicas were each listed four times, and the remaining crops (beans, corn, peas, peppers, tomatoes) were listed three times or less. Whitefly and whitefly-vectored viruses were listed three times as the limiting agent, but other insects were mentioned only twice (i.e. pepper weevil [*Anthonomus eugenii* Cano] and fire ants [*Solenopsis* spp.]) or once. The four growers who reported that an insect pest had prevented them from growing a high value crop each indicated a different crop and pest (stink bugs, fire ants, pepper weevil, whitefly/virus). These varied responses reflect not only the diversity of crops grown, but the fact that growers represented several distinct growing environments, from the Florida panhandle to subtropical Homestead.

When growers were asked to list their primary insect pests, the responses were similarly varied. Growers were allowed to list more than one pest. Whiteflies or whitefly-transmitted disease were listed by 32% of the growers. Aphids and caterpillars were each listed by 20% of the growers. Sixteen percent mentioned stink bugs or grasshoppers, and 12% listed fire ants. Thrips and the yellow-margined leaf beetle (*Microtheca ochroloma* Stal) were each listed by 8% of the growers. Grubs, the Colorado potato beetle, and various weevils were reported once.

The yellow-margined leaf beetle tends to be killed by broad spectrum pesticides, and is rarely a problem for conventional growers (Webb, 2002). Stink bugs, whiteflies, fire ants, the pepper weevil and other pests of organic growers also affect conventional growers in Florida. However conventional growers rely on synthetic chemicals to reduce losses to these pests. Target-specific compounds such as imidacloprid and hydramethylnon are crucial for the management of *B. argentifolii* and *S. invicta*, respectively, under conventional farming systems (Polston et al., 1994; Collins and Schefran, 2003). Various broad-spectrum pesticides are included in conventional management programs for stink bugs and the pepper weevil (Capinera, 2002).

DISCUSSION—The diversity of pests listed is not surprising given the diversity of crops grown by organic farmers in Florida. Visits to farms and telephone conversations with growers suggest that the overall level of crop diversity on Florida's mixed organic vegetable farms is even greater than indicated by this survey. A primary tenet of organic farming is that healthy soils produce healthy plants. A complementary concept of organic farming is that diversity lends balance to a cropping system. The philosophical attachment to crop diversity held by organic producers influences not only the insect pests they encounter, but also their responses to pest problems.

While many conventional growers identify with a given crop commodity, organic growers seem to identify instead with a system of farming. For example, conventional cabbage or potato farmers are wed to the pests of the crops they grow. By contrast, organic farmers often indicated that if a given crop had too many pest problems, they would either stop growing the crop or grow less of it. We found, as did Swisher and co-workers (1994), that many organic farmers expressed an entrepreneurial or market-driven approach to farming: they were constantly experimenting with new crops, and exploring new market niches, such as Asian greens or specialty potatoes. The diversity of organic vegetable farms, and the willingness of the growers to change what they grow, make it difficult to generalize about the insect pests of mixed organic vegetable growers.

Some organic growers reacted to pest damage with an established pesticide application regime much as would a conventional grower. Other organic growers relied very little on commercially-available organic pest control products, and had developed an array of home-made methods for reducing pests. Several organic growers in the survey stated that they never applied pesticides of any sort to their crops. On the whole, the survey and visits to farms suggest that organic growers are willing to tolerate considerably higher levels of pest damage than conventional growers. This may be from necessity, but the higher market value of organic compared to conventionally produced crops may also mean that losses to pest damage are simply less critical to organic than conventional farmers.

The need to develop pest management strategies and to define pest management research priorities for mixed organic vegetable growers presents crop protection specialists with special challenges. Agricultural entomologists tend to be trained in commodity-based research, and how to develop management programs for the pest or pest complexes of crops grown in monoculture. The population dynamics of a given arthropod may differ on a host crop that is grown in polyculture compared to when it is grown in monoculture (Stanton, 1983; Andow, 1991). It is possible therefore that sampling methods and economic thresholds for pests may have to be adjusted when crops are grown in mixed rather than simple systems. Further research is required in this area.

In addition, researchers accustomed to setting up replicated on-farm studies in large, uniform fields face unusual difficulties establishing studies in small complex polycultures. Crops on mixed vegetable farms are often grown in patches that are too small to include replicated treatment and control plots. Given the highly diverse environment in which crops are grown in polyculture, it may be difficult or impossible to remove extraneous sources of variation surrounding treatment areas of interest. More importantly, the need to reduce variability that is fundamental for meaningful treatment comparisons under most field plot research designs is contrary to research objectives in polyculture. It is often the relationship between the complexity of a polyculture and its success as an agroecosystem that the researcher wishes to evaluate. Meaningful assessment of polycultures may require multiple regression techniques, or the employment of spatial analysis programs that evaluate a variety of diversity parameters.

The survey revealed the need to develop extension material to assist mixed vegetable farmers in identifying the great variety of pest and beneficial arthropod species found in complex cropping systems. Growers might also benefit from orientation in how to increase efficacy in using sprays such as botanicals or soaps and oils by understanding better which arthropod pests can be managed with these different products. Finally, several growers requested information related to increasing the density and efficacy of beneficial insects on their farms by growing plant species that provide habitat and resources for natural enemies.

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EXTRACTION OF HEAVY METALS USING MODIFIED MONTMORILLONITE KSF

Craig A. Bowe, Nadine Krikorian, and Dean F. Martin

Institute for Environmental Studies, Department of Chemistry, University of South Florida, 4202 East Fowler Avenue, Tampa, FL 33620

Abstract: *Previous work has established the possibility of utilizing known ion exchange resins and known chelating and coordinating agents that were supported on materials such as silica gel. These studies revealed the utility of these materials in the removal of heavy metals such as lead, silver, copper and nickel from aqueous media. Montmorillonite KSF has been commonly used as an ion-exchange material in the removal of heavy metals from standard solutions. In the present study, montmorillonite KSF was modified using azeotropic distillation to condense 2-mercaptoethanol with the clay material. The resulting product was used as a coordinating agent to remove copper(II), cadmium(II), silver(I), nickel(II) and lead(II) ions from standard aqueous solutions. Without adjusting the pH, better than 90% of the metal ions could be removed. Application to other solid substrates has been considered and is pending.*

Key Words: Mercaptoethanol, cadmium, copper, lead, nickel, silver, montmorillonite, remediation

The Concentration of heavy metals from dilute solutions is a technology that is not only beneficial to the environment but a practical and economical approach to the problem of removal of toxic substances from fresh water sources. The use of montmorillonite as a nutritional-based matrix for alfalfa plants proved successful in the removal of toxic metals such as cadmium(II) and zinc(II) from aqueous media. These studies were done at different acidity contents and each proved to be beneficial in the removal of the previously stated metal ions. The investigation concluded that phytoremediation utilizing montmorillonite-based soils was a viable means of removing heavy metals by competing rhizofiltration and ion exchange processes (Peralta-Videa et al., 2002).

Related investigations in the removal of heavy metals from aqueous sources illustrate the effectiveness of using montmorillonite as an ion exchange substance. The study, completed over a period of weeks, culminated in the conclusion that montmorillonite was a proficient ion exchange material even with the interaction of humic substances (Lothenbach et al., 1997, 1999). The use of humic substances, humic and fulvic acids, had been used with the montmorillonite adsorbent to remove heavy metals from a natural aqueous system. This combination was used quite successfully in the removal of high concentrations of lead, cadmium and copper ions (Liu and Gonzalez, 1999). Montmorillonite clays have been used as well in the extraction of lead and cadmium from aqueous solution. The clay material was treated with acidic and basic washes that improved the ion exchange capacity of the clay sorbent. The results were reported as being very good with more success being observed with the lead ion than the cadmium ion (Barbier et al., 2000).

The application of using clay sorbents in the removal of heavy metals was attempted in wastewater treatment. The use of acid-treated montmorillonite facilitated the removal of the heavy metals zinc, copper, and nickel from wastewater (Vengris et al., 2001). Research has also investigated the use of montmorillonite that was impregnated with organic-based ligands. The use of di-(2-ethylhexyl)phosphoric acid (DEHPA) as a binding ligand was tested with aqueous media. These studies concluded that the use of the binding agent, DEHPA, impregnated within the layers of clay sorbents such as montmorillonite, had high chemical sorption and fast kinetics in the uptake of heavy metals such as copper(II) ions (Cox et al., 2001). Also, the use of sodium dodecylsulfate (SDS) as a binding ligand was tested with standard solutions. These studies were performed as a function of relative acidity and concluded that the sorption of metal ions copper(II) and zinc(II) increased as a function of increasing pH (Lin and Juang, 2002).

We devised a procedure for attaching suitable bifunctional coordinating agents to silica gel (Martin and Bowe, 2002). Specifically, an inexpensive, commercially available reagent, 2-mercaptoethanol, was attached covalently to silica gel by acid-catalyzed azeotropic distillation. The advantage of the ligand is that it is likely to coordinate with so-called Type B heavy metal ions, that the, tendency is pH sensitive (Eqn 1). In the present study, we describe the application of the procedure to a commercially available clay, montmorillonite. The advantages would appear to be that the material is cheaper than silica gel, that it is likely to be stable over a wider range of pH. In addition, montmorillonite has intrinsic cation-exchange properties. In fact the exchange capacity was given (Wiklander, 1964; cited by Stumm and Morgan, 1970) as 0.81 meq/g, which was significantly greater than illite (0.162) or kaolinite (0.023), which seems like an additional asset.

MATERIALS AND METHODS—*Reagents*—Calcium sulfate (anhydrous), 2-mercaptoethanol, the metal salts, and concentrated sulfuric acid were obtained from Fisher Scientific and were used without any further purification. Toluene (optima grade, Aldrich Chemical) was used without further purification as the solvent for the synthesis of modified montmorillonite that was dried over anhydrous calcium sulfate.

Synthesis of modified montmorillonite KSF agents—Montmorillonite KSF (20 g, Aldrich Chemical) was treated with 50 mL of toluene that was pre-treated with anhydrous calcium sulfate. Then, 11.7 g (0.15 moles) of 2-mercaptoethanol dissolved in 50 mL of anhydrous toluene was added to the clay–toluene suspension, and four drops of concentrated sulfuric acid were added as a catalyst to facilitate the condensation reaction between the adsorbent and the bifunctional binding agent. The resulting mixture was refluxed for a period of two hours utilizing a Dean-Stark apparatus fitted with a Barrett trap to collect the water by-product. After the reaction was driven to completion, with elimination of 1.8 mL of water, the mixture was allowed to cool to room temperature and the solvent was removed by reduced pressure evaporation using a rotatory evaporator. A separate control experiment using the clay adsorbent suspended in toluene with sulfuric acid catalyst was completed producing 0.5 mL of water during the two-hour reflux period.

Analyses—Metal analyses were obtained using a Varian SpectrAA 100 atomic absorption spectrometer coupled to a Mega computer and Hewlett Packard 520C printer. Five standards, prepared by serial dilution, 0.5 ppm, 1.0 ppm, 2.0 ppm, 5.0 ppm and 10.0 ppm, were used to determine the calibration plot. The spectrometer determined the concentration of the given metal cation, which did not exceed 20 ppm, by the calibration curve. Calibrations were performed each time an analysis was completed, and a total of five standards were used to calibrate the instrument.

Sulfur analyses were performed by Constellation Technology Corporation, Largo, Florida. The elemental analysis was performed on a sample of modified montmorillonite prepared as described above. Each analysis was performed in duplicate. Anal.: S, 22.1%.

Metal-removal studies—Aqueous solutions (1.57×10^{-3} *M*) of lead(II) nitrate, cadmium(II) acetate, nickel(II) nitrate, silver(I) nitrate and copper(II) nitrate were prepared in 1.0-L volumetric flasks. Five 100-mL aliquots of stock solution were used. Modified montmorillonite KSF (4.0g) was added to each metal solution, and the resulting mixtures were agitated at a rate of 200 rpm in a New Brunswick Scientific Company Model G76 gyrotory water bath (25°C) shaker for two hours. This is a period of time that has proven to be sufficient for quantitative removal in previous studies (Norris et al., 1997).

RESULTS—The metal removal procedure is summarized (Eqn 1), where Clay–OCH_2CH_2-SH is a representation of a mercaptoethanol-montmorillonite composite

$$\text{Clay–OCH}_2\text{CH}_2\text{–SH} + \text{MX}_2 = \text{Clay–OCH}_2\text{CH}_2\text{–SM}^+\text{X}^- + \text{H}^+ + \text{X}^- \qquad (1)$$

and MX_2 is a divalent metal salt. The amount of water eliminated suggests condensation of 5 mmoles of 2-mercaptoethanol per gram of montmorillonite KSF, and a considerable excess of composite was used in our studies. With initial pH of 7, the large excess of composite constituted a driving force, as did the loss of protons (Eqn 1) that permits driving the reaction to completion by using alkali. [It should also be possible by using acid to reverse the process in order to recycle the composite.]

Results (Table 1) indicated that it was possible to remove the selected metal ions from aqueous solutions using the modified montmorillonite with a varying degree of success. For example, removal of three metals was considered successful, *viz.*, cadmium, 97.7 ± 0.5%; silver, 90.2 ± 1.9%; and copper, 85.2 ± 2.3%. Conversely, two metals were less successfully removed, *viz.*, lead, 55.8 ± 13.8 and nickel, 30.7 ± 6.4%.

DISCUSSION—Use of modified montmorillonite KSF reagents potentially affords a relatively inexpensive and convenient method for using solid-based materials in the remediation of heavy metals. Clays, related to montmorillonite KSF, seem to be relatively less expensive than silica, which is a commonly used solid support. In addition, this clay is more stable over a wider range of pH (up to 11). Although silica gel is stable under acidic conditions and shows very little loss when placed in very acidic and basic media, it has the disadvantage of becoming more soluble at pH 10 or greater (Stumm and Morgan, 1996). In addition, the method of attachment of the β-oxyethylmercapto moiety should be both inexpensive and an example of green chemistry (provided the toluene is recycled). Making the best usage of the reversible reaction between the sulfhydryl moiety and binding metal permits us the opportunity to presumably recycle the material and concentrate the metal being removed from the aquatic source.

The results indicate that a favorable equilibrium (Eqn 1) can be obtained, resulting in a favorable degree of removal of heavy metal ions from solution without

TABLE 1. Removal of heavy metals using modified montmorillonite KSF from 1.57 m*M* solution.*

Metal	Sample	Extraction,%
Cu^{2+}	Composite	85.2 ± 2.3
	Control	10.0 ± 6.0
Pb^{2+}	Composite	55.8 ± 13.8
	Control	19.9 ± 4.5
Cd^{2+}	Composite	97.7 ± 0.5
	Control	3.84 ± 2.1
Ag^{+}	Composite	90.2 ± 1.9
	Control	9.72 ± 4.7
Ni^{2+}	Composite	30.7 ± 6.4
	Control	6.18 ± 1.0

* n = 5.

the aid of basic material to drive the reaction to completion. Adjustment of pH (Eqn 1) would be a driving force, but it would also constitute an additional cost, and it seemed worthwhile to see if significant removal would occur without such adjustment. Unfortunately, the results were successful for some, but not all metals, as indicated by the results in Table 1. All five metals are Group B–type metals, for which S-atom coordination is favored over O-atom donors. All metals fall into a group called calcogenides, indicating the tendency to be found in nature associated with sulfur.

The stability relationships that apply to various coordinating agents, including amines, and heavy metals were summarized by Fernelius (1956), who noted that for a wide variety of chelating agents and bivalent metal ions, there was a fixed order of stabilities Mn < Fe < Co < Ni < Cu > Zn, the so-called Irving-Williams Order. Martell and Calvin (1952) illustrated that for a wide variety of aliphatic amines used as chelating agents and bivalent metal ions, there was a fixed order of stabilities Ni < Cu > Cd. The same logic can be applied to ligands that are sulfur-based. These examined ligands involved having sulfur as the coordination donor, and some deviation from the Irving-Williams Order might be expected for unidentate ligands with S-donor atoms.

It is interesting to examine the amounts of metals removed. One might expect that the maximum removals should be related to the relative chelating tendencies and might be expected to follow the extended order noted by Fernelius (1956), *viz.*, Pd > Hg > UO_2 > Be > Cu > Ni > Co > Pb > Zn > Fe > Mn > Mg > Ca > Sr > Ba. For systems in deionized water, the metals and their maximum removals (in parentheses) were: Cu (85.2%), Cd (97.7%), Pb (55.8%), Ag (90.2 %) and Ni (30.7%).

Conversely, the results may be better understood in terms of a HASB (Hard acid, soft base) concept (Pearson, 1997). The results are feasible due to the fact that the donor atom and metals selected are considered to be moderately "soft" and very polarizable (Pearson, 1997), and the favorable removal of cadmium, silver, and copper is consistent with the interaction of a soft base and a soft acid (borderline for Cu^{+2}) versus a soft base and a borderline metal (lead and nickel).

The coordinating moiety, β-oxyethylmercaptan, could function as a chelating agent, but there is no evidence concerning this possibility. It is possible to compare the removal values for this composite versus those composites obtained by using mercaptans absorbed on silica gel (Bowe et al., 2002). For the latter substrates, there was no possibility of chelation. The results do not indicate a notable difference in removal *vis-a-vis* the present results. The maximum removal (Bowe et al., 2002) for copper ion was 80.7 ± 7.4 (vs 85.2 ± 2.3 for copper, Table 1). A similar trend was observed for cadmium (94.6 ± 3.6, Bowe et al., 2002 vs 97.7 ± 0.5, Table 1) and, in fact, better removal percentages were obtained by Bowe and co-workers (2002) for supported alkyl mercaptans for lead (65.6 ± 2.7) and nickel (76.9 ± 0.8) than are reported for the two metals in Table 1. Thus while one might not expect a great enhancement of coordination with β-oxyethylmercaptan versus an alkyl mercaptan, nevertheless the results do not support the expected trend, and one may suspect that β-oxyethylmercaptan behaved as a unidentate ligand in these studies.

Conclusions—Heavy metal removal using modified montmorillonite was achieved with a reliable degree of success. The removal effectiveness was independent of the type of metal used in the study. The same efficiency is presumably not specific to the valence of the metal, nor its size. The validity of the proposed model (summarized in Eqn. 1) was evident here as the pH decreased upon reaction with divalent metal ions. Further consideration of the reaction (Eqn 1) indicates that the product of reaction with metal ions and the composite could subsequently be treated with acid to regenerate the adsorbent material for subsequent extraction studies. The success of regeneration was achieved previously with silica-supported mercaptans (Bowe et al., 2002). This tendency is presumably independent of the type of metal being studied. Use of modified montmorillonite using 2-mercaptoethanol affords a relatively inexpensive, convenient and effective method for removing selected heavy metal ions, with greatest success being achieved with copper(II), cadmium(II), and silver(I) ions. The application to other ligands and solid-support materials should be considered.

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PATTERNS OF HYPOXIA IN A COASTAL SALT MARSH: IMPLICATIONS FOR ECOPHYSIOLOGY OF RESIDENT FISHES

CINDY M. TIMMERMAN[1] AND LAUREN J. CHAPMAN[1,2]*

[1]Department of Zoology, University of Florida, Gainesville, Florida 32611
[2]Wildlife Conservation Society, 185th Street and Southern Boulevard, Bronx, NY 10460

ABSTRACT: *The major objective of this study was to quantify temporal and spatial patterns of hypoxia in the Cedar Key salt marsh of northwestern Florida and identify predictors of dissolved oxygen availability. In the tidal creeks of the marsh, dissolved oxygen levels were highly variable, conforming to the general trend of coastal salt marshes. Hypoxic conditions (<2.0 mg l^{-1}) were not infrequent events at the collection site, occurring on 10 of the 13 sampling dates over a 21-mo period. Despite the number of interactive variables influencing dissolved oxygen concentration, there were clear diurnal trends, with oxygen levels being lower near sunrise. In addition, oxygen levels varied with season and tidal cycles. Daily mean dissolved oxygen measured when the morning tide was low was lowest in the summer and highest in the winter. Daily mean dissolved oxygen taken on an afternoon low tide was lowest in the summer and highest in the fall.*

Key Words: Dissolved oxygen, wetland, Florida, salinity, water quality

EFFECTIVE uptake of dissolved oxygen is critical to the long-term survival of most aquatic organisms. However, there are many systems where water may not remain saturated with oxygen, leading to hypoxia. This often occurs in waters characterized by low light and reduced mixing including, as examples, heavily vegetated wetlands (Carter and Beadle, 1931; Carter, 1934, 1955; Chapman et al., 1998, 2000); flooded forests (Gessner, 1961; Chapman and Chapman, 2003); floodplain lakes and ponds (Welcomme, 1979; Junk et al., 1983); spring boils (Odum and Caldwell, 1955; McKinsey and Chapman, 1988); and the deep waters of lakes and ponds (Wetzel, 1975; Lewis and Weibezahn, 1976; Kizito et al., 1993; Talling and Lemoalle, 1998). Anoxia or severe hypoxia can also occur when seasonally or chronically deoxygenated waters in deep-water habitats periodically well up into shallow habitats (e.g., Lake Victoria: Hecky, 1993; Chesapeake Bay: Breitburg, 1990).

Strong seasonal variation in dissolved oxygen occurs in many systems associated with seasonal fluctuations in rainfall, mixing, incident light, and water temperature. For example, in intermittent tropical streams, habitats may shift from fast flowing, well-oxygenated habitats in the wet season to small isolated hypoxic pools in the dry season (Chapman and Kramer, 1991). Spatial variation in oxygen is also evident as strong vertical gradients in many deep lakes (Kizito et al., 1993; Talling and Lemoalle, 1998) and in horizontal gradients in lakes with marginal areas

* Corresponding author: email: lchapman@zoo.ufl.edu; Phone: (352) 392-7474; Fax: (352) 392-3704.

covered by floating vegetation (Bonetto et al., 1969; Junk et al., 1983; Saint-Paul and Soares, 1987). Diurnal patterns of reduced oxygen availability, linked to photosynthetic and respiratory activity have been observed in many shallow water habitats, such as eutrophic lakes and tropical/subtropical pools (Wetzel, 1975; Kushlan, 1979; Chapman and Chapman, 1993), and many estuarine habitats (Officer et al., 1984; Rosenberg et al., 1992; Justic et al., 1993; Rabalais et al., 1998).

The pattern of hypoxia in coastal marshes is particularly complex due to changes in tidal flow, seasonal variation, and complex spatial variation in patterns of mixing. In addition, tidal influences and seasonal changes in rainfall result in strong fluctuations in other physico-chemical characters, most notably salinity (Odum, 1988). Although shallow waters of tidal marsh systems are typically well mixed and have high photosynthetic productivity, hypoxia can occur (Subrahmanyam and Drake, 1975). The receding tide may lead to hypoxia in isolated pools during the day. In addition, solar input may heat these pools to over 40 °C (Subrahmanyam and Drake, 1975), and evaporation may increase salinity (>35 ppt, Kilby, 1955), thus significantly reducing the water's ability to hold oxygen. Hypoxia is also possible during tidal isolation at night, when phytoplankton and plants consume oxygen. In addition, there is growing evidence that many coastal marine ecosystems are severely affected by the increase of nutrients loadings from land. Eutrophication of these coastal waters accelerates the factors leading to hypoxia and anoxia, resulting in more frequent and longer hypoxic episodes (Rosenberg et al., 1992).

Although there has been much interest in the tolerance of coastal salt marsh fishes to extreme physico-chemical conditions (e.g., *Cyprinodon variegatus*, Nordlie, 1985; Haney, 1995; *Fundulus* spp., Nordlie 2000; *Poecilia latipinna*, Nordlie et al., 1992), there are few studies that document patterns of hypoxia and other water-quality parameters in these systems. The major objective of this study was to quantify temporal and spatial patterns of hypoxia in the Cedar Key salt marsh of northwestern Florida and identify predictors of dissolved oxygen availability. We also report spatial and temporal variation in water temperature and salinity and discuss implications of these limnological patterns for the ecology and physiology of resident fishes.

STUDY SITE AND METHODS—The tidal marsh of Cedar Key lies where the flat shoreline of the upper peninsula of Florida meets the shallow waters of the Gulf of Mexico (Fig. 1). Distinguished from terrestrial habitats by tidal submergence, the tidal marsh hosts a diverse and characteristic biota. The shoreline near Cedar Key consists of coastal salt marsh that is more saline than fresh and about 5 km in width. The dominant emergent macrophyte, *Spartina alterniflora*, fringes many of the oyster bars and outlying small islands. Dense thickets of black mangrove, *Avicennia germinans*, as well as smooth cordgrass, *Spartina alterniflora* dominate the landscape. Closer to the mainland, black needle rush, *Juncus roemerianus*, also occurs. A sinuous maze of channels links the shallow backwaters of these mudflats to the deeper coastal waters of the Gulf. At extremes in tide, the entire tidal area may be completely submerged or consist of expanses of bare, flocculent ooze, interspersed with islands of vegetation and isolated pools.

Measurements of dissolved oxygen, water temperature, and salinity were taken at four sites in the Cedar Key salt marsh (Fig. 1). These sites were located at a large culvert 1.2 km from the town of Cedar Key on Florida State Highway 24. Site 1, a pool, was more isolated than the other sites, especially during low tide. Sites 2, 3, and 4 receive more runoff from terrestrial freshwater sources and were isolated from

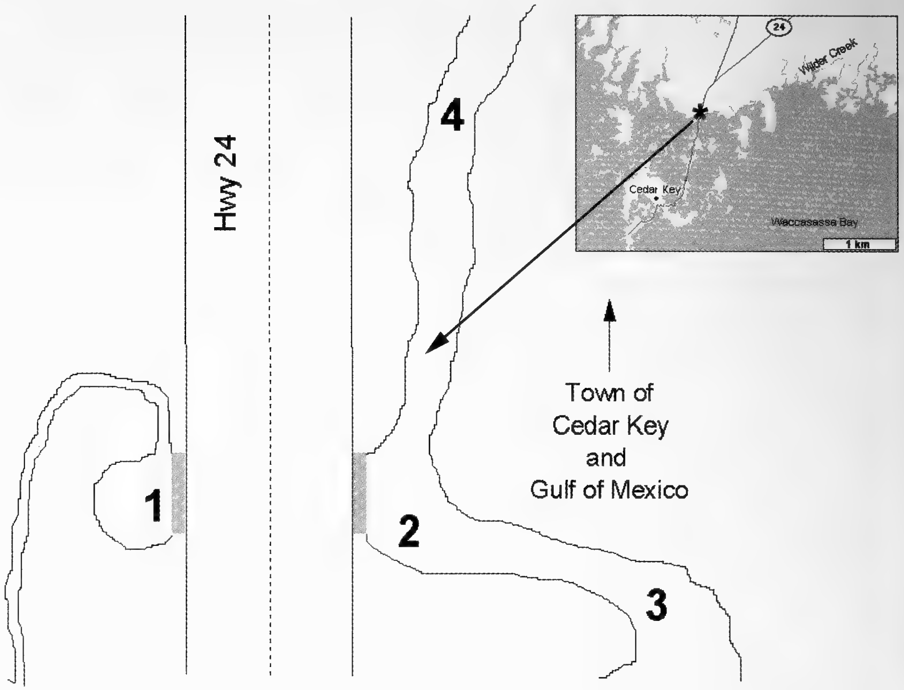

FIG. 1. Map of sites where dissolved oxygen concentration, salinity, and water temperature were measured at the salt marsh of Cedar Key, Florida.

each other only on occasional extreme low tide events. We predicted that tide might be an important factor in determining hypoxia, and therefore, measurements were taken on 2 days every 3 mo (one day with a morning low tide, one day with a morning high tide) over 21 mo to produce 6.5 quarters of data. Each sampling day, measurements were made at four sites every 2 h from 08:00 to 17:00 h. Salinity was measured using an optical refractometer. Dissolved oxygen concentration and water temperature were measured using a YSI (Yellow Springs Instruments) Model 57 meter. The average of two dissolved oxygen readings taken 10 cm below the water surface was used to estimate aquatic oxygen levels.

To test for inter-site variation in mean oxygen levels, the mean dissolved oxygen concentration over each sample day was calculated for each site producing 13 values across 21 months of sampling. Paired t-tests were used to detect differences in the mean dissolved oxygen levels between pairs of sites. The Bonferonii correction factor was used to adjust the P-value of acceptance for the multiple comparisons to $p = 0.008$. To test for a significant seasonal trend in mean dissolved oxygen levels, a repeated measures analysis of variance was conducted with the four sites representing the replicates. Data collected in morning low tide and morning high tide were analyzed separately, and each year was analyzed separately to permit sufficient replication within season ($n = 4$ sites) for the analysis of temporal trends [$n = 4$ quarters in year 1 (January 1995 to October 1995) and 2-3 quarters in year 2 (December 1995 to September 1996)]. The Mauchly's criterion was used to test for the compound sphericity of the variance-covariance matrix. When the criterion was rejected, an alternative test (Greenhouse-Geisser), which relaxes the symmetry assumption, was used to obtain a corrected significance level (Potvin et al., 1990). Repeated contrasts were used to identify when a sample period differed from the subsequent sample. Pearson correlation was used to detect relationships between mean dissolved oxygen at Sites 1 and 4 and mean salinity and water temperature at these sites over the seasonal cycle. Sites 2, 3, and 4 were very similar in mean dissolved oxygen levels, so we selected Site 4 as representative of the three sites for some analyses.

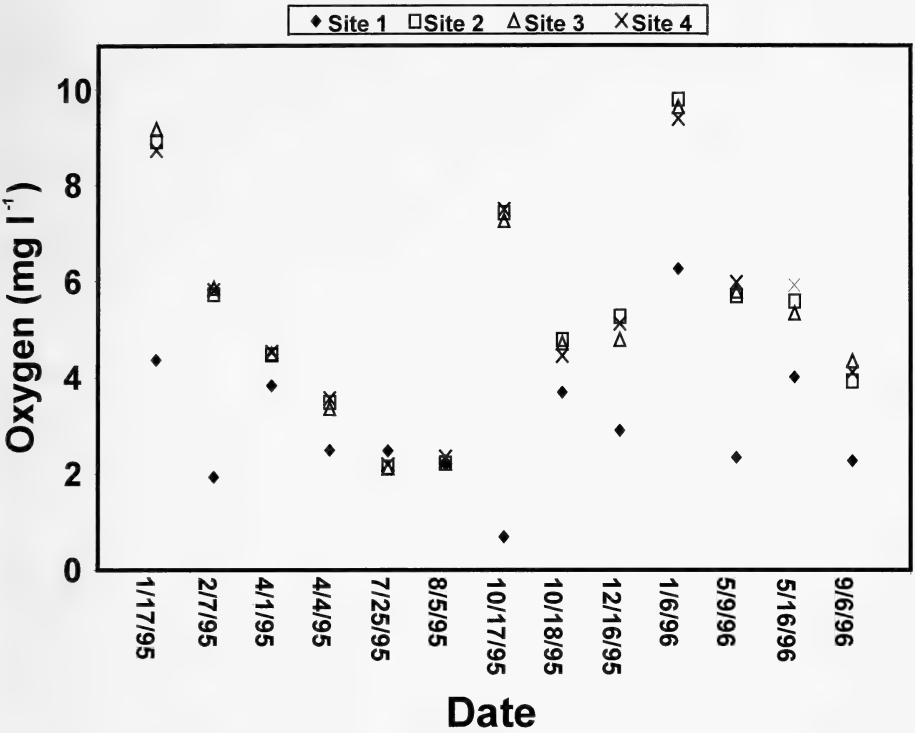

FIG. 2. Average dissolved oxygen concentration (mg l^{-1}) measured over a diurnal cycle for each of four sites measured in the Cedar Key salt marsh.

RESULTS—*Dissolved oxygen*—There were consistent differences between the physico-chemical characters measured at Site 1 and those of other sites (Fig. 1) despite their close proximity. Mean dissolved oxygen level at Site 1 was lower than at the other three sites (paired t-tests: Sites 1 vs 2: $t = 4.73$, $p < 0.001$; 1 vs 3: $t = 4.50$, $p = 0.001$, 1 vs 4: $t = 4.88$, $p < 0.001$, Figs. 2, 3, Table 1). The oxygen levels at sites 2 through 4 were highly correlated (2 vs. 3: $r = 0.995$, $p < 0.000$; 2 vs 4: $r = 0.996$, $p < 0.000$; 3 vs 4: $r = 0.993$, $p < 0.000$), and no differences in mean levels were detected between any two pairs of the three sites (paired t = tests, $P > 0.05$, Fig. 2).

Dissolved oxygen varied significantly among sampling dates in year 1 (repeated measures ANOVA, morning tide low: $F = 18.189$, $P = 0.023$; morning tide high: $F = 20.329$, $P = 0.020$). Dissolved oxygen decreased to the lowest levels in the summer (morning tide low: mean DO $= 2.23 \pm 0.08$ mg l^{-1}, SE; morning tide high: mean DO $= 2.25 \pm 0.03$ mg l^{-1}, repeated contrasts, $P < 0.05$, Table 1, Fig. 2). Highest mean levels were recorded in the winter (mean DO $= 7.80 \pm 1.15$ mg l^{-1}) when the morning tide was low and in the fall (mean DO $= 6.69 \pm 0.70$ mg l^{-1}) when the morning tide was high (repeated contrasts, $P < 0.05$, Table 1, Fig. 2). In the second year, we sampled two quarters (morning tide low) and three quarters (morning tide high). In both cases, temporal variation was significant (repeated measures ANOVA, morning tide low: $F = 56.243$, $P = 0.005$; morning tide

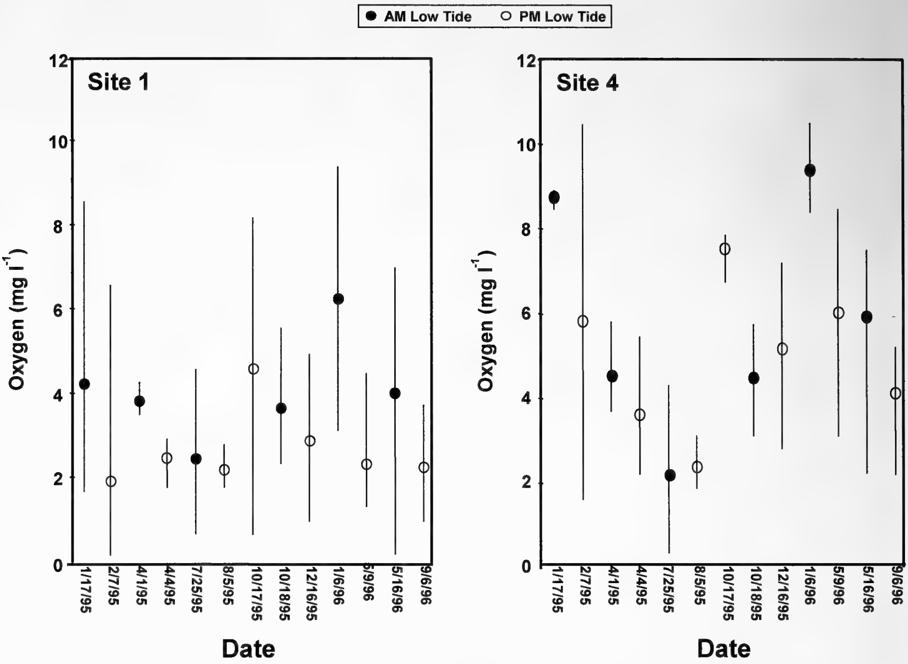

FIG. 3. The daily mean and range of dissolved oxygen concentration (mg l^{-1}) at Site 1 and Site 4 in the Cedar Key salt marsh, Florida. Sites 1 and 4 refer to locations indicated on Fig. 1.

high: F = 7.676, P = 0.046); and the pattern of DO variation was similar to the first year (Table 1, Fig. 2).

Dissolved oxygen showed very strong diurnal variation on many of the sample days ranging by an average of 4.1 mg l^{-1} for Site 1, 3.5 mg l^{-1} for Site 2, 3.5 mg l^{-1} for Site 3, and 3.4 mg l^{-1} for Site 4 (Fig. 3). Although extreme hypoxia (<1.0 mg l^{-1} oxygen) occurred at all sites, it was more common at Site 1 than at the other sites. Minimum levels averaged 1.5 (range = 0.2 to 3.5) for Site 1; 3.5 (range = 0.5 to 9.0) for Site 2; 3.5 (range = 0.4 to 8.9) for Site 3; and 3.6 (range = 0.3 to 8.4) for Site 4. In many cases, an afternoon low tide appeared to depress the mean oxygen level for the day (Fig. 3). This was reflected in lower minimum values when the low tide was in the afternoon (mean minimum DO = 2.6 mg l^{-1}) than when the low tide was in the morning (mean minimum DO = 3.8 mg l^{-1}). In many cases dissolved oxygen levels appeared to be influenced by the ebb and flow of the day's tidal currents (e.g., Fig. 4). However, on four of the 13 dates recorded, the diurnal pattern of dissolved oxygen availability did not follow tide levels. Three of these four dates shared a common tidal pattern: a moderate morning high tide followed by a weak afternoon low tide. The fourth date had a strong midday low tide, and dissolved oxygen levels were depressed throughout the day (DO range = 1.0–3.7 mg l^{-1}).

Water temperature and salinity—Water temperature varied little among sites (Site 1: mean daily average = 23.5 °C, mean daily range = 19.8–27.1 °C; Site 2: mean

TABLE 1. Dissolved oxygen concentration (mg l^{-1}, daily mean and range) measured at four sites in the Cedar Key salt marsh, Florida. The mean represents the average of samples taken every 2 h from 08:00 to 17:00 h.

Date	Morning tide	Site 1 oxygen (mg l^{-1})	Site 2 oxygen (mg l^{-1})	Site 3 oxygen (mg l^{-1})	Site 4 oxygen (mg l^{-1})
1/17/95	Low	4.36	8.92	9.17	8.73
		1.9–8.5	8.7–9.0	8.8–9.6	8.4–8.9
2/7/95	High	1.93	5.73	5.86	5.81
		0.2–6.6	1.1–10.6	1.2–10.3	1.6–10.4
4/1/95	Low	3.83	4.47	4.49	4.53
		3.5–4.3	3.4–5.8	3.6–5.6	3.7–5.8
4/4/95	High	2.49	3.49	3.35	3.56
		1.8–2.9	2.1–5.6	2.0–5.1	2.2–5.4
7/25/95	Low	2.48	2.14	2.12	2.19
		0.7–4.6	0.5–4.1	0.4–4.1	0.3–4.3
8/5/95	High	2.20	2.22	2.22	2.35
		1.8–2.8	1.6–3.1	1.6–3.1	1.8–3.1
10/17/95	High	4.6	7.42	7.25	7.49
		0.7–8.2	6.4–8.1	6.3–8.1	6.7–7.8
10/18/95	Low	3.69	4.79	4.71	4.45
		2.3–5.6	3.6–5.8	3.4–5.9	3.1–5.7
12/16/95	High	2.90	5.27	4.79	5.12
		1.0–4.9	2.8–7.4	2.6–6.3	2.8–7.2
1/6/96	Low	6.25	9.78	9.62	9.38
		3.1–9.4	9.0–10.8	8.9–10.6	8.4–10.5
5/9/96	High	2.34	5.69	5.78	5.97
		1.3–4.4	3.0–8.3	2.9–8.4	3.1–8.4
5/16/96	Low	4.01	5.59	5.33	5.92
		0.2–7.0	1.8–7.4	1.2–7.5	2.2–7.5
9/6/96	High	2.27	3.92	4.34	4.11
		1.0–3.7	2.1–5.8	2.1–5.9	2.2–5.2

daily average = 23.2 °C, mean daily range = 19.6–26.9 °C; Site 3: mean daily average = 23.4 °C, mean daily range = 19.8–28.5 °C; Site 4: mean daily average = 23.0 °C, mean daily range = 18.9–26.0 °C, Table 2). Differences in salinity among sites were greater than temperature differences and seemed to be affected by differential inland freshwater (FW) run-off among the sites (Site 1: mean daily average = 14.5 ppt, mean range = 10.8–19.0 ppt; Site 4: mean daily average = 13.5 ppt, mean range = 9.6–18.8 ppt, Table 2). The most extreme salinities were measured at Site 4 ($\leq$0.5 and 28.0 ppt). We examined relationships between dissolved oxygen concentration and both salinity and water temperature at Sites 1 and 4. Across the 13 sampling periods, mean dissolved oxygen concentration was negatively related to water temperature at Site 4 ($r = -0.680$ $P = 0.011$ and marginally related to temperature at Site 1 ($r = -0.483$, $P = 0.094$). Across the same sampling periods, mean dissolved oxygen was also negatively related to salinity at Site 1 ($r = -0.554$, $P = 0.049$), but not related to salinity at Site 4: $r = -0.347$, $P = 0.246$).

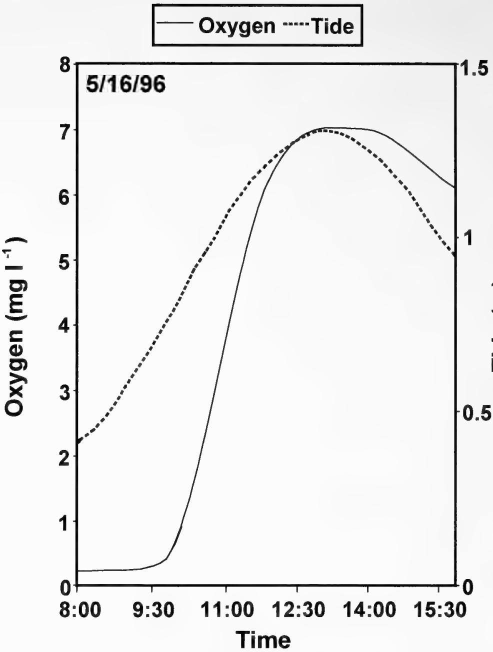

FIG. 4. The patterns of dissolved oxygen concentration (mg l^{-1}) and tide height (m) at Site 1 in the Cedar Key salt marsh, Florida (5/16/96).

DISCUSSION—The pattern of dissolved oxygen availability in the Cedar Key salt marsh system appears to be influenced by photosynthetic processes as well as seasonal and tidal conditions. Hypoxic conditions (<2.0 mg l^{-1} O_2) were frequent, occurring on 10 of the 13 dates measurements were taken. Extreme hypoxia ($\leq$1.0 mg l^{-1} O_2) was recorded on six of the 13 dates. Although there was general correlation between mean daily oxygen levels and water temperature across the 13 sampling periods, extreme hypoxia was documented at Cedar Key sites under colder conditions, when oxygen solubility is higher (2/7/95, Tables 1, 2). When hypoxia occurred, it was usually temporally associated with low tide and isolation from the surrounding system regardless of time of day. During isolation at low tide microbial metabolism of the heavy organic bottom sediments may contribute to hypoxic episodes (Belanger, 1981). However, photosynthetic processes also seem important; highest oxygen levels were often associated with high tide in the afternoon when both light and water levels were concomitantly high. Other environmental factors, such as freshwater runoff, current, and wind are also likely to influence aquatic oxygen levels and may account for the high degree of temporal and spatial variance in the patterns of hypoxia documented.

Although local generation of hypoxic conditions through pool isolation and/or nocturnal respiration appear to be factors affecting hypoxia in the Cedar Key system, other factors play an important role in other coastal systems. Seasonal severe hypoxia (<2.0 mgl^{-1} O_2) occurs in the bottom waters of the Northern Gulf of

TABLE 2. Temperature and salinity (daily mean and range) measured at two sites of Cedar Key salt marsh. The mean represents the average of samples taken every 2 h from 08:00 to 17:00 h.

Date	Morning tide	Site 1 temperature (°C)	Site 4 temperature (°C)	Site 1 salinity (ppt)	Site 4 salinity (ppt)
1/17/95	Low	14.2 13.5–14.5	13.7 11.2–15.4	7.0 6.2–8.0	1.3 ≤0.5–2.0
2/7/95	High	15.0 11.0–18.0	15.2 11.5–18.5	11.0 10.0–15.0	11.2 10.0–14.0
4/1/95	Low	21.6 17.0–24.0	21.3 17.2–24.5	15.8 8.0–25.0	17 10.0–25.0
4/4/95	High	21.0 18.5–25.0	21.6 18.2–25.0	8.8 6.0–15.0	14.2 12.0–20.0
7/25/95	Low	30.1 27.7–32.8	30.1 27.7–32.8	20.2 12.0–24.5	18.8 12.0–23.0
8/5/95	High	30.8 24.1–35.0	27.4 17.5–34.0	14.6 11.3–19.5	14.2 4.5–22.0
10/17/95	High	22.6 18.0–26.5	22.1 17.5–25.4	6.2 5.0–7.0	1.2 ≤0.5–2.0
10/18/95	Low	26.4 24.2–29.0	26.7 23.9–30.0	13.0 10.0–22.0	9.8 5.0–24.0
12/16/95	High	20.6 18.0–22.0	20.6 17.0–24.0	18.0 15.0–26.0	23.6 19.0–28.0
1/6/96	Low	14.4 11.0–17.9	13.8 9.0–18.0	7.8 5.0–10.0	4.0 ≤0.5–8.0
5/9/96	High	29.4 24.5–34.0	29.8 25.0–34.0	21.2 16.0–25.0	22.4 19.0–26.0
5/16/96	Low	27.9 24.0–33.0	28.3 24.0–33.0	23.9 20.5–26.0	23.3 19.5–26.0
9/6/96	High	29.4 26.0–34.0	29.4 26.0–33.0	20.7 16.0–24.0	14.0 5.5–24.0

Mexico. The temporal and spatial variability in hypoxic water masses in that region is correlated with the amplitude and phasing of freshwater discharge from the Mississippi River (Justic et al., 1993; Rabalais et al., 1998). Anthropogenic nutrient loads (e.g., synthetic fertilizers, detergents) appear to overwhelm homeostatic mechanisms of the marine coast ecosystem to produce hypoxic water masses below the picnocline of the Louisiana shelf (Justic et al., 1993; Rabalais et al., 1998). Strong variation in oxygen levels has also been reported in Tampa Bay, ranging from 1.4–11.6 mg l^{-1} (Simon, 1974). These hypoxic episodes were reportedly attributed to anthropogenically-induced factors, such as pollution sources, or resuspension of anaerobic bottom sediments from ship's propellers (Simon, 1974).

Hypoxia is also a common occurrence in the near-shore, shallow waters of the Chesapeake Bay and seems to be linked to stratification and an influx of oxygen-depleted lower layers into the shallows by wind and tidal currents (Carter et al., 1978; Officer et al., 1984; Tyler, 1984; Seliger et al., 1985; Breitburg, 1990).

Breitburg (1990) measured oxygen concentration, salinity, and temperature in the shallow inland waters of the Chesapeake Bay over two summers. Dissolved oxygen concentrations dipped below 2 mg l^{-1} on 45% and below 1 mg l^{-1} on 9% of summer days measured. Minimum daily DO concentrations ranged from 0.47 to 7.16 mg l^{-1}, with the extreme minima occurring during the dark and morning hours.

Temperatures recorded in our study of the Cedar Key salt marsh were similar to those of other north Florida salt marshes (Cedar Key: 9–33 °C, this study; 15–31 °C, Haney, 1995; St. Marks and Wakulla marshes, 12–36 °C, Subrahmanyam and Drake, 1975; Florida coastal marshes: 12.5–37.5 °C, Kilby, 1955). Kilby's (1955) measurements of several North Florida coastal marshes revealed an extensive salinity range in the inner tide pools (1.2–35.6 ppt), although he surmised that there was generally low fresh water influx in these systems. We found that daily salinity minima $\leq$ 5 ppt were common, occurring on five of the 13 dates recorded, and daily means of $\leq$ 5 ppt occurred on three occasions. However, the average mean daily salinity for the sites was approximately 14 ppt, in agreement with Kilby (1955). Seasonal salinity increases have been reported in other North Florida salt marshes and attributed to different rates of evaporation (e.g., 2 ppt, February, 32 ppt June, Subrahmanyam and Drake, 1975).

A feature of coastal marshes is variation in species composition with elevation. It is generally accepted that the upper inland range limit of aquatic species within these coastal habitats is determined by physiological tolerance to extremes in environmental conditions, such as salinity and temperature, while their seaward range is limited by competitive interactions (Adam, 1990). As such, there has been much effort toward quantifying the physiological tolerance of inland aquatic species in these systems. In the interior waters of the salt marsh, where the environmental measurements were taken, the ichthyofauna is dominated by the sailfin molly, *Poecilia latipinna* (Kilby, 1955; pers. obs.), with diamond killifish (*Adinia xenica*), longnose killifish (*Fundulus similis*), gulf killifish, (*Fundulus grandis*), and southern sheepshead minnow (*Cyprinodon variegatus*) also present in great numbers. The euryhaline characteristics of many of these species have been studied, and they generally show a very broad range of salinity tolerance (Table 3).

Fishes commonly found in the protected inland areas of the mudflat are likely to share not only tolerance of euryhaline conditions, but also tolerance to periodic extreme hypoxia. Surprisingly little has been published on aquatic hypoxia as a factor for survival in tidal marsh environments. Those fish commonly occurring in the inland waters of Cedar Key probably use aquatic surface respiration among other strategies to survive these periods of hypoxia. The surface film of water contains high concentrations of oxygen, and many fish can take advantage of this by skimming the surface film, a behavior referred to as surface skimming or aquatic surface respiration (ASR; Kramer and Mehegan, 1981). However, since ASR in some circumstances increases exposure to aerial predation (Kramer et al., 1983), salt marsh fishes are likely to have developed other behavioral, or physiological, mechanisms to minimize their time at the surface. In her study of the ecophysiology of sailfin mollies from the Cedar Key salt marsh, Timmerman (2001) found that short-term behavioral and physiological responses appear to permit *P. latipinna* the

TABLE 3. Summary of salinity tolerances of species that commonly occur at the Cedar Key salt marsh sites.

Species	Salinity Tolerance	Reference
Adinia xenica	0.5-95+ ppt	Nordlie, 1987
Cyprinodon variegatus	≤0.5-142 ppt	Simpson and Gunter, 1956; Kilby, 1955, 1980
Fundulus confluentus	≤0.5-100 ppt	Nordlie, 2000
Fundulus grandis	≤0.5-110 ppt	Nordlie, 2000
Fundulus similis	≤0.5-100 ppt	Nordlie, 2000
Mugil cephalus	≤0.5-54 ppt	Nordlie and Leffler, 1975
Poecilia latipinna	≤0.5-70 to 80 ppt	Nordlie et al., 1992

flexibility to deal with extreme hypoxia. Sailfin mollies acclimated to chronic extreme hypoxia (6 wk at 1.0 mg l^{-1} O_2) exhibited higher hemoglobin [Hb] and red blood cell concentrations (RBC), and a 15–20% lower critical oxygen tension (P_c) than fish acclimated to normoxia. Timmerman (2001) noted that ASR was an immediate response to hypoxia that decreased over the acclimation period suggesting that physiological compensation reduces the threshold for ASR. Reproduction continued under extreme hypoxia, and there was no effect on survivorship. However, the oxygen requirements of female mollies (measured as routine metabolic rate) increased with development of their gestating brood, as did time allocated to ASR under hypoxic stress, which may affect vulnerability to aerial predators (Timmerman, 2001).

In our study of the Cedar Key salt marsh, dissolved oxygen concentration was measured in the upper 10 cm of the water column. It should be noted that hypoxic bottom water commonly occurs in many estuarine and coastal systems, and, had this been measured, overall hypoxia would have been greater. Sediment oxygen levels are typically anoxic in coastal and estuarine systems, and many studies of adaptations to hypoxia (such as anaerobic capacity, reduced metabolic rate [VO_2], or critical tension [P_c]) have focused on benthic or burrowing fishes (e.g., Innes and Wells, 1995; Quinn and Schneider, 1991).

In summary, the temporal and spatial patterns of hypoxia in coastal salt marshes like the Cedar Key salt marsh are extremely complex reflecting the complex interactions of tide, photosynthesis, and water input, as well as local habitat characteristics that may affect mixing and rates of production and respiration. Despite the number of interactive variables influencing dissolved oxygen availability, there were seasonal and diurnal trends. The latter seem to be affected by tidal cycles. Water-breathing organisms that survive in these systems throughout the year must show either strong diel migrations or very high tolerance to fluctuating physico-chemical conditions.

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