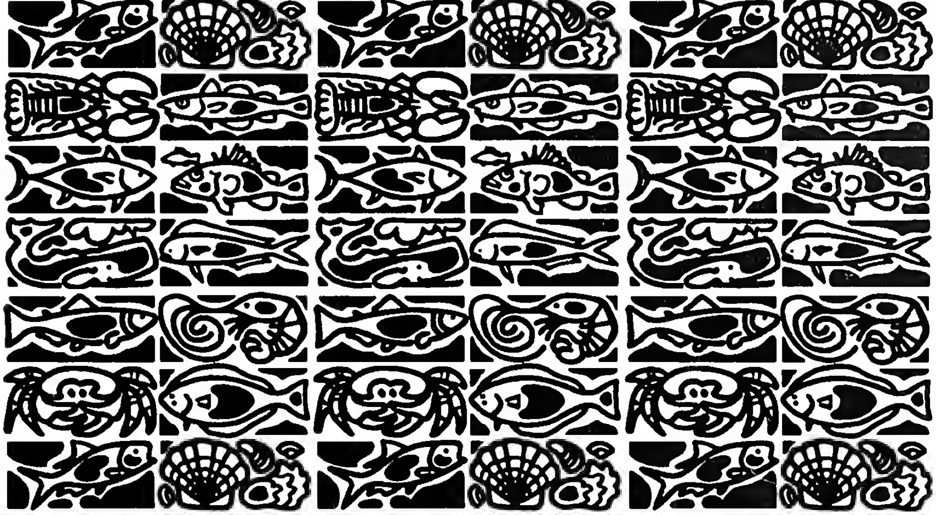

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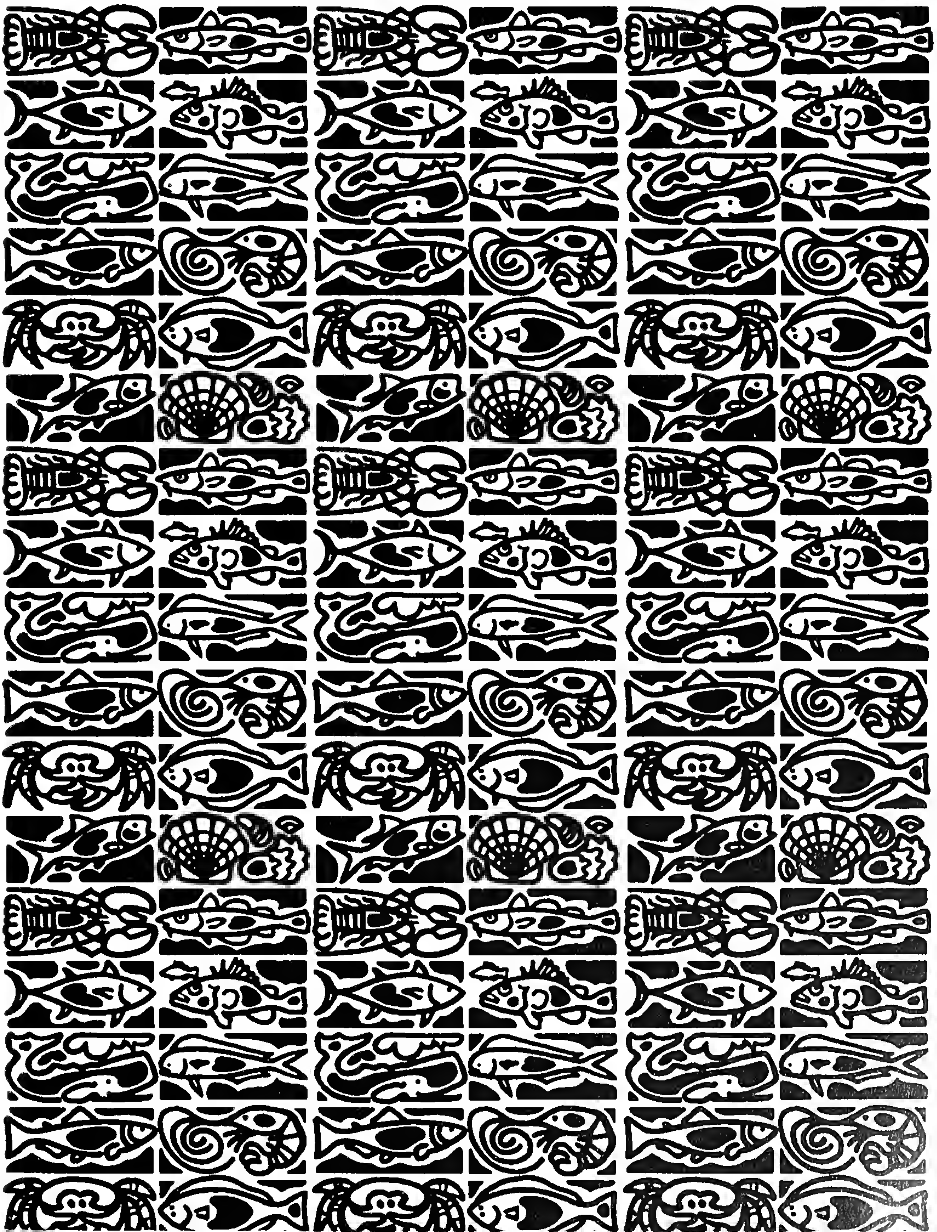

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Scientific Editor
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National Marine Fisheries Service
Northeast Fisheries Science Center
Maine Field Station
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Orono, ME 04473

Managing Editor
Sharyn Matriotti

National Marine Fisheries Service
Scientific Publications Office
7600 Sand Point Way NE
Seattle, Washington 98115-0070

Associate Editor
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National Marine Fisheries Service
Office of Science and Technology
1845 Wasp Blvd., Bldg. 176
Honolulu, Hawaii 96818

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established 1881

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of *Fishery Bulletin*

Identification of a nonlethal method for aging tautog (*Tautoga onitis*)

Scott P. Elzey (contact author)
Kimberly J. Trull

Email address for contact author: scott.elzey@state.ma.us

Fish Biology Program
Massachusetts Division of Marine Fisheries
30 Emerson Avenue
Gloucester, Massachusetts 01930

Abstract—Aging of tautog (*Tautoga onitis*) has historically required sacrificing fish to obtain opercula and otoliths. Use of these structures for age determination has hindered researchers from obtaining samples from fish that were to be released alive, as well as from commercially collected fish that are commonly sold whole. In this study we evaluated the use of scales, dorsal-fin spines, pelvic-fin spines, opercula, whole sagittal otoliths, and sectioned sagittal otoliths as structures for age determination of tautog. Our results indicate that pelvic-fin spines provide high-precision age estimates without bias. Dorsal-fin spines had well-defined annuli, but vascularization near the core prevented consistent identification of the first annulus and led to biased ages. Scales were difficult to read and provided highly biased ages in older (>age 7) fish. The precision of age estimations derived from pelvic-fin spines was better than the precision of age estimations derived from the other structures. Pelvic-fin spines provide suitable age estimates for tautog, and these structures can be collected easily from a wider variety of sample sources than can the structures currently being collected for age determination of this species.

The tautog (*Tautoga onitis*) is a species of fish from the family Labridae that ranges from Nova Scotia (Scott and Scott, 1988) to South Carolina (Grimes et al., 1982). It is a commercially and recreationally important species from Massachusetts to Virginia (ASMFC[1]). Tautog grow to approximately 90 cm in total length (TL) and 10.2 kg in weight (Bigelow and Schroeder, 1953). It is a slow growing and long-lived species that reaches maturity at age 3 (Hostetter and Munroe, 1993) and has been estimated to live up to age 34 (Cooper, 1965, 1967; Hostetter and Munroe, 1993). Cooper (1965, 1967) and Hostetter and Munroe (1993) were able to estimate the age of tautog from marks on their opercula. Hostetter and Munroe (1993) were also able to justify the assumption that marks on the opercula were deposited annually and, therefore, justify the use of those marks for age determination through marginal increment analysis. For these reasons, opercula have been the primary and recommended structure for estimating the age of tautog (ASMFC[1]).

In 2012, representatives from 10 different laboratories attended a workshop on aging tautog (ASMFC[2]). Although staff at the majority of the laboratories had considerable experience aging tautog, with the use of the operculum as the structure for determining age, and staff at a few of the laboratories had experience with otoliths of tautog, the precision of age estimates between laboratories was similar for both structures. The results from that workshop indicated that, with increased experience by the staff, the use of sectioned otoliths from tautog may yield age estimates of higher precision than the use of opercula. After that workshop, sectioned otoliths have been used as a supplementary method for age determination (ASMFC[2]).

Although current methods for age determination of tautog are based on opercula and otoliths, multiple structures have been used to age other fish species, including opercula, otoliths, vertebrae, fin rays, fin spines, and scales (Beamish and McFarlane, 1987; Panfili et al., 2002). Fin rays, fin spines, and scales have the distinct advantage in that their collection is nonlethal. Phelps et

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[1] ASMFC (Atlantic States Marine Fisheries Commission). 2015. Tautog benchmark stock assessment and peer review reports, 283 p. AFMFC, Arlington, VA. [Available at website.]

[2] ASMFC (Atlantic States Marine Fisheries Commission). 2012. Proceedings of the tautog ageing workshop, 88 p. AFMFC, Arlington, VA. [Available at website.]

al. (2007) and Watkins et al. (2015) were able to successfully determine the age of common carp (*Cyprinus carpio*) using cross sections of fin rays. Carbines (2004) compared ages of blue cod (*Parapercis colias*) derived from otoliths and fin spines and determined that spines yielded precise estimates. In comparisons of otoliths, dorsal-fin spines, and teeth of the leopard coralgrouper (*Plectropomus leopardus*), Hobbs et al. (2014) found that the most cost- and time-efficient structure for age determination was the dorsal-fin spine. Fin rays or spines also have been found to be useful by Sylvester and Berry (2006) for white sucker (*Catostomus commersonii*), by Zymonas and McMahon (2009) for bull trout (*Salvelinus confluentus*), by Burton et al. (2015) for gray triggerfish (*Balistes capriscus*), by Keller Kopf et al. (2010) for billfishes (*Kajikia spp.*), and by Murie et al. (2009) for Atlantic goliath grouper (*Epinephelus itajara*).

Management recommendations for tautog stocks are a product of the stock assessment process, which currently is based on values derived from an age structured assessment model. The benchmark stock assessment for tautog, conducted in 2015 by the Atlantic States Marine Fisheries Commission (ASMFC) and incorporating an external peer review, gave evidence that tautog in all management areas (Southern New England, which includes Massachusetts and Connecticut; New York–New Jersey; and Delaware, Maryland, and Virginia) were overfished and that overfishing was occurring for the stock in Southern New England (ASMFC[1]). The assessment came with several research recommendations that included 1) examination of differences in tautog growth rates by using data that are representative of the full size–age structure of the species, 2) expanded biological sampling of the commercial catch (including collection of structures for age estimates), 3) enhanced collection of age information for smaller fish (<20 cm TL), and 4) maintaining and improving the precision of age readings between state agencies that are estimating the ages of tautog (ASMFC[1]).

To address the aforementioned research recommendations from this stock assessment is difficult with current aging methods, primarily because removal of opercula and otoliths from tautog require sacrificing and disfiguring the fish. In Massachusetts, many of the commercially captured fish are sold whole, both alive and dead. Many of the commercial dealers do not want their fish damaged by the removal of opercula or otoliths; therefore, the collection of age samples from the commercial harvest is not feasible without the expense of purchasing fish. Identification of a structure that could be used for age determination without the need of sacrificing or altering the marketability of the fish would enable more samples to be collected across a variety of sources. In this study, the precision of age estimates generated from multiple structures was examined to establish an alternative to the current use of opercula and sectioned otoliths as the primary aging structures.

Materials and methods

Tautog were collected by rod and reel, as well as from the trawl survey of the Massachusetts Division of Marine Fisheries in the waters of Buzzards Bay, Massachusetts, in May, September, and October 2014. Specimens were transported frozen or on ice to the Annisquam River Marine Fisheries Station of the Massachusetts Division of Marine Fisheries in Gloucester, Massachusetts, for further processing. Total length in millimeters, weight in grams, and sex were recorded. Scales were removed from the side of each fish just posterior to the pectoral fin and placed in an envelope. The fourth dorsal-fin spine and the first pelvic-fin spines were removed with wire cutters as close to the body of the fish as possible and stored frozen in plastic bags. Both opercula were removed with a knife and stored frozen in plastic bags. Sagittal otoliths were removed with a serrated knife and fine forceps and then rinsed, dabbed dry on a paper towel, and stored dry in microcentrifuge tubes.

Opercula were placed into boiling water for 2 min and a small brush was used to remove any flesh still adhering to the bone. Opercula were allowed to air dry for a minimum of 24 h before being examined without magnification by using a combination of reflected and transmitted light. Annuli were defined as alternating pairs of translucent and opaque growth zones. Both left and right opercula were examined together to aid in discriminating between annuli and checks. As noted by both Cooper (1967) and Hostetter and Munroe (1993), the thickness of the bone in some opercula obscured the area of earliest growth, occasionally hiding the first annulus.

Dorsal- and pelvic-fin spines were placed into boiling water for 2 min, and a small brush was used to remove any flesh still adhering to the spines. Spines were allowed to air dry for a minimum of 24 h before being placed in bullet molds and embedded in epoxy. The epoxy block with the embedded spine was sectioned with an IsoMet Low Speed Saw[3] (Buehler, Lake Bluff, IL) affixed with 4 blades and a 0.75-mm-thick spacer between each blade. Sections were affixed sequentially (from the spine base to the tip) to labeled glass microscope slides with Flo-Texx liquid coverslip (Thermo Fisher Scientific, Waltham, MA). Sections of pelvic- and dorsal-fin spines were examined through a compound microscope with transmitted light at 100× magnification. Each section from each spine was examined to determine the age of the structure. Annuli were considered to consist of alternating pairs of opaque and translucent growth zones.

Whole sagittal otoliths were cleaned with water as needed before being placed in a black dish filled with mineral oil and were viewed through a dissecting microscope with reflected light at 30–40× magnification.

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[3] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

Left and right otoliths were examined side by side to aid in discerning between annuli and checks. The distal surface of the otoliths provided the clearest view of the annuli, which were identified as alternating bands of opaque and translucent growth zones.

One sagittal otolith from each fish was randomly selected for sectioning. Otoliths that showed any sign of malformation were not sectioned. Otoliths were placed on a porcelain tray that had been heated to 400°C for approximately 30 s or until they reached a caramel brown color. Otoliths were then embedded in epoxy in silicon bullet molds, and each epoxy block was marked with a pencil through the core of the otolith perpendicular to the sulcal groove. This mark was used as a guide to cut a section with an IsoMet Low Speed Saw affixed with 2 blades and a 0.5-mm-thick spacer between them. The resulting section was affixed to a microscope slide with a Flo-Texx liquid coverslip and labeled. If the first otolith produced an undesirable section, the second otolith was cut. Otolith sections were examined through a compound microscope with transmitted light at 100× magnification. Annuli were counted as alternating bands of opaque and translucent growth zones.

Scales were briefly soaked in water to soften any attached tissue before being rubbed clean with a paper towel. Impressions of the scales were made by pressing them into acetate sheets for 3 min with a heat press set at 100°C and with 5.5 metric tons of pressure. Impressions were made for scales in which the anterior portion of the scale appeared to make a "v" shape, signaling a nonregenerated scale. Regenerated and nonregenerated scales were counted to create an estimation of the percentage of regenerated scales. Scale impressions were examined under a microfiche reader at 25× magnification. Breakages in the circuli that continued around the anterior portion of the scale were counted as the outer margin of annual growth zones. True annuli were differentiated from false annuli by confirming that the circuli breakages continued through the transitional area between the anterior and posterior portion of the scale.

All ages were assigned on the basis of year class. Fish captured in May, before annulus deposition was complete, had the edge counted as the final annulus. Fish captured in September and October had growth past the final annulus; therefore, the edge was not counted. In all structures, the outside edge of the winter growth was treated as the end of one annual growth zone and the beginning of the next. All structures were independently assigned an age by 2 readers and each individual read each structure twice. All ages were assigned without knowledge of fish size, sex, or previously assigned ages. When ages assigned for a structure of a fish did not agree between the 2 readers, both readers examined the structure together and reached a consensus-based age. A final age for each fish was reached by the 2 readers considering consensus-based ages of all structures, as well as the quality of each structure. For example, a fish determined to be age 7 with the use of opercula, age 8 with the use of whole otolith, age 8 with the use of sectioned otolith, age 7 with the use of dorsal-fin spine, age 8 with the use of pelvic-fin spine, and age 6 with the use of a scale would be assigned a final age of 8 years if the opercula was thick at the base, the dorsal-fin spine was vascularized, and the scale was of poor quality.

Precision of readings was measured by using percent agreement and coefficient of variation (CV) (Chang, 1982). Estimates of precision were generated for comparisons 1) within reader for each structure, 2) between readers for the first reading of each structure, 3) between readers for the second reading of each structure, 4) between consensus-based ages for sectioned otoliths and consensus-based ages for each other structure, and 5) between consensus-based ages for each structure and final ages assigned to a fish. The following equation was used to calculate CV, as shown in Campana (2001):

$$CV_j = 100\% \times \frac{\sqrt{\sum_{i=1}^{R} \frac{\left(x_{ij} - x_j\right)^2}{R-1}}}{x_j}.$$

This equation gives the CV for the *j*th fish,

where x_{ij} = the *i*th age estimate of the *j*th fish;

x_j = the average age estimate of the *j*th fish; and

R = the number of times that that a fish was read.

For all CV analyses, consensus-based ages were treated as a single reading. Coefficients of variations listed in this article were averaged across all fish aged.

Tests of symmetry (Bowker, 1948; Evans and Hoenig, 1998) were used to examine bias between consensus-based ages for each structure and the final age assigned to the fish, as well as between consensus-based ages for sectioned otoliths and consensus-based ages for each other structure. McBride (2015) suggested that Bowker's test (Bowker, 1948) has a lower type-1 error rate at high levels of precision than the type-1 error rate with Evans and Hoenig's test (Evans and Hoenig, 1998). We, therefore, used Bowker's test when the CV was less than 5% and Evans and Hoenig's test when the CV was above 5%.

Results

In this study, 119 tautog were collected and analyzed (52 female, 51 male, and 16 of unknown sex). Fish ranged from 35 mm TL to 506 mm TL (average: 313 mm TL). Males were slightly larger on average (346 mm TL) than females (335 mm TL), and fish of both sexes were larger than fish of unknown sex (140 mm TL).

All structures examined contained annuli (Fig. 1). Scales yielded ages 0–10, opercula yielded ages 0–11, and all ages estimated for other structures ranged from 0 to 12 years. For all structures, average consensus-based ages of structures agreed within 1 year of

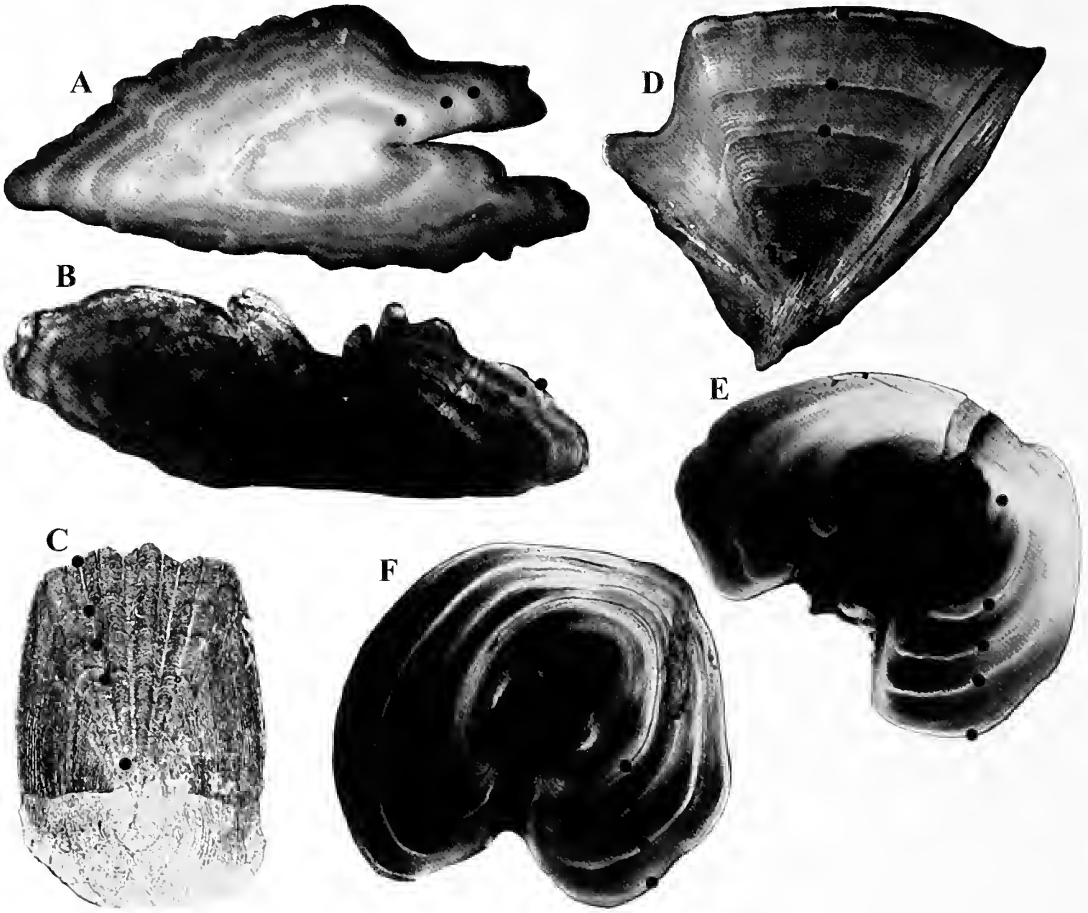

Figure 1

Multiple structures were used in this study for age determination of tautog (*Tautoga onitis*) captured in Buzzards Bay Massachusetts, in 2014. This figure shows (**A**) a whole sagittal otolith, (**B**) a sectioned sagittal otolith, (**C**) a scale, (**D**) an operculum, (**E**) a dorsal-fin spine, and (**F**) a pelvic-fin spine from an age-5 tautog captured in June 2014. Black dots represent the location of annuli.

the final ages of fish up to final age of 7 years. Beyond age 7, ages derived from scales diverged by more than a year, and ages derived from whole otoliths diverged at the final age of 12 years (Table 1). The percentage of regenerated scales ranged from an average of 59% at age 1 to an average of 91% at age 11 (average estimate: 74.3% for all fish examined).

Tests of symmetry against ages derived from sectioned otoliths yielded no bias for ages from whole otoliths, dorsal-fin spines, or pelvic-fin spines. Opercula and scales produced biased ages compared with ages from sectioned otoliths. The bias seen in ages from scales increased with the age of the fish, whereas the bias observed in ages from opercula appeared to be systematic because the age for a portion of the fish was underestimated by 1 year (Fig. 2). Comparisons between final ages and consensus-based ages for structures showed that only pelvic-fin spines and whole otoliths yielded ages that were not biased in comparison with the final ages determined for fish (Fig. 2). All other structures yielded ages that were biased younger than the final ages (Fig. 2). The bias in age estimates from scales became greater with fish age, whereas the other structures appeared to produce ages with a systematic bias of underestimating a portion of the fish by 1 year.

Within-reader comparisons showed reader 1 had the best precision (CV=1.51%, 89.9% agreement) with pelvic-fin spines (Table 2). Reader 2 had the best precision (CV=2.39%, 80.7% agreement) with opercula, followed closely by that for pelvic-fin spines (CV=2.69%, 79.0% agreement). Between-reader precision was best in the second reading of whole otoliths (CV=1.96%, 84.0% agreement). For 4 of the 6 structures, between-reader precision increased between the first and second readings. Precision decreased only between readings for scales (CV changed from 6.99% to 8.97%) and pelvic-fin spines (CV changed from 4.68% to 5.28%).

Table 1

Average age and sample size for each type of structure examined for each final age assigned for tautog (*Tautoga onitis*) collected in Buzzards Bay, Massachusetts, in 2014. Final ages were assigned by 2 age readers taking into account ages assigned, and quality of all structures examined for each fish. Ages for each structure are ages based on the consensus of 2 readers, each performing 2 readings. Standard errors of the mean appear in parentheses after the average ages.

Final age	*n*	Scale	Opercula	Dorsal-fin spine	Pelvic-fin spine	Whole otolith	Sectioned otolith
0	6	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
1	6	1.17 (0.17)	1.50 (0.22)	1.00 (0.00)	1.17 (0.17)	1.00 (0.00)	1.00 (0.00)
2	3	2.33 (0.33)	2.00 (0.00)	2.33 (0.33)	2.00 (0.00)	2.00 (0.00)	1.67 (0.33)
3	3	2.67 (0.33)	2.33 (0.33)	2.67 (0.33)	3.33 (0.33)	3.00 (0.00)	2.67 (0.33)
4	5	4.00 (0.32)	4.00 (0.00)	4.00 (0.00)	4.20 (0.20)	4.00 (0.00)	4.00 (0.00)
5	11	4.91 (0.16)	4.82 (0.12)	4.82 (0.12)	5.09 (0.09)	5.00 (0.00)	5.00 (0.00)
6	14	5.71 (0.13)	5.71 (0.16)	5.79 (0.11)	6.00 (0.00)	5.93 (0.07)	6.00 (0.00)
7	32	6.53 (0.13)	6.69 (0.08)	6.94 (0.04)	6.97 (0.03)	7.00 (0.04)	6.97 (0.05)
8	20	6.70 (0.16)	7.45 (0.14)	7.60 (0.15)	8.00 (0.07)	7.75 (0.12)	7.70 (0.11)
9	6	8.17 (0.31)	8.33 (0.21)	8.67 (0.21)	9.00 (0.00)	9.00 (0.00)	8.50 (0.22)
10	9	7.89 (0.42)	9.22 (0.22)	9.56 (0.18)	10.00 (0.17)	9.78 (0.15)	9.56 (0.29)
11	2	5.50 (0.50)	11.00 (0.00)	10.00 (0.00)	11.00 (0.00)	10.50 (0.50)	11.00 (0.00)
12	2	8.50 (1.50)	11.00 (0.00)	11.50 (0.50)	12.00 (0.00)	10.50 (0.50)	11.00 (0.00)

Consensus-based ages from sectioned otoliths had the best precision when compared with consensus-based ages from whole otolith ages (CV=2.11%, 87.4% agreement), followed by consensus-based ages from pelvic-fin spines (CV=2.99%, 79.0% agreement). Consensus-based ages from pelvic-fin spines had better precision for final ages than any other structure (CV=1.17%, 92.4% agreement). The CV estimate for comparisons between final ages and consensus-based ages from whole otoliths was identical to that for consensus-based ages from pelvic-fin spines, but the agreement was not as high (CV=1.17%, 87.4% agreement) (Fig. 2).

Discussion

In this study, we made age determinations based on the examination of multiple structures from tautog in an effort to find a nonlethal aging method for this species and, thereby, increase the availability of sample sources. Current standard aging techniques for tautog are based on opercula and otoliths, which necessitates sacrificing and damaging the fish. Scales were evaluated as possible aging structures for tautog by Cooper (1967) and Hostetter and Munroe (1993). Before this study, no one had evaluated fin spines for aging tautog.

Fin spines have proven useful for aging a variety of fish species (e.g., Carbines, 2004; Hobbs et al., 2014; Burton et al., 2015; Watkins et al., 2015). Among the structures that could be removed in a nonlethal way, we found pelvic-fin spines to be the best structure for aging tautog. Pelvic-fin spines had strong annular marks (Fig. 1), yielded high-precision age estimates (Table 2) and a lack of bias (Fig. 2). The precision of age estimates was higher for pelvic-fin spines in comparison with final ages than from any other structure tested. Furthermore, with the pelvic-fin spines used in this study, we did not find the core to be obscured by vascularization as has been seen in fin spines of other species (e.g., Keller Kopf et al., 2010; Kopf and Davie, 2011; Landa et al., 2015). Although the tautog in our study reached only age 12, we believe that the growth bands in the spines would be discernible in tautog of considerably older ages. To support this assumption, we examined a whole otolith, sectioned otolith, opercula, and a pelvic-fin spine from an 895-mm-TL tautog. Using the opercula and pelvic-fin spine, we determined that the fish was age 20. The whole and sectioned otoliths, however, indicated that the fish was age 21 (senior author, unpubl. data). Annuli on all structures examined were clear all the way to the edge. The discrepancy in ages between structures is presumed to be related to difficulty in finding the first annulus.

Dorsal-fin spines had strong annular marks that were very similar to those on pelvic-fin spines (Fig. 1), but the dorsal-fin spines had more vascularization near the core than the pelvic-fin spines. The vascularized core left the readers unsure at times whether the first visible annulus was the age-1 or the age-2 annulus. This uncertainty led to decreased precision and systematic bias in the age estimates.

Scales have been a primary structure for nonlethal age determination in many other fish species (e.g., Penttila and Dery, 1988; Welch et al., 1993; Secor et al., 1995; Elzey et al.[4]), but our data support the find-

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[4] Elzey, S. P., K. J. Trull, and K. A. Rogers. 2015. Massachusetts Division of Marine Fisheries Age and Growth Laboratory: fish aging protocols. Massachusetts Div. Mar. Fish. Tech. Rep. TR-58, 43 p. [Available at website.]

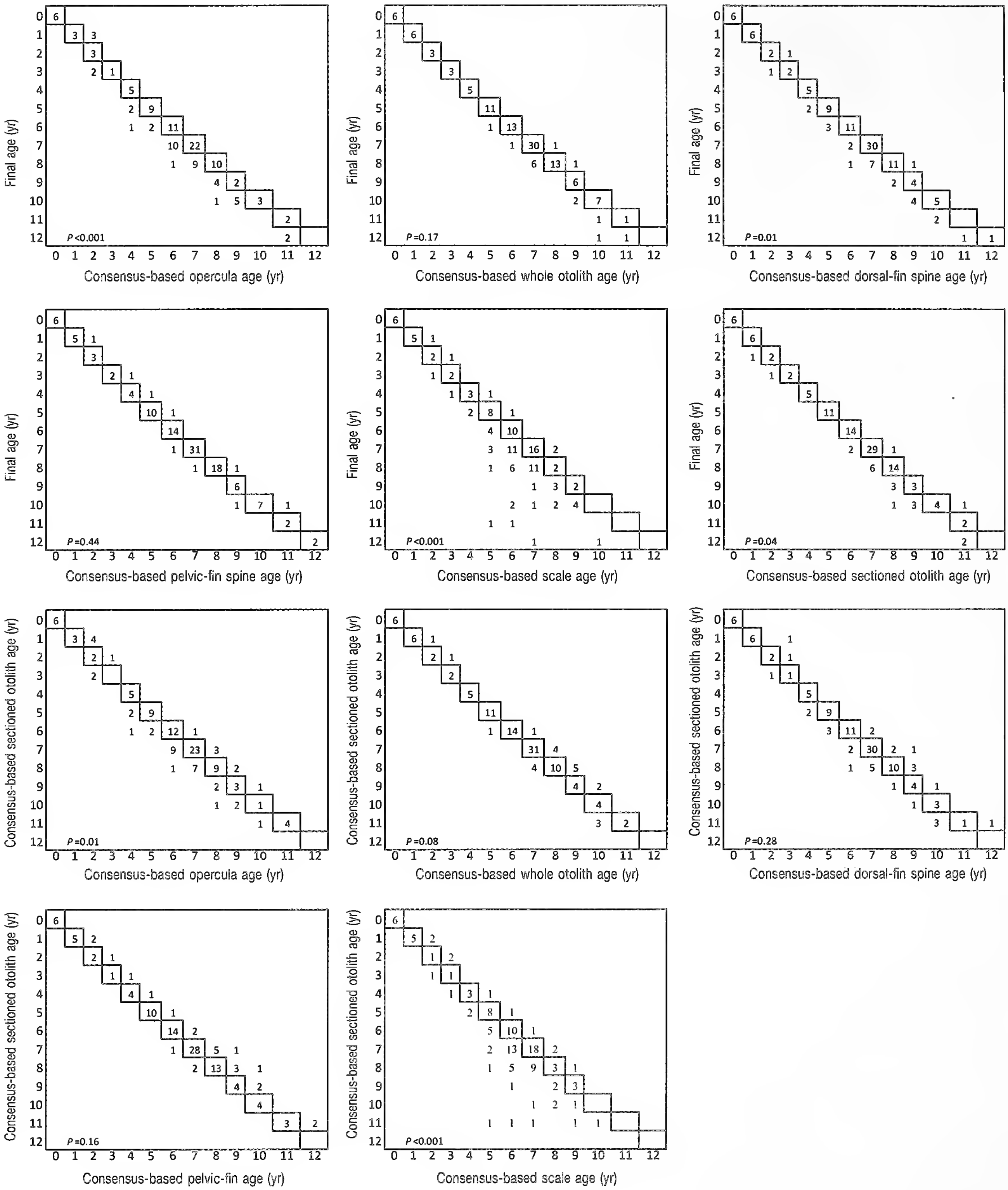

Figure 2

Age bias plots for tautog (*Tautoga onitis*) collected in Buzzards Bay, Massachusetts. Final age versus consensus-based age for each structure, as well as sectioned otolith consensus-based age versus consensus-based age for each other structure are presented. Numbers within each plot represent the number of fish assigned each age. *P*-values are the results of Bowker's (1948) (whole otoliths, dorsal-fin spine, pelvic-fin spine, and sectioned otoliths) or Evans and Hoenig's (1998) (opercula and scales) test of symmetry.

Table 2

Percent coefficient of variation (CV%) and percent agreement were used to examine the precision of age estimates for tautog (*Tautoga onitis*) collected in 2014 in Buzzards Bay, Massachusetts. Comparisons were made within reader for each structure, between readers for each of 2 readings of each structure, between consensus-based readings from sectioned otoliths and consensus-based readings from other structures, and between consensus-based readings from each structure and final readings.

Comparison	Aging structure	CV%	Agreement (%)
Between readings, reader 1	Opercula	4.02	82.4
	Whole otolith	1.58	84.9
	Dorsal-fin spine	3.02	79.0
	Pelvic-fin spine	1.51	89.9
	Scale	5.94	65.5
	Sectioned otolith	1.93	84.0
Between readings, reader 2	Opercula	2.39	80.7
	Whole otolith	3.05	76.5
	Dorsal-fin spine	3.12	76.5
	Pelvic-fin spine	2.69	79.0
	Scale	6.56	56.3
	Sectioned otolith	7.55	69.5
Between readers, reading 1	Opercula	4.91	79.8
	Whole otolith	2.60	79.0
	Dorsal-fin spine	4.36	71.4
	Pelvic-fin spine	4.68	64.7
	Scale	6.99	54.6
	Sectioned otolith	8.53	66.1
Between readers, reading 2	Opercula	3.85	79.0
	Whole otolith	1.96	84.0
	Dorsal-fin spine	3.32	76.5
	Pelvic-fin spine	5.28	65.5
	Scale	8.97	45.4
	Sectioned otolith	3.92	73.1
Consensus-based readings from sectioned otolith vs. consensus-based readings from other structures	Opercula	5.55	64.7
	Whole otolith	2.11	81.5
	Dorsal-fin spine	3.52	73.9
	Pelvic-fin spine	2.99	79.0
	Scale	8.61	48.7
Consensus-based readings from each structure vs. final readings	Opercula	5.01	64.7
	Whole otolith	1.17	87.4
	Dorsal-fin spine	2.60	77.3
	Pelvic-fin spine	1.17	92.4
	Scale	8.79	47.1
	Sectioned otolith	2.06	82.4

ings of Cooper (1967) and Hostetter and Munroe (1993) that indicate that scales are not suitable for use with tautog. The percentage of regenerated scales ranged from an average of 59% at age 1 to an average of 91% at age 11 (overall average: 74.3%). The large amount of regenerated scales in this species made it difficult to attain an adequate sample for age determination. Additionally, annuli on scales were not well defined (Fig. 1), and discrimination between true and false annuli was problematic, all of which led to poor precision and bias toward underestimating ages of fish older than age 7 (Table 1, Fig. 2).

Currently, the majority of age data for tautog is gathered from the examination of opercula (ASMFC1). Cooper (1967) and Hostetter and Munroe (1993) were both able to use marginal increment analysis to justify that growth marks on opercula were deposited annually. We found that growth marks on opercula were distinct (Fig. 1), and we were able to achieve good precision with age estimates (CV<5%) between and within readers (Table 2). However, in the comparison between final ages of fish and consensus-based ages for opercula, the CV was slightly higher (5.01%) and we found significant bias. Furthermore, we found a similar CV (5.55%) and bias between consensus-based ages from sectioned otoliths and consensus-based ages from opercula. The bias we observed in our data appears to be systematic and most prevalent in fish age 4 and older (Fig. 2). The most likely explanation for such a bias would be a failure to correctly identify the first annulus because of the thickness of the bone in older fish. As was found by both Cooper (1967) and Hostetter and Munroe (1993), this thickening can obscure the first annulus.

Otoliths can be viewed whole or cross-sectioned and have been the most reliable structure for age determination in many fish species (e.g., Barnes and Power, 1984; Boxrucker, 1986; Welch et al., 1993; Secor et al., 1995; Sipe and Chittenden, 2001; Robillard et al., 2009; Zymonas and McMahon, 2009; Stolarski and Sutton, 2013; Elzey et al., 2015). In this study, we examined whole and sectioned sagittal otoliths. Ages derived from whole otoliths provided good precision within readers, between readers, and between consensus-based ages for structures and final ages of fish (Table 2). No evidence of bias was observed for the consensus-based ages from whole otoliths and the consensus-based ages from sectioned otoliths or final ages (Fig. 2). Hostetter and Munroe (1993) found that whole otoliths were useful only in young fish because, as the otolith grew thicker, the annuli near the core of the otolith became obscured; however, we did not often encounter this problem. As the age of the fish increased and growth increments decreased, we found it increasingly more difficult to distinguish between annuli near the edge of the otolith. The oldest age assigned as a final age in this study was age 12, but ages from whole otoliths were assigned to age 11. A larger sample size that includes older fish would give us the ability to determine where ages from whole otoliths diverge from ages determined from other, more accurate structures.

Precision of the consensus-based ages from sectioned otoliths in comparison with final ages of fish was good (CV=2.06%). Although bias was detected, the percent agreement was more than 80%, indicating that the bias may have been less severe than the bias seen from other structures. Because otoliths of tautog are small (~5-mm in length), cutting a section exactly through the origin and getting the sectioning plane correct is difficult. If the cut is not made correctly through the core, the first annulus can be missed. Because the sections are aged with transmitted light, a section that is not perpendicular to the annual growth can lead to difficulties in interpreting annuli close to the edge. Both of these problems that can be encountered with sectioned otoliths can introduce bias.

Before this study, age determination of tautog was based on methods that require sacrificing fish to harvest the structures used for aging. Removal of these structures alters the appearance of the fish, thereby affecting the marketability of a species that is largely sold as whole fish. The need to kill and alter fish to obtain age data negatively affects the sample sources available and the costs associated with collecting adequate samples from juvenile and commercially captured fish. The use of pelvic-fin spines for age determination should allow samples to be taken from more diverse sources covering a wider selection of the stock of tautog. Strong annuli on the pelvic-fin spines also lead to high-precision age estimations. The availability of high-precision age data will strengthen the stock assessment of this species. The use of pelvic-fin spines as a primary structure for age determination would allow researchers to more easily gather the information that is necessary to successfully manage tautog with an aged-based assessment model.

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A comparison of density and length of Pacific groundfishes observed from 2 survey vehicles: a manned submersible and a remotely operated vehicle

Thomas E. Laidig (contact author)
Mary M. Yoklavich

Email address for contact author: tom.laidig@noaa.gov

Southwest Fisheries Science Center
National Marine Fisheries Service, NOAA
110 Shaffer Road
Santa Cruz, California 95060

Abstract—Visual surveys of seafloor communities in deep water are becoming more common and provide fishery-independent abundance estimates that could improve stock assessments for some groundfish species. However, limitations of the survey vehicle must be considered when developing methods. To that end, we estimated densities of demersal fishes from 28 paired strip-transect surveys, using a manned submersible (a human-occupied vehicle, HOV) and a remotely operated vehicle (ROV) in 3 types of habitats (high-relief rock, low-relief mixed rock, and soft sediments) at water depths of 75–315 m off central California. Differences in fish detection, identification, and measurements were observed between vehicles (e.g., densities of unidentified fishes, unidentified rockfishes, and unidentified species of *Sebastomus* were significantly higher in ROV surveys). Species most closely associated with the seafloor were observed at higher densities in HOV surveys than in ROV surveys—a result possibly due to the greater reactions of fish to the ROV. The percentage of fish for which we could not estimate size was greater from video images collected with the ROV than from in situ observations made from the HOV. Results of our study will be useful for evaluation of the limitations and biases of these survey vehicles in assessments of demersal fishes.

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Visual surveys of seafloor communities in deep water (depths >50 m) are becoming more common, and the results are used to provide fishery-independent estimates of abundance and to improve stock assessments for some demersal fish species. All survey vehicles are associated with assumptions, biases, and limitations, which must be considered when selecting the type of vehicle and developing a survey design. Biases in underwater visual surveys can result from the influence of illumination, noise, and movement of a vehicle on fish behavior (Stoner et al., 2008). Changes in fish behavior caused by these influences can affect the sampling efficiency of survey vehicles, thereby leading to over- or underestimation of fish abundance.

Various types of vehicles have been used to conduct visual surveys of demersal fish abundance in both trawlable and untrawlable habitats. Adams et al. (1995) used a relatively large remotely operated vehicle (ROV) to estimate population size of several groundfish species on soft sediments, and Krieger (1993) and Krieger and Sigler (1996) estimated density of rockfishes (*Sebastes* spp.) surveyed with a bottom trawl and a human-occupied vehicle (HOV), also known as a manned submersible, in low-relief habitats. Abundance of rockfishes that live in high-relief rock habitats has been determined by using an HOV (O'Connell and Carlile, 1993; Yoklavich et al., 2007), an ROV (Stierhoff et al., 2013), and a combination of hydroacoustics and observations from an ROV (Demer, 2012) as well as by using an ROV, towed stereo-camera sled, and catch composition from a bottom trawl (Jones et al., 2012).

The capabilities and limitations of visual survey vehicles need to be considered when interpreting information obtained from them for management purposes. Estimates of fish abundance from different visual survey vehicles have been compared in only a few studies. O'Connell and Carlile (1994) conducted surveys in Alaska, using an HOV and a MiniROVER MKI[1] ROV (Teledyne Benthos, North Falmouth, MA); however, the ROV was effective only in low-relief areas and not useful for quantitatively surveying their target

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[1] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

species, the yelloweye rockfish (*Sebastes ruberrimus*) in its primary habitat of high-relief rock. Uzmann et al. (1977) used a towed camera sled (TCS) and HOV to estimate fish densities on Georges Bank. Higher densities of flounders, hakes, and dogfish were found in the HOV surveys, and density of goosefish was greater in the TCS surveys. Cailliet et al. (1999) used a TCS and an HOV to characterize fish species from 3 areas off California and found that species observed in deep surveys were similar between type of vehicle, but 4 species were observed from the HOV that were not seen from the TCS at shallower depths (<2200 m). More rockfish species were identified by using an ROV then by using a TCS in Alaska (Rooper et al., 2012); however, a larger number of the measurements of fish length were estimated with the stereo cameras on the TCS than with a single camera with paired lasers on the ROV. Understanding the limitations of survey vehicles in providing accurate species detection, identification, and length measurements can help to design effective surveys with a consideration of the specific capabilities of the vehicle and to improve abundance and biomass assessments of the target species.

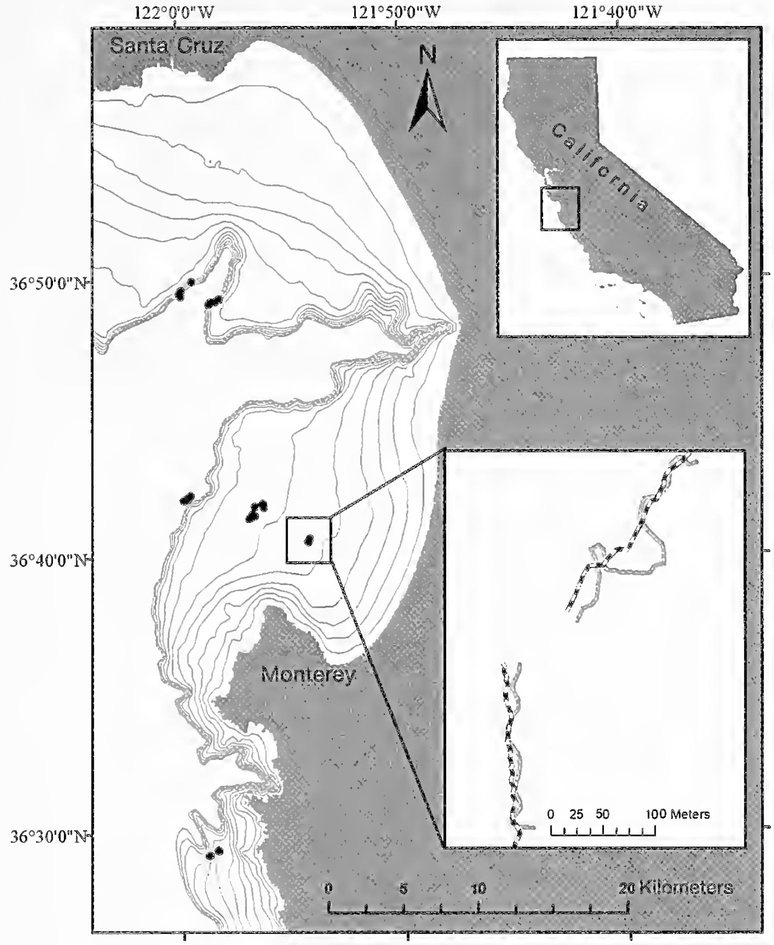

Figure 1

Location of 28 paired transects of surveys conducted with a human-occupied vehicle and a remotely operated vehicle to survey groundfishes off central California in 2007. Bottom inset is an enlargement of 2 sets of paired transects; the dark dashed line represents the HOV track and the light solid line is the ROV track.

In this study, we evaluated habitat-specific capabilities of 2 visual survey vehicles (i.e., an HOV and an ROV) to detect, identify, and determine the length of a variety of demersal fishes. Fish density was estimated by using each vehicle in 3 different types of seafloor habitats: high-relief hard rock, low-relief mixed rock, and soft sediments. In an earlier study, we examined the reactions of fishes to these same vehicles (Laidig et al., 2013), concluding that avoidance reactions to the ROV were greater than those to the HOV and that avoidance of both vehicles was greater by fishes above the seafloor than by fishes on the seafloor. Surveying fishes that display large avoidance reactions to either vehicle could result in inaccurate estimates of density. Here we expand our work to consider the differences in fish densities in relation to reactions of the fish to each vehicle. Information from this study, and that from our previously published work, can be used to evaluate potential limitations and biases of these underwater vehicles and will be useful in developing sampling strategies for surveying demersal fishes.

Materials and methods

Fish surveys were conducted off the coast of central California, from Monterey Bay to south of Carmel Bay (Fig. 1), with a 2-person HOV and an ROV. Sampling was conducted in the fall of 2007 at depths ranging from 75 to 315 m for both vehicles. Sampling took place from 12 October through 4 November for the HOV and from 18 November through 23 November for the ROV. All surveys were conducted during daylight hours (0800–1700), when our species of interest are known to be active (Love et al., 2002).

The 2-person *Delta* HOV was launched from the FV *Velero IV* and was operated by experienced pilots from Delta Oceanographics (Torrance, CA). An experienced scientific observer accompanied 1 pilot inside the untethered HOV. The yellow-orange HOV measured 1.8 m tall, 4.6 m long, and from 0.4 m wide at its forward-most point to 1.1 m wide at mid-vehicle. A single 24-volt motor and propeller provided thrust. An external color video camera (custom-built by DeepSea Power and Light, San Diego, CA), with 400 lines of resolution and an illumination range of 2–100,000 lux, was mounted on the starboard side above the observer's viewport. The HOV was equipped with ten 150-watt halogen lights, 4 of which were used during surveys (1 forward light by which the pilot navigated and 3 that illuminated the starboard-side survey area). Two parallel lasers were mounted 20 cm apart on either side of the camera.

The position of the HOV was tracked from the support vessel with WinFrog integrated navigation software (Fugro Pelagos, San Diego, CA) and an ORE Trackpoint II ultra-short baseline (USBL) acoustic tracking system (EdgeTech, West Wareham, MA). The distance traveled was estimated with a MiniRLG2 measurement unit based on ring laser gyro technology (Teledyne CDL, Houston, TX) and a NavQuest 600 Micro Doppler Velocity Log (DVL; LinkQuest Inc., San Diego, CA) mounted externally on the HOV.

An unmanned *Phantom DS4* ROV (Deep Ocean Engineering Inc., San Jose, CA) was operated by experienced pilots from the Southwest Fisheries Science Center (La Jolla, CA), National Marine Fisheries Service, aboard the NOAA Ship *David Starr Jordan*. The ROV had a yellow body and black frame and measured 1 m tall, 2 m long, and 1.4 m wide. Six electric thrusters (2 angled and 4 that were perpendicular to the seafloor) provided propulsion for the ROV. The ROV was equipped with a forward-facing, color video camera with 470 lines of horizontal resolution and an 18× optical zoom (FCB-IX47C, Sony Corp., Tokyo) and a Coolpix 995 digital still camera with 3.2 megapixel resolution and 4× optical zoom (Nikon Corp., Tokyo). Illumination was provided by 2 forward-facing 250-watt Multi SeaLite halogen lights (DeepSea Power and Light) mounted on the ROV camera tray. Two sets of parallel lasers (20 and 61 cm apart) and 1 crossing laser were mounted to the front of the ROV and used to determine depth of field. The position of the ROV also was determined with WinFrog software and an ORE Trackpoint II *plus* USBL tracking system.

Strip-transect surveys were conducted with each vehicle, and the resultant video footage was recorded onto MiniDV (HOV) or DVCAM (ROV) tapes. During 10-min surveys of transects, the HOV traveled along a depth contour at an average speed of 0.5 m/s (standard error [SE] 0.04) and an average altitude of 1.1 m (SE 0.04) above the seafloor. The ROV transect surveys were conducted at an average of 1.2 m (SE 0.08) above the seafloor and at an average speed of 0.6 m/s (SE 0.03), and covered a path comparable to that of the HOV.

During these transect surveys, the observer inside the HOV identified and counted all fish that occurred within 2 m on the starboard side. The observer also estimated size of fish to the nearest 5 cm in total length (TL), using the paired lasers. The observer's comments were captured on an audio channel of the video footage for later review.

Video footage collected from the HOV and ROV and digital still images collected from the ROV were reviewed by one person in the laboratory after the surveys. Fish were identified to the lowest possible taxon and counted. Some species were not considered in our analyses. For instance, pelagic schooling fishes, such as the northern anchovy (*Engraulis mordax*), jack mackerel (*Trachurus symmetricus*), and Pacific chub mackerel (*Scomber japonicus*), swam around the vehicles for extended periods of time, increasing the possibility that these fish would be counted more than once. From the ROV images, fish size was estimated to the nearest 5 cm TL by using the parallel lasers. An effort was made to estimate the size of all fish in both surveys, with the exception of fish in holes, partially obscured by objects, perpendicular to the plane of the laser spots, or in midwater (providing no target for the lasers). We also used the laser spots in the images from the ROV to estimate width of transects. The space between the paired 20-cm lasers (*Measured laser width*) was measured with a ruler on the video screen once during each minute of a transect survey. *Transect width* was estimated with the following equation:

$$Transect\ width = \left(\frac{Measured\ screen\ width}{Measured\ laser\ width} \right) \times Laser\ width,$$

where *Laser width* = 20 cm; and
Measured screen width = the horizontal width of the video screen through the laser spots.

We examined only video footage that was collected while the vehicles were traveling forward in survey mode (i.e., the vehicle was considered to be on transect). Fish were counted when they were observed within 2 m of the starboard side of the HOV (as estimated with a handheld dive sonar inside the HOV) or 2 m in front of the ROV. Brief stops by the ROV to capture still images for identification of species (at a rate of about 1 image/min) and to avoid obstacles were considered to occur on transect. No fish observations were counted in instances where the seafloor was not seen in the video footage for >5 s (for example, when a vehicle transited over small canyons or the ROV was pulled backwards by the ship). Information on identification and size of fish was augmented by comments from the observer inside the HOV, and data from the ROV surveys were derived only from video and still images.

We determined the amount and type of primary and secondary seafloor habitat from the video footage taken along each transect. Primary habitat covered >50% of the seafloor, and secondary habitat covered >20% of the remaining seafloor. We used 4 main habitat types: bedrock (R; large in-place rock), boulders (B; unattached rock >25 cm), cobble (C; unattached rock <25 cm), and mud (M). We reduced the 16 habitat combinations to 3 categories: hard was any combination of rock, boulder, and cobble in both the primary and secondary groupings (RR, RB, RC, BR, BB, BC, CR, CB, CC), mixed was a combination of mud as the primary or secondary habitat and one of the other habitat types (MR, MB, MC, RM, BM, CM), and soft was entirely mud (MM). Habitat categories were assigned to patches of distinct substratum types, delineated by time. Length of each patch was then estimated from time-specific cumulative distance (as measured with the DVL [HOV] or USBL [ROV]) along each transect. The areas of all habitat

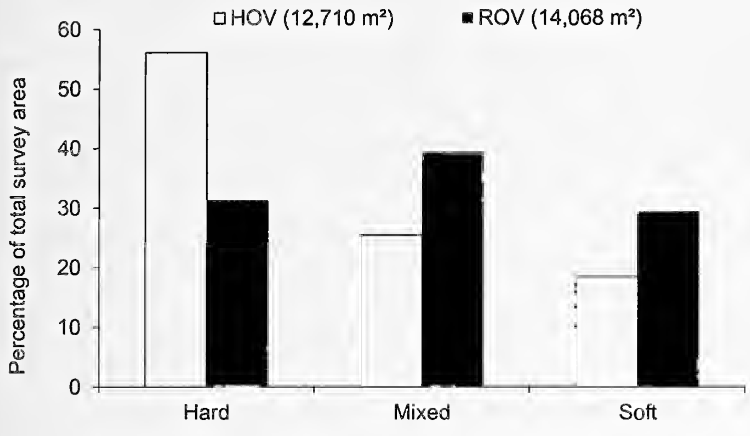

Figure 2

Percentage of survey area classified as hard, mixed, and soft habitat for surveys conducted off central California in 2007 with a human-occupied vehicle (HOV) and a remotely operated vehicle (ROV). Numbers in parentheses are the total area surveyed by each vehicle.

patches (length×width) in each habitat category were summed for each transect.

We selected corresponding HOV and ROV transects for our analyses. We began with a 10-min HOV transect and then matched the corresponding ROV track with the start and end points of the HOV survey using ArcGIS 9.2 (ESRI, Redlands, CA). This approach resulted in transects of similar lengths. We excluded sections from the paired transects that were >10 m apart. If sections were discarded, the remaining sections of the survey track were combined for each vehicle to form the sample transect used for analyses. Total area for each of the 3 habitat categories (average transect width×total length of each habitat category) was estimated per transect. All 3 habitats were not encountered on every transect, and some habitats were present in only small amounts. To further refine comparisons between the HOV and ROV transect surveys, only total areas >20 m^2 for each habitat category on each of the paired transects were considered in the analyses. Using this method, we used 21 of the 28 paired transects for the hard habitat category, 22 transects for the mixed habitat category, and 14 transects for the soft habitat category for our comparisons.

Fish densities were determined for each habitat category (hard, mixed, and soft) within each transect. Density was estimated as the total number of fish of a particular taxon per total area of a habitat category on each transect. A pairwise (by transect) unbalanced analysis of variance (ANOVA), with unequal sample size, was used to compare mean densities (of each fish taxon and of all taxa combined) between the 2 vehicles, among the 3 habitat categories, and to compare interactions between vehicles and habitats. This work was done with the statistical program SAS 9.4 (SAS Institute Inc., Cary, NC). The distribution of residuals did not differ significantly from normal for these analyses. We used a Tukey–Kramer post-hoc test to determine which taxa accounted for significant differences. We used a Kolmogorov–Smirnov 2-sample test to examine differences in length distributions of fishes between vehicles for each habitat. Further, we examined the differences in mean length for each habitat, using a Student's *t*-test.

Results

We analyzed 28 pairs of transect surveys conducted with the HOV and the ROV. The estimated width of an HOV transect was 2 m, and the average width of an ROV transect was 1.9 m (SE 0.07). The total survey area (transect width×total length of all transects) was 12,710 m^2 for the HOV and 14,068 m^2 for the ROV. For HOV surveys, hard habitat was the category with the greatest amount of survey area (56%), followed by mixed and soft habitats (26% and 18%, respectively) (Fig. 2). For the ROV, mixed habitat was most abundant category (39%), and hard and soft habitats were found in similar proportions (31% and 29 %, respectively) (Fig. 2).

During HOV surveys, 4489 fish were counted, and 6480 fish were counted from the ROV. For the analyses, we included only 23 common taxa (i.e., taxa that accounted for at least 1% of the total number of fish observed from either the HOV or ROV), which included 4235 fish from the HOV surveys and 6253 from the ROV surveys. Total fish density was not significantly different for the 2 vehicles in surveys of any of the 3 habitat categories: hard (Tukey-Kramer; $P>0.05$), mixed (Tukey-Kramer; $P>0.05$), and soft (Tukey-Kramer; $P>0.05$).

Most fish occupied hard habitat (73% and 57% of all fishes observed in the HOV and ROV surveys, respectively; Table 1A). Average density of all fish in hard habitat was 42.1 individuals/100 m^2 (HOV) and 53.8 individuals/100 m^2 (ROV). The pygmy rockfish (*Sebastes wilsoni*), the halfbanded rockfish (*S. semicinctus*), and young-of-the-year (YOY) rockfishes (*Sebastes* spp., 5 cm TL or less) were the 3 most abundant taxa in the HOV surveys. The most abundant taxa on ROV transects were the pygmy rockfish, the halfbanded rockfish, unidentified rockfishes (*Sebastes* spp. >5 cm TL), and unidentified species of *Sebastomus* (a subgenus of 11 similar-looking species of rockfishes [Love et al., 2002], 4 members of which are shown in Figure 3). Densities of YOY, rosy (*S. rosaceus*), and rosethorn (*S. helvomaculatus*) rockfish in hard habitat were significantly greater on HOV transects than on ROV transects (Tukey-Kramer: $P<0.05$). Densities of unidentified rockfishes and unidentified *Sebastomus* were significantly greater in the ROV surveys than in the HOV surveys (Tukey-Kramer: $P<0.01$).

Table 1

The number and mean density of fish taxa or groups observed during visual surveys conducted with a human-occupied vehicle (HOV) and a remotely occupied vehicle (ROV) in 2007 off central California. Data are reported as (**A**) individual taxa and (**B**) combined fish groups per habitat category (hard, mixed, and soft). Common names in bold text are considered benthic taxa. Densities are the number of fish per 100 m^2. An asterisk (*) indicates significantly greater fish density for that vehicle compared with the other. A superscript^1 indicates taxa that are in the *Sebastomus* group. SD=standard deviation; YOY=young-of-the-year; TL=total length.

			HOV								
Common name	Scientific name	Total fish	No.	Hard density	SD	No.	Mixed density	SD	No.	Soft density	SD
A Individual taxa											
Bank rockfish	*Sebastes rufus*	124	114	1.3	5.3	8	0.3	0.7	2	0.1	0.2
Blackeye goby	*Rhinogobiops nicholsii*	235	119	1.9	3.9	81	4.3*	6.6	35	1.8	3.4
Bocaccio	*Sebastes paucispinis*	97	94	1.6	5.3	3	0.2	0.7	0	0.0	0.0
Flatfishes	Pleuronectiformes	108	22	0.3	0.7	26	0.8	1.5	60	3.0	3.0
Greenspotted rockfish^1	*Sebastes chlorostictus*	89	58	0.7	1.9	27	1.0	1.8	4	0.2	0.3
Greenstriped rockfish	*Sebastes elongatus*	47	29	0.4	1.8	9	0.4	1.7	9	0.5	1.3
Hagfishes	*Eptatretus* spp.	157	3	0.0	0.1	5	0.1	0.3	149	4.3	15.6
Halfbanded rockfish	*Sebastes semicinctus*	779	538	8.1	14.0	207	4.7	8.6	34	1.8	5.3
Pink seaperch	*Zalembius rosaceus*	16	8	0.1	0.2	6	0.3	0.6	2	0.1	0.3
Poachers	Agonidae	77	9	0.1	0.3	39	0.9	1.9	29	1.6	2.2
Pygmy rockfish	*Sebastes wilsoni*	971	919	11.6	15.4	50	1.0	2.2	2	0.1	0.2
Rosethorn rockfish^1	*Sebastes helvomaculatus*	99	58	0.9*	1.4	38	0.9*	1.3	3	0.2	0.1
Rosy rockfish^1	*Sebastes rosaceus*	128	111	1.7*	2.1	15	0.6*	0.9	2	0.1	0.6
Splitnose rockfish	*Sebastes diploproa*	145	43	1.8	5.6	91	1.7	5.2	11	0.5	1.2
Squarespot rockfish	*Sebastes hopkinsi*	171	156	2.1	2.8	14	0.5	1.5	1	0.0	0.1
Starry rockfish^1	*Sebastes constellatus*	49	44	0.6	1.1	5	0.2	0.5	0	0.0	0.0
Thornyheads	*Sebastolobus* spp.	77	8	0.2	0.8	43	0.8	1.6	26	1.4	3.6
Unidentified fishes	Osteichthyes	24	13	0.2	0.5	5	0.1	0.4	6	0.6	1.6
Unidentified rockfishes	*Sebastes* spp.	60	44	0.5	0.6	14	0.2	0.5	2	0.1	0.5
Unidentified *Sebastomus*^{1}	*Sebastes* spp.	101	79	1.1	1.0	19	0.6	0.9	3	0.2	0.5
Widow rockfish	*Sebastes entomelas*	73	73	0.7	2.7	0	0.0	0.0	0	0.0	0.0
Yellowtail rockfish	*Sebastes flavidus*	79	78	1.0	1.9	1	0.1	0.3	0	0.0	0.0
YOY rockfishes	*Sebastes* spp. (YOY)	529	473	5.2*	9.6	34	1.7	6.3	22	1.0	1.2
Total		4235	3093	42.1		740	21.4		402	17.6	
B Combined fish groups											
All benthic fish		1167	540	8.0	6.6	307	10.4*	6.9	320	14.8	17.6
All large fish (≥30 cm TL)		496	436	5.8*	9.6	59	1.6	2.7	1	0.0	0.1
All *Sebastomus*	*Sebastes* spp.	466	350	4.7	3.4	104	3.3	1.9	12	0.7	0.7

Table continued

In mixed habitats, 740 fish were observed and counted in the HOV surveys (an average of 21.4 individuals/100 m^2) and 2208 fish were counted in the ROV surveys (27.5 individuals/100 m^2; Table 1A). The halfbanded rockfish was the most abundant taxon identified from each vehicle in mixed habitats (an average of 4.7 individuals/100 m^2 on HOV transects and 11.4 individuals/100 m^2 on ROV transects). The densities of blackeye goby (*Rhinogobiops nicholsii*) and rosethorn and rosy rockfish in mixed habitats were significantly greater in HOV surveys than in ROV surveys (Tukey-Kramer: P<0.05). Densities of unidentified fishes (Osteichthyes), unidentified *Sebastomus*, and unidentified rockfishes were significantly greater on ROV transects than on HOV transects (Tukey-Kramer: P<0.05).

The lowest density of fish in surveys from both vehicles occurred in soft habitats (17.6 individuals/100 m^2 for the HOV surveys and 11.8 individuals/100 m^2 for the ROV surveys; Table 1A). Hagfishes (*Eptatretus* spp., most likely Pacific hagfish [*E. stoutii*]), flatfishes (Pleuronectiformes), halfbanded rockfish, blackeye goby, and poachers (Agonidae) were relatively abundant in soft habitat on both HOV and ROV surveys. Unidentified *Sebastomus* was the only group having significantly greater density (Tukey-Kramer: P<0.05) on the ROV surveys than on HOV surveys over soft habitats.

Densities of rosy rockfish and unidentified rockfish differed significantly between the 2 survey vehicles in the 3 habitat categories (i.e., there was a significant interaction between survey vehicle and habitat; un-

Table 1 continued

			ROV								
				Hard			Mixed			Soft	
Common name	Scientific name	Total fish	No.	density	SD	No.	density	SD	No.	density	SD
A Individual taxa											
Bank rockfish	*Sebastes rufus*	48	4	0.3	1.3	42	0.7	3.3	2	0.1	0.2
Blackeye goby	*Rhinogobiops nicholsii*	158	42	0.8	1.3	59	1.1	1.8	57	1.4	2.8
Bocaccio	*Sebastes paucispinis*	32	28	0.7	2.5	4	0.1	0.3	0	0.0	0.0
Flatfishes	Pleuronectiformes	81	2	0.0	0.2	14	0.4	0.6	65	1.9	1.8
Greenspotted rockfish[1]	*Sebastes chlorostictus*	73	24	0.5	0.8	37	0.8	1.3	12	0.4	0.7
Greenstriped rockfish	*Sebastes elongatus*	45	6	0.2	0.5	23	0.3	0.8	16	0.3	0.5
Hagfishes	*Eptatretus* spp.	44	0	0.0	0.0	5	0.2	0.7	39	1.0	4.9
Halfbanded rockfish	*Sebastes semicinctus*	1906	729	15.2	38.7	1080	11.4	24.1	97	1.0	2.1
Pink seaperch	*Zalembius rosaceus*	67	5	0.1	0.1	57	1.3	5.6	5	0.2	0.4
Poachers	Agonidae	72	2	0.0	0.3	20	0.6	1.3	50	1.3	1.6
Pygmy rockfish	*Sebastes wilsoni*	2090	1928	20.8	60.0	159	3.0	5.8	3	0.1	0.2
Rosethorn rockfish[1]	*Sebastes helvomaculatus*	12	6	0.2	0.6	6	0.1	0.3	0	0.0	0.0
Rosy rockfish[1]	*Sebastes rosaceus*	42	27	0.4	0.6	13	0.2	0.4	2	0.1	0.2
Splitnose rockfish	*Sebastes diploproa*	88	11	1.0	3.9	32	0.8	2.4	45	1.2	4.3
Squarespot rockfish	*Sebastes hopkinsi*	104	59	1.0	2.0	44	0.9	3.2	1	0.0	0.1
Starry rockfish[1]	*Sebastes constellatus*	22	16	0.3	0.3	6	0.1	0.3	0	0.0	0.0
Thornyheads	*Sebastolobus* spp.	33	4	0.2	0.6	16	0.4	0.9	13	0.3	0.9
Unidentified fishes	Osteichthyes	195	133	1.8	4.3	38	0.6*	0.9	24	0.5	0.7
Unidentified rockfishes	*Sebastes* spp.	320	164	3.9*	3.7	102	1.8*	2.8	54	1.1	1.9
Unidentified *Sebastomus*[1]	*Sebastes* spp.	307	162	3.9*	4.0	122	2.1*	2.6	23	0.8*	1.2
Widow rockfish	*Sebastes entomelas*	14	14	0.3	0.6	0	0.0	0.0	0	0.0	0.0
Yellowtail rockfish	*Sebastes flavidus*	71	55	1.0	2.0	16	0.3	0.8	0	0.0	0.0
YOY rockfishes	*Sebastes* spp. (YOY)	429	113	1.2	4.2	313	0.3	1.2	3	0.1	0.2
Total		6253	3534	53.8		2208	27.5		511	11.8	
B Combined fish groups											
All benthic fish		889	291	6.4	4.2	321	6.4	4.8	277	7.8	5.2
All large fish (≥30 cm TL)		211	81	1.9	2.4	94	1.7	2.1	36	1.0	1.9
All *Sebastomus*	*Sebastes* spp.	456	235	5.0	4.3	184	3.4	3.4	37	1.1	1.6

balanced ANOVA: $P<0.05$). The interaction effect was based on the observations of greater densities in the HOV surveys for rosy rockfish and in the ROV surveys for unidentified rockfish in both hard and mixed habitats and based on the observations of low densities of each of these taxa in soft sediments in surveys conducted with both vehicles. There was no significant interaction among vehicles and habitat types in our comparisons of densities of the other taxa.

We grouped taxa typically occurring on the seafloor (Love et al., 2002; Love, 2011) into a category called *benthic fish* (bolded common names in Table 1A). We grouped them in this way because, in a related study, we found that fishes living on the seafloor reacted least to both vehicles than fishes that occurred above the seafloor (Laidig et al., 2013). Benthic fish represented 25% of the total number of fish observed in the HOV survey and about 14% of all fish seen in the ROV survey (Table 1B). Densities of benthic fish were similar for both the vehicles in surveys of hard and soft habitats, but there were significantly more benthic fish in mixed habitat in the HOV survey (Tukey-Kramer: $P<0.01$).

We grouped the 4 identified species of *Sebastomus* (i.e., the greenspotted [*S. chlorostictus*], starry [*S. constellatus*], rosethorn, and rosy rockfish) with the unidentified *Sebastomus* to investigate the degree of difficulty in identification of these similar-looking species (Fig. 3). Densities for the category "*all Sebastomus*" were not significantly different from densities for any of the 3 habitat categories in either the HOV or ROV surveys (Table 1B).

Most fish in the HOV and ROV surveys were small (≤15 cm TL), a size group that represented about 73% of all fish with length estimates observed from the HOV (3027 of 4146 fish) and 85% of fish seen from the ROV (4706 of 5537 fish; Fig. 4). The most abundant groups with fish ≤15 cm TL in surveys from both vehicles were pygmy, halfbanded, and YOY rockfishes. Pygmy rockfish accounted for 70% of the 5-cm-TL fish and 51% of 10-cm-TL fish from the ROV transects but for only 26% of 5-cm-TL fish and 40% of 10-cm-TL fish from the HOV transects. Most small fish were observed in hard habitats (79% and 62% of all fish with length estimates on HOV and ROV transects, respectively).

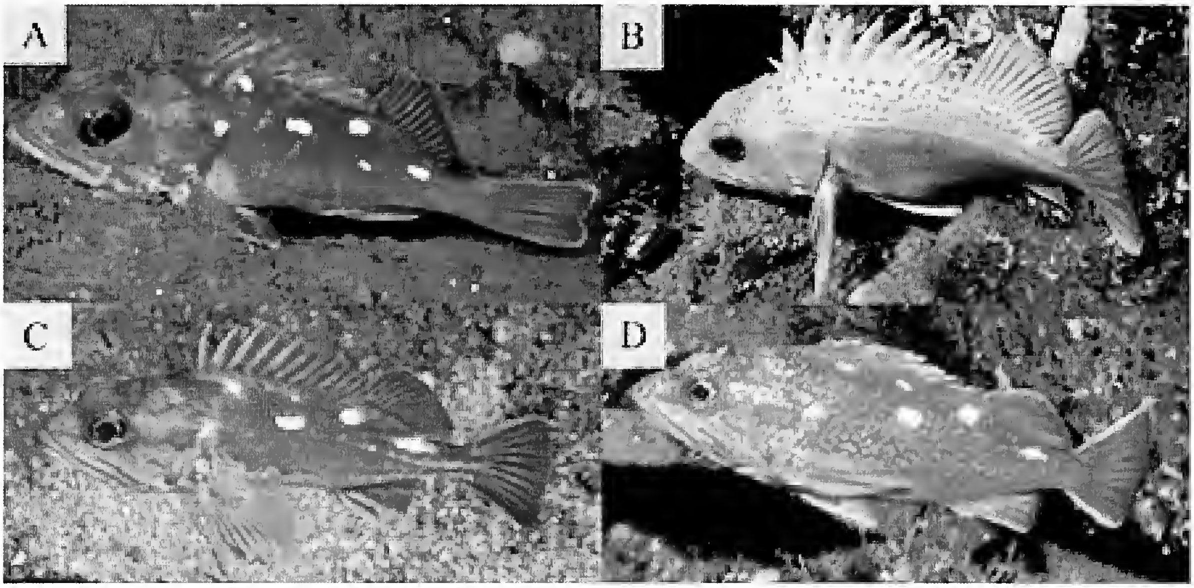

Figure 3

Underwater images of 4 similar-looking species of rockfish in the subgenus *Sebastomus*: (**A**) rosethorn rockfish (*Sebastes helvomaculatus*) identified by green pigmentation along dorsal surface (photograph by J. Butler); (**B**) greenspotted rockfish (*S. chlorostictus*), identified by green spots along the dorsal surface (photograph by J. Field); (**C**) rosy rockfish (*S. rosaceus*), identified by purple on the head (photograph by L. Snook); and (**D**) starry rockfish (*S. constellatus*), identified by the numerous tiny white speckles on the body (photograph by R. Starr).

The lowest number of small fish (mostly blackeye goby and halfbanded rockfish) occurred on soft sediments (5% of all fish with length estimates on both HOV and ROV transects).

The group called *large fish* (≥30 cm TL) accounted for 12% and 4% of all fish observed from the HOV and the ROV (Table 1B). Bocaccio (*Sebastes paucispinis*; n=97), as well as bank (*S. rufus*; n=108), yellowtail (*S. flavidus*; n=67), and widow (*S. entomelas*; n=63) rockfish were abundant large fish in the HOV surveys, and the most abundant large fish in the ROV surveys were unidentified *Sebastomus* (n=54), as well as yellowtail (n=47), greenspotted (n=34), and splitnose (*Sebastes diploproa*; n=29) rockfish. The density of large fish in hard habitats was 3 times greater on HOV transects than on ROV transects (Tukey-Kramer: $P<0.05$).

Fish length could not be estimated for 89 fish representing 10 taxa (2% of all fish) in the HOV surveys and for 716 fish in 22 taxa (11% of all fish) in the ROV surveys (Table 2). The most abundant taxa among fish of unknown size in the HOV surveys were hagfishes (n=56), unidentified rockfishes (n=11), and unidentified *Sebastomus* (n=7). For the ROV surveys, the most abundant taxa without size estimates were unidentified rockfishes (n=191), halfbanded rockfish (n=180), and unidentified *Sebastomus* (n=70). The greatest percentage of fish without size estimates was from surveys with both vehicles in soft habitat (14% of fish from the HOV surveys and 23% of fish from the ROV surveys). This finding was mainly a result of hagfishes that could not be measured because they were observed in partial view (i.e., in holes or under rocks). However, disregarding hagfishes, lengths of 15% of other fish in soft habitats (e.g., unidentified rockfishes and other fish species, and halfbanded rockfish hovering above the seafloor) could not be estimated from the ROV images compared with <1% of fish from the HOV surveys. Length distributions of fish were significantly different for both vehicles for each habitat category (Kolmogorov–Smirnov test: $P<0.005$). The HOV and ROV surveys yielded a different average fish size for hard habitats (Student's t-test: $P<0.03$; 17.0 cm TL for HOV transects and 18.0 cm TL for ROV transects) and for soft habitats (Student's t-test: $P<0.005$; 15.1 cm TL for HOV surveys and 16.7 cm TL for ROV surveys) but average fish size was not significantly different for mixed habitats (16.4 cm TL for HOV transects and 17.0 cm TL for ROV transects).

Discussion

The ability to accurately identify fishes is a necessity when conducting meaningful visual surveys underwater. Without the fish in hand, some taxa are difficult to identify to species (e.g., 11 similar-looking *Sebastomus* rockfishes co-occur on the central California coast) and others are practically impossible to discern (e.g., the small cryptic species of poachers [particularly the 4 species of the deep-dwelling genus *Xeneretmus*]). Densi-

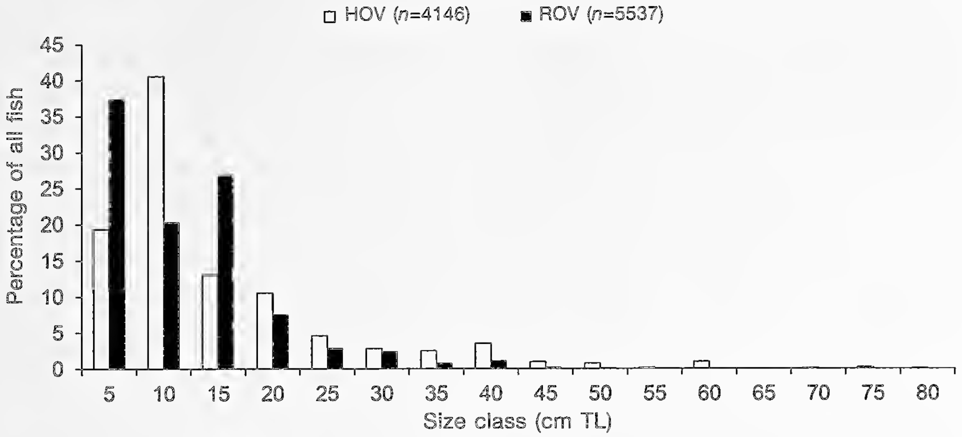

Figure 4

Distribution of size classes of fish observed on transect surveys conducted with a human-occupied vehicle (HOV) and a remotely operated vehicle (ROV) off central California in 2007. Numbers in parentheses are total number of fish used to estimate fish size from surveys conducted with each vehicle.

ties of unidentified rockfishes, unidentified *Sebastomus*, and unidentified fishes were all significantly greater in the ROV surveys than in the HOV surveys. In a study off southern California with the use of the same ROV as that used in this study (Demer, 2012), 12% of all rockfishes could not be identified to species. Rockfishes also were difficult to identify by using an ROV off British Columbia (8% unidentified; Du Preez and Tunnicliffe, 2011) and Alaska (9% unidentified; Rooper et al., 2012), areas where there are far fewer species of rockfishes than off the central California coast (Love et al., 2002). Our ability to identify species from video images should improve with the availability of advanced camera technology (e.g., light-field cameras in which an array of lenses can collect information, such as distance to, and size of, targets) and increased video resolution (moving from high definition with 1440 lines of resolution to ultra-high definition with 2000, 4000, 8000, or 16,000 lines).

Nonetheless, not all fish can be identified to species by observers inside an HOV. Unidentified rockfish (not including YOY rockfishes) varied from <1% to 4% of all fish observed by using HOVs in benthic surveys off Oregon and California (Pearcy et al., 1989; Stein et al., 1992; Yoklavich et al., 2002); unidentified rockfishes accounted for 5% of all fishes in the HOV surveys in our study. Although HOV surveys have relatively low numbers of unidentified rockfishes, identification to species can be extremely difficult, no matter what visual survey vehicle is used, in areas of high rockfish diversity and high numbers of small individuals. For example, Love et al. (2009) counted more than 700,000 fish from a minimum of 137 species (with at least 50 *Sebastes* species) using an HOV off southern California. Because of the high diversity and small size of the fish (over 60% of fish were <15 cm TL) in that region, many rockfish could not be identified to species (unidentified rockfish and unidentified *Sebastomus* composed 15% and 3% of all fish, respectively).

Our ability to accurately estimate the length of fish was limited when the ROV was used. In particular, without a reference surface, lasers on the ROV were not helpful as a measurement tool for fish that hovered or swam above the seafloor and for fish that were oriented perpendicular to the laser spots. Rochet et al. (2006) examined accuracy of lasers to measure fish and concluded that the major difficulty in measuring fish lengths was caused by the orientation and position of fish. Measuring fish with laser arrays also has proved problematic in other ROV surveys (Johnson et al., 2003; Rooper et al., 2012). However, the increasing use of stereo camera systems on visual survey vehicles is improving the accuracy of underwater measurements of fish, including fish in the water column that cannot be measured by using lasers alone. In a study of rockfishes in Alaska, researchers were able to measure 35% of all fish by using a towed stereo-camera sled, compared with 10% of the fish observed from an ROV with paired lasers and a single camera (Rooper et al., 2012).

The presence of an in situ observer, whether in an HOV in deep water or with scuba at shallow depths, is more effective than using only video footage when detecting, identifying, and measuring fish species. The in situ observer in an HOV has a 3-dimensional view and a wider depth of field than the depth of the view from a 2-dimensional video monitor. The in situ view allows the observer to distinguish a cryptic fish from the background more easily than a fish in the same background seen in a video image. As the HOV passes an area, the observer can look in multiple directions in contrast to

Table 2

Number of all fish observed, number of unmeasured fishes observed, and proportion of all observed fish that were unmeasured from surveys of groundfish conducted over hard, mixed, and soft habitats with a human-occupied vehicle (HOV) and a remotely operated vehicle (ROV) off central California in 2007.

	HOV				ROV			
	All habitats	Hard	Mixed	Soft	All habitats	Hard	Mixed	Soft
Total unmeasured	89	23	10	56	716	283	315	118
Total fish	4235	3093	740	402	6253	3534	2208	511
Proportion unmeasured (%)	2	1	1	14	11	8	14	23

the single, fixed direction of a video camera. This practice creates greater opportunity to detect and identify fishes within the transect area. For example, Marliave and Challenger (2009) conducted paired strip-transect surveys with scuba off British Columbia, in which one diver surveyed by eye and the other used a video camera. They found that fish counts per dive hour estimated in situ by the divers (i.e., by eye) were as much as double the counts estimated per dive hour from the diver's video footage. They attributed this difference to the ability of divers to survey in multiple directions (forward, left, and right) and thereby could detect fish more easily within the transect area. Further, Stein et al. (1992) and O'Connell and Carlile (1994) suggested that video and still images were not as effective as the human eye for accurate fish identifications.

Estimating size of fish also can be improved with an in situ observer in the water (by using either scuba or an HOV). Length of fish in midwater is nearly impossible to estimate from a video image, but a human can use natural stereo vision and the laser reference dots to help estimate size. In our study, lengths were unknown for only 2% of fish in the HOV surveys, compared with 11% in the ROV surveys. Advances in video technology have increased the ability to detect, identify, and estimate the size of fish. Interestingly, most of these advances, including stereo cameras with increased field of view and high definition in 3-dimensional space, imitate attributes of the human eye.

Difficulty in species identification and fish measurement during ROV surveys occurred in all habitat types, indicating that these issues are not habitat specific. When using the ROV, we were less able to identify or estimate the size of fish on soft sediment than we were in mixed or hard rock habitats. However, the greater number of fish of unknown size is partially the result of hagfishes viewed in holes. Other studies in which fish densities have been compared between vehicles have focused effort on a single seafloor habitat type, such as mud (Uzmann et al., 1977; Krieger, 1993; Adams et al., 1995) or rocky outcrops (O'Connell and Carlile, 1994). Our study is unique in its comparison of fish densities estimated by using an HOV and ROV in 3 different habitats.

Small fish, in particular, can be difficult to detect and identify with visual survey vehicles. In a study in which fish abundance near 3 gas platforms was estimated with an ROV and compared with fish abundance estimated by scuba divers, Andaloro et al. (2013) found that 9 taxa of small benthic species were reported in the diver surveys but none of those taxa were observed with the ROV. Small rockfishes <20 cm TL could not be identified to species during nearshore ROV surveys in the waters of southeast Alaska (Johnson et al., 2003), and Love et al. (2009) suggested that densities of small fish taxa, such as the bluebanded goby (*Lythrypnus dalli*), could be underestimated in their HOV surveys. In our study, densities of small benthic fishes (such as blackeye goby, poachers, hagfishes, and YOY rockfishes) were greater from HOV surveys than from ROV surveys in all habitats. This difference is also reflected in the significantly smaller sizes of fishes in HOV surveys conducted over hard and soft habitats, compared with sizes of fishes in ROV surveys. The observer inside the HOV was able to see, identify, and measure many small species that otherwise would have been difficult to detect in video footage alone. Consequently, an ROV may not be the vehicle of choice to assess the importance of nursery grounds, predator–prey interactions, or ecosystem functions, all of which require an ability to detect and identify small fish species.

When selecting a survey vehicle for visual assessments, associated assumptions, biases, and limitations must be considered, along with logistic variables, such as cost and availability of the tools and optimal survey design (Yoklavich et al., 2015). For example, bias in estimating abundance can result from fish avoidance or attraction to particular survey vehicles (Adams et al., 1995; Trenkel et al., 2004; Lorance and Trenkel, 2006). Laidig et al. (2013), using the same HOV and ROV as those used in our study, assessed the reactions of fishes and found that more fishes reacted to the tethered ROV than to the autonomous HOV (57% versus 11% avoidance, respectively). They also found that the proportion of fishes residing on the seafloor that reacted negatively to the vehicle was 2% for the HOV compared with 18% for the ROV.

Considering this result, we expected higher fish densities in the HOV surveys. Indeed, blackeyed goby and YOY, rosethorn, and rosy rockfish had significantly higher densities in the HOV surveys, and only the unidentified taxa had significantly higher densities in the ROV surveys. Additionally, benthic species (as a group) had higher densities in the HOV surveys. Results from our study, and those from our companion work on fish reactions (Laidig et al., 2013), indicate that abundance of at least some demersal fish species will be underestimated depending on which vehicle is used for a survey. This type of information improves understanding of the limitations and biases associated with visual survey methods used to identify and count fish species underwater.

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Skipped spawning in the Patagonian stock of Argentine hake (*Merluccius hubbsi*)

Gustavo J. Macchi (contact author)[1,2]
Marina V. Diaz[1,2]
Ezequiel Leonarduzzi[2]
María Inés Militelli[1,2]
Karina Rodrigues[1,2]

Email address for contact author: gmacchi@inidep.edu.ar

[1] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)
Instituto de Investigaciones Marinas y Costeras (IIMyC)
Deán Funes 3250
B7602HSA Mar del Plata
Buenos Aires, Argentina

[2] Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP)
Paseo Victoria Ocampo Número 1
B7602HSA Mar del Plata
Buenos Aires, Argentina

Abstract—We analyzed the macroscopic and histological maturity data of Argentine hake (*Merluccius hubbsi*) collected off Patagonia in the spawning area of the Patagonian stock of this species between 2005 and 2014 to determine the frequency of skipped spawning. This study revealed that the only evidence of skipped spawning observed for Argentine hake was the resting stage. Analysis of 58,891 adult females of Argentine hake sampled during their reproductive peak indicates that, annually, between 6% and 22% of them had skipped spawning. These females were located mostly in the periphery of the spawning area, in deeper (>90 m) and colder waters (7–8°C). Individuals that had skipped spawning had a more intense feeding activity and a better nutritional condition (K=0.68–0.75) than females collected in the main spawning area according to the Fulton's condition index. In contrast, postspawning females showed the poorest condition (K=0.62–0.68) because of the energy cost involved with reproduction. Females that had skipped spawning were mostly young individuals with a modal age of 3 years and a modal size of 38 cm TL. These results indicate that a significant proportion of females that had completed their first annual spawning could skip the next spawning event and stay on the periphery of the reproductive area to feed.

Recruitment (i.e., the number of progeny that will survive each year to be incorporated into the population) depends (in most traditional fishery assessment models) directly on the abundance of the parental stock, or spawning stock biomass (SSB), calculated from the maturity ogive (Mace and Sissenwine, 1993; Rodgveller et al., 2016). In iteroparous fish species that spawn repeatedly, it is assumed that once they have reached sexual maturity, all individuals reproduce on an annual cycle, i.e., the total adult fraction of the population. In recent years, evidence from many fish species indicates that this pattern does not occur for at least part of the spawning stock (Rideout and Tomkiewicz, 2011). This phenomenon, known as skipped spawning (SS), implies that during the breeding season a proportion of the adult population does not spawn during that year. This "irregularity" in the reproductive cycle would have consequences for the estimate of the SSB because not every adult would contribute to the reproductive potential of the population. This potential issue with SSB estimates could introduce greater variability in the stock-recruitment relationship. Including the nonreproductive fish in calculations of the SSB could lead to an overestimation of the number of spawning fish and, therefore, would lead to an overestimation of expected recruitment. The degree of effect would depend on the fraction of the population affected by skipped spawning, the magnitude of which may vary between years.

Different hypotheses explain the origin of skipped spawning, but most of them are associated with food deficiencies that affect energy storage before spawning activity or with unfavorable physical conditions that prevent or delay ovaries from ripening (Holmgren, 2003; Jørgensen et al., 2006; Rideout et al., 2006; Rideout and Tomkiewicz, 2011). It has been suggested that, in general, poor nutritional conditions may not allow fish to accumulate enough energy to support egg production in consecutive years (Kennedy, 1953; Dutil, 1986). However, the main problem in determining the origin of this

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"anomaly" in the annual spawning cycle is the lack of reliable historical information about a stock (i.e., data from a histological examination of gonads, as well as condition indices during the reproductive cycle) and on the oceanographic or physical features associated with the population data. Moreover, to be able to estimate a reliable index that represents the fraction of the population that does not reproduce during the year, data are needed for the entire distribution of the species, collected at different times during the reproductive cycle. For this reason, despite evidence of skipped spawning for several species, there are very few cases where this information has been incorporated into assessment models (Rideout et al., 2011). Rideout et al., 2005, on the basis of the morphological examination of the ovaries, have suggested that there are 3 categories of skipped spawning depending on when maturation is interrupted: retaining, reabsorbing, and resting.

Skipped spawning has been associated primarily with species that have determinate annual fecundity (Rideout and Tomkiewicz, 2011), which is characterized by a fixed fecundity at the onset of the spawning season, when recruitment of previtellogenic oocytes to the secondary growth stage ceases (Hunter et al., 1992). On the other hand, in species with indeterminate annual fecundity, unyolked oocytes mature continuously and are spawned throughout an extended reproductive season. This pattern of oocyte development and the extended breeding season of such species make it difficult to evaluate the reproductive history of a fish and to determine whether spawning has been skipped. Nevertheless, the identification of adult fish with ovaries in the regenerating stage during the reproductive season and the presence of adults outside of the spawning area were considered evidence of skipped spawning in fish with indeterminate annual fecundity (Rideout and Tomkiewicz, 2011).

The Argentine hake is a batch spawner with indeterminate annual fecundity (Macchi et al., 2004). In the Argentine sea, the Patagonian stock, located from 41°S to 55°S and with a biomass of about 1,000,000 metric tons, is economically the most important fishery resource according to a virtual population analysis in 2013 (Villarino and Santos[1]). The reproductive activity of this stock occurs during austral spring and summer (from November through March) peaks in January (Macchi et al., 2004, Pájaro et al., 2005). Although on several occasions the presence of nonreproductive adult individuals has been noted during the spawning season of Argentine hake (Macchi et al., 2004), information on the incidence of skipped spawning has not been reported for this species. The main goal of this study was to analyze the phenomenon of skipped spawning in the Patagonian stock of Argentine hake, from macroscopic and histological analyses of samples collected during research surveys during the main reproductive season of this stock over a period of 10 years. Specific objectives were 1) to determine the dominant category of skipped spawning in Argentine hake, 2) to estimate the incidence of this phenomenon in the population, and 3) to study the relationship of skipped spawning with the size and nutritional status of fish, as well as its possible relationship to environmental factors.

Materials and methods

Sample collection and laboratory processing

During 10 research surveys conducted by the Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), carried out in the north Patagonian area in Argentina between 2005 and 2014, samples of Argentine hake were obtained from hauls of bottom trawls. These surveys were performed during the time of peak spawning of the Patagonian stock (January) in the main reproductive area of Argentine hake and in the nursery ground of this species in San Jorge Gulf (Fig. 1). Although the area sampled during these surveys is smaller than the overall area of the distribution of the Patagonian stock of Argentine hake, we are confident that most of the adult population was sampled because individuals congregated in shallow coastal waters of the north Patagonia area during the peak spawning period (Macchi et al., 2007). Trawling was conducted along transects regularly separated by about 37 km (20 nmi) and was oriented perpendicularly to the coastline. The same stations were visited annually. Specimens of Argentine hake were captured at depths between 50 and 120 m by employing a bottom trawl with a mouth width of about 20 m, a height of about 4 m, and a net with 20-mm mesh at the inner lining of the codend. At each sampling station, salinity and temperature data were collected with an SBE 19 SeaCAT Profiler CTD[2] (Sea-Bird Electronics Inc., Bellevue, WA). Data series were filtered and reduced to values of temperature and salinity to a 1 meter interval approximately.

Samples of Argentine hake were weighed, and total lengths (TLs in centimeters), sex, and maturity stage (Table 1) were recorded for each fish. A visual maturity key of 5 stages was used: 1) immature, 2) developing, 3) spawning, 4) postspawning or spent, and 5) resting or recovering (Macchi and Pájaro, 2003). Because the phenomenon of skipped spawning involves adult females, only data from fish in maturity stages 2–5 were analyzed (Table 2). In addition, complementary biological data collected from subsamples of Argentine hake were also used. These data included information on the individual total weight (TW) in grams and on the degree

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[1] Villarino, M. F., and B. A. Santos. 2014. Evaluación del estado de explotación del efectivo sur de 41° S de la merluza (*Merluccius hubbsi*) y estimatión de las captura biológicamente aceptable para 2015. INIDEP Inf. Tec. Of. 30, 39 p. Instituto Nacional de Investigación y Desarrollo Pesquero, Mar del Plata, Argentina.

[2] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

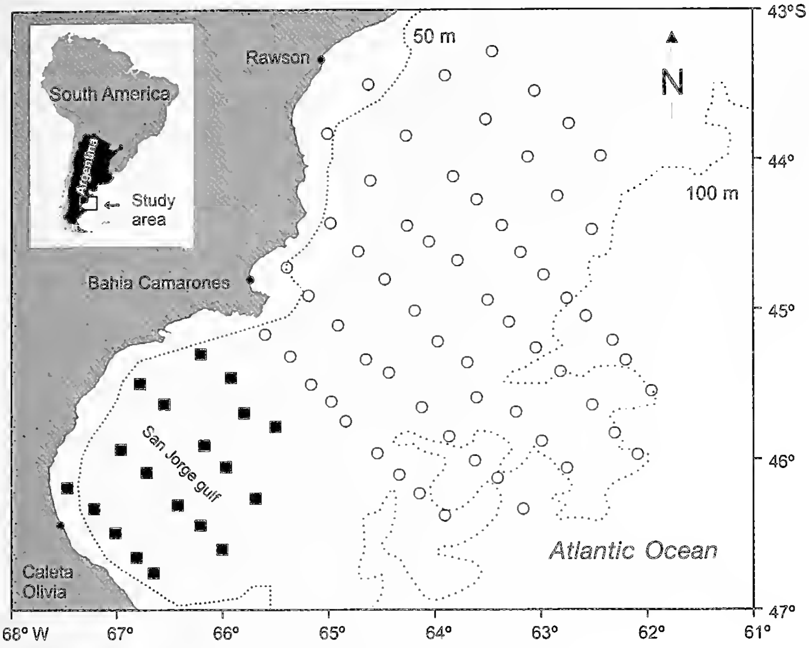

Figure 1

Locations where Argentine hake (*Merluccius hubbsi*) were collected during January from 2005 through 2014 in waters of the north Patagonian area off Argentina. Open circles indicate sampling locations in the area of Argentine hake reproduction, and black squares indicate sampling locations in the nursery area for juvenile Argentine hake in San Jorge Gulf.

of stomach fullness (Table 2). Moreover, from theses subsamples, we collected sagittal otoliths for age determination (Table 2). Ages were determined by the methods described by Renzi and Pérez (1992).

The macroscopic maturity scale was validated by histological analysis of 11,494 gonads of adult females collected from different trawl hauls and preserved in 10% formalin during the research cruises (Table 2). Ovaries were weighed to the nearest 0.1 g to obtain gonad weight (GW), and a portion (about 2.0 g) of each gonad was removed, dehydrated in ethanol, cleared in xylol, and embedded in paraffin. Sections were cut at a 5-µm thickness and stained with Harris's hematoxylin, followed by eosin counterstain. Histological staging of ovaries was based on the stage of oocyte development and on the occurrence of postovulatory follicles and atresia, described by Macchi et al. (2004) and Brown-Peterson et al. (2011).

Data analysis

To estimate the incidence of skipped spawning from the macroscopic information, we calculated the percentage of rest-

Table 1

Visual maturity scale (1–5) and microscopic characteristics of the different stages in the reproductive cycle of female Argentine hake (*Merluccius hubbsi*) collected from the Patagonian stock during 2005–2014. Comparison with the phases of reproductive development described by Brown-Peterson et. al. (2011). The last column shows which females are in reproduction during the season. POFs=postovulatory follicles.

Maturity stage		Main histological features	Brown-Peterson et al. (2011)	Reproduction during season
1	Immature	Only oogonias and oocytes in primary growing stage. Some little cortical alveoli can be observed. Thin and transparent ovarian tunica.	Immature	No reproduction
2	Developing	Oocytes in secondary vitellogenesis present. Atresia can be observed.	Developing	Reproduction
		Yolked oocytes, with or without POFs. Atresia can be present	Spawning capable (mature)	Reproduction
3	Spawning	Hydrated oocytes, with or without POFs. Atresia can be present.	Spawning capable (spawning)	Reproduction
4	Postspawning	Flaccid ovaries with abundant atresia of oocytes in vitellogenesis. POFs and residual hydrated oocytes may be present.	Regressing	Reproduction
5	Resting	Only oocytes in primary growing stage. Thick ovarian tunica.	Regenerating	Skipped spawning

Table 2

Number of samples and subsamples of adult female Argentine hake (*Merluccius hubbsi*), as well as the number of ovaries that were analyzed for this study. Fish were obtained from the Patagonian stock during research surveys conducted off Argentina in January between 2005 and 2014.

Year	Samples	Subsamples	Ovaries for histological examination
2005	4879	1253	1263
2006	3110	1766	1388
2007	5067	1507	1253
2008	5154	1163	1203
2009	6412	1163	1223
2010	7558	1434	1192
2011	4446	1036	731
2012	7076	2112	1261
2013	7763	1947	1093
2014	5810	1944	887

ing females (Table 1, maturity stage 5) within the total number of adult females (Table 1, maturity stages 2–5) for each sample, and that percentage was weighted by the abundance of Argentine hake (number of individuals/km^2) estimated for each trawl haul. This information was used to determine the spatial distribution of females that had skipped spawning and to establish a percentage of this phenomenon in the studied area during peak spawning. These data were contrasted with the spatial distribution of females in the spawning stage (with hydrated oocytes) obtained from each survey by using the same method.

Size distributions of individuals that had skipped spawning and reproductive females (i.e., developing, spawning, and postspawning) obtained from the visual and histological diagnoses were analyzed. Data collected during each survey were grouped in 2 categories, SS and reproductive females, which were compared by using a Kolmogorov–Smirnov test.

The age data obtained from the subsamples of Argentine hake were used to determine the age structure of females that had skipped spawning and reproductive females for each survey. These age distributions were compared in the same way as that used to compare total lengths, but only for the period 2005–2013, because 2014 data were not available.

Data on stomach contents were used to estimate Argentine hake feeding intensity associated with size, condition, and maturity of females. The percentage of stomachs with contents (SC) was estimated for females at different maturity stages, according to the visual scale, and for each survey. Individuals with everted stomachs as a consequence of pressure changes during capture were not used in this analysis.

To assess female nutritional condition and its relationship with maturity stages (histologically determined), we used the gonadosomatic index (GSI) and Fulton's condition factor (K) for the samples collected during each survey. Because gutted weight data were not available, female TW (without ovaries) was used, according to the following equations:

$$GSI = (GW / (TW - GW)) \times 100 \qquad (1)$$

and

$$K = (TW - GW) / (TL^3), \qquad (2)$$

where GW = gonad weight; and
TW = total weight.

The mean values of GSI and K obtained for the different maturity stages were compared by using the Kruskal–Wallis test, after analysis for normality of the data. Generalized linear modeling was used to determine if year, TL, K, or SC significantly influenced the probability of skipped spawning during the season. Skipped spawning was analyzed as a variable with a binomial distribution: females in the resting stage were nonreproductive (probability of skipped spawning [SS]=1), and the other stages (developing, spawning, and postspawning) were considered representative of reproductive individuals (probability of SS=0). Stomach contents were analyzed as a binary variable: with (1) or without (0) content. Therefore, year and SC were modeled as categorical variables, and TL and K were modeled as continuous variables. In this study, to avoid problems of multicollinearity, age was excluded from the model because it was correlated with length. Analyses were restricted to adult fish greater than 32 cm TL because very few smaller fish were available; the length at maturity for the Patagonian stock is estimated to fall within 32–33 cm TL (Macchi et al., 2007).

The model had a logit link function and a binomial error structure. Pseudo values of the coefficient of determination (r^2) were calculated to compare the proportions of the deviation by year, TL, K, and SC. This pseudo r^2 was constructed by expressing the deviance of the model as a proportion of deviance for the null model. Pseudo r^2 was estimated as the deviance in the model individually containing only the intercept (null deviance) minus the deviance after adding the mains factors, divided by the null deviance of the model containing only the intercept.

To analyze the effect of different variables in the model, the probability of skipped spawning was plotted against TL (by using a mean K of 0.67) and against K (by using a mean TL of 51.14 cm) for females with or without SC. This analysis was performed with data from all sampled trawl hauls, but because the pattern was similar between years, we provide the results only from 2012, because that year was the year with the most sampling information (Table 2). All statistical analyses were conducted with the statistical software R, vers. 3.2.3 (R Core Team, 2015).

A principal component analysis was used to determine the possible relationship between the incidence of skipped spawning and some physical environmen-

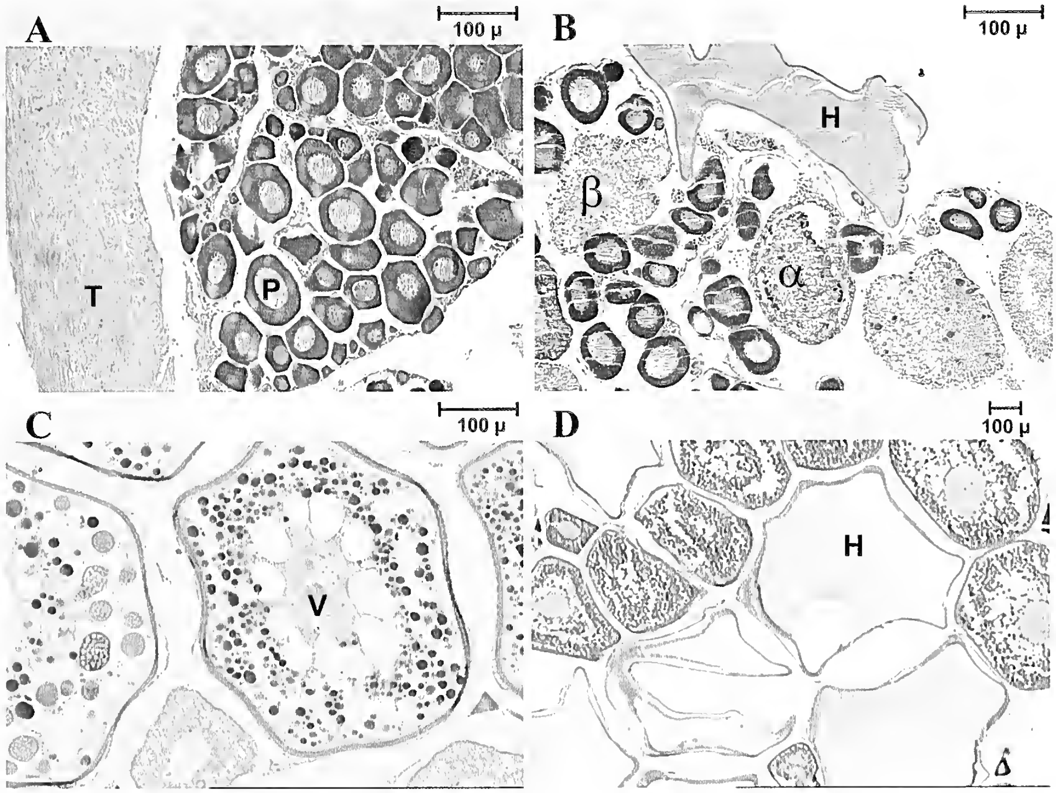

Figure 2

Histological sections of ovaries of Argentine hake (*Merluccius hubbsi*) at different maturity stages: (**A**) resting or skipped spawning, (**B**) postspawning, (**C**) developing, and (**D**) spawning. Oocytes at the primary growing stage (P), in vitellogenesis (V), and in a hydrated stage (H) are shown. Also labeled are the ovarian tunica (T), and the conditions of alpha atresia of yolked oocyte (α), and beta atresia (β).

tal characteristics in the reproductive area. This study also included information on incidence of spawning, for comparison with data from females that had skipped spawning. The variables used were the percentages of females that had skipped spawning and the percentages of females in the spawning stage (with hydrated oocytes) and depth, temperature, and salinity estimated for the surface of the water and at the seafloor at sampling stations. Data collected in the nursery area of San Jorge Gulf (see Fig. 1) were excluded from this study. This analysis was performed with statistical software InfoStat, vers. 2009 (Grupo InfoStat, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, Argentina).

Results

Histological analysis and designation of maturity stages

Histological analysis of 11,494 adult females, sampled during the peak spawning period (January) of the Patagonian stock between 2005 and 2014, confirmed that of the 3 categories of skipped spawning (retaining, reabsorbing, and resting), only the resting stage was observed in Argentine hake. The main characteristic of this stage is that only oocytes in the primary growth stage can be observed in the ovaries, and there is no evidence of recent maturation (Fig. 2A). Unlike that in juveniles, the ovarian tunic in adults is markedly thick because of the completion of previous maturation cycles (Table 1). The other category of skipped spawning that has been described often for fish in the wild, reabsorbing or massive atresia, was not observed in Argentine hake. However, it was very common to see ovaries with oocytes in atresia, both in cortical alveoli or vitellogenesis stages, with different degrees of incidence, but all such cases corresponded with females with evidence of postspawning (Fig. 2B). The rest of the observed maturity stages included developing ovaries with oocytes in the second growth phase, with or without postovulatory follicles and low atresia (Fig. 2C), and active spawning, with hydrated oocytes, with or without postovulatory follicles (Fig. 2D).

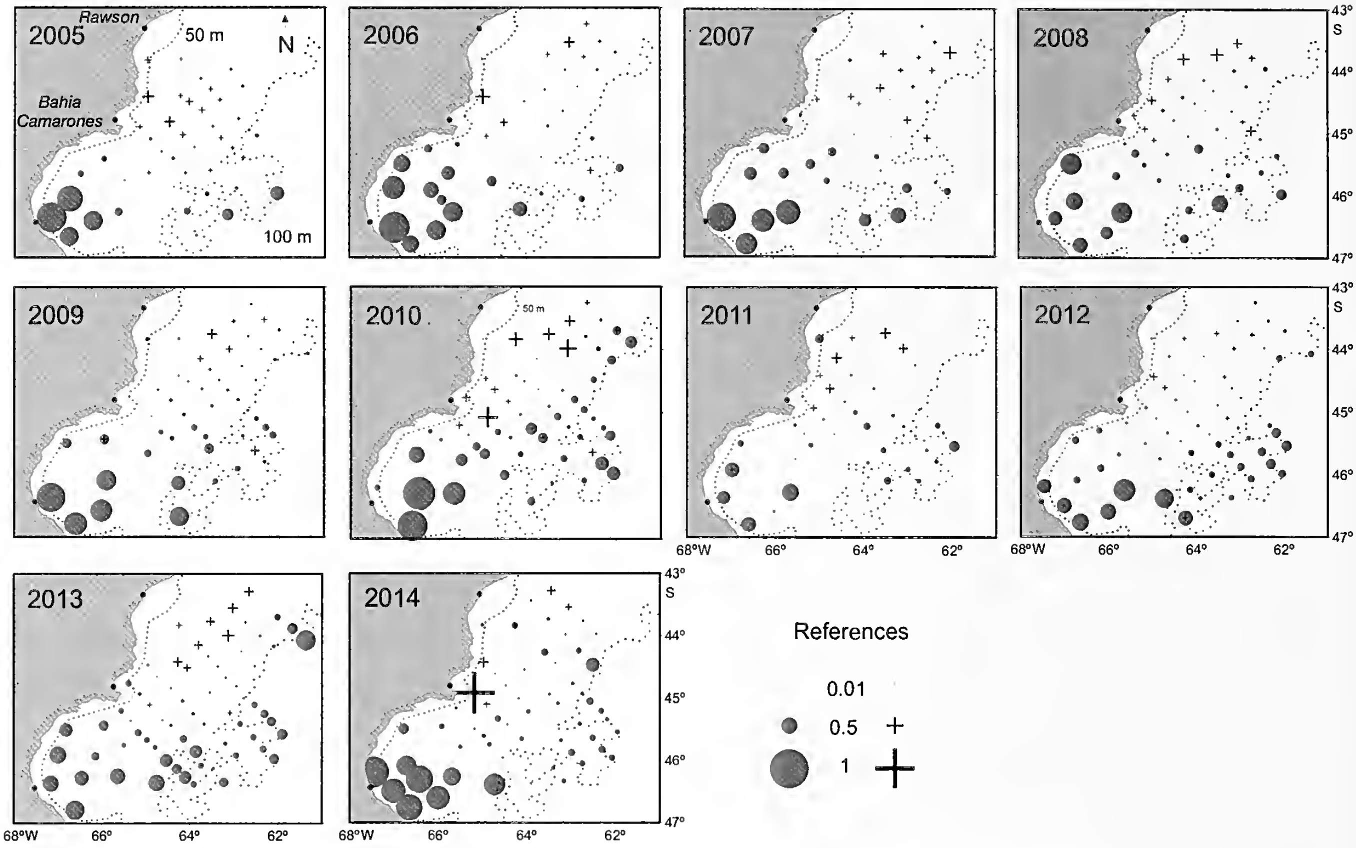

Figure 3

Spatial distribution of skipped spawning (gray circles) and spawning females (plus signs) of Argentine hake (*Merluccius hubbsi*) sampled in the north Patagonian area off Argentina during January from 2005 to 2014. The size of the symbols is proportional to the percentage of each stage weighted by the abundance of Argentine hake.

On the basis of our histological findings, we grouped adult female ovaries of Argentine hake into 4 main categories that represented the reproductive cycle: developing, spawning (with hydrated oocytes), postspawning, and resting. Table 1 shows how the features used in our histological analysis are related to the 5 stages of the visual maturity scale used in our macroscopic analysis and that the resting stage corresponds to skipped spawning.

Spatial variation with skipped spawning and with spawning

Macroscopic information collected during research surveys was used to analyze the annual spatial variation of females that had skipped spawning during the sampling period. This analysis also incorporated the percentages of spawning females (with hydrated oocytes) for comparison of the spatial variation of females that had skipped spawning and females that are in reproduction in the areas of spawning aggregation (Fig. 3).The maps show that females that had skipped spawning predominated in the external sector outside the spawning area of the Patagonian stock, with particular abundance near the 100-m isobath and within the San Jorge Gulf. In January, when the main reproductive peak occurs, this spatial pattern remained relatively constant over the years.

The percentages of females that had skipped spawning for each survey within the area of reproductive activity of Argentine hake (see Fig. 1) ranged from 4% to 10%, but when the data obtained in the San Jorge Gulf were incorporated, the percentages of females that had skipped spawning increased, ranging in most cases between 10% and 15% (Fig. 4). In January 2006 and 2014, the percentage of such females for the whole area studied reached a value close to 22%—a level that is probably due to the very large catches of Argentine hake that were recorded in the San Jorge Gulf during those years.

Size and age of females that had skipped spawning

Figure 5A shows the length distributions, grouped for all years of the study that correspond with females that had skipped spawning and with females in reproductive condition (developing, spawning, and postspawning) determined from visual analysis. In comparisons of both

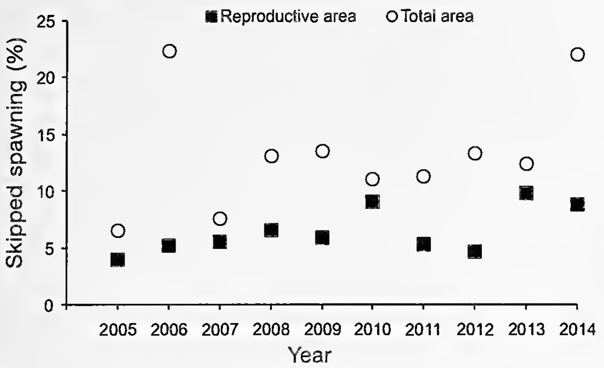

Figure 4

Percentages of female Argentine hake (*Merluccius hubbsi*) that had skipped spawning among all females of this species collected from the Patagonian stock, estimated for the reproductive area of this stock and for the total area sampled during the surveys conducted in January from 2005 through 2014.

distributions, highly significant differences ($P<0.001$) were observed, and females that had skipped spawning were mostly composed of smaller specimens with a modal size of about 38 cm TL. On the other hand, reproductive females showed higher frequencies of individuals larger than 50 cm TL. To corroborate these results, the length distributions of females based on our histological results were also analyzed by grouping all surveys. The result was similar to that obtained by using the visual information: highly significant differences were observed in comparisons of the 2 distributions ($P<0.001$) and nonreproductive females generally smaller than 50 cm TL.

The analysis of the age distribution obtained from subsamples of Argentine hake between 2005 and 2013 confirmed that females that had skipped spawning were primarily younger specimens with a modal age of 3 years and that the reproductive females were mainly represented by older individuals (Fig. 5B).

Condition indices and feeding activity

The mean GSI estimated for females that had skipped spawning was the lowest value given for females in a given maturity stage, followed by the GSI estimated for postspawning females, for developing females, and finally for spawning females (Table 3). The interannual variation in GSI values for different stages was relatively stable, with the widest range observed for spawning females.

When the nutritional condition, represented by K, was considered, females that had skipped spawning had mean values significantly higher ($P<0.0001$) than those estimated for other maturity stages (Table 3). In comparison, female Argentine hake that had recently completed spawning showed the poorest condition.

Results of the analysis of stomach fullness in adult female Argentine hake during the reproductive peak indicated that both females that had skipped spawning and females that recently had completed spawning (postspawning) had a higher frequency of stomachs with contents (Fig. 6) and that females in the spawning stage had the lowest percentages of feeding activity. We observed an increasing trend in the frequency of stomachs with contents toward the end of the study period (2005–2014), particularly in the case of females that had skipped spawning.

Results from the generalized linear model confirmed that year, TL, K, and SC influenced the probability of skipped spawning during the reproductive season of the Patagonian stock of Argentine hake (Table 4). However, although these variables were statistically significant, the amount of variation explained by these factors was very low. The model explained only 10% of the variability for the probability of skipped spawning. Total length had a negative effect in the model, which provided evidence of an increase of the probability of skipped spawning in small (<60 cm TL) adult females (Fig. 7A). In contrast, the effect of K on the probability of skipped spawning trended positively, indicating that females that had skipped spawning were characterized by higher values and, therefore, better condition (Fig. 7B). The effect of SC, as a binary variable, indicated that the probability of skipped spawning is higher when females have food in their stomachs, than in its relationship with both K and TL. Nevertheless, in the last case, the model results tended to merge when females were larger than 60 cm TL (Fig. 7A).

Skipped spawning and physical variables

The first 2 principal components of the principal component analysis explained 64% of total variance (Table 5). Principal component 1 explained 42% of total variance, and the variables most closely related to this component were the percentage of females that had skipped spawning and depth. Principal component 2 explained 22% of total variance, primarily the result of the percentage of spawning females and bottom temperature. This analysis indicated an opposite trend with the remaining studied variables (Table 5). An association between the highest proportion of females that had skipped spawning and deeper (>90 m) and colder (7–8°C) waters was observed. Conversely, spawning females were primarily associated with shallower coastal waters, where temperatures near the bottom were higher (9–13°C). Surface temperature and both bottom and surface salinity seem to have had no influence on the spatial distribution of female Argentine hake.

Discussion

By the year 2000, some authors mentioned the importance of considering the effect of skipped spawning

Table 3

Mean values and confidence intervals (CIs) of the gonadosomatic index (GSI) and Fulton's condition factor (*K*) estimated for each maturity stage, assessed by visual analysis, in adult female Argentine hake (*Merluccius hubbsi*) collected from the Patagonian stock between 2005 and 2014. Resting stage=skipped spawning.

Visual maturity stage	Mean GSI (CI)	Mean *K* (CI)
Developing	6.55 (6.48–6.62)	0.6724 (0.6705–0.6743)
Spawning	17.34 (19.94–17.74)	0.6540 (0.6501–0.6579)
Postspawning	2.70 (2.64–2.76)	0.6517 (0.6481–0.6553)
Resting	1.18 (1.15–1.21)	0.6937 (0.6322–0.6418)

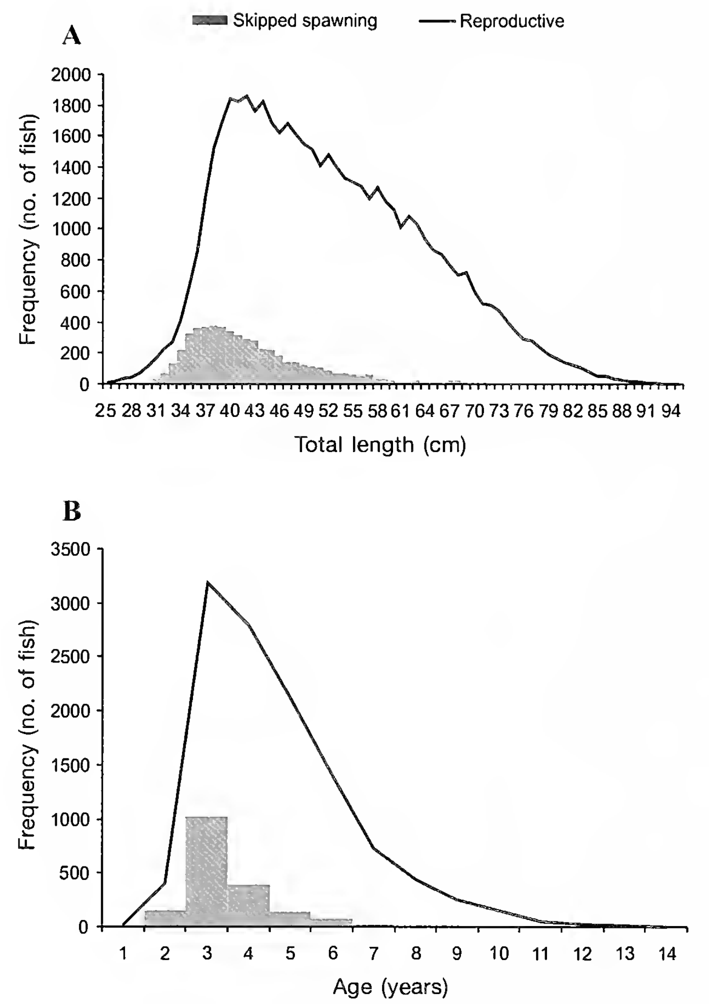

Figure 5

Grouped for all years analyzed, (**A**) size distribution and (**B**) age distribution of females of Argentine hake (*Merluccius hubbsi*) that had skipped spawning (gray bars) and reproductive (black line) Argentine hake sampled from the Patagonian stock off Argentina in January during 2005–2014.

in estimates of the reproductive potential of fish stocks (Livingston et al., 1997; Trippel, 1999; Rideout et al., 2000). However, the analysis of this phenomenon in general has been focused largely on experimental studies, under controlled conditions, and on the morphological description of fish during this process (Rideout et al., 2005; Skjæraasen et al., 2009). In a recent review about skipped spawning, the presence of this phenomenon was reported for at least 31 species, including marine teleosts (demersal and pelagic), freshwater fish, and anadromous and catadromous species (Rideout and Tomkiewicz, 2011).

On the basis of when maturation is interrupted, 3 categories of skipped spawning have been suggested (Rideout et al., 2005): retaining, reabsorbing, and resting. In the first category, ovaries have completed the maturation process but eggs are not released because ovulation is stopped. This interruption usually happens in specimens kept in captivity and may be due to unfavorable physical or chemical conditions in the environment or to changes in the sex ratio or sexual behavior during the reproductive process. The second category includes those cases in which all oocytes in the growing phase are reabsorbed through massive follicular atresia after oogenesis is interrupted during vitellogenesis. In the resting category, the ovaries of adult specimens capable of spawning during the reproductive season remain in a nonreproductive condition.

Among the 3 categories described for the process of skipped spawning by Rideout et al. (2005), only the resting stage was observed in Argentine hake from the Patagonian stock in our study. Ovaries in the resting stage are characterized by the presence of oocytes in the primary growth stage and show no evidence of maturation or recent spawning. This diagnostic observed in adult specimens during the breeding peak in the repro-

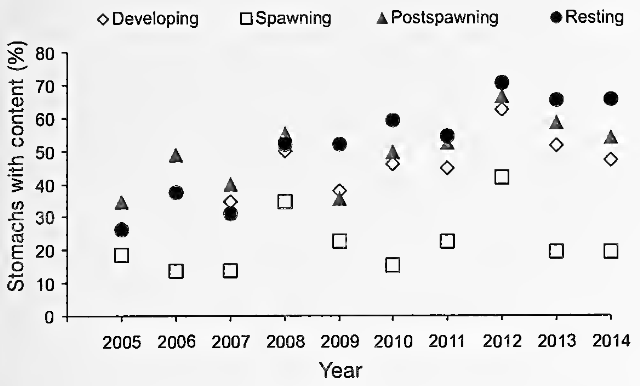

Figure 6

Annual variation of the mean percentages of stomachs with content for each maturity stage of female Argentine hake (*Merluccius hubbsi*) sampled from the Patagonian stock off Argentina during January between 2005 and 2014. Resting stage=skipped spawning.

Table 4

Results of the generalized linear model, with the effect of year (Y), total length (TL), stomach content (SC), and Fulton's condition index (K) on the probability of skipped spawning by Argentine hake (*Merluccius hubbsi*) collected from the Patagonian stock in 2005–2014. df=degrees of freedom.

	df	Deviance	*P*-value
NULL		12,931	
Y	8	85.20	<0.0001
TL	1	880.54	<0.0001
SC	1	51.93	<0.0001
K	1	9.94	<0.01

ductive area is evidence of skipped spawning, as was suggested for other species (Rideout and Tomkiewicz, 2011; Rodgveller et al., 2016). The other category of SS commonly described in natural conditions, reabsorbing in prespawning fish, has not been observed in Argentine hake, indicating that once vitellogenesis begins, most of the oocytes would develop through to the end of maturation. Massive atresia probably would be rather associated with species that have a short life cycle because the disruption of gonadal maturation is more common when the environmental conditions are not suitable for spawning. This mechanism would allow fish to divert energy for reproduction to other biological processes, such as growing and migration, until optimal conditions for reproduction are ensured (Rideout et al., 2005; Rideout and Tomkiewicz, 2011).

During January, the female Argentine hake that had skipped spawning were most often observed on the periphery of the main reproductive area, in depths close to 100 m. Considering the entire area covered during the research surveys, the highest proportions of SS females were recorded within the San Jorge Gulf, coinciding with the locations of the main aggregations of juvenile Argentine hake (Álvarez-Colombo et al., 2014). These results may indicate a spatial segregation of SS females during the reproductive season, similar to that reported for Atlantic cod (*Gadus morhua*) (Rose, 1993). Some authors have suggested that a location of spatial segregation could act as a reservoir for adult specimens of species that are exploited mainly in their spawning areas (Livingston et al., 1997).

The fact that females that had skipped spawning were primarily located on the periphery of the main spawning area, in deeper waters, indicates that the low temperature in this region may be associated with the interruption of the reproductive cycle, if the role of temperature is taken into account in triggering gonadal maturation in fish (Bye, 1984; Rideout et al., 2005). However, it is unknown why these individuals do not move into the coastal area where ripening and spawning of Argentine hake occurs. One possible explanation is that these females choose to remain in the offshore zone in order to feed, as was suggested for a population of Atlantic cod, referred to as "Northeast Arctic cod" (Skjæraasen et al., 2012). In fact, the migratory circuit described for the Patagonia stock of Argentine hake indicates that by the beginning of the reproductive season, Argentine hake move from deeper waters to the coast to spawn and that, once the reproductive activity is completed, spent females return to deeper waters to feed (Macchi et al., 2007).

Our analysis of the incidence of stomachs with contents confirms this hypothesis, given that females that had skipped spawning and postspawning females had the highest percentages of stomachs with food. Results indicate more intense feeding activity by these individuals, but with a very different nutritional condition between maturity stages. Postspawning females had the poorest condition (K=0.62 to 0.68) because of the energetic cost of reproduction, and females that had skipped spawning had the highest K values (0.68–0.75), partly as a result of the energy saving and reinforced by the sustained feeding activity.

Results from the analysis of the size and age structure for both females that had skipped spawning and reproductive females indicate that females of Argentine hake that had skipped spawning are composed mostly of young fish, 3 years old with a modal size of about 38 cm TL. The histological information confirms these results with a greater certainty than does the macroscopic diagnosis of gonads alone.

This outcome indicates that a significant proportion of females that have already experienced their first annual spawning could skip the next spawning cycle because age at first maturity for female Argentine

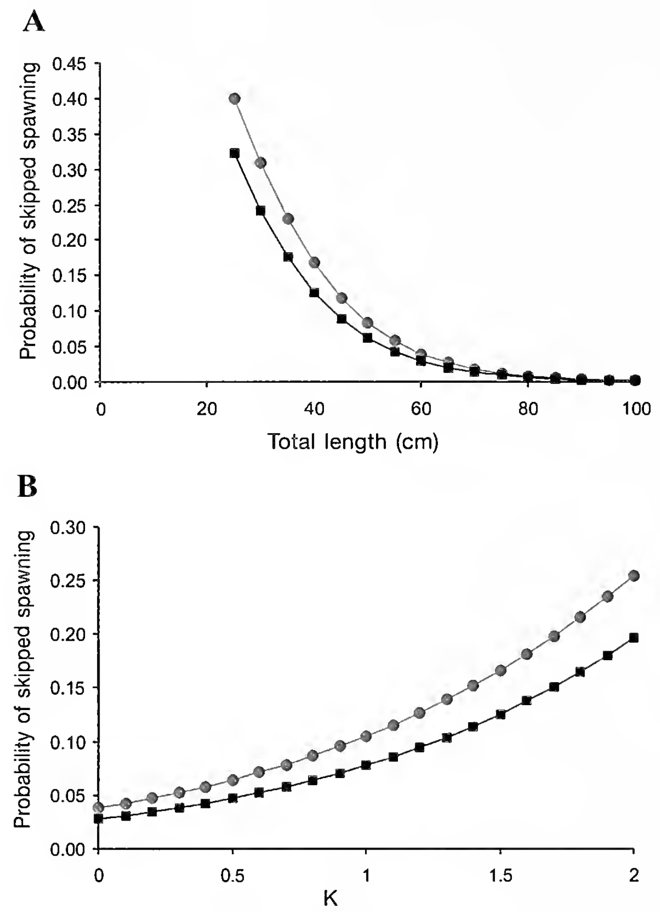

Figure 7

Probability of skipped spawning in female Argentine hake (*Merluccius hubbsi*), collected from the Patagonian stock off Argentina during January, 2005–2014, (**A**) versus total length and (**B**) versus Fulton's condition factor (*K*), estimated with a mean *K* of 0.67 and a mean total length of 51.14 cm, respectively. The line with gray circles represents the model with data for fish with content in their stomachs, and the dark line with black squares represents the model with data for fish with empty stomachs.

hake was estimated to be 2.6 years (Simonazzi[3]). Jørgensen et al. (2006) suggested that the high proportion of females of Atlantic cod that had skipped spawning corresponds with potential second-time spawners that were unable to fully recover from their first spawning. This pattern of skipped spawning, which mainly affects second-time spawners, has also been suggested for *Solea solea* (Ramsay and Witthames, 1996), for Atlantic herring (*Clupea harengus* [Engelhard and Heino, 2005]), and for Atlantic cod from the Northeast Artic (Skjæraasen et al., 2012) and from Canadian waters (Rideout and Rose, 2006). In the latter case, the authors reported that suppression of reproductive activity for this stock of Atlantic cod ranged annually from 8.4% to 55.6% of the stock, affecting primarily younger females between 40 and 49 cm TL. Such results indicate an overestimation of reproductive potential that could reach near 40%, if the proportion of skipped spawning is not considered.

A nutritional deficiency before the beginning of the reproductive season (October–November) could explain why young adult Argentine hake do not begin the annual maturation cycle and stay offshore in the feeding area during the spawning period. In January, during the reproductive peak, these fish will attain a better condition and continue feeding in deeper water. For this reason, the results of generalized linear modeling indicate higher probabilities of females that had skipped spawning with stomach contents because these females were observed mainly offshore, where the feeding activity is more intense. The relationship between the proportion of females that had skipped spawning and total length also showed a higher probability that individuals would not spawn while having food in their stomachs for fish with lengths smaller than 60 cm TL. In specimens larger than 60 cm TL, the probability of skipped spawning decreased for both females with food in their stomachs, as well as for those with empty stomachs.

These results indicate that not only larger females are reproductively active during January but also that some of them are actively feeding. In other words, larger female Argentine hake had a higher incidence of both spawning and feeding during the reproductive season than had young females, as has been previously suggested for this stock (Macchi et al., 2013).

The analyzed data do not allow an accurate estimation of the percentage of adult females that skip the annual spawning because the research surveys did not cover the entire spatial distribution of the Patagonian stock of Argentine hake. Estimates of the percentage of females that skip spawning for the reproductive area (see Fig. 1) ranged between 4% and 10% of the adult females sampled during January between 2005 and 2014, but these values markedly increase when data from San Jorge Gulf were included, reaching as high as 22% in some years. However, it is important to mention that, because females that skip spawning were primarily represented by young females, the overestimation of reproductive potential may have been lower than expected because both relative fecundity and egg quality are lower in female Argentine hake smaller than 50 cm TL (Macchi et al., 2013).

The Argentine hake is a species with indeterminate annual fecundity, i.e., with the capacity to continually recruit oocytes into the vitellogenic phase in an extended breeding season; therefore, it is possible

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[3] Simonazzi, M. 2003. Relación largo-peso y largo-edad de primera madurez sexual de la merluza. *In* Aportes para la evaluación del recurso merluza (*Merluccius hubbsi*) al sur de los 40° S. Año 1999* INIDEP Inf. Téc. 51 (L. S. Tringali and S. I. Bezzi), p. 11–26. Instituto Nacional de Investigación y Desarrollo Pesquero, Mar del Plata, Argentina.

Table 5

Results of the principal component (PC) analysis of environmental data collected during the research surveys conducted in the spawning area of the Patagonian stock of Argentine hake (*Merluccius hubbsi*) between 2005 and 2014. Values of the eigenvectors and percentage of the variance explained by the original data set (coefficient of determination [r^2]). Employed variables were percentage of skipped spawning (% SS), percentage of spawning females (% S), bottom temperature (BT), surface temperature (ST); bottom salinity (BS), surface salinity (SS), and depth (D).

Variables	PC1	PC2
% SS	0.39	0.13
% S	−0.38	−0.12
BT (°C)	−0.42	−0.39
ST (°C)	−0.36	0.35
BS	−0.22	0.60
SS	−0.39	0.43
D	0.45	0.38
r^2	0.42	0.22

that some females complete their gonadal maturation and their spawning outside of the normal reproductive period, as suggested by Rideout and Tomkiewicz (2011). However, we consider it unlikely that females that skipped spawning found in January could begin the process of maturation and spawning before the environmental conditions become adverse for the growth and survival of the Argentine hake larvae. Moreover, it is noteworthy that previous histological studies performed throughout the breeding season documented the existence of females that had skipped spawning during the entire spawning period and an increase in skipped spawning toward the end of the reproductive season (Macchi et al., 2004; Pájaro et al., 2005).

Data presented here indicate that skipped spawning may be a common phenomenon in the Patagonian stock of Argentine hake and that it may be a mechanism that allows a species to regulate its energy needs for future spawning, as has been suggested for many other fish (Rideout and Tomkiewicz, 2011; Skjæraasen et al., 2012, 2015). However, from a fisheries assessment point of view, the effect of skipped spawning on SSB estimates should be considered. For this reason, it is necessary to continue and expand studies of this phenomenon in Argentine hake, a species that is a resource of great economic importance for Southwest Atlantic fisheries. Understanding this process, together with estimates of its incidence throughout the entire Patagonian stock of Argentine hake, may help to explain the wide variation in the stock-recruitment relationships reported for this species. Moreover, further field research is needed to understand why some individuals decide to skip spawning and some do not. For this reason, an analysis of the biochemical composition of different tissues and determination of the physiological status and feeding activity of individuals before the beginning of maturation may help to improve understanding of the mechanisms that regulate the origin and frequency of skipped spawning in Argentine hake.

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Life history characteristics and stock status of the thumbprint emperor (*Lethrinus harak*) in Saipan Lagoon

Michael S. Trianni

Email address for contact author: michael.trianni@noaa.gov

Pacific Islands Fisheries Science Center
National Marine Fisheries Service, NOAA
P.O. Box 8216
Saipan, Northern Mariana Islands 96950

Abstract—Analysis of the life history of the thumbprint emperor (*Lethrinus harak*) sampled from Saipan Lagoon during 2005–2006 revealed sex-specific differences in von Bertalanffy age and growth parameters. Length at 50% reproductive maturity was estimated as 19.6 cm fork length (FL) for females and as 18.7 cm FL for males for the corresponding ages of 2.6 and 2.4 years. Available data from several sources for this data-poor coral reef fish were analyzed to assess its population status in Saipan Lagoon, Northern Marianna islands. Estimates of total mortality (Z) within the period 2005–2011 were derived by using length-converted catch-curve analysis and the Chapman–Robson estimator. Natural mortality (M) was estimated from 3 models based on applicable observations and parameter estimates derived from data for thumbprint emperor in Saipan Lagoon. Exploitation ratios (E) derived with combinations of Z and M estimators within the period 2005–2011 showed a wide disparity, although no estimate of average annual E exceeded 0.5 (an E over that threshold would have indicated overexploitation). Location-specific life history studies should be pursued to support local research and management goals. Standardized methods for the estimation of life history parameters would allow empirical comparisons between regions.

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Coral reef fishes of the family Lethrinidae, the emperors, are widely distributed throughout the tropical and subtropical Indo-Pacific, where they are primary targets of important commercial and noncommercial fisheries (Carpenter, 2001). Annual global landings of emperors rose sharply beginning in the mid-1970s and started to level off at about 84,000 metric tons (t) in the mid-1990s, according to statistics accessed through the Food and Agriculture Organization of the United Nations, FAO application FishStatJ, vers. 2.2.0 (Capture Production data set, available at website). As a result of the increase in landings, numerous studies have been directed at estimating life history characteristics for lethrinid species that are targeted in commercial and noncommercial fisheries (Brown and Sumpton, 1998; Laursen et al., 1999; Grandcourt, 2002; Kulmiye et al., 2002; Sumpton and Brown, 2004; Ebisawa, 2006; Williams et al., 2007; Ebisawa and Ozawa, 2009; Trianni, 2011; Currey et al., 2013), and additional studies have focused on other topics, including demographics (spatial and temporal), status designations, or both (Dalzell et al., 1992; Williams et al., 2003; Williams et al., 2007; Grandcourt et al., 2010; Taylor and McIlwain, 2010).

For most coral reef fishes caught in the central and western Pacific under U.S. jurisdiction, landings data are inconsistent or unreliable and there is a lack of estimates for jurisdiction-specific life history parameters (WPRFM[1]). As a result, these data often are insufficient to support formal stock assessments. The issue of data-poor fishery resources has in recent years led to an array of techniques designed to address data shortfalls. Examples of such approaches were specific natural mortality estimates, extended time-series of landings, or both (MacCall, 2009; Dick and MacCall, 2011); stock assessments with hierarchical Bayesian models that allow data for poor-data species to be borrowed from data for similar species for which "good-quality data" exist in single (Jiao et al., 2011) or multispecies fisheries (Punt et al., 2011); and density ratios that reflect both larval dispersal and adult movement patterns for species that occur within and outside of functional marine protect areas (McGilliard et al., 2011). The data required for these methods tend to differ from or exceed existing information for coral reef fisheries in U.S. jurisdictions in

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[1] WPRFMC (Western Pacific Regional Fishery Management Council). 2009. Fishery ecosystem Plan for the Mariana Archipelago, 231 p. Western Pacific Regional Fishery Management Council, Honolulu. [Available at website]

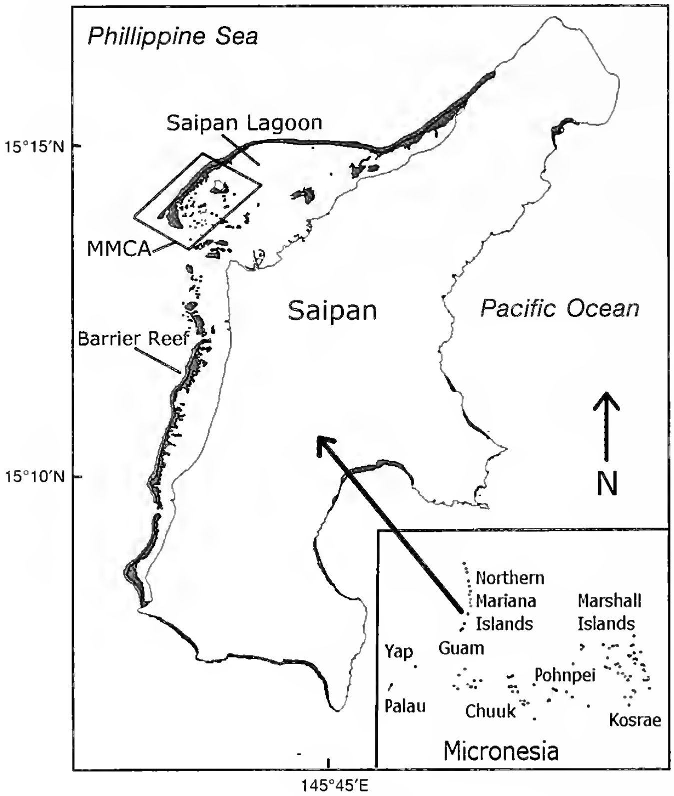

Figure 1

Map of the island of Saipan in the Mariana Archipelago showing the spatial extent of Saipan Lagoon and the boundaries of the Mānagaha Marine Conservation Area (MMCA).

the central and western Pacific; therefore, the application of these techniques for U.S. Pacific Island fisheries is limited. Species-specific evaluation in U.S jurisdictions will need to occur on a case-by-case basis and incorporate appropriate levels of analysis.

A protogynous hermaphrodite, the thumbprint emperor (*Lethrinus harak*), ranges throughout the Indo-Pacific region, including the Red Sea, where it is found in various habitats, including mangroves, lagoon seagrass habitats (Carpenter, 2001; Shibuno et al., 2008; Nakamura et al., 2009), expanded reef flats (Taylor and McIlwain, 2010), and sand environments on protected outer reef slopes (Myers, 1999). This species occurs solitarily or in groups of a few individuals and exhibits high diurnal site fidelity (Nanami and Yamada, 2009). Its diet consists of benthic invertebrates and small fishes (Carpenter, 2001). The thumbprint emperor can be a dominant species in landings from nearshore fisheries for which the primary methods of capture include gill nets, surround nets, and hook and line (Dalzell et al., 1996; Carpenter, 2001; Ebisawa, 2006; Taylor and McIlwain, 2010).

At Saipan in the Commonwealth of the Northern Mariana Islands (CNMI), the thumbprint emperor is a numerically dominant reef fish species in Saipan Lagoon. Historically, this fish was primarily harvested with gill and surround nets and, to a lesser extent, with hook and line and by free-dive spearfishing. Since December 2003, the use of gill nets, drag nets (which are gill nets dragged along the bottom in a manner similar to that used with beach seines), and surround nets in the CNMI have been restricted by the CNMI Department of Lands and Natural Resources (DLNR), and their use is allowed only for annual cultural fiestas through a special exemption granted by the DLNR. Hook and line has become the primary harvest method, followed by free-dive spearfishing. Given the importance of the thumbprint emperor as a major component of the coral reef fish community in Saipan Lagoon and the lack of biological data for this species, the DLNR Division of Fish and Wildlife (DFW) conducted research from 2005 through 2006 during which period age and growth and reproductive data were collected. This data collection effort corresponded with the commencement of a DFW creel survey program in 2005 that was designed to reflect fishing activity in Saipan Lagoon.

The objective of the present study was to use the thumbprint emperor as an example species in an examination of the challenges in assessing data-poor fisheries. This work drew on available data pertinent to the thumbprint emperor from Saipan Lagoon and this article presents estimates of life history parameters and mortality rates from 2 models for total mortality (Z) and 3 models for natural mortality (M). Generated rates of exploitation of the thumbprint emperor are provided here, along with biological and fishery metrics, to examine the status of this species in Saipan Lagoon.

Materials and methods

Study site

Saipan Lagoon extends along the western side of the island of Saipan and is bordered by a nearly continuous barrier reef (Fig. 1). The habitat that covers the largest amount of area in Saipan Lagoon is composed of unconsolidated sands, followed, in descending size, by a deeper (>7 m average depth) sandy area interspersed with consolidated coral reef structure (deep patch-reef), seagrass beds (*Halodule* spp. and *Enhalus* spp.), mid-lagoon zones of coral rubble, and backreef. Depths in Saipan Lagoon range from <1 m in nearshore areas to about 15 m in the main shipping channel. The lagoon serves as the primary area for a variety of recreational activities that do not involve the extraction of resources, as well as the primary grounds for noncommercial and commercial fishing for the island of Saipan. The principal fishing gears include hook and line, free-dive fishing with a spear gun, and cast net or talaya. Spearfishing with scuba gear is prohibited throughout the CNMI by public law (CNMI Admin. Code § 85-30.1-401). The restrictions on net use issued by DLNR allow a total annual catch of reef fish of about 907 kg (2000 lb). The no-take Mañagaha Marine Con-

Table 1

Macroscopic sex-specific gonad criteria used to assign maturity class to thumbprint emperor (*Lethrinus harak*) collected from Saipan Lagoon during 2005–2006.

Stage	Male	Female	Maturity class
1 Immature	Gonads long and slender, threadlike, and translucent.	Gonads long and slender, transparent or pinkish in color. Oocytes not discernible.	Inactive
2 Developing	Grayish-white in color, beginning to swell in girth.	Ovaries elongated, some beginning to swell in girth. Individual oocytes not discernible. Some blood vessels apparent. Color pink to light pink.	Active
3 Mature or ripe	Thickened, white to beige in color, blood vessels visible, milt exudes when gonad is squeezed. For gonads with further thickening, milt exudes on slight pressure.	Ovaries long and swollen with large oocytes visible through thin ovary wall. Blood vessels easily visible or disappearing. Color dark pink to red. Ova easily exuded upon application of pressure. Blood vessels disappearing. Color yellow to orange.	Active
4 Spent	Flaccid in appearance, thickened gonad wall loose and furrowed. Little to no milt exudes upon application of pressure.	Ovaries flaccid, partially empty in appearance, and color faded from dark yellow to brown. Ovary wall thick.	Active

servation Area (MMCA) surrounds Mañagaha Island, a cay within Saipan Lagoon, and includes fringing reef, coral and rubble, deep patch-reef, and sand-dominated habitats.

Data sources: fishery independent

Sampling During 2005–2006, staff working in the DFW life history program obtained monthly samples of thumbprint emperor, using hook and line, to develop estimates of reproduction, age, and growth. Specimens were measured to the nearest 1 cm in fork length (FL) and weighed to the nearest 0.1 g. Sex of fish was determined and fish were assigned to a status of either immature or sexually active (mature) on the basis of macroscopic examination (Table 1). Excised whole gonads were weighed to the nearest 0.01 g with an electronic scale. To process them for reading, sagittal otoliths were embedded in epoxy resin, cut transversely into 300–500 µm sections through the otolith core by using a low-speed saw, and were smoothed with abrasive paper of 600–1200 grit until the annuli were visible. These sections were then mounted on a glass slide with thermoplastic cement and hand polished with 0.3-µm alumina powder (Choat et al., 1996).

With the use of a dissecting microscope with transmitted light, each sectioned and mounted otolith was read randomly 3 times for counts of annual increments between the core and outer otolith edge.

The final age was decided when 2 of the readings agreed (Choat and Axe, 1996). The margins of otoliths were assessed and assigned to one of the 3 classifications based on the relative stage of marginal increment formation. Margins were classified as *thin* if opaque material was visible but not necessarily continuous around the otolith margin; *medium wide* if there was a continuous increment of translucent material that was less than two-thirds complete (based on the width of the prior translucent increment) and visible on the outermost margin of the opaque increment; and *wide* if the marginal translucent increment was more than two-thirds complete. A classification of *thin* was indicative of a new annual mark and counted as a year. Otolith processing, age determination, and marginal edge analysis were conducted by the fish aging laboratory at James Cook University, Townsville, Australia.

Biomass surveys The DFW conducted underwater visual census (UVC) surveys in 2004, 2007, and 2011 in the southern portion of Saipan Lagoon to measure the influence of restrictions on net use imposed in December 2003. In these surveys, 4 major lagoon habitat units were surveyed on the basis of dominant substrate by using belt transects of 25×5 m (counts of 10–15 min) for fish <20 cm FL and by using stationary point counts with a 10-m radius (8-min duration) for fish ≥20 cm FL. For these surveys, standard DFW fishery-independent UVC techniques were used and the surveys were conducted by using a proportional allocation design (Cochran, 1977). Additionally, the same UVC survey techniques were used to estimate biomass of thumbprint emperor for the deep patch-reef habitat in the MMCA. Results from these surveys were used to obtain estimates of total biomass of thumbprint emperor from Saipan Lagoon for 2007 and 2011 by using the following equations (Cochran, 1977):

$$\bar{X}_{\mathrm{H}} = \sum_h^H \bar{x}_{\mathrm{h}} \times \tfrac{h}{H} \text{ and} \tag{1}$$

$$Y = \bar{X}_{\mathrm{H}} \times H, \tag{2}$$

where $\bar{X}_{\mathrm{H}}$ = overall habitat mean (g/m^2);
$\bar{x}_{\mathrm{h}}$ = habitat unit mean (g/m^2);
H = total size all habitats (m^2);
h = habitat unit size (m^2); and
Y = estimated total (g).

Biomass estimates of thumbprint emperor were not available from 2007 because most individuals of *Lethrinus* were recorded only at the genus level. As a result, although there is uncertainty in the amount of error associated with this approach, the biomass of species of *Lethrinus* estimated for 2007 was multiplied by the proportion of species of *Lethrinus* identified as thumbprint emperor in surveys in which UVC methods were used during 2011.

Data sources: fishery dependent

Inshore creel survey The DFW currently operates an inshore creel survey (ICS) that targets Saipan Lagoon. The ICS collects data on nonvessel fishing activities that involve gear that includes hook and line, free-dive with a spear gun (pachinko and cast net [talaya]). For this study, length-frequency data and estimated annual landings of the thumbprint emperor for the years 2005–2011 were obtained from the ICS database.

Exemptions from net-use restrictions In December 2003, the DLNR established regulations that prohibit the use of gill, drag, and surround nets, except as exempted by the DLNR for special cultural events, such as annual fiestas. Catch quotas of 68–136 kg (from 150 to 300 lb) with a 2-to-3-day time limit were established for exemptions (net-use exemptions, NUE, under an annual limit of 907 kg [2000 lb.]) For all fishing activities exempted from DLNR regulations through 2011, surround nets (*chenchulun umesugon*) were used and the landings taken under these exemptions were monitored by the DFW. In this study, landings taken under the NUE were used in estimating total annual landings and recording length-frequency measurements for thumbprint emperor.

Tracking commercial landings In the CNMI, the primary means of tracking commercial landings of coral reef fishes is the DFW Commercial Purchase Database System (CPDS), a database that tracks commercial purchases and sales recorded on trip tickets provided by DFW and voluntarily submitted by vendors. Additionally, in 2011, a biosampling program (BSP) for coral reef fish commenced in the CNMI. Several times a week, the BSP sampled reef fish for species identification, length frequency in centimeters in FL, and body weight in grams from randomly selected catches of fishermen before commercial sale. The BSP also collected daily data on vendor purchases from fishermen, in an effort similar to that of the CPDS. In 2011, the BSP sampled 19% of total reef fish landings from the coral reef spear fishery. The proportion of thumbprint emperor among the total biomass of coral reef fishes recorded during the BSP sampling in 2011 was used to estimate total landings of thumbprint emperor by multiplying the proportion of thumbprint emperor by the estimated CPDS annual landings of lethrinids during 2006–2011.

Data analyses

Size at maturity Data from the macroscopic determination of sex and reproductive status were partitioned into 1-cm-FL classes for the analysis of female length at 50% maturity (L_{50}), female age at 50% maturity (A_{50}), and length (L_{T}) and age (A_{T}) at 50% sex transition from female to male. A logistic model was used for these estimations:

$$P = 1/(1 + e^{(a - bX_f)}), \tag{3}$$

where a and b = the fitted model constants;
P = the percent mature or transitioned in length or age class,
X_{f} = length or age class; and
L_{50} or A_{50} = $-\frac{a}{b}$.

This model was weighted by the square root of the numbers of fish in each length class, and the regression parameters were estimated by using maximum likelihood with a binomial logistic link function. Bias-corrected percentile confidence intervals were produced around all estimates from 1000 resamples drawn with replacement (R Core Team, 2015).

Age and growth A nonlinear least squares model was used to fit the standard von Bertalanffy growth function (VBGF) (von Bertalanffy, 1938) to length-at-age data. The VBGF is defined with the following equation:

$$L_t = L_\infty (1 - e^{-k(t - t_0)}), \tag{4}$$

where L_t = the length at time t;
L_∞ = the asymptotic length;
k = the Brody growth coefficient; and
t_0 = the theoretical age at which length is equal to 0.

Data were fitted both by not constraining t_0 and by constraining it to an estimated age at settlement from Japan (Nakamura, et al. 2010). Growth curves generated from the VBGF fitting procedure were subsequently tested for equality by using the analysis of residual sum of squares (ARSS) method developed by Chen et al. (1992) for male and female comparisons. Parameters of the length–weight relationship were obtained by fitting the following power function

$$W = a \times FL^b \tag{5}$$

to length and weight data,

where W = the total wet weight; and
a and b = the associated equation constants (R Core Team, 2015).

Total mortality The expectation–maximization algorithm approach developed by Hoenig and Heisey (1987) was used to create length–age keys from the 2005 and 2006 life history data, and those keys were used to estimate age groups from length-frequency data collected in subsequent years from the ICS and NUE data. The annual instantaneous rate of Z was subsequently determined with the age-based catch-curve (CC) method (Beverton and Holt, 1957) in its linearized form:

$$\ln(C_t) = \ln(N_0) - Zt, \tag{6}$$

where C_t = the catch in year t;
N_0 = recruitment into cohort; and
Zt = the instantaneous rate of Z at age t.

The descending limb of the plot of the natural logarithm of relative abundance versus age (time) was used to estimate Z with exclusion of derived age groups beyond the first age class where one or fewer individuals were observed (Dunn et al., 2002). Additionally, Z was estimated by using the maximum likelihood estimator for catch curves developed by Chapman and Robson (Chapman and Robson, 1960; Robson and Chapman, 1961):

$$Z = -\log\left(\frac{T}{n+T-1}\right), \tag{7}$$

where n = the total number of fish observed on the descending limb of the catch curve, defined as fully recruited; and
T = the fish ages on the descending limb of the catch curve, where the first fully recruited age is set to 0.

Estimates of Z from the use of both methods, and both age–length keys, were generated yearly as permitted by the availability of data from the ICS, as well as the availability of the NUE data.

Natural mortality Three models were used to estimate the instantaneous rate of M. For one model, the predictive equation developed by Hoenig (1983) was used; the equation relates the maximum age observed in a stock to M:

$$\ln(M) = 1.46 \times 1.01 \times \ln(t_{\max}), \tag{8}$$

where $t_{\max}$ = the maximum age observed in the population.

Another model was Lorenzen's (1996) power function of weight for natural populations:

$$M = 3W^{-0.288}, \tag{9}$$

where W is weight (g).

Finally, the third model was the mortality estimator based on the empirical equation by Gislason et al. (2010):

$$\mathrm{Log}M = 0.55 - 1.61\log L + 1.44L_{\infty} \log k, \tag{10}$$

where L = the fish fork length;
L_{∞} = the asymptotic length; and
k = the instantaneous growth coefficient.

Spawning potential ratios Goodyear (1993) summarized the concept of spawning potential ratio (SPR) and evaluated it against other traditional fisheries reference points, defining SPR as a ratio of the potential recruit fecundity (P) in an exploited stock to the potential recruit fecundity in an unexploited stock:

$$SPR = \frac{P_{\text{fished}}}{P_{\text{unfished}}}, \tag{11}$$

where $P = \sum_{i=j}^{n}(E_i * Fr_i) * \exp^{(-(\mathrm{F_i}+\mathrm{M_i}))}$, (12)
n = the number of female length classes;
E = the mean fecundity of females at length i;
Fr_{i} = the fraction of females at length i that were mature;
Fi = the fishing mortality rate of females at age i; and
M = the natural mortality rate of females at age i.

The SPR was estimated by calculation of P in the absence of fishing mortality (F=0) and with various levels of fishing mortality (F>0). Length classes included the smallest mature female and the largest measured female observed during sampling by the DFW during 2005–2006.

Mean fecundity per size class for thumbprint emperor was estimated by using the fecundity relationship (batch fecundity=$1.58\times\ 0^{-3}\times\mathrm{FL}^{5.2}$) provided by Ebisawa (1990) for the spangled emperor (*Lethrinus nebulosus*), a phylogenetically related species (Lo Galbo et al., 2002) with similar trophic-level characteristics (Carpenter, 2001). The fraction of females that were mature for a specific FL was estimated from collected length-at-maturity data. The fishing mortality rate was multiplied by a selection coefficient that was assumed to increase linearly with length from 0.0 at 1 cm less than the smallest length sampled to 1.0 at 1 cm greater than the modal length in the length distribution. Through the use of growth parameter estimates derived from the VBGF, M was estimated from the empirical equation of Gislason et al. (2010) and applied as estimated for each length class. The ratio of estimated total annual landings of thumbprint emperor from fishery-dependent data sources over estimated biomass from the fishery-independent surveys conducted in 2007 and 2011 approximated estimates of fishing mortality (Beddington and Kirkwood, 2005), and those estimates of fishing mortality were then used to assess SPR for the period 2005–2011.

Exploitation The exploitation ratio (E) was computed as E=F / Z, where Z=M +F (Gulland, 1971) and F is instantaneous fishing mortality. An E value at or near 0.5 is considered indicative of full exploitation, al-

Table 2

Number of male and female fish sampled, percentage of fish that were male, and sex ratio by length bin for thumbprint emperor (*Lethrinus harak*) collected during 2005–2006 from Saipan Lagoon. *n*=sample size.

Fork length (cm) ratio(M:F)	*n*	Male	Female	Male (%)	Sex
10.0–11.9	6	3	3	0.50	1.0:1.0
12.0–13.9	9	1	8	0.11	1.0:8.0
14.0–15.9	37	8	29	0.22	1.0:3.6
16.0–17.9	88	19	69	0.22	1.0:3.6
18.0–19.9	109	19	90	0.17	1.0:4.7
20.0–21.9	98	16	82	0.16	1.0:5.1
22.0–23.9	116	38	78	0.33	1.0:2.1
24.0–25.9	80	36	44	0.45	1.0:1.2
26.0–27.9	18	5	13	0.28	1.0:2.6
28.0–29.9	5	0	5	0.00	–
30.0–31.9	2	1	1	0.50	1.0:1.0
32.0–33.9	2	1	1	0.50	1.0:1.0

though Pauly (1984) suggested a more conservative value of 0.2 for lower-trophic-level, small-size fish with high recruitment variability. Although the thumbprint emperor is a higher-trophic-level species, the value of 0.2 could serve as a lower bound for E for this species. To obtain uncertainties around estimates of E, in this study, uncertainties around Z and M were first obtained by sampling with replacement (1000 resamples). For Z, obtaining uncertainties was accomplished by resampling within the ranges of the estimated annual values from both the CC and the Chapman–Robson (CR) methods. Estimates of M uncertainty were derived by resampling within boundaries of applicable model parameters: within the uncertainties (confidence intervals) of estimated VBGF age and growth parameters for thumbprint emperor from this study with the Gislason model; within the range of weights (Lorenzen model) or lengths (Gislason model) observed from sampling thumbprint emperor from this study; within the maximum ages (Hoenig equation) of thumbprint emperor estimated from this study and from Taylor and McIlwain (2010) for Guam.

Estimates of F were subsequently derived by resampling (1000 resamples) the equivalency $Z=M+F$, rearranged as $F=Z-M$, by using results from the resampled M and Z estimators. All analyses were conducted with R statistical software (R Core Team, 2015).

Results

Reproduction

The overall observed male-to-female sex ratio for the thumbprint emperor from the CNMI for the period 2005–2006 was 1.0:2.9, and females were predominant in most size classes (Table 2). The estimated L_{50} and A_{50} for females were 19.6 cm FL and 2.6 years, respectively (Table 3). Values of the gonadosomatic index for female thumbprint emperor began to increase concomitant with a decrease in the percentage of immature individuals at size, elevating noticeably at around 19–20 cm FL (Fig. 2), with fewer samples from size classes greater than 27 cm FL (Table 2). Estimates of age and length at the transition from females to males were not obtained because the expected change in sex ratio—a change to more males and fewer females that would indicate sex transition—was not observed (Table 2).

Age and growth

Results from the marginal edge analysis completed in 2005 and 2006 indicated that otolith accretion with a margin classification of new (opaque material visible on otolith margin), occurred from June through October (Fig. 3, A and B). The oldest individuals collected were an age-9 male at 27.6 cm FL and an age-9 female at 29.3 cm FL. The estimates of unconstrained overall, sex-specific, and constrained VBGF parameters are provided in Table 4. Growth curves for the combined unconstrained, sex-specific, and constrained model fits are displayed in Figure 4. Estimates of L_∞ and k for male and female thumbprint emperor from Saipan differed substantially; females were estimated to have a larger L_∞ and lower k. The ARSS model used to analyze the sex-specific growth curves revealed significant differences between the sexes (P=0.016) and years (P=0.004).

Length frequency, biomass, and landings

Percent contributions of measured thumbprint emperor per length class generated from ICS and NUE data were graphically compared with data from female and male size histograms developed from DFW sampling in

Table 3

Reproductive life history parameters for thumbprint emperor (*Lethrinus harak*) as estimated from locations in the Indo-Pacific: female size at 50% maturity (L_{50}) with confidence intervals (CIs), female age at 50% maturity (A_{50}) with CIs, length at transition from female to male (L_T), age at transition from female to male (A_T), and sex ratio (M:F). The number of male and female thumbprint emperor sampled appear in parenthesis below each location. All lengths are fork lengths measured to the nearest centimeter.

Location	Female L_{50} (CI)	Female A_{50} (CI)	L_T	A_T	M:F
Saipan (150,420)	19.6 (19.1–19.9)	2.6 (2.4–2.8)	–	–	1.0:2.9
Guam[1] (85,328)	20.8	3.8	24.1	5.38	1.0:3.8
Ryukyu Islands (194,254)	19.5[2]	1.0–2.0[3]	25.5[2]	–	1.0:1.3[2]
Kenya[4] (386,426)	24.3	–	–	–	1.0:1.1

[1] Taylor and McIlwain, 2010.
[2] Ebisawa, 2006.
[3] Ebisawa and Ozawa, 2009.
[4] Kulmiye et. al., 2002.

Saipan Lagoon (Fig. 5). There was a distinct difference in the length classes targeted by the ICS and NUE fishermen. The ICS landed mostly smaller thumbprint emperor in the length classes from 12 to 15 cm FL, and NUE fishermen targeted fish in length classes from 22 to 25 cm FL. When length-class percentages from the different data sources were overlain, NUE data covered nearly the entire male length distribution derived from DFW sampling, whereas ICS landings consisted primarily of smaller (Fig. 5) females and males. The highest percentages of thumbprint emperor in annual landings corresponded with hook-and-line landings, as estimated from the ICS. In contrast, landings of thumbprint emperor in 2011 from the BSP came from free-dive spearfishing, composing about 0.8% of the estimated total annual BSP landings. Table 5 provides

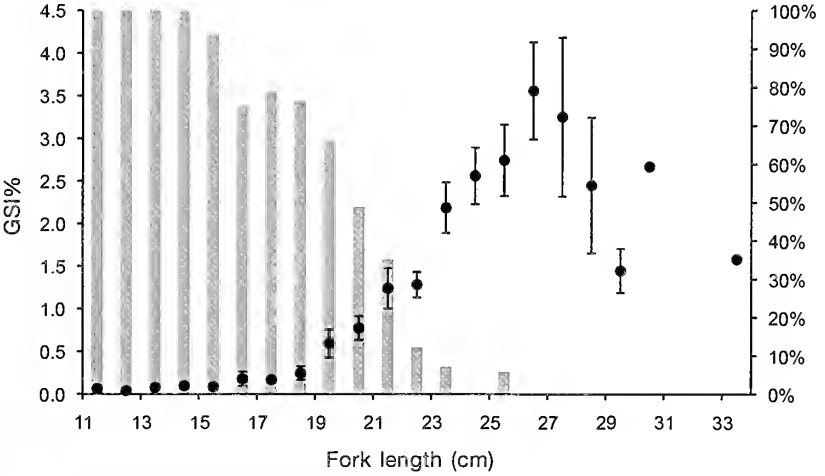

Figure 2

Gonadosomatic index (GSI) values, indicated with black dots, and percentages of individuals that were immature at a given fork length (cm), indicated by gray bars, for thumbprint emperor (*Lethrinus harak*) collected from Saipan Lagoon during 2005–2006. Error bars on the black dots indicate standard errors of the mean.

Table 4

Age and growth parameters, with standard errors of the mean (SEs), for thumbprint emperor (*Lethrinus harak*), as estimated for Saipan Lagoon with models that used the von Bertalanffy growth function: asymptotic length (L_∞), Brody growth coefficient (k), the theoretical age at which length is equal to 0 (t_0), and maximum age estimated (t_{max}). The combined, constrained model was constrained to an estimated length at settlement of 15.8 mm in fork length. All values of L_∞ are fork lengths given in centimeters. CI=confidence interval. n=sample size.

Estimated categories	n	L_∞ (SE)	k (SE)	t_0	t_{max}
Male	127	27.3 (1.6)	0.377(0.035)	-1.11	9
Female	369	37.2(5.7)	0.139(0.049)	-2.92	9
Combined	522	30.1 (1.4)	0.259 (0.041)	-1.65	9
Combined CIs		27.2–32.9	0.180–0.340		
Combined constrained	522	24.5 (0.288)	0.688 (0.024)		9

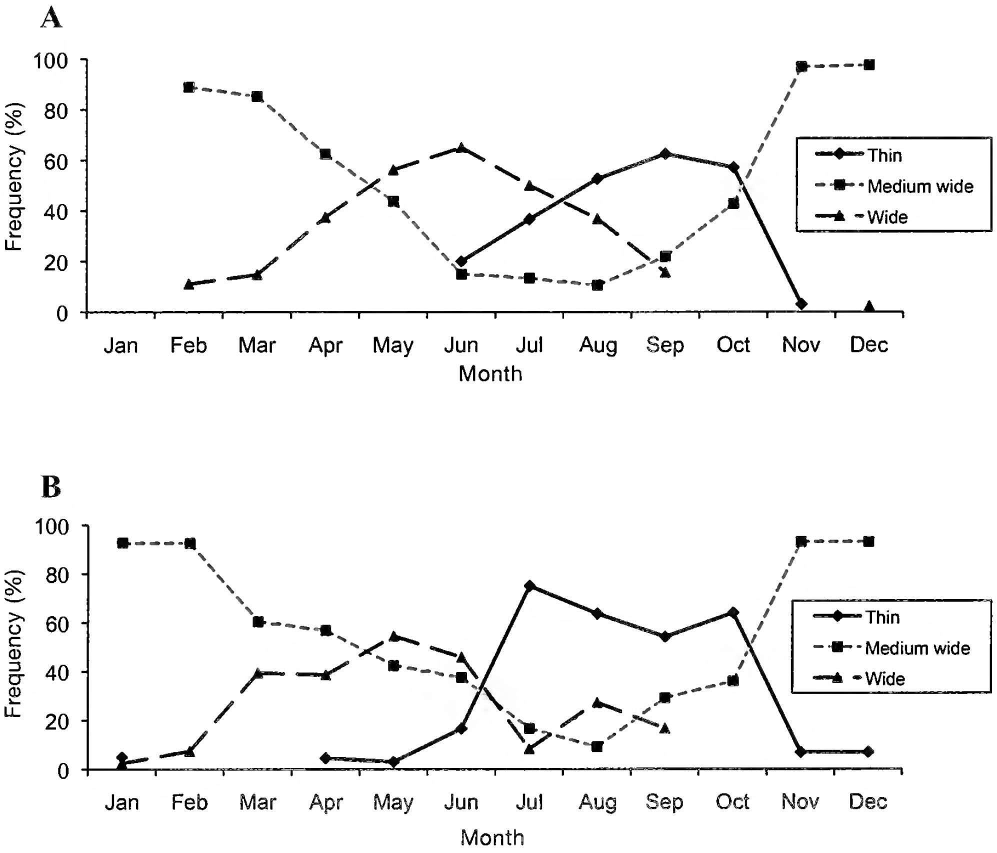

Figure 3

Monthly classification of otolith margins in thumbprint emperor (*Lethrinus harak*) collected from Saipan Lagoon during (**A**) 2005 (n=281) and (**B**) 2006 (n=337). The 3 classifications were 1) *thin*, opaque material visible but not necessarily continuous around otolith margin, 2) *medium wide*, continuous increment of translucent material visible on the outermost margin of the opaque increment that was less than two-thirds complete; and 3) *wide*, marginal translucent increment more than two-thirds complete.

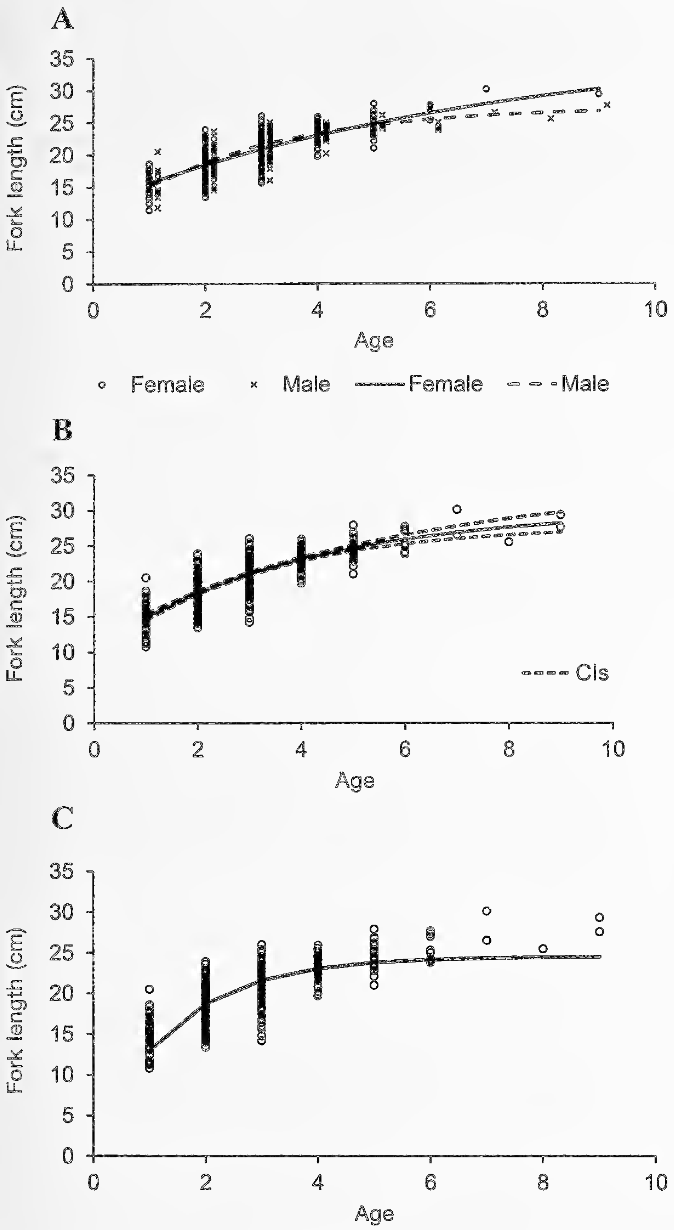

Figure 4

The von Bertalanffy growth curves fitted to observed fork length over age for thumbprint emperor (*Lethrinus harak*) collected from Saipan Lagoon during 2005–2006 (**A**) by sex (females, n=369; males, n=127), (**B**) by both sexes combined (n=522) with confidence intervals (CIs), and (**C**) by both sexes combined and with the model constrained to length at settlement. In, graph A, open circles represent female length at age and × symbols represent male length at age. In graphs B and C, open circles represent length at age.

biomass values from fishery-independent surveys in 2007 and 2011 and the estimated annual landings for the period 2005–2011 that were derived from pooling estimated landings from the ICS, NUE, and CPDS and BSP data.

Spawning potential ratios

Approximated baseline values of F for 2005–2011 from the ratio of estimated total annual landings of thumbprint emperor from fishery-dependent data sources over estimated biomass from the 2007 and 2011 fishery-independent surveys are given in Table 5. Estimated SPR values at varying levels of F along with approximated values of F and corresponding estimated SPR values from 2005 through 2011 are listed in Table 6. Estimated SPR values for the period 2005–2011 ranged from 0.91 to 0.98 (Table 6).

Mortality

Estimates of Z with the CC and CR methods had similar ranges: 0.326–0.867 and 0.473–0.871, respectively. The CR model generally produced higher estimates of Z than those produced with the CC model for any given combination of source data, age–length key, and year (Table 7). The estimates of the models were significantly different (t-stat=6.57, df=17, $P<0.00$). In Figure 6, age frequencies derived from DFW samples, as well as from age–length keys for the period 2005–2006, are overlain with estimated M derived from average length and average weight for the Gislason and Lorenzen estimators. The M estimates from the Gislason model were lower than the estimates derived from the Lorenzen model for all corresponding ages (Fig. 6).

Exploitation rate

Variability between the 3 estimators of M resulted in a wide range of estimates of exploitation rate (Fig. 7). The E estimates from the use of M from the Lorenzen model were negative in 4 years when Z from the CC method was used and negative for 2 years when Z from the CR method was used (Fig. 7). The E estimates were positive when Z from the CC and CR methods and M from the Gislason et al. (2010) and Hoenig (1983) methods were used. Average values of E from the 3 yearly estimates are depicted as circles in Figure 7. These estimates of E ranged from −0.082 in 2009 (Fig. 7A) to 0.341 in 2011 (Fig. 7B).

Estimates of M from the Gislason model in which VBGF parameter t_0 was constrained to size at settlement from Japan (15.8 mm FL; Nakamura et al., 2010) produced high estimates of k, which led to high estimates of M, and no positive E values for estimates derived from Z with the CC and CR methods.

Discussion

Fishing pressure has been found to result in truncated length and age frequencies (Friedlander and DeMar-

Table 5

Estimated landings (in kilograms) of thumbprint emperor (*Lethrinus harak*) from Saipan Lagoon for the period 2005–2011 from the Commonwealth of the Northern Mariana Islands Division of Fish and Wildlife inshore creel survey (ICS), commercial reef fish biosampling program (BSP), commercial purchase database system (CPDS), and records of net-use exemptions (NUE), as well as estimated fishing mortality (*F*) and estimated biomass from fishery-independent surveys (FIS) conducted in 2007 and 2011. Activities covered in the ICS include free-dive spearfishing (Spear) and fishing with hook and line (H&L) and cast nets. Total landings are given in kilograms and metric tons. The BSP did not begin until 2011.

	ICS											
Year	Reef fishes	*L. harak*	Spear	H&L	Cast net	BSP	CPDS	NUE	Total (kg)	Total (t)	*F*	FIS
2005	23,139	1310	595	711	4	–	648	108	2066	2.06	0.050	41.5[2]
2006	25,360	2493	680	1808	5	–	940	119	3552	3.55	0.085	41.5[2]
2007	23,349	2285	536	1748	1	–	665	231	3181	3.18	0.085	37.5[1]
2008	18,523	1915	197	1710	9	–	623	69	2607	2.61	0.063	41.5[2]
2009	27,201	2904	236	2653	15	–	535	33	3472	3.47	0.084	41.5[2]
2010	11,267	471	65	405	1	–	444	5	920	0.92	0.022	41.5[2]
2011	9342	477	0	472	5	408	[3]	64	948	0.95	0.021	45.4[1]

[1] Estimated biomass from FIS.

[2] Averaged biomass from FIS estimates.

[3] BPS used.

tini, 2002; Graham et al., 2005; Lewin et al., 2006) and in skewed sex ratios of hermaphroditic species (Birkeland and Dayton, 2005; Fenberg and Roy, 2008; Shepherd et al., 2010), and potential effects on stock recruitment (Alonzo and Mangel, 2004; Alonzo et al., 2008) and ecosystem functioning (Zhou et al., 2010; Garcia et al., 2012). The CNMI employs a complete ban on spearfishing with scuba gear, as well as restrictions on the use of gill, drag, and surround nets. Fishermen use nets toward the central parts of Saipan Lagoon in areas dominated by soft sediments, whereas hook-and-line fishing occurs mostly from shore near seagrass

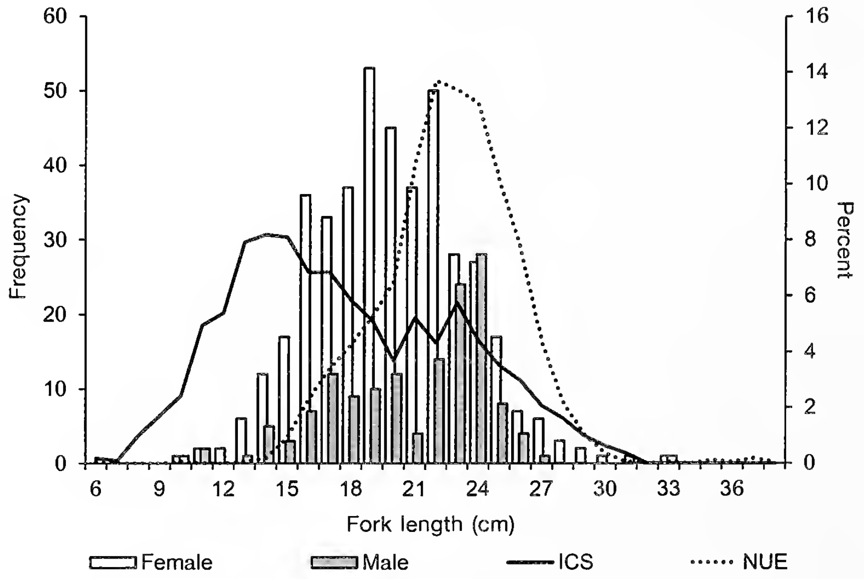

Figure 5

Length-frequency histograms for female and male thumbprint emperor (*Lethrinus harak*) from Saipan Lagoon during 2005–2006, measured by staff working in the life history program of the Commonwealth of the Northern Mariana Islands Division of Fish and Wildlife (DFW), overlain with percentages of length classes from the DFW inshore creel survey (ICS) and recorded net-use exemptions (NUE) for the period 2005–2011.

Table 6

Approximate annual values of fishing mortality (F) and estimated spawning potential ratio (SPR) for thumbprint emperor (*Lethrinus harak*) collected from Saipan Lagoon during 2005–2011. Values were derived from estimated total landings from fishery-dependent data sources and estimated biomass from fishery-independent survey and were compared with estimated SPRs at differing levels of F.

Estimated from annual landings			Derived	
Year	F	SPR	F	SPR
2005	0.05	0.95	0.00	1.00
2006	0.09	0.91	0.25	0.78
2007	0.08	0.91	0.50	0.61
2008	0.06	0.94	0.75	0.47
2009	0.08	0.92	1.00	0.37
2010	0.02	0.98	1.25	0.29
2011	0.02	0.98	1.50	0.22
			1.75	0.17
			2.00	0.14
			2.25	0.11

beds, which have been shown to be a preferred habitat for juvenile thumbprint emperor in southern Japan (Nakamura et al., 2009). The difference in length frequencies derived from the Saipan Lagoon ICS and in those derived from NUE data indicate that shoreline fishing targets smaller (Fig. 5) thumbprint emperors and that fishing pressure on larger thumbprint emperor was probably reduced as a result of regulations on net use.

Length-frequency data in published studies from Kenya (Kulmiye, et al. 2002), Guam (Taylor and McIlwain, 2010), Ryukyu Islands (Ebisawa and Ozawa, 2009), as well as from this study in Saipan Lagoon, show that females were prominent in the largest size classes and, except for females in Saipan Lagoon, were the largest recorded individuals (Table 2). Kulmiye et al. (2002) concluded that identification of hermaphroditism in thumbprint emperor would require further study, but Ebisawa (2006) considered the thumbprint emperor a protogynous hermaphrodite. Sadovy de Mitcheson and Liu (2008) did not designate the thumbprint emperor as a protogynous hermaphrodite, and, although the seemingly complex sexuality of this species may require further inquiry, data from Guam provide evidence for protogyny in that population (Taylor and McIlwain, 2010). Ebisawa (2006) stated that the observed difference in sex ratio in the largest size classes in the Ryukyu Islands, where females outnumbered males, was a result of females attaining a larger maximum size than that of males, although Ebisawa and Ozawa (2009) did not distinguish L_∞ between the sexes in their study. The

Table 7

Estimates of total instantaneous mortality (Z) and standard errors of the mean (SEs) from analysis with the linearized catch-curve (CC) method and the Chapman–Robson (CR) method for thumbprint emperor (*Lethrinus harak*) from Saipan Lagoon. Values are listed by fishery source; inshore creel survey (ICS) or net-use exemption (NUE), year of age–length key (2005–2006), and year of data collection (2005–2011)

Source	Year of age–length key	Year of data collection	Z (CC)	SE	Z (CR)	SE
ICS	2005	2006	0.549	0.051	0.598	0.036
ICS	2005	2007	0.454	0.110	0.509	0.041
ICS	2005	2008	0.521	0.056	0.573	0.037
ICS	2005	2009	0.326	0.046	0.473	0.033
ICS	2006	2006	0.725	0.071	0.845	0.064
ICS	2006	2007	0.674	0.094	0.760	0.072
ICS	2006	2008	0.720	0.074	0.806	0.064
ICS	2006	2009	0.544	0.127	0.626	0.051
NUE	2005	2005	0.431	0.150	0.664	0.036
NUE	2005	2006	0.575	0.101	0.753	0.040
NUE	2005	2007	0.389	0.083	0.663	0.047
NUE	2005	2009	0.368	0.127	0.639	0.790
NUE	2005	2011	0.446	0.086	0.691	0.049
NUE	2006	2006	0.867	0.120	0.871	0.046
NUE	2006	2007	0.659	0.142	0.756	0.052
NUE	2006	2009	0.415	0.069	0.671	0.084
NUE	2006	2011	0.493	0.182	0.758	0.055

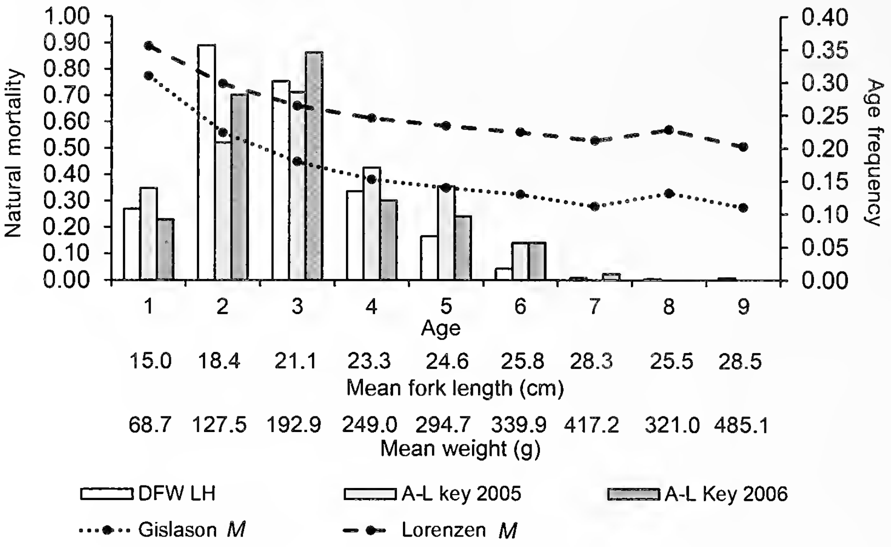

Figure 6

Estimates of natural mortality (*M*) for thumbprint emperor (*Lethrinus harak*) from Saipan Lagoon during the period 2005–2006, derived from average weight-per-age class in the Lorenzen model and from the average length-per-age class in the Gislason model, expressed against age frequencies determined from data provided by the life history (LH) program of the Commonwealth of the Northern Mariana Islands Division of Fish and Wildlife (DFW) and age–length keys for 2005 and 2006.

issue of female maximum size in the thumbprint emperor is important given that, in general, larger female fish are exponentially more fecund and potentially more beneficial to population recruitment (Birkeland and Dayton, 2005; Fenberg and Roy, 2008).

The L_{50} value for thumbprint emperor in Saipan Lagoon (19.6 cm FL) was lower than L_{50} values for thumbprint emperor from east Kenya, 23.4 cm FL (Kulmiye et al, 2002) and Guam, 20.8 cm FL (Taylor and McIlwain, 2010). Estimated L_{50} from the Ryuku Islands, 19.5 cm FL (Ebisawa, 2006), was similar to the estimate from this study in Saipan Lagoon. In assessing regional differences in female A_{50}, the value of A_{50} for thumbprint emperor in Saipan (2.6 years) was intermediate between the estimate for Guam (Taylor and McIlwain, 2010) and the estimate for the Ryukyu Islands (Ebisawa and Ozawa 2009); see Table 2. Although L_T and A_T were not estimated for thumbprint emperor in Saipan, Taylor and McIlwain (2010) estimated L_T at 24.1 cm FL and A_T at 5.38 years for this species in Guam and Ebisawa (2006) estimated L_T at 25.5 cm FL for thumbprint emperor from the Ryukyu Islands.

Variability in estimates of L_{50} could be introduced by the differing approaches to evaluation of the maturity status of gonads (e.g., gonad histology vs. macroscopic evaluation), by differences in the criteria used to classify maturity status, and by differing analytical approaches to estimation of L_{50} (e.g., graphical [Kulmiye et al., 2002] vs. statistical [Ebisawa, 2006; Taylor and McIlwain, 2010]). In this study of thumbprint emperor in Saipan Lagoon, gonad maturation was evaluated macroscopically and was found to be similar to the macroscopic stages documented by Kulmiye et al. (2002). Taylor and McIlwain (2010) compared macroscopic and microscopic gonad evaluation, and they found that macroscopic staging was adequate 92% of the time in determining sex of thumbprint emperor and adequate 76% of the time in determining maturity status. The macroscopic approach provides approximate estimates of L_{50} that need to be calibrated with gonad histological examination to evaluate the accuracy of maturity status classifications, particularly for species that undergo sex change. Such studies would lend support toward the goal of refining macroscopic techniques for use in circumstances in which microscopic staging is not possible, as well as toward the goal of understanding the uncertainties associated with macroscopic staging among families of reef fishes.

Otolith annuli formation in thumbprint emperor from Saipan Lagoon during June through October (Fig. 3, A and B) was similar to that observed by Ebisawa and Ozawa (2009) in thumbprint emperor from the Ryuku Islands. The similarity in estimates of L_{50} and the timing of otolith annuli formation between thumbprint emperor from Saipan and the Ryukyu Islands seems to indicate some level of demographic correspondence in the western Pacific. However, differences in

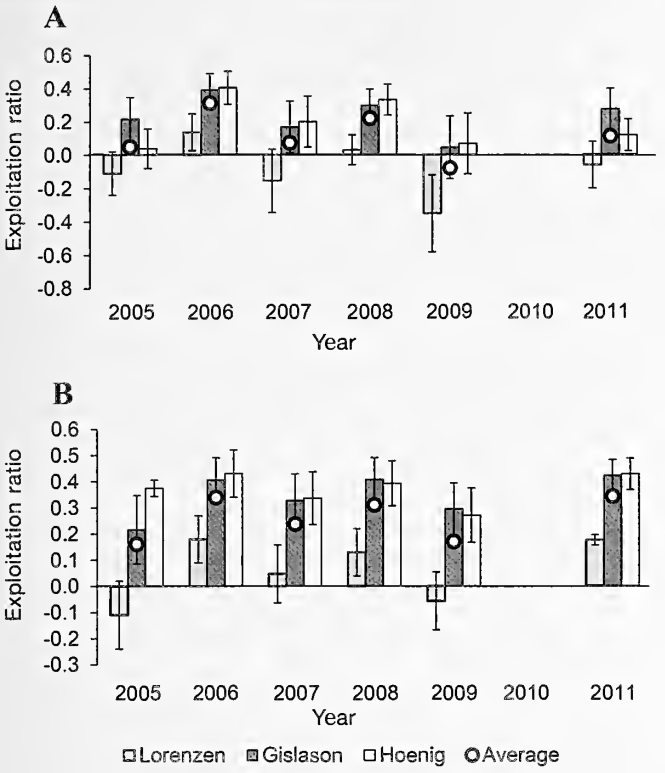

Figure 7

Estimated exploitation ratios for thumbprint emperor (*Lethrinus harak*) in Saipan Lagoon for the period 2005–2011, derived from estimates of natural mortality (M) generated from the Lorenzen and Gislason models and from the Hoenig equation, estimates of total mortality based on results from the use of the linearized catch curve and Chapman–Robson methods, and estimates of fishing mortality (F) derived as $F = Z - M$. Exploitation ratios estimated by using total mortality (**A**) with the linearized catch curve method and (**B**) with the Chapman–Robson method are shown. The bars depict the means of exploitation estimates, from 1000 simulations, with the use of the stated natural mortality model, and error bars indicate standard deviation. The circles are the averaged values from the listed exploitation rate estimates for that year.

other life history parameters (e.g., A_{50}) among thumbprint emperor from Saipan, Guam, and the Ryukyu Islands support the need for standardized methods to study reproductive maturity, as well as age and growth for coral reef species. Demographic plasticity for age and growth and in reproductive parameters pertaining to maturity, sex change, or both (Adams et al., 2000; Gust, 2004; Munday et al., 2006; Mariani et al., 2013) has been documented in both gonochoristic (Choat and Robertson, 2002; Robertson et al., 2005) and hermaphroditic (Gust et al., 2002; Donovan et al., 2012) species of coral reef fish.

In addition to altering sex ratios, fishing intensity can alter life history traits, such as length and age at maturity (Heino and Dieckmann, 2008; Sharpe and Hendry, 2009), the timing of sex transition in hermaphrodites (Hamilton et al., 2007; Götz et al., 2008), size and biomass spectra (Friedlander and DeMartini, 2002; Graham et al., 2005) and, in some cases, coral reef fish community structure through compensatory effects from decreased predation (Dulvy et al., 2004; Ruttenberg et al., 2011). The governments of Guam and Saipan have implemented marine protected areas as means to enhance fishery resources within and outside of those areas, and the CNMI has instituted a ban on spearfishing with scuba gear and has implemented restrictions on the use of gill, drag, and surround nets.

The NUE data for 2005–2011 show that thumbprint emperor composed about 35% of NUE landings in the CNMI (Tenorio[2]), but historical landings of thumbprint emperor in Saipan Lagoon by net fishing were not available for comparison. The commencement of the life history project in Saipan Lagoon shortly after implementation of net-use regulations, coupled with the 22.2% decline of the Saipan population observed in the CNMI census from 2000 to 2010, suggests that a life history study of thumbprint emperor should be repeated for comparative purposes. Additionally, it would be useful to obtain life history data from an unexploited or lightly exploited population of thumbprint emperor in the CNMI, although acquisition of such data would be logistically challenging within the populated islands of the CNMI where unfished reef flats or embayments are limited. Such a study would provide estimates of Z directly from natural mortality models.

The estimation of k with the VBGF was important in the estimation of M in one of the 3 M models used in this study and, therefore, in the subsequent estimation of E. Constraining the VBGF equation to the settlement size for this species produced a high estimate of k and, consequently, a lower estimate of L_∞ in comparison with the estimates from the unconstrained equation (Table 4). Constraining the VBGF model to an estimated settlement size resulted in larger estimates of M and in estimates of E that were subsequently negative, indicating that the current levels of fishing mortality were not having a detrimental influence on the thumbprint emperor. In anchoring the fitted VBGF models, in the absence of smaller specimens in the sample, a lack of variability was assumed in the length–age relationship below the smallest fish aged. As shown in this study, constriction had the effect of altering the curve from the fitted VBGF model. Obtaining samples from individuals after settlement, as well

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[2] Tenorio, M. 2012. Personal commun. Division of Fish and Wildlife, Department of Land and Natural Resources, Commonwealth of the Northern Mariana Islands, Saipan, MP 96950.

as more samples from fish in larger size classes to enable more accurate estimation of variability of length at age for these population segments, should remain a goal of life history research, although obtaining fish of those sizes remains a challenging task.

The ratio of estimated annual landings to fishery-independent biomass estimates from 2005 through 2011 resulted in low values of F and subsequently to high estimates of SPR. Also, these estimates of F form what can be considered a "rough" baseline, and the high SPR values tend to reflect the nature of fishing for this species. The thumbprint emperor was (and is) not a primary target of the nighttime commercial free-dive spear fishery in Saipan, and this species appeared to be primarily harvested by hook and line. Data presented here indicate that larger thumbprint emperors were harvested during fishing activities under net-use exemption—activities that were employed frequently in the past and that have declined since implementation of net-use regulations. One of the purposes of the Magnuson-Stevens Fishery Conservation and Management Act is to achieve and maintain the optimum yield from each fishery through the implementation of fishery management plans, in which the optimum yield is prescribed under certain circumstances on the basis of maximum sustainable yield (MSY). Because MSY is often difficult to estimate for data-moderate and data-poor fisheries, U.S. Fishery Management Councils have used SPR as a proxy for MSY-based precautionary control rules (Restrepo et al., 1998). The exploration of new approaches in the use of SPR for evaluating the status of data-poor fisheries has begun (Hordyk et al., 2015).

Estimates of E were variable because they were derived by sampling within the range of annually derived estimates of Z from 2 common estimation methods (Table 7): by resampling within the M model parameter uncertainty, and through the use of derived F estimates. Results indicate that thumbprint emperor from Saipan Lagoon, with estimated values of average annual E falling below 0.4, were likely not fully exploited. Still, the use of the more conservative value of 0.2 for E, as suggested by Pauly (1984), for small fish at lower trophic levels with high recruitment variability, would have resulted in values above 0.2 for 4 of the 6 estimates of E determined with the CR method to estimate Z.

In simulations where the CR method and a variety of approaches to CC analysis were evaluated in the presence of stochastic error, Dunn et al. (2002) found that the CR estimator generally was more precise in estimating true Z values, although any advantage of the CR method over the CC method decreased with increasing Z estimates and stochastic error. The values of Z generated in this study with the CR model were greater than those derived with the the CC approach, and those higher values of Z resulted in larger estimates of F and subsequently higher estimates of E. The use of 3 equations for M and 2 approaches for Z resulted in a range of E values, with uncertainty, for any given year as opposed to the singular values generally produced when E is estimated. All M models come with caveats (Kenchington, 2014), and modifications and improvements in M estimation continue (Gislason et al., 2010). The use of a few M models that resulted in a range of E estimates provides ample uncertainty that may be useful in ascertaining a relative understanding of species status, given a data-poor scenario as described here.

One aspect of the results of this study that should be highlighted is the use of size limits as a fish mortality control for protogynous species in coral reef fisheries. As noted, larger thumbprint emperor are captured in nets than those captured with the fishing methods used in the ICS. If a size limit for thumbprint emperor coincident with L_{50} (e.g., 20 cm FL) is implemented, about 65% of the fish landed in the ICS and 17% of the fish landed as NUE would be prohibited from harvest. Conversely, more than 58% of males and more than 43% of females would be harvested, including those females with the greatest fecundity and greatest importance to population recruitment.

Additionally, with respect to estimated ages, lengths, and weights of thumbprint emperor, size limitations would force increased fishing mortality on older and larger size classes of females that have lower estimated rates of M and greater reproductive potential and would eliminate fishing mortality on classes with the highest rates of M and, therefore, the best ability to absorb fishing mortality in the population. Heppell et al. (2006) concluded that catch and effort controls with spatial closures should be enacted to enhance the sustainability of hermaphroditic species, and Fenberg and Roy (2008) noted the potential negative biological effects of size-selective harvesting. The existence of a large marine protected area in Saipan Lagoon, the MMCA, and restrictions on the use of nets appear to be beneficial for thumbprint emperor and may be for other coral reef fish species in Saipan Lagoon.

Standardized methods for estimating life history parameters should be implemented for the macroscopic staging of gonads and be calibrated against histological staging (Brown-Peterson et al., 2011) because dervied life history parameters directly influence estimates of L_{50} and L_{T}, as well as age estimation from sectioned otoliths (Marriott et al., 2010). A standardized approach for estimation of life history parameters will make jurisdictional and regional comparisons practical. In the case of thumbprint emperor from Saipan Lagoon, deficiencies in sample sizes existed for individuals less than 1 year old and for those fish beyond age 6, in comparison with other age classes. The recent implementation of a BSP, as well as similar programs in other U.S. jurisdictions of the central and western Pacific, provides a useful platform for representative sampling of the fisheries and for the standardization of life history parameters.

The thumbprint emperor in Saipan Lagoon, unlike many other coral reef species in the CNMI, is a data-poor species for which relatively considerable data were available or estimable from a number of sources. More species-specific or species-complex data will be required before formal stock assessments can be engaged for coral reef fishes in the CNMI.

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Swimming depth of dolphinfish (*Coryphaena hippurus*) associated and unassociated with fish aggregating devices

Nicholas M. Whitney (contact author)[1]
Marc Taquet[2]
Richard W. Brill[3]
Charlotte Girard[4]
Gail D. Schwieterman[1]
Laurent Dagorn[4]
Kim N. Holland[5]

Email address for contact author: nwhitney@mote.org

[1] Behavioral Ecology and Physiology Program
Mote Marine Laboratory
1600 Ken Thompson Parkway
Sarasota, Florida 34236

[2] Unités Mixtes de Recherché (UMR)
Ecosystèmes Insulaires Océaniens (EIO)
Institut de Recherche pour le Développement
BP 529
98713 Papeete, Tahiti, Polynésie française

[3] Pacific Islands Fisheries Science Center
National Marine Fisheries Service, NOAA
2570 Dole Street
Honolulu, Hawaii 96822-2396

[4] Unités Mixtes de Recherché (UMR) Marine
Biodiversity, Exploitation, and
Conservation (MARBEC)
Institut de Recherche pour le Développement
Avenue Jean Monnet CS 30171
34203 Sète Cedex, France

[5] Hawaii Institute of Marine Biology
University of Hawaii at Manoa
P.O. Box 1346
Kaneohe, Hawaii 96744

Abstract—Dolphinfish (*Coryphaena hippurus*), large pelagic predators and important fishery targets, frequently associate with floating debris or manmade fish aggregating devices (FADs). We tagged 8 dolphinfish with pressure-sensitive ultrasonic transmitters and actively tracked individuals continuously for up to 40 h to elucidate the vertical movement patterns and differences between FAD-associated (FAD-A) and FAD-unassociated (FAD-U) fish. Four additional fish were equipped with acoustic transmitters and passively monitored for several days with receivers attached to FADs. When not associated with FADs, dolphinfish used the upper 75–100 m of the water column during the day and made descents up to 160 m during the night. In contrast, FAD-A fish generally stayed within the upper 10 m of the water column and tended to make deeper excursions during the day rather than at night. Water temperature data from expendable bathythermographs deployed during active tracking showed that fish only descended to depths where temperatures were ≤3°C cooler than the uniform-temperature surface layer. The use of vertical behavior to determine whether a dolphinfish is associated or not with a floating object opens the possibility for new, large-scale research aimed at investigating the role of floating objects in the ecosystem inhabited by this species and at assessing the impacts of FADs on its ecology.

Dolphinfish (*Coryphaena hippurus*) are large pelagic fish that are common globally in tropical and warm temperate seas (Palko et al., 1982). They have diverse diets composed of floating debris-associated organisms, such as portunids (crabs) and epipelagic cephalopods (Olson and Galván-Magaña, 2002; Rudershausen et al., 2010), as well as neritic and demersal fish (Tripp-Valdez et al., 2010). The fast growth rate (Schwenke and Buckle, 2008; Furukawa et al., 2012), early sexual maturity (less than one year; Trippel, 1995; Furukawa et al., 2012), and high food value (Beardsley, 1967; Rodríguez-Ferrer et al., 2004) of this species makes it an increasingly important target of commercial, sport, and artisanal fisheries (Rodríguez-Ferrer et al., 2004). The tendency of dolphinfish to aggregate around floating objects and flora, such as sargassum (Oxenford, 1999; Rooker et al., 2006; Casazza and Ross, 2008; Farrell et al., 2014; Merten et al., 2014a), logs (Baughman, 1941), and manmade fish aggregating devices (FADs; Rose and Hassler, 1974; Massutí et al., 1998, Oxenford and Hunte, 1999; Dempster, 2004; Dagorn et al., 2007; Taquet et al., 2007), facilitates their exploitation by both commercial and recreational fisheries throughout the world.

Recent estimates put annual catch of dolphinfish at more than 102,986 metric tons (t) (FAO, 2013), which excludes unreported bycatch (common in longline tuna fisheries) and underreported artisanal landings. A recent study estimated that 5382 t of illegal or unreported dolphinfish were imported from Ecuador to the United States in 2011 alone (Pramod et al., 2014). The high exploitation rate and commercial importance of this species justify a better foundational knowledge of the life history and behavioral

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Table 1

Characteristics of the tracking or monitoring of dolphinfish, including which method was used: active (boat-based) or passive (receiver attached to a fish aggregating device [FAD]). Behavior categories are day (D) or night (N) and either FAD-unassociated (U) or FAD-associated (A). FAD types (drifting or anchored) are listed only for animals that were actually associated with a FAD for a part of the time they were tracked.

Method	Fish	Fork length (cm)	Tracking start date	Duration of tracking (h)	Behavior category	FAD type	Location
Active	1	125	4-Mar-86	40.1	DU/NU		Hawaii
	2	108	3-Feb-87	6.0	DU		Hawaii
	3	103	7-Mar-87	22.5	DU/NU		Hawaii
	4	104	24-Mar-87	27.2	DU/NA	drifting	Hawaii
	5	116	21-Jul-03	8.0	DA	anchored	Réunion Is.
	6	124	6-Aug-03	8.0	DU		Réunion Is.
	7	58	12-Apr-05	4.3	DU[1]		Seychelles
	8	69	21-Apr-05	7.5	DU[1]		Seychelles
Passive	9	110	13-Dec-01	38.4	DA/NA	drifting	S. Indian
	10	77	12-Oct-03	81.6	DA/NA	drifting	S. Indian
	11	86	8-Feb-04	87.8	DA/NA	drifting	Seychelles
	12	102	16-Oct-04	84.5	DA/NA	drifting	Seychelles

[1] Fish tracked in shallow areas (depths of 50–60 m) were not included in statistical analyses.

patterns of dolphinfish in order to ensure development of appropriate measures for fishery management and resource conservation.

Previous studies of the movements of dolphinfish have focused either on broadscale migrations or on movements in highly specific contexts. For example, Merten et al. (2014a) used mark-recapture data to describe broadscale migrations along the East Coast of the United States, from the Florida Keys to Long Island, New York. Merten et al. (2014b) used plastic dart tags and pop-off satellite tags to determine regional patterns of horizontal movement, and Norton (1999) described a global poleward shift of dolphinfish that corresponded with an increase in ocean temperatures.

Individual dolphinfish have been shown to home to a specific moored FAD (Girard et al., 2007) and to remain associated with an anchored FAD for a mean duration of 3.98 days (Dagorn et al., 2007) and with drifting FADs for a mean duration of 6.25 days (Taquet et al., 2007). Dolphinfish in the western Atlantic generally maintain swimming depths in the uniform-temperature surface layer and descend beyond the surface layer (0–10 m) longer at night than during the day, possibly to forage (Merten et al., 2014c). FAD-U fish in the northern East China Sea often remain above the thermocline (Furukawa et al., 2011), although Merten et al. (2014c) found that the thermocline was not a barrier to vertical movements for dolphinfish in the Atlantic. In general, dolphinfish have the shallowest vertical distribution of other sympatric, mid-trophic level predators, such as the striped marlin (*Kajikia audax*; Brill et al., 1993) and sailfish (*Istiophorus platypteru;* Hoolihan, 2005).

To date, the movement patterns between FAD-A and FAD-U dolphinfish have not been compared. We analyzed the vertical swimming behavior of both FAD-A and FAD-U dolphinfish, tracked by using active and passive telemetry, with particular regard to diurnal patterns. Understanding the vertical movement patterns of this commercially important species is increasingly essential for sustainable fisheries management, especially in the face of climate change. The use of a comprehensive ethology will enable managers to predict changes in distribution of dolphinfish as seawaters warm and to implement bycatch reduction regulations, such as a mandate that would limit the depths at which gear can be set to depths outside the range preferred by dolphinfish.

Materials and methods

Study sites

A total of 12 dolphinfish were tagged and released at locations 1) in the central Pacific (near the main Hawaiian Islands, n=4) in 1986 and 1987, 2) in the southwestern Indian Ocean (near Réunion Island; n=4) in 2005, and 3) in the Western Indian Ocean (near Seychelles, n=4) in 2003 (Table 1). In the central Pacific, active tracking of 4 fish was conducted off the northeast (windward) coast of Oahu near an array of FADs that consisted of surface buoys anchored at depths from 670 to 3660 m. The tracks of 4 more fish were recorded with active telemetry around FADs made of about 10 surface buoys, a metal cable, and strap bands located on the cable at a depth of ~20 m, anchored with concrete blocks on the Seychelles Plateau in relatively shallow (50–60 m) water or around drifting FADs of similar construction. In the open sea off Réunion Island, 2 fish were passively monitored in depths of 2000–5000 m, one fish in 2001 and another in 2003, by using drifting FADs, made of bamboo rafts with trailing netting panels, that were deployed specifically to study pelagic fish aggregations (Taquet, 2004). Off the Seychelles Plateau in 2004, 2 more fish were passively monitored near FADs made of surface buoys anchored at depths of 500–1500 m (Girard et al., 2007). Fish from all locations were caught by trolling and pole techniques and were brought aboard the vessel for tagging.

Active telemetry

A detailed account of the tracking methods employed in Hawaii are described in Holland et al. (1985), and those used off Réunion Island and in the Seychelles are detailed in Girard et al. (2007). In Hawaii, pressure-sensitive V16 ultrasonic transmitters (16.0 mm in diameter, 27.7 g in air and 11.7 g in seawater; Vemco Inc.[1], Bedford, Nova Scotia, Canada) were attached externally to a fish by passing 2 nylon cable ties through the dorsal or ventral pterygiophores and trunk musculature adjacent to the second dorsal fin (we switched to the latter procedure after one fish was observed swimming at the surface with its dorsal tag carried above the surface of the water). The transmitters were equipped with pressure sensors that modulated the rate of pulses transmitted in response to changes in water pressure (depth). Therefore, vertical movements of the fish were determined by measuring the time between signal pulses (e.g., Holland et al., 1990b). Individuals were held out of the water for approximately 1 min during the tagging procedure. For tracking, we used a 12V DC amplifier and receivers (CR-40, Vemco Inc.) and a directional hydrophone (Vemco Inc.), which was mounted to a pole extending about 1.5 m below the surface. Attempts were made to keep the tracking vessel 200 m away from the tagged individual to maintain maximum signal amplitude and not disturb natural behavior.

The fish actively tracked off Réunion Island were caught at FADs and held in a container of flowing seawater, and the fish caught in the Seychelles were placed in a padded cradle for a few minutes and provided with flow of water to oxygenate their gills. Fish tracked in the Seychelles and off Réunion Island were moved distances of 70–1720 m from the FAD during tagging, as part of a study on dolphinfish homing abilities around FADs (Girard et al., 2007). For fish tracked in these 2 areas, V16P-4H transmitters (16.0 mm in diameter, 25 g in air and 11 g in seawater, with a random delay between 40 and 120 s, depth resolutions from 0.3 to 1.5 m; Vemco Inc.) were attached by means of a hook that passed through the pterygiophores of the anal fin. Four hydrophones were towed below the tracking vessel on a V-fin depressor, and the signal emitted by a tag was processed with a VR28 receiver system (Vemco Inc.) connected to a laptop computer to record the data that were automatically stored in the receiver. Whenever possible, a distance of at least 100 m was maintained between the tagged fish and the boat to reduce the effect of the tracking vessel on fish behavior.

During active tracking, data for seawater temperature were acquired through periodic deployments of an expendable bathythermograph system (Lockheed Martin Sippican, Marion, MA.). We defined association with a FAD as a fish remaining within 360–655 m of a FAD or floating debris for at least 30 min, as determined bf direct observation.

Passive telemetry

Fish were passively monitored with V13P-1H acoustic transmitters that would transmit on a random delay of 40–120 s (fish 9, 11, and 12; 13 mm in diameter, 11 g in air and 6 g in seawater; Vemco Inc.). Tags were inserted into the peritoneal cavity through an incision of 1–2 cm made to the side of the ventral centerline of the fish and 2–3 cm anterior to the cloaca. The wound was closed by using sterile needles and suture material; the entire procedure was completed in less than 3 min. Fish 10 was equipped with a V16P-4H acoustic transmitter on a delay of 10–30 s (18 mm in diameter, 36 g in air and 16 g in seawater; Vemco Inc.) attached above their anal fin with a hook. To monitor tagged fish around FADs, VR2 receivers (Vemco Inc.) were suspended 5 m below a buoy that was tied to the FAD. Following Girard et al. (2007), we defined association of passively monitored fish as fish remaining within the detection range of the transmitters on the FAD (distances of 360 and 655m for V13P-4H and V16P-4H receivers, respectively). After a few days of observation, VR2 receivers were removed from the FADs so that data could be downloaded.

Analyses

Pooled time-at-depth histograms were constructed with 5- or 10-m bins as described by Holland et al. (1990b). These data subsequently were expressed as a fraction of the total time each fish was followed, and the fractional data bins were averaged across all fish. Fish 7 and 8 were excluded from statistical analyses because their vertical movements were limited by a bottom depth of 50–60 m. A linear mixed-effects model was used to examine the effects of time of day and FAD association on swimming depth. Our model included fixed effects for tagging method, FAD association (associated or unassociated), and time of day (day or night). Additionally, individual fish and location of study site were random effects because it was not our aim to study intraspecific variability or differences that resulted from study site. Our model did not include interaction terms. Models were run with the lmer function in the lme4 package (Bates et al., 2014) in R, vers. 2.15.3 (R Core Team, 2013). The candidate model was selected by using the Akaike information criterion, with factor significance determined by pairwise analysis of variance (ANOVA) tests (α=0.05). *P*-values for pairwise comparisons were computed by using

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[1] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

Markov chain Monte Carlo methods from the LMERConvenienceFunction package (Tremblay and Ransijn, 2013) in R.

Results

A total of 8 dolphinfish (mean fork length:; 100.9 cm [standard deviation (SD) 24.7]) were actively tracked, and 4 fish (mean fork length: 93.8 cm [SD 15.0]) were monitored with passive acoustic receivers (Table 1). The duration of active tracking of individual fish (n=8) ranged from 4.3 to 40.1 h (mean duration of tracking: 15.5 h). Actively tracked fish were unassociated with FADs for the majority of the time that they were tracked, with the exception of fish 4 and 5 (Table 1). Passively monitored fish (n=4), and therefore FAD-A fish, were monitored for periods of 38.4–87.8 h (mean duration of tracking: 73.1 h).

Four dolphinfish (fish 5, 6, 9, and 11) immediately after release made quick descents that lasted less than 1 h before they resumed shallower swimming behavior for the remainder of the time that they were tracked. No other response to tagging was observed.

Our analysis revealed that study location and monitoring method did not have a significant effect on swimming depth (variance approached 0 for both effects (P>0.05), and these effects were removed from the model. We did not find significant interaction between time of day (day or night) and FAD association (associated or unassociated) (ANOVA: P>0.05); therefore, we present values from a reduced model in which depth was dependent on the fixed effects of time of day and FAD association, with individual (fish) as a random effect. We found mean daytime depth of FAD-A fish to be 1.8 m (SD 8.1), mean nighttime depth of FAD-A fish to be 0.8 m (SD 1.7), mean daytime depth of FAD-U fish to be 49.5 m (SD 0.3), and mean nighttime depth of FAD-U fish to be 28.3 m (SD 2.9).

During the day when not associated with FADs or floating debris, dolphinfish remained within the uniform-temperature surface layer (above the thermocline) and made only limited vertical excursions to depths of 75–100 m. At night, FAD-U fish swam at depths between 30 and 160 m and ventured into cooler water. However, fish reached depths that were no more than 3°C cooler than the uniform-temperature surface layer (Fig. 1). Although FAD-U fish spent an average of 29.4% of their time in the upper 10 m of the water column during both day and night, there was another peak of time-at-depth at depths between 30 and 40 m, their overall depth distribution during nighttime was broader and deeper than their daytime distribution (P<0.001; Fig. 2).

When associated with FADs or floating debris, dolphinfish spent an average of 94.8% of their time in the

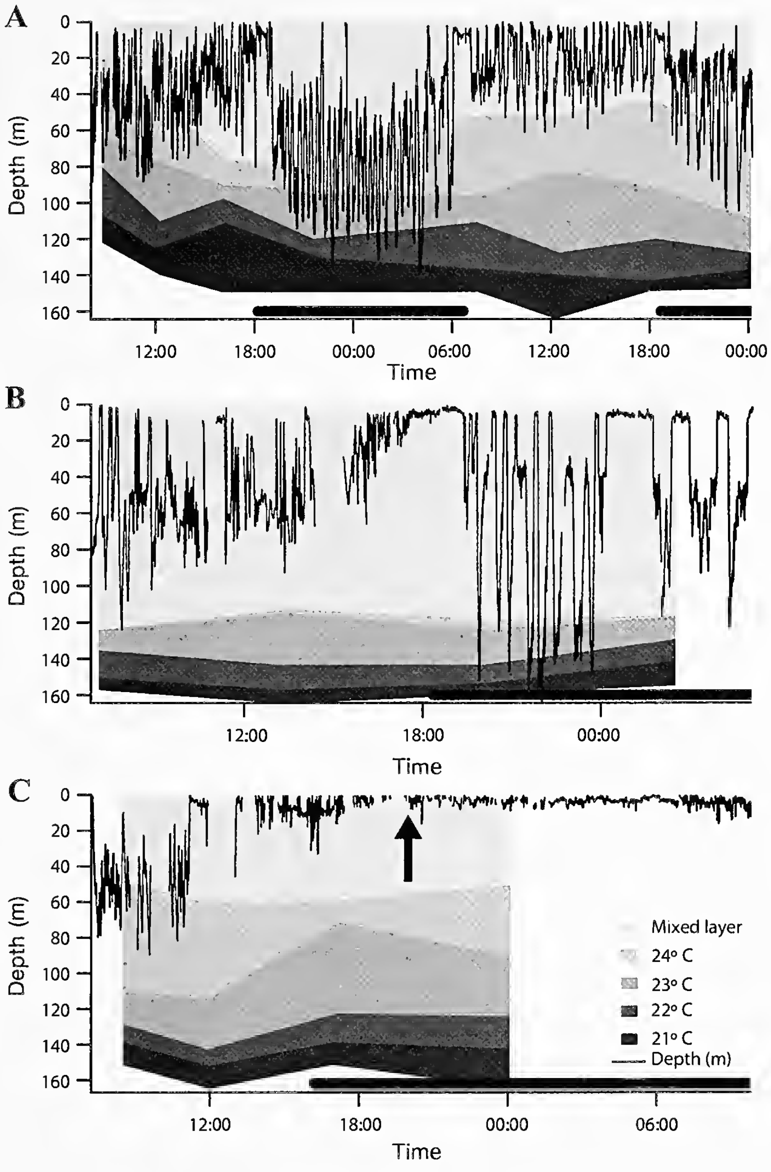

Figure 1

Swimming depth of dolphinfish (*Coryphaena hippurus*) actively tracked off Oahu, Hawaii, in March 1986 and March 1997: (**A**) fish 1 during tracking for a period of 40.1 h, (**B**) fish 3 during tracking for a 22.5-h period, and (**C**) fish 4 during tracking for a period of 27.2 h. Shades of gray distinguish temperature isotherms. Bold black lines above the x-axes indicate nighttime. The arrow in graph C indicates the time at which fish 4 became associated with drifting debris and numerous other (untagged) dolphinfish. The isotherms are based on temperature recordings from the active acoustic tracking and extend to the last recorded temperature reading.

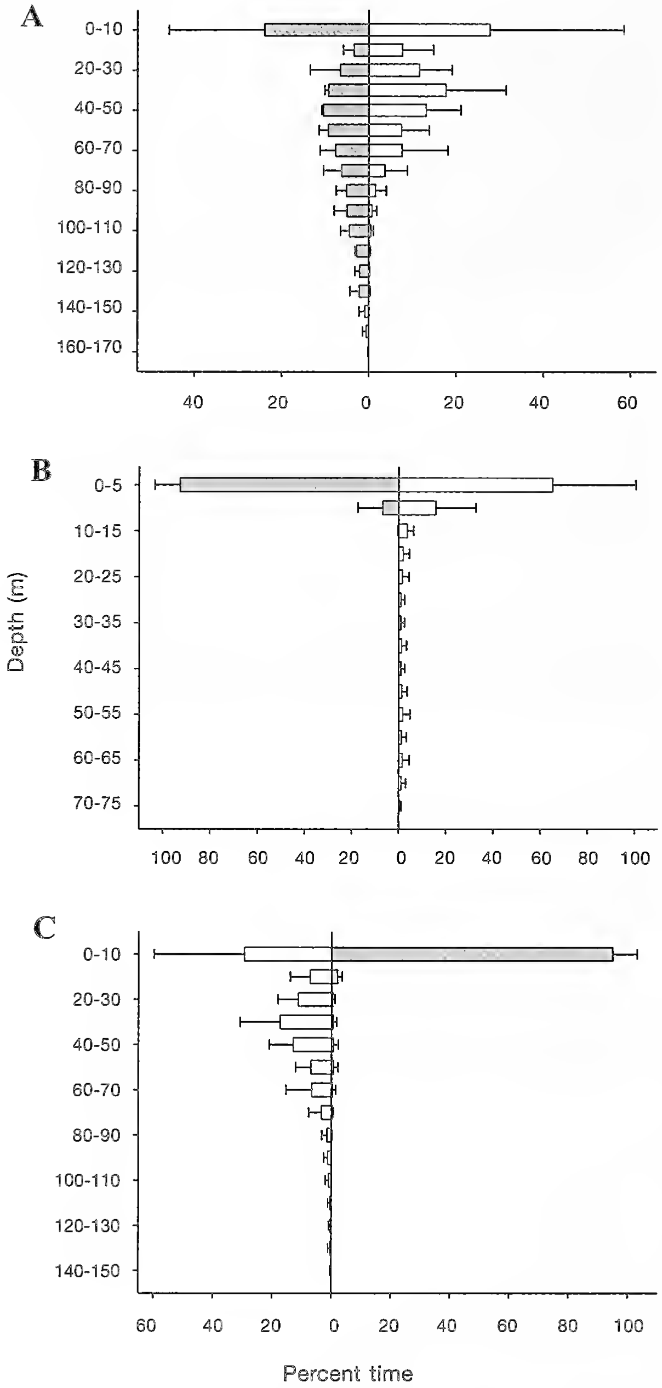

Figure 2

Percent time at depth (A) for all dolphinfish (*Coryphaena hippurus*) unassociated with a fish aggregating device (FAD) during day (white bars: n=7) and night (gray bars: n=2), (B) for FAD-associated fish during day (white bars: n=5) and night (gray bars: n=5), and (C) for fish unassociated with FADs (white bars: n=7) and fish associated with FADs (gray bars: n=6). Sample sizes differ because not all individuals were actively tracked or monitored during full 24-h cycles (see Table 1.). Dolphinfish were actively tracked in Hawaii, Reunion Island, and the Seychelles Plateau between 1986 and 2005 and were passively monitored in the Seychelles Plateau and the Indian Ocean between 2001 and 2004.

upper 10 m of the water column. During the night, fish typically remained within 5 m of the surface, venturing into depths that were only slightly deeper during the day (Fig 3); this difference was not significant. Only 1 fish (fish 11) exceeded a depth of 30 m while associated with a FAD (Fig 3). Fish 4 became associated with an abandoned net and other drifting debris while being tracked (Fig. 1). It swam at depths between 25 and 75 m for the first 4 h after it was tagged and while it was unassociated with a FAD or debris. After becoming associated with floating debris, this fish remained within the upper 20 m of the water column for the final 17 h that it was tracked, and it did not have the deeper nighttime swimming behavior observed in FAD-U fish. These results from active telemetry were confirmed by direct observations of the tagged fish near the surface on several occasions.

By the end of the active tracking of fish 4, approximately 300 dolphinfish, a large school of small yellowfin tuna (*Thunnus albacares*), and at least 1 marlin (*Istiophoridae)* were observed to also be associated with debris. No fish were observed to leave a FAD once it became associated with one, and passive monitoring ended when tracked fish were recaptured or the receivers were retrieved. At the end of its passive monitoring, fish 11 showed a rapid descent to more than 200 m and then exceeded the range of the receiver (not shown in Fig. 3), likely as a result of shedding its tag or possibly mortality.

Actively tracked fish 1 and 3, which were unassociated with floating objects during crepuscular periods, interrupted their vertical movement patterns and remained within the upper 10 m of the water column for several minutes during the dawn or dusk periods (n=4). These shallow periods lasted from 39 to 169 min.

Discussion

We present a first examination of the effect of FAD association on diel movement patterns of dolphinfish. Overall, individuals remained in the uniform-temperature surface layer in congruence with results of other studies on tracking dolphinfish (Furukawa et al., 2011, 2014; Merten et al., 2014c), as well as with results from studies on tracking wahoo (*Acanthocybium solandri*; Sepulveda et al., 2011) and sailfish (Chiang et al., 2011). However, we found differences in the vertical movement patterns between FAD-A fish and FAD-U fish. The distributions of the former were extremely shallow, with deeper excursions observed during the day. In contrast, the latter ranged throughout the upper 100 m of the water column and made deeper descents at night. The differences in behavior of FAD-A and FAD-U fish can be noted particularly in the behavior of fish 4, which became associated partway through the period in which it was actively tracked (Fig. 1). These results are similar to those from studies of bigeye tuna (*Thunnus obesus*): deeper diving occurred when fish were not associated with surface objects and shallow distribution was generally uniform during day and night when fish were associated with surface objects (Holland et al., 1990b; Musyl et al., 2003).

The difference in depth distribution between FAD-A and FAD-U dolphinfish may be due to differences in feeding strategy. Although Taquet (2004) found that only 27% of the diet of FAD-A dolphinfish comes from FAD-associated organisms, dolphinfish are primarily visual predators (Massutí et al., 1998) and would be expected to forage primarily during the daytime. For FAD-A fish, deeper daytime dives may represent attempts to forage for prey species distributed throughout the uniform-temperature surface layer. If this is the case, the motivation to make deeper descents would be lessened at night because reduced light levels make foraging difficult.

The broad patterns of vertical movement that we observed in FAD-U dolphinfish are similar to the behaviors observed in other large pelagic fishes, such as tropical tunas and marlin, unassociated with a FAD or debris (e.g., Holland et al., 1990a, 1990b; Brill et al., 1993, Musyl et al., 2003). Such movement patterns may allow fish to explore the water column for prey. During the day, FAD-U dolphinfish may target prey that occupy the uniform-temperature surface layer—prey species that likely are not part of the deep scattering layer. However, at night, dolphinfish may forage on prey of the deep scattering layer that rise to occupy the uniform-temperature surface layer. Although we did not observe the dissociation of any fish from a FAD, Taquet et al. (2007) suggested the need to forage may prompt dolphinfish to leave FADs or other floating objects.

The rapid, deep descents of 4 fish immediately after release were likely a response to tagging because these fish quickly resumed vertical movement patterns that were maintained for extended periods and, therefore, presumably represent natural behavior (Girard et al., 2007). The remaining 8 individuals showed no such initial response. Hoolihan et al. (2011) suggested that stress from capture and handling affects the behavior of large pelagic fishes for periods that span from days to weeks after release. However, a comparison of vertical movement behaviors from our study with those from other acoustic telemetry studies (with tracking conducted for ~24-48 h after release) and with those from studies that employ im-

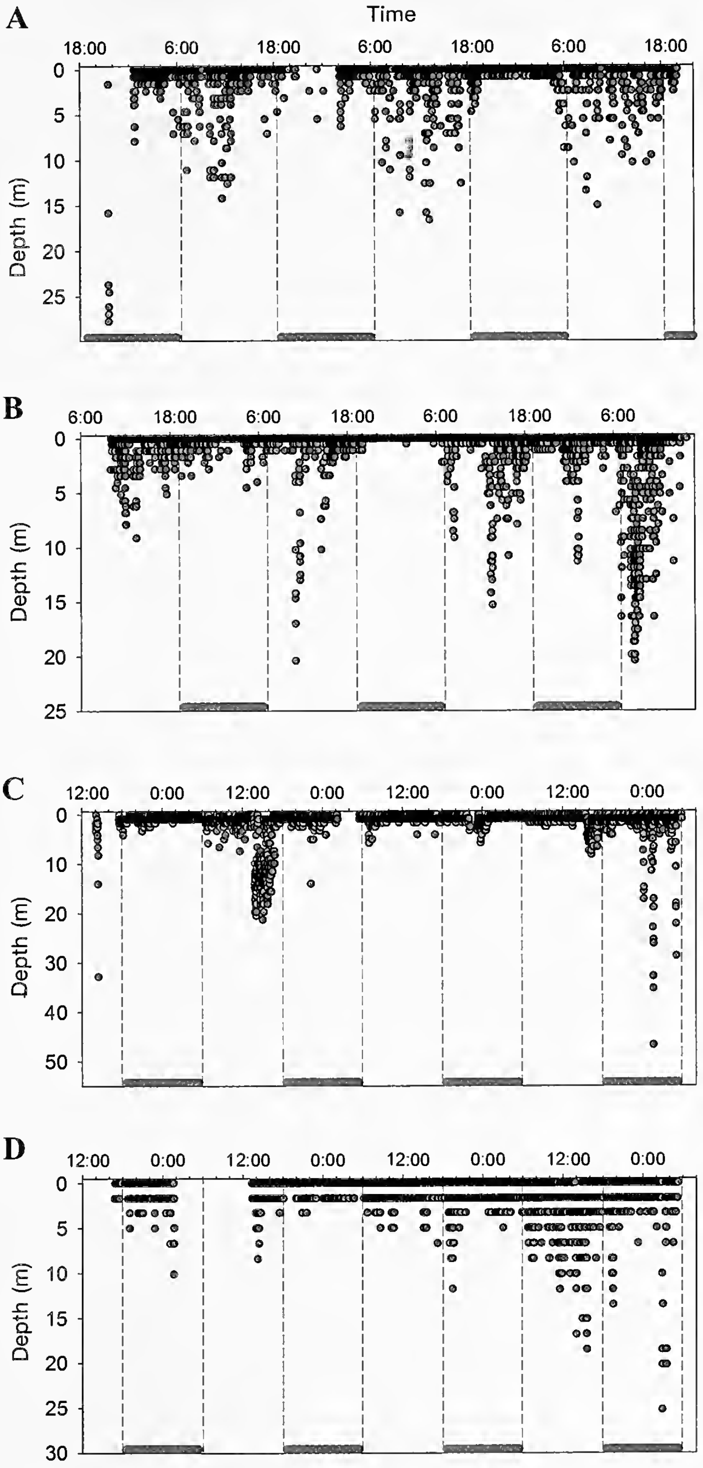

Figure 3

Swimming depths of 4 dolphinfish (*Coryphaena hippurus*) associated with a fish aggregating device and monitored passively with V13P-1H or V16-4H transmitters over periods of several days in the open sea in the southwestern Indian Ocean or near the Seychelles Plateau. (**A–C**) Fish 9, 11, and 12 were tracked in 2001 and 2004. (**D**) Fish 10 was tracked in 2003. Individual dots represent detections by the acoustic receiver. Bold gray lines above the *x*-axes indicate nighttime.

planted archival tags and pop-up satellite archival tags (with tags often containing months long data records) showed remarkable similarity and indicates that 1) recovery after release for pelagic teleosts, such as tunas and billfishes, requires only 2–6 h (Holland et al., 1990a, 1990b; Brill et al., 1993) and 2) differences in vertical movement patterns can be explained largely by differences in oceanographic conditions and prey distributions (e.g., Dewar et al., 2011; Schaefer et al., 2007, 2011).

We observed 2 fish that interrupted their regular patterns of vertical movement during crepuscular periods to remain instead in the upper 10 m of the water column. These shallow periods may enhance the ability of fish to use sunrise and sunset as zeitgebers to entrain a circadian clock (Aschoff, 1965; Takahashi and Zatz, 1982; Neilson and Perry, 1990) or may represent optimal times for feeding on shallow-water prey, such as flying fish, a main portion of the diet of dolphinfish (Olson and Galván-Magaña, 2002). Merten et al. (2014c) also posited that dawn and dusk were periods of transition that represent the end and start, respectively, to more extensive vertical movements. Bluefin tuna (*Thunnus thynnus*) have been observed to occasionally interrupt their regular day or night dive behavior at crepuscular periods (Gunn and Block, 2001). However, rather than remaining at shallow depths, these fish make “spike dives” that may have a navigational role through the detection of polarized light patterns or magnetic anomalies (Willis et al., 2009).

The results presented here should be examined further in future studies. Because of resource constraints, our sample size is relatively small and does not encompass different life stages. Although location was determined to be an insignificant factor in our model, there may be differences between populations that were undetectable here. To help determine how vulnerable dolphinfish are to fishing pressure, more work is needed to investigate the original drivers behind FAD association if they are not a source of prey and to determine sexual or life-stage preference for FAD associations. Further studies may incorporate the use of accelerometry (e.g., Furukawa et al., 2011) or further vertical movement profiles from time series data (e.g., Merten et al., 2014c) in order to further elucidate proximate drivers of the difference in behavior between FAD-A and FAD-U fish that was observed in our study.

Our results show that, as with results reported for bigeye tuna (Holland et al., 1990b, Schaefer and Fuller, 2005, 2010), it may be possible to use information on vertical behavior to assess when an individual dolphinfish is associated with a floating object. Combined with the long-term data-recording capabilities of archival tag studies (Gunn and Block, 2001), information on the impact of FAD association on swimming depth could help elucidate the dependence of dolphinfish on floating objects. This information is key in assessing the effects of fisheries on populations of dolphinfish given the increasing use of FADs by the purse-seine fisheries that target tunas and given the substantial bycatch of dolphinfish (Dagorn et al., 2013, Fonteneau et al., 2013, Leroy et al., 2013). The findings presented here regarding the vertical distributions of FAD-A and FAD-U dolphinfish may help to develop sustainable fishing techniques and management regulations of FAD-based fisheries.

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Abstract—The Atlantic silverside (*Menidia menidia*) is extremely abundant in estuaries in eastern North America, is a significant component of food webs, and is the subject of many laboratory studies; however, the ecology of the larvae of this species in estuaries is poorly known. Using 4 simple collecting gears, we sampled Atlantic silverside larvae in 2 estuaries in Rhode Island that differ in anthropogenic inputs, Pettaquamscutt River estuary and Point Judith Pond, to assess the distribution and abundance of larvae of this species. These larvae occur predominantly in waters less than 1 m deep and are patchily distributed. Larvae collected at depths of 0.2–0.6 m were significantly shorter than those collected at depths of 0.6–0.8 m—a difference in mean total length of ~2 mm. We also compared diets and growth rates of larvae in the 2 estuaries, using gut content analysis and otolith analysis, respectively. Copepod eggs made up 76% of the diet of larval Atlantic silverside in Pettaquamscutt River, whereas copepod nauplii made up 73% of their diet in Point Judith Pond. Growth rates of the larvae did not differ between estuaries.

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First assessment of the field ecology of larval Atlantic silverside (*Menidia menidia*)

Miranda Lopez[1]
Gavino Puggioni[2]
David A. Bengtson (contact author)[1]

Email address for contact author: dbengtson@uri.edu

[1] Department of Fisheries, Animal, and Veterinary Sciences
University of Rhode Island
113 Woodward Hall
9 East Alumni Avenue
Kingston, Rhode Island 02881

[2] Department of Computer Science and Statistics
University of Rhode Island
246 Tyler Hall
9 Greenhouse Road, Suite 2
Kingston, Rhode Island 02881

The Atlantic silverside (*Menidia menidia*) is one of the most abundant estuarine species along the east coast of North America from Nova Scotia, Canada, to Florida (Middaugh et al., 1981). Although this species has no commercial fishery value, it serves as a forage species for commercially important fish, such as bluefish (*Pomatomus saltatrix*), striped bass (*Morone saxatilis*), and Atlantic mackerel (*Scomber scombrus*) (Fay et al., 1983). It is perhaps best known scientifically because of the important laboratory experiments of Conover and Kynard (1981), whose work demonstrated that sex was determined by environmental factors, and of Conover and Munch (2002), whose investigation showed multigenerational reductions in fish size after size-selective "fishing" in experimental tanks. Because the adults of this species are easy to spawn in captivity (Barkman and Beck, 1976; Middaugh and Lempesis, 1976), larvae have been the subject of laboratory studies for decades. However, surprisingly, the larval ecology of this species in the field is poorly known.

In Rhode Island, Atlantic silverside occupy estuaries from March through December and migrate to open water during the winter months. Adults return after winter in an emaciated condition, then feed heavily on zooplankton in March and April to become reproductively mature, which occurs between May and early July (Huber and Bengtson, 1999). Spawning throughout the species range occurs with semilunar periodicity, and eggs are deposited in discrete areas in the upper intertidal reaches of salt marshes (Middaugh, 1981; Middaugh et al., 1981; Conover and Kynard, 1984; Conover, 1985), the latter of which reduces egg predation by open-water predators (Tewksbury and Conover, 1987).

In the upper reaches of 2 estuaries in Rhode Island, the upper Pettaquamscutt River (UPR) and upper Point Judith Pond (UPJP), zooplankton communities are quite different in early spring when Atlantic silverside adults return to feed and prepare for spawning (Bengtson, 1982; Huber, 1995; Volson, 2012). The zooplankton community in the UPR is

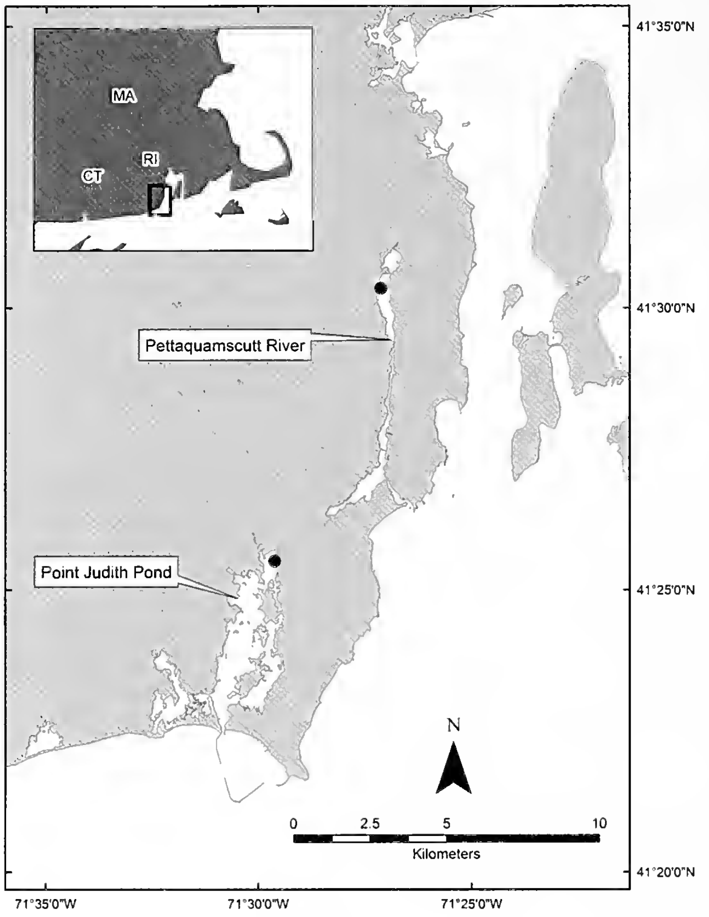

Figure 1

Map of the upper portions of the Pettaquamscutt River estuary and Point Judith Pond, the 2 estuaries in Rhode Island where larvae of Atlantic silverside (*Menidia menidia*) were sampled in 2012 for this study. The rectangle on the smaller map indicates the location of both estuaries in southern New England. The dots on the larger map show the approximate sampling locations in each estuary.

dominated by crustaceans during early spring; their presence indicates a fairly pristine environment. The UPJP is dominated by polychaete larvae, which require a eutrophic environment. Given the general propensity of marine larval fish to feed on copepods, an a priori assumption might be made that Atlantic silverside in the UPR feed on higher quality prey than those in the UPJP. Volson (2012) examined effects of the nutritional quality of zooplankton prey from these 2 estuaries on adult Atlantic silverside and on their eggs, along with the hatching length of their larvae after incubation in the laboratory. Surprisingly, in each of 2 years, length at hatching was greater for fish from the UPJP than for fish from the UPR. It has remained unclear whether a greater length at hatching translates into different larval growth rates during the first 2 weeks of life in the field. Therefore, in this study, we examined whether it does.

Very little is known about the habitat ecology of Atlantic silverside larvae during their first 2–3 weeks of life in an estuary. Because newly hatched larvae in the laboratory are attracted to the interface between the water surface and the tank edge (D. Bengtson, personal observ.) and because adults spawn (and embryos hatch) in the upper intertidal, we suspected that larvae might be found in extremely shallow water. The feeding ecology of larval Atlantic silverside in the field is undocumented (Fay et al., 1983). Therefore, knowledge of critical elements of the field ecology of this important species during the larval period is lacking. The goals of our study, therefore, were 1) to determine the depth distribution of Atlantic silverside larvae, 2) to compare abundance and distribution of Atlantic silverside larvae between estuaries, 3) to compare feeding habits of the larvae in the 2 estuaries by analyzing gut contents, and 4) to compare growth of larvae in the 2 estuaries through age–length relationships based on otolith analysis.

Materials and methods

Study sites

Field collections took place in the UPJP and UPR (Fig. 1). These estuaries are approximately 5 km apart, located in Washington County, Rhode Island, and have different physical characteristics (Table 1). Point Judith Pond is a shallow coastal lagoon, 1 of 7 along the southern coast of Rhode Island, connected to Block Island Sound by a breachway (Lee[1]). The Pettaquamscutt River is an annual flooded river valley that drains into Narragansett Bay (Gaines, 1975).

Abundance and distribution

To determine distribution patterns and densities (abundance per cubic meters) of Atlantic silverside larvae in the field, 4 sampling devices were used: 1) a cylindrical polycarbonate quadrat (with a diameter of 0.5 m to sample the land–water interface); 2) an aquarium net, 19.05×26.03 cm with 500-µm mesh, to collect larval samples in water that was 0.05–0.80 m deep; 3) a plankton net with a diameter of 0.2 m, length of 0.6 m, and 200-µm mesh to collect samples in water 0.15–0.91 m deep, and 4) a second plankton net with a diameter of 0.5 m, length of 1.8 m, and 100-µm mesh to collect samples in water with depths slightly greater than 1 m.

At both estuaries, collections occurred 7 days after the new moon of 20 May 2012 and continued for 2 weeks. A second 2-week period of sampling occurred 7 days after the full moon of 4 June 2012. These dates were chosen to collect larvae hatched during those pre-

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[1] Lee, V. 1980. An elusive compromise: Rhode Island coastal ponds and their people. Univ. Rhode Island, Coast. Resour. Cent., Mar. Tech. Rep. 73, 65 p. [Available at website.]

Table 1

Physical parameters of 2 estuaries, the upper Pettaquamscutt River (UPR) and upper Point Judith Pond (UPJP), from May through July 2012 documented within the program of the University of Rhode Island Watershed Watch (website).

Parameters	Time	Depth (m)	UPR	Depth (m)	UPJP
Temperature (°C)	May 2012	0.1	19.5	0.5	19.8
	June 2012		24.0		21.0
	July 2012		27.0		25.3
		Average:	24		22
Practical salinity	May 2012	0.1	16.5	0.5	–
	June 2012		15.0		27.5
	July 2012		16.0		30.5
		Average:	16		29
Fecal coliform (per 100 mL)	May 2012		<10		478
	June 2012		<10		189
	July 2012		<10		84
Enterococci (per 100 mL)	May 2012		<10		124
	June 2012		<10		20
	July 2012		124		30
Dissolved phosphorus (µg/L)	May 2012	0.5	5	0.5	7
	June 2012		<3		8
	July 2012		4		29
Ammonium-nitrogen (µg/L)	May 2012	0.5	45	0.5	60
	June 2012		40		45
	July 2012		25		75
Total phosphorus (µg/L)	May 2012	0.5	16	0.5	42
	June 2012		23		72
	July 2012		35		107

sumed semilunar spawning periods Sampling began at 0630, an important time for determining foraging habits because this is the time when Atlantic silverside begin feeding for the day. Initially, each sampling device was used at 4 locations within each estuary to try to identify microhabitat differences. Collections on and after 14 June in the UPJP were sampled from one location only because we determined that this estuary had undifferentiated microhabitat structure. At each of the locations within the UPR and UPJP, the quadrat, aquarium net and 2 plankton nets were each used 4 times (replicates) daily. Once sampling began, it continued throughout the day until all tows and plots were complete or until weather conditions prohibited further sampling.

The quadrat was haphazardly tossed at the land–water interface. Both plankton nets were pulled along 10-m transects by means of a rope; the senior author deployed the nets in the water, walked around the transect with the rope, waited for any disturbed sediment to settle out of the water column, and quickly pulled the net over the 10-m distance. Finally, the aquarium net was pushed along a 10-m transect. However, on and after June 14, the aquarium net was pushed along a 1-m transect. Immediately before each sampling event, the water depth at the sampling point was measured in meters with a yard stick. In between each tow with the plankton nets and aquarium net, a 15-min waiting period allowed suspended sediment from the previous tow to settle. The quadrat on average sampled 0.010 m^3 of water per sample at the land–water interface. For field collections made before 14 June, the volume of water filtered by the aquarium net was 0.496 m^3 per sample. For field collections made on and after 14 June, the aquarium net filtered 0.049 m^3 of water per sample because of the shorter sampling transect. The small plankton net filtered 0.324 m^3 of water per sample, whereas the large plankton net filtered 1.980 m^3 of water per sample. Although it was not possible to determine the sampling efficiency of each device, we assumed that the swimming speed of Atlantic silverside larvae is insufficient for them to avoid these sampling devices in any meaningful way.

Larvae that were collected for laboratory analysis

Table 2

Catch data for each sampling device used in this study of Atlantic silverside (*Menidia menidia*) larvae in the upper Pettaquamscutt River (UPR) and upper Point Judith Pond (UPJP), Rhode Island, before 14 June 2012 (**A**), as well as on and after 14 June 2012 (**B**). Also included in each table are descriptions of the volume of water filtered by each sampling device. Average densities of larvae, measured in number of fish per cubic meter, are provided with standard error (SE) values in parentheses.

		Total number of larvae collected		Average density of larvae (fish/m^3) (SE)	
Sampling device	Volume of water sampled per tow (m^3)	UPR	UPJP	UPR	UPJP
A					
Quadrat	0.01	33	2	0.18 (SE 0.12)	0.01 (SE 0.01)
Aquarium net	0.49	152	311	2.55 (SE 0.55)	10.11 (SE 3.21)
Small plankton net	0.32	35	3	0.90 (SE 0.38)	0.11 (SE 0.08)
Large plankton net	1.98	0	1	0	0.01 (SE 0.01)
B					
Quadrat	0.01	8	1	0.05 (SE 0.03)	0.02 (SE 0.02)
Aquarium net	0.049	25	24	6.00 (SE 3.70)	20.16 (SE 9.71)
Small plankton net	0.32	2	0	0.07 (SE 0.05)	0
Large plankton net	1.98	0	0	0	0

were euthanized with tricaine methanesulfonate (MS-222) mixed in seawater (90 g/mL) and preserved in either 95% ethanol (for otolith analysis) or 10% formalin (for gut content analysis). Each larva collected in the field was measured to the nearest 0.01 mm for total length (TL) with a dial caliper.

Gut content analysis

Foraging habits of Atlantic silverside larvae were determined by gut content analysis of preserved larvae collected from the field. In the laboratory, the gut was gently pulled apart and examined in a 50-mm Sedgewick-Rafter counting cell under a compound microscope. Each prey item was tallied and identified to the lowest possible taxon. From fish collected from the UPJP, 58 guts were examined. From fish sampled from the UPR, 51 guts were examined. All larvae dissected were between 4.18 and 9.36 mm TL for both estuaries. The number method was used to show food type as a percentage of the total gut contents of each larva (Hyslop, 1980). Each taxon was represented as a percentage of the total gut contents for all the larvae dissected for each estuary.

Otolith analysis

In the laboratory, one sagittal otolith was extracted from each larva, placed on a microscope slide with one drop of immersion oil (Grade A, Cargille-Sacher Laboratories[2], Cedar Grove, NJ), photographed by using a light microscope camera (at 100× or 400× magnification), and its rings were counted by using the methods of Barkman (1978). Otoliths were first examined by using light microscopy (400× magnification) to count daily growth rings and in turn to determine age (in days). Measurements of the diameters of the sagittae were taken as a proxy for growth. At a later time, a second reading was completed by the same observer from the photographs taken of the sagittal otoliths. Six prehatching rings were subtracted from the total number of daily rings on each sagittal otolith because Barkman determined that the rings start to be laid down 6 days before hatching (Barkman, 1978). The sagittae did not require additional processing because the core was visible. The relation of the number of daily rings (age in days) to larval length was determined for fish from each estuary. The slopes of these linear relationships provide an estimate of growth (in millimeters per day) of larvae in each of the estuaries. All measurements were made in micrometers with the computer software program ImageJ (Abràmoff et al., 2004). We examined 17 larvae from the UPJP and 19 larvae from the UPR.

Statistical analysis

The relationship between age and length of larvae was determined for each estuary and the slopes of these regressions were analyzed with an analysis of covariance (ANCOVA). A chi-square analysis was applied to the gut content data to determine a significant difference between the feeding habits of Atlantic silverside larvae from UPJP and the feeding habits of Atlantic silverside larvae from UPR.

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[2] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

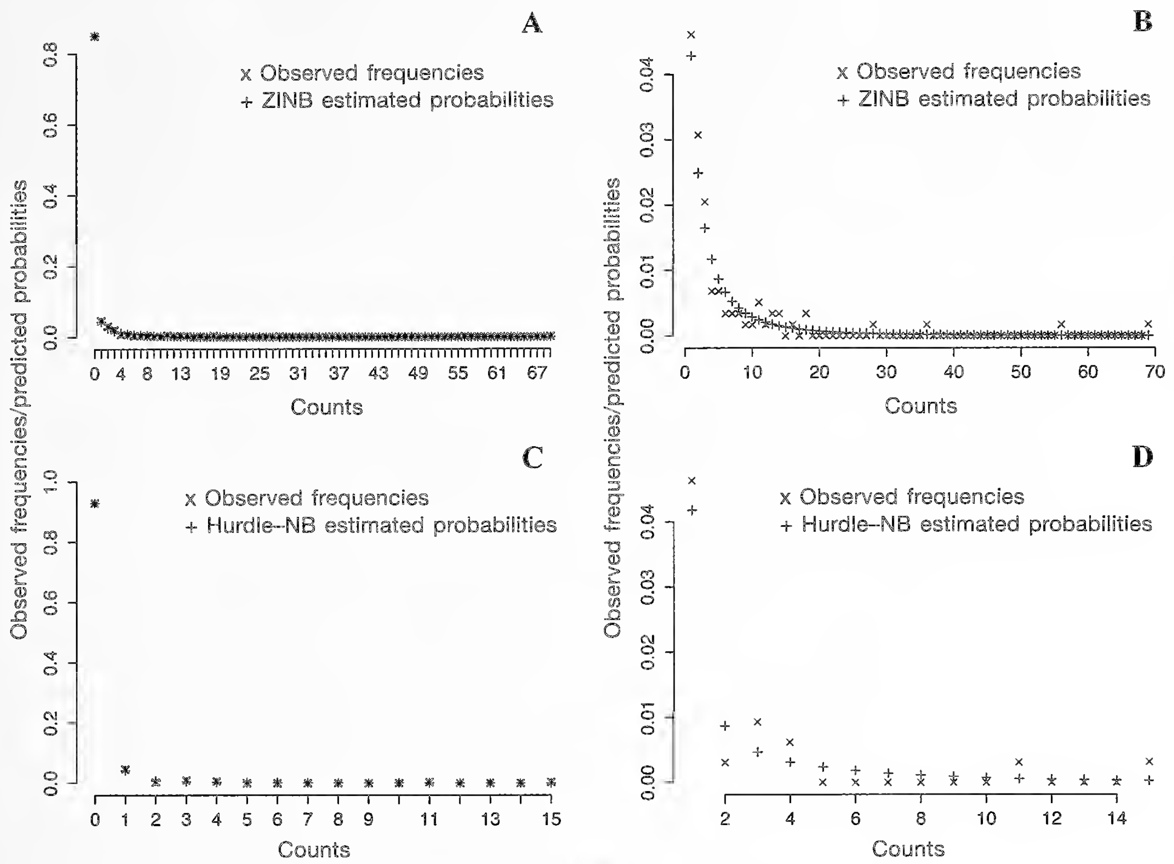

Figure 2

The frequency of occurrence of the total number of larvae of Atlantic silverside (*Menidia menidia*) collected from both the upper Point Judith Pond and the upper Pettaquamscutt River (**A** and **B**) before 14 June 2012 and (**C** and **D**) on and after 14 June 2012. For all graphs, the gray symbols represent the probability estimates following the zero-inflated negative binomial (ZINB) or the hurdle negative binomial (hurdle-NB) models used for estimation. The black symbols in each graph represent the observed relative frequencies of the total number of larvae collected from the field. Both models take into account the probability of observing a zero (absence of Atlantic silverside larvae), as well as observing a positive value (presence and abundance of Atlantic silverside larvae). Panels B and D differ from panels A and C in that the former lack the zero probability estimates and observed values. The components of both models depend on covariates, and that is why occupancy and abundance are modeled jointly. All graphs also show how many Atlantic silverside larvae were collected from the field per tow.

Given the large presence of zeros in the abundance data collected from the field, we worked with 2 different classes of generalized linear models that explicitly take into account this feature: zero-inflated count models and hurdle count models. A zero-Inflated model is a mixture of 2 distributions: a binary distribution for structural zeros and a distribution for the counts (typically Poisson or negative binomial) that can be zero or positive. A hurdle model is similar, except that all the zeros are modeled with the binary distribution and the distribution for the counts models only the positive values. For each class of models, we fitted 2 different distributions: the Poisson and the negative binomial (White and Bennetts, 1996). The Poisson distribution is appropriate when mean and variance of the data are similar, and the negative binomial features an additional parameter to measure overdispersion (variance larger than the mean) in the data.

All these models allow specifications of covariates that can be different for the zero-inflated part and the count part. Possible covariates that could be predictors of abundance were depth of the measurements, date, site effect (UPJP versus UPR), type of collection gear or net effect (quadrat, aquarium net, small plankton net, and large plankton net). Estimation was performed by using maximum likelihood methods and implemented in the package pscl, vers. 1.4.9 (Jackman, 2015; Zeileis et al., 2008) in the statistical software R, vers. 3.2.3 (R Core Team, 2015). Statistical significance for the parameters was assessed by using Z-tests. Given the relatively small amount of nonzero data, we had to choose the predictors carefully, using Akaike infor-

Table 3

Parameter estimates from the zero-inflated negative minomial (ZINB) model for field samples of larval Atlantic silverside (*Menidia menidia*) collected before 14 June 2012. The top part of this table includes parameters from the ZINB model that analyzes all data values greater than zero (i.e., when a larva was collected). Site refers to the upper Pettaquamscutt River and upper Point Judith Pond in Rhode Island. Date represents the duration of sampling, 30 May 2012 through 13 June 2012. The Z statistic tests whether the probability of collecting a larva is significantly influenced by site, depth, the quadrat, the aquarium net, and the small plankton net. The bottom table includes the parameters influencing the probability of having a count of zero in the data; the Z statistic tests whether the probability of not collecting a larva is significantly affected by 2 of the sampling devices. SE=standard error.

	Estimate	SE	Z-value	P-value	Significance
Count model coefficients (negative binomial with log link)					
Date	−0.0405	0.0506	−0.802	0.4226	NS
Depth	0.5182	1.2352	0.420	0.6748	NS
Aquarium net	1.6155	0.7151	2.259	0.0239	**
Quadrat	−1.9364	1.0629	−1.822	0.0684	*
Small plankton net	−2.0110	1.2911	−1.558	0.1193	NS
Site [2 sites]	−1.2817	0.4146	−3.092	0.0020	***
Quadrat:Site[2 sties]	3.8550	1.1643	3.311	0.0009	****
Small plankton net:Site[2 sites]	3.5268	0.9957	3.542	0.00040	****
Log(theta)	−1.6682	0.1635	−10.206	<0.0001	****
Zero-inflated model coefficients (binomial with logit link)					
Aquarium net	−10.8903	381.4482	−0.029	0.9772	NS
Quadrat	1.4663	0.5074	2.89	0.0039	***
Small plankton net	1.0366	0.5092	2.036	0.0418	**

Significance codes:
**** ($P<0.001$);
*** (P<0.01);
** ($P<0.05$);
* ($P<0.1$);
NS=not significant.

mation criterion (AIC). We analyzed the data collected before 14 June separately from data collected on and after that date.

Finally, to determine whether the sizes of the larvae changed with depth of capture, we used analysis of variance (ANOVA) to compare the lengths of larvae collected from the following 5 depth strata: 0.0–0.2 m; 0.2–0.4 m; 0.4–0.6 m; 0.6–0.8 m; and >0.8 m.

Results

Abundance and distribution in the field 2

Average density and the number of larvae collected by each sampling device (Table 2) indicated that only one Atlantic silverside larva was collected with the large plankton net; therefore, this device was excluded from the remainder of the analysis. The quadrat, aquarium net, and small plankton net all collected more larvae than the large plankton net. This finding indicates that Atlantic silverside larvae are generally not found in waters greater than 1 m deep but can be found in waters less than 1 m deep in the littoral zone. The quadrat, the aquarium net, and small plankton net can all be used to collect Atlantic silverside larvae from the littoral zone of estuaries.

Distribution and abundance of Atlantic silverside larvae have a discrete distribution with a high frequency of zeros on the basis of our analysis of field collections (Fig. 2), indicating that Atlantic silverside larvae have a patchy distribution in the littoral zone. In terms of maximum numbers per tow, for field collections made before 14 June, up to 69 larvae were collected per tow (Fig. 2, A and B). For field collections made on and after 14 June, up to 15 larvae were collected per tow (Fig. 2, C and D).

The results of model analysis provided the predictors that influenced the number of larvae collected, as well as the predictors that influenced the structural zeros in the data (Tables 3 and 4). For the data collected before 14 June, the use of AIC indicated that the chosen model was a zero-inflated negative binomial. The presence of larvae in the littoral zone in both estuaries correlated with date, depth, and all sampling devices, as well as with interaction terms (Table 3). Date was found to be

Table 4

Parameter estimates for the negative binomial hurdle model obtained from field samples of larval Atlantic silverside (*Menidia menidia*) collected on and after 14 June 2012. The top table includes parameters from the model that analyzes all data values greater than zero (i.e., when a larva was collected). Date represents the duration of sampling, from 14 June 2012 through 25 June 2012. The Z statistic tests whether the probability of collecting a larva is significantly influenced by depth and site. The bottom table includes the parameters influencing the probability of having a count of zero in the data. Site 1=Upper Point Judith Pond; site 2=Upper Pettaquamscutt River. SE=standard error.

	Estimate	Standard error (SE)	Z-value	P-value	Significance
Count model coefficients (truncated negative binomial with log link)					
Depth	13.272	5.614	2.364	0.0181	**
Site 1	−14.943	64.922	−0.230	0.8180	NS
Site 2	−9.885	64.827	−0.152	0.8788	NS
Log(theta)	−9.077	64.822	−0.140	0.8886	NS
Zero-inflated hurdle model coefficients (binomial with logit link)					
Depth	6.7017	2.0211	3.316	0.0009	****
Aquarium net	−3.4220	0.6359	−5.381	<0.0001	****
Quadrat	−3.9519	1.0082	−3.920	<0.0001	****
Small plankton net	1.5323	2.2454	0.682	0.4950	NS
Depth : Quadrat	1.4099	9.3280	0.151	0.8799	NS
Depth : Small plankton net	−22.8333	7.4331	−3.072	0.0021	***

Significance codes:
****($P<0.001$);
***($P<0.01$);
**($P<0.05$);
NS=not significant.

nonsignificant ($_{\mathrm{date}}=-0.04$, $Z=-0.802$, $P=0.422$; Table 3). The number of larvae collected did not increase with depth in the littoral zone ($_{\mathrm{depth}}=0.518$, $Z=0.420$, $P=0.674$; Table 3). The different nets all had significant effects for the count part of the model. The quadrat and the small plankton net influenced the presence of zeros in the data to a larger extent than that of the aquarium net. Notice that all the estimated coefficients (Table 3) need to be interpreted, considering that the model is in the log scale (the negative binomial part) and in the logit scale (the zero-inflated part).

For field collections made on and after 14 June, given the smaller number of positive counts, we needed to implement a more parsimonious model, with fewer predictors. We tried several different specifications. For simplicity, we report that the negative binomial hurdle model offered the best performance in terms of AIC. Depth influenced the counts; depth and net type influenced the number of structural zeros. The number of larvae collected increased significantly with depth in the littoral zone ($_{\mathrm{depth}}=13.272$, $Z=2.364$, $P=0.0181$; Table 4).

One of the goals of our project was to determine whether the density of Atlantic silverside larvae differed between the 2 estuaries. The results of the zero-inflated Poisson analysis for site indicated that, for the entire sampling period, the UPR had a higher density of Atlantic silverside larvae than the UPJP.

Results of the ANOVA of larval lengths in the depth strata from the shoreline to depths >0.8 m indicated that, at the UPR, larvae captured at the depth stratum 0.2–0.4 m were significantly shorter (mean: 10.1 mm TL [standard deviation (SD) 3.4]) than larvae captured at the depth stratum 0.6–0.8 m (mean: 14.2 mm TL [SD 2.7]). At the UPJP, larvae captured at both the depth strata of 0.2–0.4 m and 0.4–0.6 m were significantly shorter (means: 7.7 mm TL [SD 2.5] and 7.8 mm TL [SD 2.2], respectively) than larvae captured at the depth stratum 0.6–0.8 m (9.5 mm TL [SD 3.1]).

Gut content analysis

Feeding habits of Atlantic silverside larvae between estuaries were significantly different ($\chi^2=622.7$, $P<0.0001$). In the UPJP, copepod nauplii made up 72.5% of total gut contents (Fig. 3). In the UPR, Atlantic silverside larvae consumed mostly copepod eggs, which made up 76.2% of the total gut contents (Fig. 3).

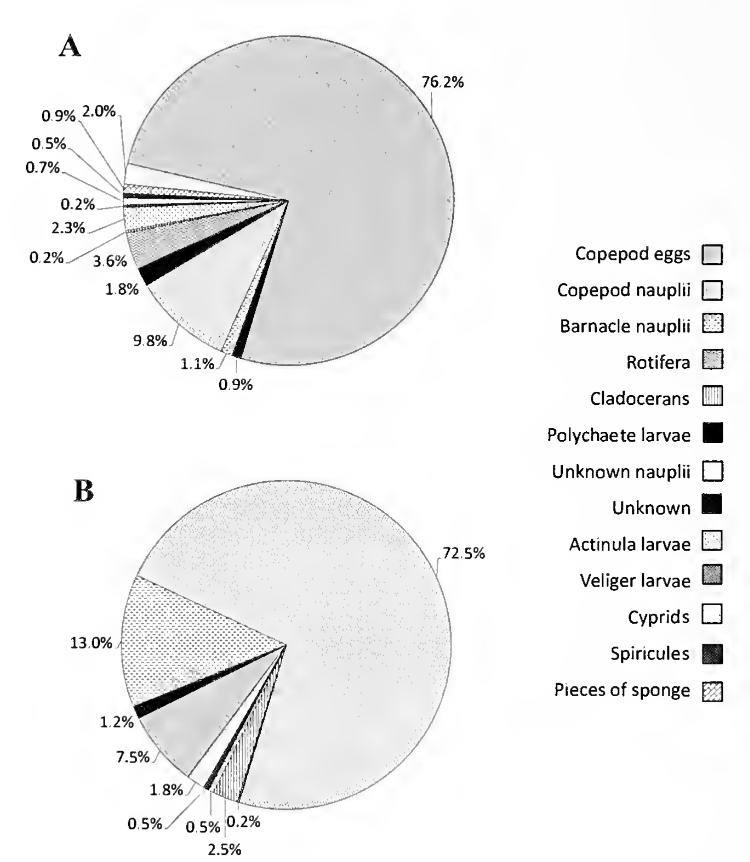

Figure 3

Gut contents of larvae of Atlantic silverside (*Menidia menidia*) collected in 2012 (**A**) in the upper Pettaquamscutt River (*n*=51) and (**B**) in the upper Point Judith Pond (*n*=58). Each taxon is represented as a percentage of the total gut contents for all larvae collected in each of these 2 estuaries in Rhode Island. Results from the chi-square analysis indicate a significant difference in feeding habits of Atlantic silverside larvae ($P<0.0001$).

Otolith analysis

Results from the ANCOVA showed a significant relationship between length of larvae and age for fish from both estuaries ($P<0.0001$; Fig. 4) The age–length regressions indicated that larvae grow 0.65 mm/d in the UPR and 0.66 mm/d in the UPJP (Fig. 4).The results from the ANCOVA showed no significant difference ($P=0.8147$) between estuaries in the age–length relationship of larvae (Fig. 4).

Discussion

This article provides the first detailed report of the field ecology of Atlantic silverside larvae, although laboratory studies on this larval species have been conducted for decades (e.g., Austin et al., 1975; Middaugh and Lempesis, 1976; Morgan and Prince, 1977; Deacutis, 1978; Bengtson, 1985; Lankford et al., 2001). Field collections from the littoral zone of the UPR and UPJP during the summer of 2012 indicate that this larval fish can be captured at the shoreline interface to waters 1 m deep, can be collected with a variety of sampling devices, and has a patchy distribution. The 2 estuaries sampled differed in abundance of Atlantic silverside larvae and in the prey consumed by the larvae, but the larvae grew at the same rates regardless of those differences.

The quadrat, aquarium net, and small plankton net collected more larvae than the large plankton net; however, the aquarium net collected the most Atlantic silverside larvae both in absolute and per-volume-sampled terms. We acknowledge that our use of different sampling devices, chosen by necessity because of the shallow depths, and the methods we used for deploying those devices add a degree of uncertainty to our results. Nevertheless, it is clear that Atlantic silverside larvae can be collected from the shallowest water out to about a 1-m depth. Middaugh (1981), Middaugh et al. (1981), Conover and Kynard (1984), and Conover (1985) documented Atlantic silverside adults depositing eggs en masse at very discrete spawning sites in the upper intertidal zone of salt marshes. The results of our study indicate that Atlantic silverside larvae stay in the very shallow littoral zone after hatching. The distribution of Atlantic silverside larvae collected from the field in this study followed a zero-inflated Poisson model, indicating that these larvae are not distributed evenly in the littoral zone and have a patchy distribution.

The patchiness that we found in the distribution of Atlantic silverside larvae may be related to the patchiness of the egg deposition sites, although we did not specifically try to test that idea. Hewitt (1981) proposed that fish larvae have a patchy distribution because it benefits schooling. Shaw (1960, 1961) reported that Atlantic silverside begin to school at a size around 11–12 mm standard length. Lindsay et al. (1978) sampled ichthyoplankton in the Indian River, Delaware, in deeper waters than those that we sampled, and noted that the low abundance of atherinid larvae was not representative of their high abundance as juveniles and adults. Occupation of very shallow waters by Atlantic silverside larvae likely provides them with protection from predators. The fact that we found shorter larvae in the shallower waters and larger larvae in slightly deeper waters within the depth stratum of <1 m within both estuaries indicates that these larvae are avoiding predators, or just diffusing very slowly from the hatching sites, or doing both.

Gut content data indicated that Atlantic silverside larvae in the UPR consume mostly copepod eggs, whereas larvae in the UPJP consume mostly copepod nauplii. Volson (2012) found a high abundance of cal-

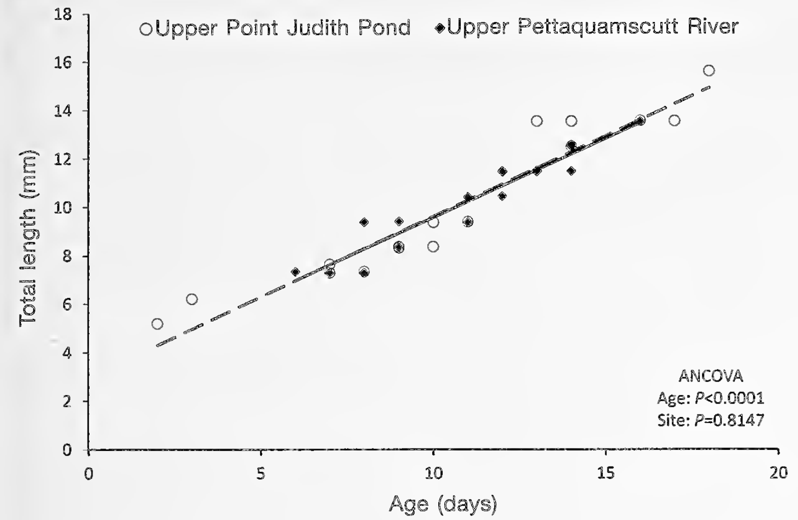

Figure 4

Growth of larvae of Atlantic silverside (*Menidia menidia*) collected in 2012 from the upper Point Judith Pond (UPJP) and upper Pettaquamscutt River (UPR) in Rhode Island. Linear regressions represent the age–length relationship of Atlantic silverside larvae from the UPJP (dashed line), $y=0.66x+2.98$, and the UPR (solid line), $y=0.65x+3.06$. In the linear equations, y is total length in millimeters and x is age in days. Results from the analysis of covariance (ANCOVA) indicate no significant differences in the slopes of the age–length relationship of larvae between estuaries ($P=0.8147$). However, age is a significant indicator of the size of larvae ($P<0.0001$).

anoid copepods in the UPR and a varying zooplankton community in the UPJP, where from early spring (April through early May) through late spring (June), the dominant zooplankton present switches from polychaete larvae to copepods. Most of the sampling for our study took place in late spring, when the polychaete larvae had already settled and were not available to, or not preferred by, the larvae. The exact species of copepod from which the eggs came from, for our study, was not determined.

The significant age–length relationship for Atlantic silverside larvae in our study has been shown previously in work by Barkman (1978). Between estuaries, there was no significant difference in the age–length relationship of Atlantic silverside larvae. According to the regression coefficients in the age–length equations, the larvae in our study grew at 0.65–0.66 mm/d. Barkman et al. (1981) found that over a length range of about 12–90 mm TL Atlantic silverside grew at 0.84 mm/d, on the basis of an age–length relationship determined with otolith analysis, whereas Mulkana (1966) estimated a growth rate of 7–11 mm/month (0.23–0.37 mm/d) on the basis of length–frequency analyses of a cohort. Volson (2012) found that larval length at hatching was significantly greater for Atlantic silverside larvae in the UPJP than for larvae in the UPR.

The results from our study indicate that a greater length at hatching does not translate into faster growth for larval Atlantic silverside. Temperature influences the growth of fish. Water temperatures in the UPJP are cooler than the water temperatures in the UPR, even during the summer months (Volson, 2012) when sampling occurred for our study (Table 1). Despite a greater length at hatching for Atlantic silverside in the UPJP, the cooler water temperature in this estuary may have resulted in a slower growth rate for the larvae. As a result, larval growth was not greater in the UPJP than larval growth in the UPR.

The larval life stage is important for recruitment of adult populations. Studies on Atlantic silverside are important not only because of the abundance of this species but also because of its role as a forage fish for fisheries species and the way in which these fish influence the energetics of estuaries. We hope that the initial information presented here will stimulate researchers of estuaries to further examine the larval ecology of this important species of estuarine ecosystems.

Acknowledgments

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Burrowing behavior, habitat, and functional morphology of the Pacific sand lance (*Ammodytes personatus*)

Joseph J. Bizzarro (contact author)[1]
Ashley N. Peterson[2]
Jennifer M. Blaine[3]
Jordan P. Balaban[2]
H. Gary Greene[4]
Adam P. Summers[4]

Email address for contact author: jbizzarro@mlml.calstate.edu

[1] School of Aquatic and Fishery Sciences
University of Washington
Box 355020
Seattle, Washington 98195-5020
Present address: Center for Habitat Studies
Moss Landing Marine Laboratories
8272 Moss Landing Road
Moss Landing, California 95039

[2] Department of Ecology and Evolutionary Biology
University of California, Irvine
321 Steinhaus Hall
Irvine, California 92697

[3] Washington Department of Fish and Wildlife
600 Capitol Way N.
Olympia, Washington 98501

[4] Friday Harbor Laboratories
University of Washington
620 University Road
Friday Harbor, Washington 98250
Kingston, Rhode Island 02881

Abstract—The Pacific sand lance (*Ammodytes personatus*) is a small, elongate forage fish that spends much of its life buried in the seafloor. We determined that the Pacific sand lance can burrow in a wide variety of sediments from silt to gravel, but it prefers coarse sand (0.50–1.00 mm grain size). In the absence of coarse sand, the Pacific sand lance chooses larger grain sizes over smaller ones. These preferences are independent of light or the compaction of sediment, and therefore indicate that visual cues and ease of entry are not primary means of choosing burial substrate. Instead, we speculate that the Pacific sand lance is morphologically adapted for rapid mobility in coarse sand and that coarse sand has enough interstitial spaces to enable respiration during protracted immersion. As an obligate burrower in specific sediments, the Pacific sand lance is a good candidate for habitat-based management. Substrate maps of 3 fishing grounds in southeast Alaska where the Pacific sand lance is abundant and where habitat-based management is practiced were used to create potential habitat maps. Different geologic histories have resulted in variable amounts of preferred (sand–gravel), suitable (sand mixed with silt, cobble–boulder, or rock outcrop), and unsuitable (mud, pebble–boulder) habitat for this species among regions.

Burrowing presents biomechanical and ecological challenges but enables access to expanded trophic opportunities and protection from many predators. The selective pressures surrounding burrowing are expressed in the morphological features of burrowing animals, as in the stout forearms of moles and armadillos and in the heavily reinforced skull of caecilians and dibamids (Kleinteich et al., 2012; Rose et al., 2013). A subterranean lifestyle is uncommon for aquatic vertebrates but has been observed in a taxonomically diverse group of marine fish taxa. Flounders and skates routinely cover themselves in substrate; jawfishes, tilefishes, and garden eels excavate permanent burrows; and some fishes (e.g., Pacific sandfish, *Trichodon trichodon*; sand lances, *Ammodytes* spp.) spend a majority of their life beneath the substrate after creating a tunnel that collapses behind them. The terrestrial equivalent of this behavior is seen in the “sand-swimming” skink species (Mushinsky and Gans, 1992; Maladen et al., 2011). Penetrating friable substrates in a completely aqueous environment is fundamentally different from terrestrial sand swimming by virtue of the density of water and its potential contribution to the excavation process.

The Pacific sand lance (*Ammodytes personatus*) is an elongate, burrowing forage fish with a wide distribution in the eastern North Pacific and a history of taxonomic confusion. Only *A. hexapterus*, formerly the Pacific sand lance and now assigned the common name of Arctic sand lance (Orr et al., 2015), and *A. personatus* were considered historically as valid North Pacific species, but the number of *Ammodytes* species in the North Pacific region and the extent of their

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distributions have long been debated (Ohshima, 1950; Han et al., 2012; Turanov and Kartavtsev, 2014). Recent genetic and morphological evidence has resolved this issue and indicates the presence of 2 additional congeners (Orr et al., 2015). Furthermore, the only species with an expansive eastern North Pacific distribution, formerly considered to be *A. hexapterus*, was redescribed as *A. personatus* (Orr et al., 2015). The range of *A. personatus* was established from southern California to the western Aleutian Islands and may extend to the Sea of Okhotsk in the western Pacific (Mecklenberg et al., 2011; Orr et al., 2015). *Ammodytes personatus* mainly occurs in coastal intertidal and subtidal waters but has been reported at depths of 172 m (Love et al., 2005).

The Pacific sand lance is a source of energy transfer between secondary producers and upper-trophic-level species and pelagic and benthic regions because it grazes on zooplankton in the water column and has an obligate affiliation with sediments. The structure and dynamics of nearshore ecosystems are heavily influenced by the biomass of forage fishes (Gaichas et al., 2010), and species of *Ammodytes*, including the Pacific sand lance, are of vital importance for the energetics and breeding success of a variety of marine mammals (Weinrich et al., 1997), seabirds (Curry et al., 2011), and fishes (Arnott et al., 2002). For instance, humpback whales (*Megaptera novaeangliae*) at Stellwagon Bank, Massachusetts, excavate bottom sediments at night to forage on dense aggregations of buried sand lances (*Ammodytes* spp.) (Friedlander et al., 2009). Off British Columbia, growth rates of rhinoceros auklet (*Cerorhinca moncerata*) chicks are positively correlated with abundance of Pacific sand lance (Bertram and Kaiser, 1993). Several groundfishes (e.g., starry flounder [*Platichthys stellatus*]; great sculpin [*Myoxocephalus polyacanthocephalus*] have been reported to feed on schools of Pacific sand lance as they move from foraging to burial grounds off southeast Alaska (Hobson, 1986).

Where species of *Ammodytes* are exploited in commercial fisheries, the associated loss of forage biomass can have ecosystem-level effects. In the North Sea, overfishing of the sand eel (*A. marinus*) has been linked to poor breeding success of several seabird species (Arnott et al., 2002), and the prosecution of a fishery for the sand eel has negatively impacted the breeding productivity of the population of black-legged kittiwake (*Rissa tridactyla*) (Frederiksen et al., 2008). Conversely, an abundance of *Ammodytes* species can enhance the productivity and efficiency of groundfish fisheries. For instance, when biomass of *Ammodytes* species is relatively high, Atlantic cod (*Gadus morhua*) form feeding aggregations in small, predictable areas (i.e., over burial habitat) where they can be targeted easily (Richardson et al., 2014). Given the importance of the Pacific sand lance to the trophic dynamics of nearshore systems (Beacham, 1986; Borstad et al., 2011) and current concern over ecosystem-level effects of exploiting forage fishes (Smith et al., 2011; Essington et al., 2015), determining the specific habitat and burrowing requirements of the Pacific sand lance are necessary steps toward the development of ecosystem approaches to the management of this species.

Like the sandfish (*Scincus scincus*), a lizard found in sandy habitats in North Africa and Southwestern Asia (Maladen et al., 2009), the Pacific sand lance is able to burrow rapidly into the substrate (Gidmark et al., 2011). It is tempting to suppose that this fish takes advantage of the viscosity and density of water to stir, or fluidize, the sand before burrowing; however, this behavior has not been observed. Instead, high-speed video of Pacific sand lance burrowing in the laboratory indicates that this fish dives headfirst into the sand, beating its tail and driving the head and anterior two-thirds of its body underground. At this point, the buried part of its body undulates and draws the remaining part of the fish beneath the sediment (Gidmark et al., 2011). Models show that the sandfish uses substantial force to burrow into dry sand. It is not possible to extrapolate this type of movement to the Pacific sand lance because there are no data to indicate the relative ease of penetrability through dry sand and sand inundated with water. Results from isolated laboratory and field studies, however, indicate that burial preferences range from fine to very coarse sands (Pinto et al., 1984; Haynes et al., 2007; Robinson et al., 2013). If these burial preferences can be refined further through more complete testing, this information could 1) be used in habitat-based management plans for Pacific sand lance and 2) may reveal morphological and behavioral adaptations that explain the preference of Pacific sand lance for a particular grain size or sizes.

The Pacific sand lance is an accomplished burrower that spends a large percentage of its time in sediment of unknown characteristics. Because it is an important forage fish with a strong benthic association, the habitat preferences of this species has direct implications for the development of ecosystem approaches to management and conservation. This fish is also an excellent model for studying locomotion by an elongate, anguilliform burrower in an aquatic environment. The goals of our study were fivefold: 1) to assess the grain sizes, ranging from silt to very fine gravel, that are potential burial habitats for Pacific sand lance; 2) to determine whether Pacific sand lance prefer substrates of a particular size; 3) to use lighting and sediment compaction to gain insight into the factors that favor the selection of a burial substrate; 4) to use field sampling and habitat mapping to link sediment preferences of the Pacific sand lance in the laboratory with substrate associations in the field; and 5) to reveal specialized morphological features for burrowing.

Materials and methods

Data collection

Pacific sand lance were collected at Jackson Beach, San Juan Island, Washington, (48.520°N, 123.011°W). Fish

Table 1

Size range, Wentworth (1922) grade, and phi (ϕ) scale of uniform sediment types used in laboratory experiments to determine the preferred habitat of Pacific sand lance (*Ammodytes personatus*). Experiments were conducted in 2010 and 2012.

Size range	Wentworth grade	Phi scale
2.0–4.0 mm	Very fine gravel	–1 to –2
1.0–2.0 mm	Very coarse sand	0 to –1
0.5–1.0 mm	Coarse sand	1 to 0
0.25–0.5 mm	Medium sand	2 to 1
125–250 µm	Fine sand	3 to 2
62.5–125 µm	Very fine sand	4 to 3
3.9–62.5 µm	Silt	5 to 4

were captured in a boat-deployed bag seine in subtidal and intertidal waters (depths ≤10 m) during the summers of 2010 and 2012. Within an hour of capture, individual fish in good condition were taken to the University of Washington's Friday Harbor Laboratories, where they were maintained in an 1136-L (300-gallon) aquarium with running seawater and a mixture of sediments to enable burrowing. The entire size range of sampled Pacific sand lance (5.0–14.5 cm in total length [TL]), that corresponded to juveniles, subadults, and adults (Wyllie-Echeverria[1]), was used to determine the burrowing capabilities of this species. However, specimens used in sediment preference trials were restricted to individuals corresponding to subadult sizes (8.5–11.0 cm TL) to account for possible scaling effects and because they were the dominant size class in catches. All laboratory experiments were conducted within a month after fish were collected. Approximately 300–500 individuals were maintained throughout the experiments and periodically fed with locally caught mysids and copepods. Only fish that appeared to be in good physical condition (i.e., were active, had no obvious abrasions or injuries, and their fins were intact) were used in experimental trials.

Marine sediments were collected from local beaches throughout San Juan Island to obtain a variety of grain sizes. Sediments were dried and sorted into 7 uniform grain sizes ranging from silt (0.4 mm) to very fine gravel (4.0 mm) by using a Ro-Tap sediment analyzer (W. S. Tyler, Mentor, Ohio) (Table 1). For all laboratory trials, sediments were placed in paired aluminum trays with a volume of 0.014 m^2 and a depth of approximately 15 cm that corresponded to the maximum depth at which Pacific sand lance have been observed locally. A dark, plastic divider measuring 7.5 cm in width separated the trays to minimize an arbitrary sediment choice.

Laboratory experiments

To determine the grain sizes that represent potential burial habitats, 10 fish were placed in a 76-L (20-gallon) aquarium with running seawater and a uniform substrate consisting of 1 of the 7 grain sizes (described in Table 1). Experimental fish were netted out of the larger holding tank after the sediment was stirred to mobilize burrowed individuals. Seawater was fed directly through a screen placed over the top of the tank so that water flow did not create bottom currents that could influence burrowing. Fish were not introduced into the tank until the water was no longer turbid. Individuals spanning the entire observed size range of the species were used in each trial, periodically observed, and continuously filmed for 6 consecutive daylight hours. The Pacific sand lance typically shelters within an hour of exposure to a suitable sediment type (Pinto et al., 1984). If at least one individual burrowed into sediment of a particular grain size during the trial period, sediment suitability was verified and the grain size was advanced for use in experiments of preferred sediment types.

For experiments on sediment preference, 50 individuals were introduced into a 76-L tank with paired sediment types configured as previously indicated. Fish were allowed to acclimate for 4 h, after which covers were placed over the sediment trays, all mobile individuals were removed, and the tank was drained. The number of buried fish in each sediment type was then recorded and all fish in good condition were returned to the holding tank. Daytime trials (n=41) were conducted between 1100 and 1700 h, and illumination was provided simultaneously by sunlight and ambient room lighting. Nighttime trials (n=16) occurred between 2300 and 0300 h in complete darkness. Eight replicates were planned for all trials; however, diurnal trials involving medium sand—a grain size that was largely avoided by fish—consisted only of either 5 or 6 replicates because of time and logistical constraints (e.g., condition of captive Pacific sand lance).

Preferred sediment was determined by using replicated G-tests of goodness of fit from an expected 1:1 ratio (Connallon and Jabukowski, 2009; McDonald, 2009). A G-test of goodness of fit was first conducted for each replicate in a comparison of paired sediments (individual test). To determine whether all of the data from the different experiments fit the expected 1:1 ratio, the individual G-values from these replicates were then added to assess the significance of the aggregate G-value (total test). A pooled G-test was then conducted by adding all the observations among experiments and by testing the resulting G-value (pooled test). Finally, a G-test of independence was used to determine whether the individual trials had significantly different relationships from one another (heterogeneity test). The totality of these results was synthesized to determine burrowing preference and to explain burrowing behavior.

Laboratory experiments were combined with the testing of compaction by using resin models of Pacific

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[1] Wyllie-Echeverria, T. 2010. Personal commun. Friday Harbor Laboratories, Univ. Wash., Friday Harbor, WA 98250.

sand lance to determine whether burrowing preference and penetration force of the Pacific sand lance differed in uncompacted and compacted sediments. Full compaction was achieved by vibrating a plexiglass plate over one of the paired sediment trays until readings, made with a penetrometer (Forestry Suppliers Inc., Jackson, MS), peaked. In experiments of preferred sediments, compacted and uncompacted sediments consisting of the 4 largest grain sizes were paired, as described in Table 1, and were conducted (only) during daylight hours (Table 1). Models of subadult Pacific sand lance were created by making dental wax (President light body[2]; Coltene, Altstätten, Switzerland) molds of euthanized fish and then filling the molds with Spurr resin. These models were pressed into inundated sediment until a third of the body was covered, corresponding with the penetration stage of burial (Gidmark et al., 2011). Force was calculated with a force gauge (MTS Systems Corp., Eden Prairie, MN) at increments of 1.0 mm, and 5 replicates were conducted for each tested grain size and compaction level.

The force necessary to penetrate uncompacted and compacted sediments of different grain sizes was plotted against penetration depth, and the data were log-transformed to achieve linearity. Slopes of the relationship between force and penetration depth were compared by using a 2-way analysis of variance (ANOVA), with compaction and sediment size as independent variables. Where ANOVAs were significant, a Tukey's honestly significant difference test was run to determine the groups that contributed to these differences.

Field collections

A comprehensive fish and sediment sampling effort was conducted at the central San Juan Channel sand wave field, a region where bottom currents have shaped the seafloor geomorphology into a series of successive crests and troughs at depths of 60–80 m to determine sediment associations of Pacific sand lance in offshore waters. Twenty-one target sites with a minimum separation distance of 70 m were chosen randomly throughout the sand wave field, excluding areas near cable crossings (Fig. 1). By using the RV *Centennial*, fish and sediment were collected with a Van Veen bottom grab, a clamshell-type sampler with long lever arms and sharp cutting edges that enable deep (up to 22 cm) penetration into seafloor sediment. The Van Veen grab has a rapid, powerful closing mechanism, with overlapping flaps, that allows the jaws to excavate 0.12 m^2 of relatively undisturbed sediment while preventing the loss of sediment or fish. This sampling method has been extremely successful for obtaining significant numbers of live sand eel and representative seafloor sediment (Freeman et al., 2004).

Samples were collected at each site during both nighttime and daytime low-tide periods; nighttime sampling was conducted during 2000–2400 on 6 November 2006, and daytime sampling was conducted during 1030–1430 on 7 November 2006. The nighttime samples were collected as close to the target sites as possible given drift and current conditions, and the actual vessel coordinates were recorded when the grab hit bottom. These coordinates then became the target sites for the daytime samples. After each sample was retrieved, all fish were removed and frozen, and a 400-600-mL subsample of sediment was collected. Fish were later counted, measured, and dissected to determine sex and maturity stage (after Macer, 1966; Nelson and Ross, 1991). Sediment was dried and sorted by using a Ro-Tap sediment analyzer, as previously described, and the total weight and relative proportion of each grain size was recorded (according to the method of Wentworth, 1922). Data were evaluated for normality and homoscedasticity, and a paired t-test was conducted to investigate the difference in the mean number of Pacific sand lance collected during day and night grabs that occurred at the same target locations. Field results of habitat preference were used for comparison with results from laboratory experiments.

Habitat mapping

Sediment preferences determined from laboratory experiments and field collections were applied to previously constructed seafloor-substrate maps of common fishing grounds for groundfish (especially for rockfish) off southeast Alaska (Fairweather Ground, Cape Ommaney, and Edgecumbe). Substrate maps were created during 1998–2004 by 2 of the authors (H. Greene and senior author) for use in habitat-based fishery management by the Alaska Department of Fish and Game, Sitka office. Source data included a combination of side-scan sonar and multibeam imagery. Map interpretations were verified with data from dives of a human-occupied submersible.

The mapped depths of the Fairweather Ground and Edgecumbe fishing grounds were within the known depth range of Pacific sand lance (Love et al., 2005). The substrate map for the Cape Ommaney fishing ground, however, extended to a depth of 305 m, well beyond the approximate maximum depth of known occurrence of Pacific sand lance (Love et al., 2005). A 175-m depth contour, therefore, was extracted from the multibeam imagery for this region and used to create a deepwater boundary. All mapping and spatial analysis were conducted in ArcMap, vers. 10.2.2 (Esri, Redlands, CA). Ostrand et al. (2005) determined that depth was the primary factor associated with offshore distribution of Pacific sand lance and that the population in Prince William Sound was largely restricted to depths <60 m. However, the Pacific sand lance is extremely common to depths of at least 80 m off the San Juan Islands, and our predictions are of potential habitat dis-

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[2] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

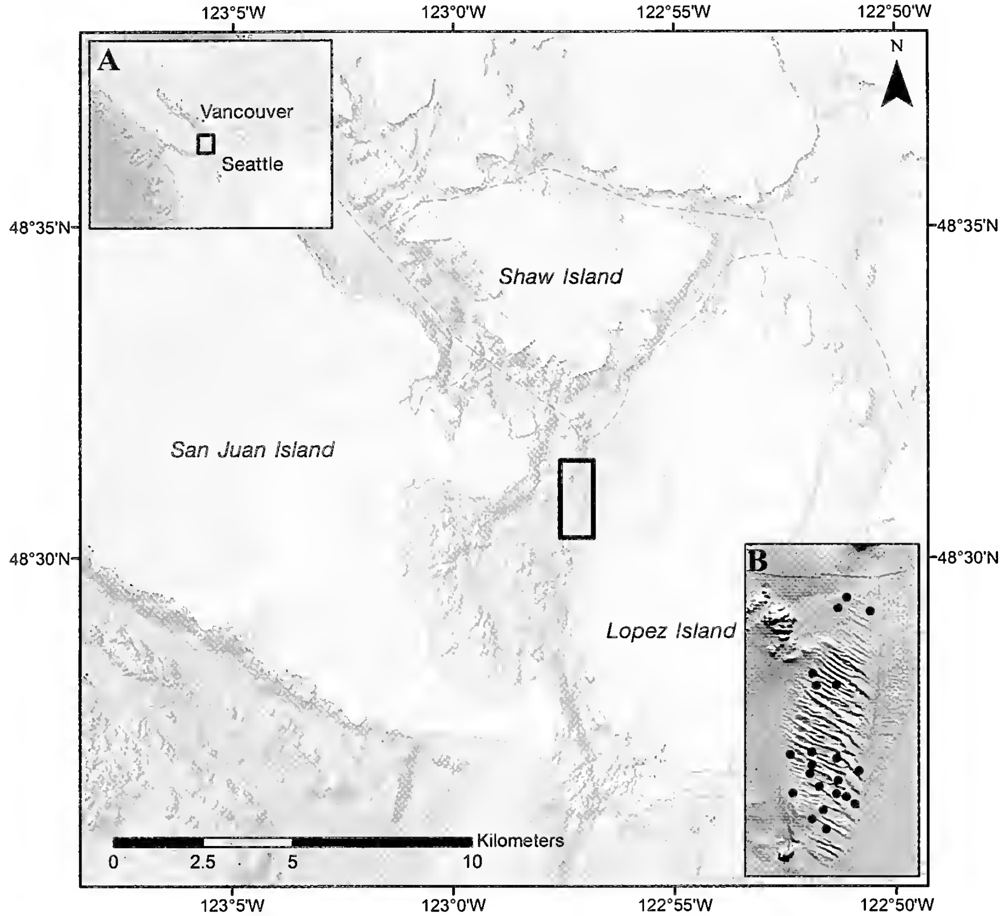

Figure 1

Location of field effort to sample Pacific sand lance (*Ammodytes personatus*) in the San Juan Channel, Washington, in 2006. (**A**) The inset map in the top left corner shows the general location of the study site among the San Juan Islands (black box) at the Pacific border between the United States and Canada (dashed line). The main map highlights the main onshore and offshore features of the region, including the location of the San Juan Channel and the sand wave field (black box) where field collections were focused. The other (**B**) inset map provides a multibeam image of the San Juan Channel sand wave field; black dots indicate the sites where Van Veen grabs (*n*=42) were made.

tribution, not fish distribution. Habitat quality, therefore, was considered to be consistent across the entire known depth range of occurrence of Pacific sand lance. Substrate data were converted into 3 potential habitat categories (preferred, suitable, unsuitable) on the basis of a synthesis of laboratory and field results.

Morphology

A neotype of *A. personatus* in the University of Washington Burke Museum collection was scanned with 5.6-μm resolution on a SkyScan 1173 micro-CT scanner (Bruker AXS GmbH, Karlsruhe, Germany) at the Karel F. Liem Bioimaging Facility at Friday Harbor Laboratories. Cross-sectional 2-dimensional images (i.e., slice data), generated across the 3-dimensional volume of the fish, were reconstructed and rendered in Amira software (FEI Co., Hillsboro, OR), and the image stack was uploaded to MorphoSource (website) as an open access resource (Godersky and Summers, 2016). A 5-mm-by-10-mm section of skin from the lateral aspect of the body, just behind the opercular cover, was excised, dehydrated in ETOH, and dried according to the critical point drying method. The skin sample was then sputter-coated with gold-palladium and visualized with a JCM-5000 NeoScope scanning electron microscope (SEM) (JEOL, Ltd., Tokyo). Digital images were made from the combined data of the secondary electron and backscatter electron detectors. To identify structural and morphological characteristics that may contribute to burrowing success, CT- and SEM-generated images were inspected.

Table 2

Results of experiments with paired sediment types in relation to the burrowing activity of Pacific sand lance (*Ammodytes personatus*). Experiments were conducted in 2010 and 2012. Data were calculated by using replicated *G*-tests of goodness of fit. Comparisons were made between medium sand (MS), coarse sand (CS), very coarse sand (VCS), and very fine gravel (VFG). In all comparisons, the grain size that contained the greater number of buried fish is listed first. The number of experimental trials (*N*) and the number of trials in which dominant grain size had significantly more buried fish (*N**) are given. The power of the test for each nonsignificant result is provided in parentheses.

			Total		Pooled		Heterogeneity	
Comparison	*N*	*N**	*G*	*P*	*G*	*P*	*G*	*P*
Diurnal								
CS–MS	6	5	94.31	<0.001	75.46	<0.001	18.85	0.002
VCS–MS	5	5	166.48	<0.001	152.39	<0.001	14.09	0.007
VFG–MS	6	4	29.28	<0.001	13.94	<0.001	15.34	0.009
CS–VCS	8	5	75.01	<0.001	11.78	<0.001	62.23	<0.001
CS–VFG	8	6	126.73	<0.001	77.54	<0.001	49.19	<0.001
VCS–VFG	8	2	69.33	<0.001	<0.01	0.957 (5)	69.33	<0.001
Nocturnal								
CS–MS	8	6	79.31	<0.001	73.76	<0.001	5.55	0.593
CS–VCS	8	0	7.98	0.435 (48)	0.22	0.636 (8)	7.76	0.354 (49)

Results

Laboratory experiments

Pacific sand lance were capable of burrowing into all 7 provided sediment types (see Table 1). However, in the smaller grain sizes (silt–fine sand), motility was considerably reduced and fish soon reoriented themselves so that their heads were exposed. Large individuals, corresponding to adult sizes, were more likely to burrow into the larger grain sediments, whereas more small individuals (juveniles) penetrated the smaller grain sediments.

During daylight hours, Pacific sand lance preferred coarse sand to all other sediment types (total *G*-value, pooled *G*-value; Table 2). Very coarse sand was selected significantly more than medium sand and significantly more than very fine gravel on the basis of the total, but not pooled, *G*-test. Correspondingly, only 2 individual tests yielded significant results and supported a preference for very coarse sand over very fine gravel, and the total number of buried individuals was nearly identical (n=174 and n=173, respectively). A significantly greater number of fish burrowed into very fine gravel than into medium sand (total *G*-value, pooled *G*-value; Table 2), which was largely avoided in all trials. Individual *G*-tests generally were consistent with the results from total and pooled tests; however, heterogeneity *G*-tests for all paired-preference trials indicated significant variation among individual trials in the observed ratio of burrowed individuals (Table 2).

At night, Pacific sand lance maintained a strong preference for coarse over medium sand, but no preference was found for either coarse sand or very coarse sand (Table 2). The total number of fish buried in coarse (n=147) and very coarse (n=139) sand was similar at night, and no individual tests revealed a significant preference for either grain size. The power to detect a significant difference was, however, low for these comparisons (Table 2). For both paired trials, results of the heterogeneity *G*-test indicated that results of individual experiments were consistent (Table 2). Night experiments were restricted to sediment types that directly bounded the preferred grain size (coarse sand) determined from daytime experiments (Table 1). Therefore, very fine gravel was not included in night experiments.

The number of buried individuals among (pooled) paired-sediment preference trials further supports a preference for coarse sand over other sediment types but indicates that results of individual trials are variable. The median number of buried individuals and intertrial variability were similar for comparisons of very coarse sand and very fine gravel (daytime) and of coarse sand and very coarse sand (nighttime) (Fig. 2). Correspondingly, results of pooled *G*-tests were insignificant for these comparisons (Table 2). The greatest median number of buried fish was observed for very coarse sand, from trials in which it was paired with medium sand during the day. The most highly variable result was also that of very coarse sand (range of nearly 40 individuals), from its paired trials with very fine gravel during daytime hours (Fig. 2). The most pronounced grain-size preferences were observed between coarse sand and very fine gravel (daytime), very coarse sand and medium sand (daytime), and coarse sand and medium sand (daytime and nighttime) (Fig. 2). Obvious preferences of grain size, therefore, were evident when

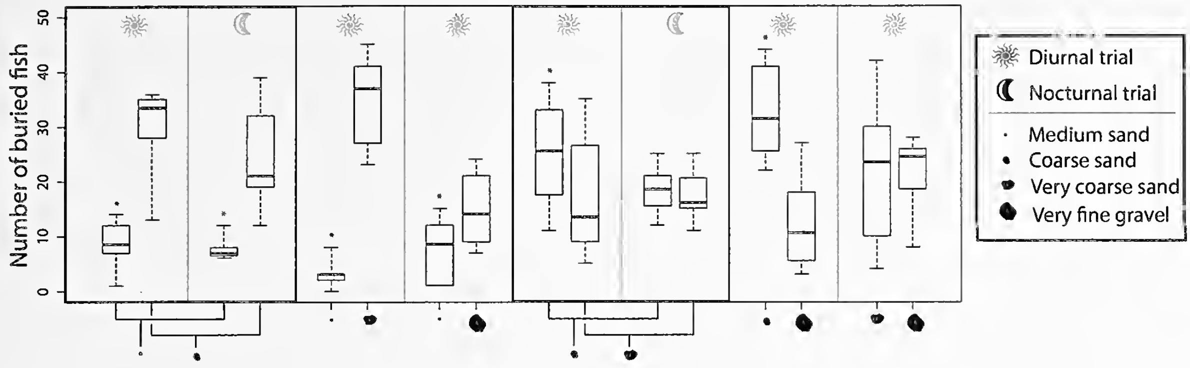

Figure 2

Box-and-whisker diagrams displaying results of pooled *G*-tests for each paired sediment preference trial conducted with Pacific sand lance (*Ammodytes personatus*) in 2010 and 2012. Displayed are the minimum and maximum values (whiskers), first and third quartiles (box), and median values (bold horizontal line).

all trials were considered for a comparison, but preferences were not generally consistent among trials.

The average proportion of Pacific sand lance buried among pooled experimental trials was greatest for coarse sand (Fig. 3). Among diurnal trials, the proportion of individuals buried in coarse sand was nearly 20% greater than expected by chance. Very coarse sand and very fine gravel were selected at ratios that were very close to the expected 1:1, whereas medium sand was selected in only about 20% of pooled trials (Fig. 3). Coarse sand also had the greatest average proportion of individuals buried among nocturnal trials, but the preference for this grain size was somewhat reduced compared with results from diurnal trials (Fig. 3). A similar situation was observed for very coarse sand, whereas the opposite trend was observed for medium sand (Fig. 3). Variability in the average proportion of buried sand lance among experimental trials was pronounced during diurnal trials but reduced in relation to medium sand and very coarse sand at night (Fig. 3).

Compacted sediments of all grain sizes required significantly more force to penetrate than did uncompacted sediments, as indicated by values for the mean slope of penetration depth (measured in millimeters) in relation to log force (measured in Newtons): compacted=0.071 (standard deviation [SD] 0.015), uncompacted=0.061 (SD 0.014) (Table 3). However, the mean percentage of buried fish in uncompacted (20.8% [SD 4.8]) and compacted (21.5% [SD 5.2]) coarse sand was similar. Correspondingly, no significant differences were detected in the selectivity for these substrates (total test: G=8.19, P=0.22; pooled test: G=8.12, P=0.80; heterogeneity test: G=0.06, P=0.15, N=6). The power to detect a difference in selectivity was low, however, especially for the heterogeneity test (total=58, pooled=57, heterogeneity=5). In comparisons of burrowing force among grain sizes, smaller grain sizes generally required more force to be penetrated regardless of compaction level (mean slope: very fine gravel=0.052 [SD 0.007], very coarse sand=0.059 [SD 0.001], coarse sand=0.075 [SD 0.008], medium sand=0.078 [SD 0.017]; Table 3). The interaction between sediment size and compaction was not significant (P=0.17).

Field collections

Field collections in the San Juan Channel yielded 421 Pacific sand lance during 21 daytime and 21 nighttime samples collected with a Van Veen bottom grab. Fish abundance, and therefore density, varied considerably among grab samples collected during both time periods. During the day, Pacific sand lance were caught in every grab, with a range of 2–62 individuals/grab and a median value of 13 individuals/grab (quartile 1 [Q1]=6, Q3=18). By contrast, Pacific sand lance were caught less frequently (66.7% of grabs, n=14) and in lower abundance (range=0–26 individuals, median=3, Q1=0, Q3=11) at night. Between paired samples collected at the same locations, a significantly greater mean number of individuals was collected during the day than during the night (day=14.4 individuals [SD 13.2]; night=5.7 individuals [SD 7.0]; t=2.47, df=20, P=0.023). Consequently, the density of buried fish among these grabs also was much greater during the daytime (119.8 individuals/m^2 [SD 110.0]) than during nighttime (47.7 individuals/m^2 [SD 58.7]).

The deepwater population of Pacific sand lance in San Juan Channel and of subtidal individuals used in laboratory experiments were consistent in size and maturity stage. The mean size of fish captured at San Juan Channel was 8.5 cm TL (SD 0.6) (range=6.5–12.3 cm TL, n=415), and 92.7% of all sampled fish (n=356) were determined to be immature on the basis of internal inspection. Mean sizes and maturity stage, the determination of which requires sacrificing individuals, were not recorded for laboratory specimens col-

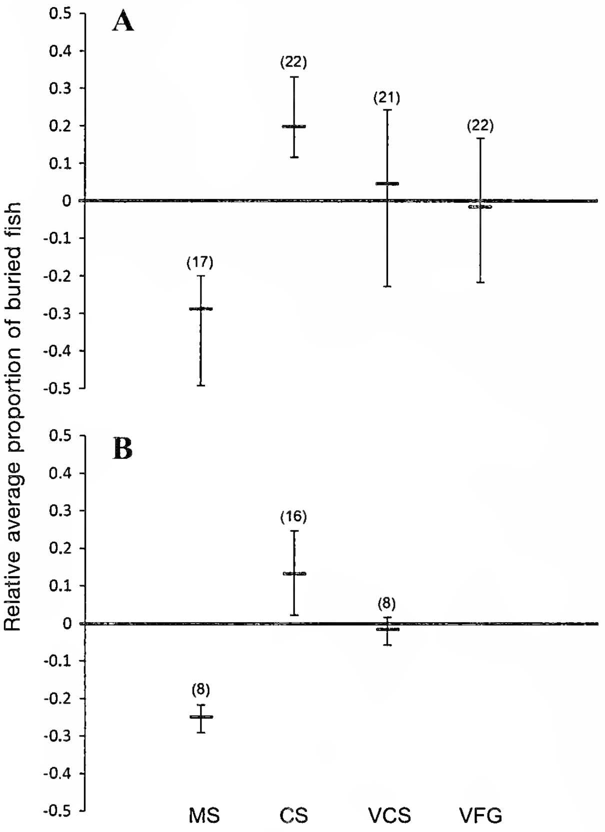

Figure 3

Average proportion of Pacific sand lance (*Ammodytes personatus*) buried in each grain size (medium sand [MS], coarse sand [CS], very coarse sand [VCS], and very fine gravel [VFG]) in relation to all grain sizes for (**A**) diurnal and (**B**) nocturnal trials conducted in 2010 and 2012. Horizontal bars depict the average deviation from the expected 1:1 ratio, and the zero line, therefore, is equivalent to a proportion of 0.50. The number of replicates for each grain size is indicated in parentheses. Error bars represent first and third quartiles.

lected from intertidal and subtidal depths at Jackson Beach; however, the size range (8.5–11.0 cm TL) that was used was skewed toward smaller specimens. This size range was chosen to correspond to large subadult specimens—a supposition that was validated through field sampling.

The sediment composition of the sampled sand wave field consisted primarily of coarse sand (51.4%) and similar proportions of very coarse sand (14.5%), gravel (16.2%), and medium sand (17.7%) (n=42 grab samples). Very coarse sand and medium sand never contributed >33% to sample weight, but 8 samples were composed of at least one-third gravel (Fig. 4). Fine sand (0.2%) and silt (0.1%) contributed trivial amounts to overall sample weight. No grain sizes larger than that of gravel were encountered. Linear regression indicated no relationship between fish abundance and the proportions of medium sand (t=0.718, P=0.477, coefficient of correlation [r^2]=0.01, df=40), coarse sand (t=0.365, P=0.717, r^2=0.02, df=40), very coarse sand (t= −0.079, P=0.717, r^2=0.02, df=40), or gravel (t= −0.553, P=0.583, r^2=0.01, df=40) (Fig. 4). However, limited variability in sample composition may have influenced results (Fig. 4).

Table 3

Mean slope of the relationship between log force (measured in Newtons) and penetration depth (measured in millimeters) determined from models of different sediment types used by Pacific sand lance (*Ammodytes personatus*), including uncompacted (U), compacted (C), very fine gravel (VFG), very coarse sand (VCS), coarse sand (CS), and medium sand (MS). The number of experimental trials (N) and standard deviation of the mean (SD) are given.

Sediment	N	Slope	SD
U	20	0.061	0.014
C	20	0.071	0.015
VFG	10	0.052	0.007
VCS	10	0.059	0.001
CS	10	0.075	0.008
MS	10	0.078	0.017

Habitat mapping

The relative amount of preferred habitat for Pacific sand lance (i.e., sand; coarse sand, very coarse sand, and gravel; and sand wave fields), varied among 3 important commercial fishing grounds of southeast Alaska (Fig. 5). Preferred habitat types were predicted to be extremely rare at Cape Ommaney (1.9% of the total mapped seafloor, 168.9 km^2) and uncommon at Fairweather Ground (13.8% of the total mapped seafloor, 288.0 km^2) but common at Edgecumbe (36.1% of the total mapped seafloor, 538.2 km^2). The total amount of potential habitat, consisting of habitat types that were preferred and suitable (i.e., sand mixed with mud, pebble, boulder, or rock outcrops) was greatest at Fairweather Ground (66.9%) and similar at Cape Ommaney (39.4%) and Edgecumbe (39.0%). Nearly all unsuitable habitats (i.e., grain sizes ≤3.9 µm [silt] or >4.0 mm [pebble]) in all regions were composed of rocky substrates or sediment of large grain sizes (Edgecumbe: 100% of 328.4 km^2; Cape Ommaney: 95.7% of 102.3 km^2; and Fairweather Ground: 100% of 95.4 km^2).

Description of morphological features

Images of the cranial and axial skeleton were produced with CT scans of the Pacific sand lance (Fig. 6, A–C). The dermatocranial elements are thin and lightly mineralized. The parasphenoid and occipital bones are well

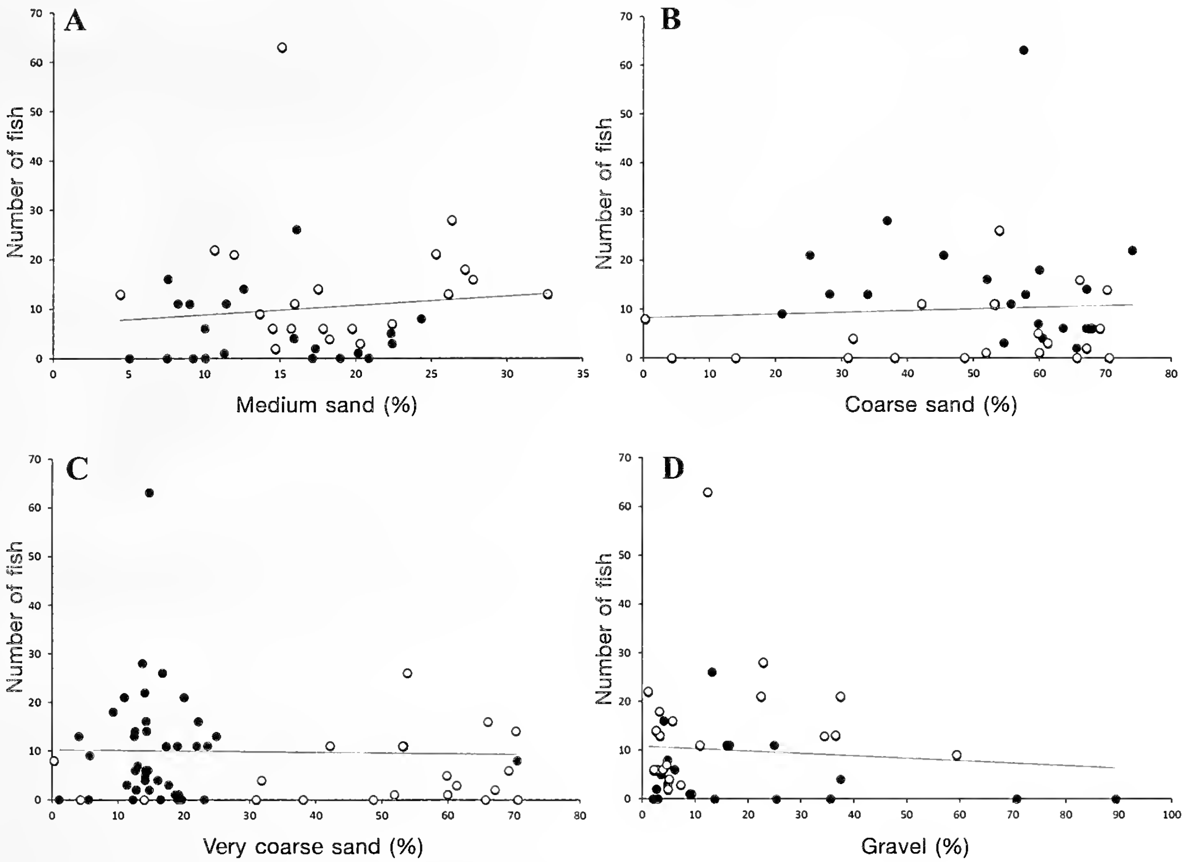

Figure 4

Relationship between percentage of grain size—(**A**) medium sand, (**B**) coarse sand, (**C**) very coarse sand, (**D**) gravel (after Wentworth, 1922)—and number of Pacific sand lance (*Ammodytes personatus*) captured during day (white dots, n=21) and night (black dots, n=21) field collections conducted in the San Juan Channel, Washington, in 2006.

mineralized but are not extremely dense. The skull of the Pacific sand lance does not exhibit a reduced number of bones, nor is there fusion of dermal roofing elements (Fig. 6, A–C). The lower jaw and premaxilla are well mineralized, and there are anterior flanges at the symphysis of the dentaries. These flanges are located at the most anterior part of the skeleton and would contact the substrate first in head-first burrowing. Analysis of the images from the SEM confirmed that the body scales are fused into bands oriented slightly obliquely to the dorsoventral axis (Fig. 6D). The spacing of these bands is approximately 0.25 mm.

Discussion

In laboratory trials, the Pacific sand lance showed remarkable habitat specificity, consistently choosing to burrow into coarse sand sediment, a grain size that spans a mere 0.50 mm. These findings refine those of a prior study in pinpointing the preferred burial grain size for this important forage species. Pinto et al. (1984) found no difference in preference between mixtures of fine–medium and coarse–very coarse sand in a similar laboratory experiment. However, Pinto et al. (1984) maintained seawater flow to the benthos during trials, and they found that significantly more fish buried in sediment types that were oriented nearest to the incoming water than in those types that were more distant. The amount of porewater exchange in sediments, and therefore of oxygen input, is an important aspect of burial habitat of *Ammodytes* species (Robards and Piatt, 1999; Høines and Bergstad, 2001; Behrens et al., 2007). It is likely that the permeability of smaller grain sizes (e.g., fine to medium sand), which allow oxygen penetration of only a few millimeters under quiescent conditions (de Beer et al., 2005), was enhanced in the Pinto et al. (1984) study because of a continuous flow of benthic seawater. On the basis of our compaction results, smaller grain sizes are easier to penetrate for Pacific sand lance. Therefore, if oxygen is not a limiting factor, habitat preferences will probably shift to include smaller grain sizes (e.g., Pinto et al., 1984). By contrast, if oxygen is limiting, preferences will probably

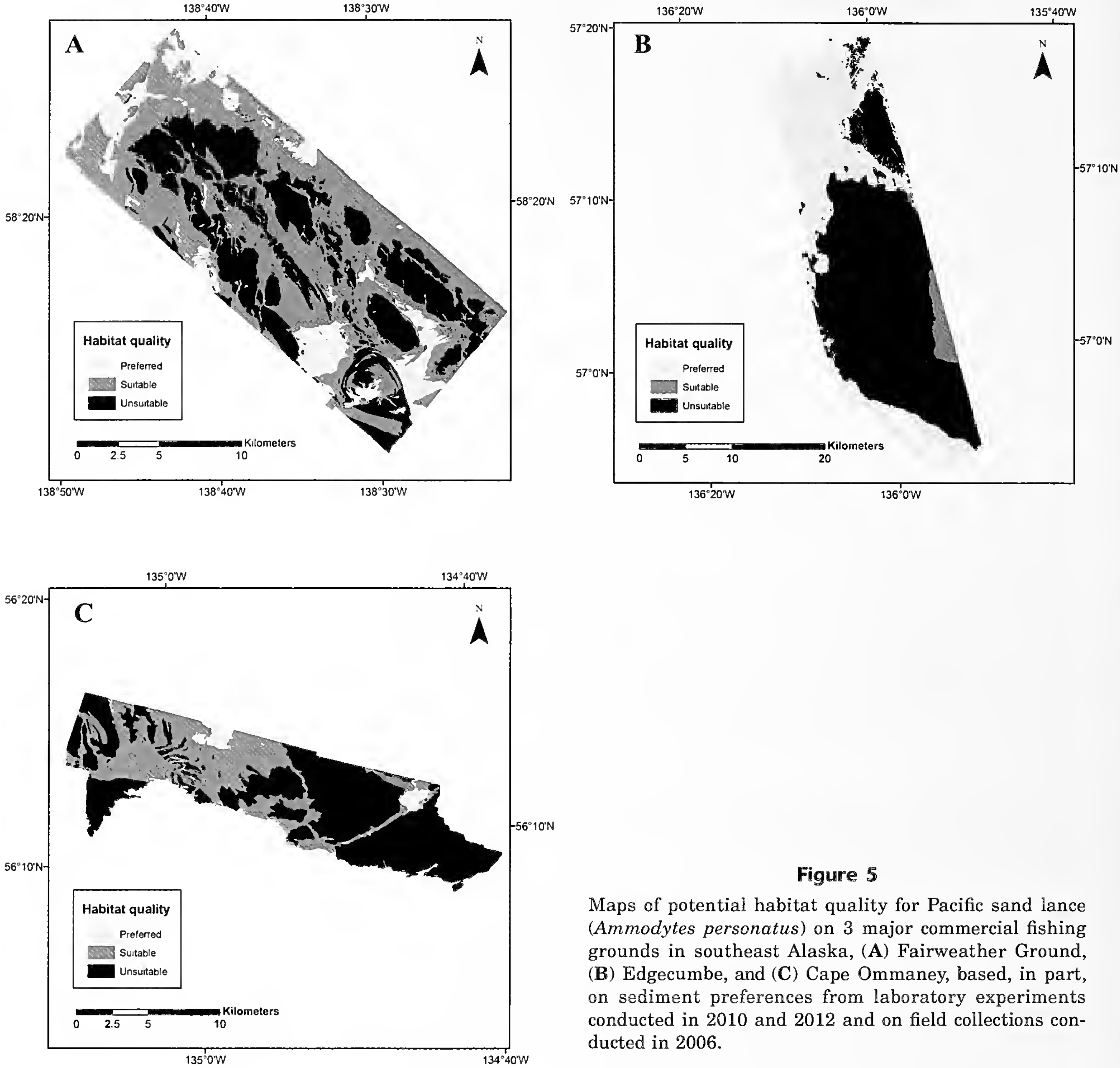

Figure 5

Maps of potential habitat quality for Pacific sand lance (*Ammodytes personatus*) on 3 major commercial fishing grounds in southeast Alaska, (**A**) Fairweather Ground, (**B**) Edgecumbe, and (**C**) Cape Ommaney, based, in part, on sediment preferences from laboratory experiments conducted in 2010 and 2012 and on field collections conducted in 2006.

shift to larger grain sizes that enable a greater degree of advective porewater exchange.

Field surveys of subtidal and inner-continental-shelf regions have also provided evidence of the association of Pacific sand lance primarily with coarse sand habitats (this study; Holland et al., 2005; Haynes et al., 2007) Coarse sand was the dominant sediment type in the San Juan Channel sand wave field, where the Pacific sand lance was common and abundant; however, grain-size composition was relatively consistent among samples. Although the homogeneity of such samples limits our conclusions on preferred field sediments, other research has associated late life stages of *Ammodytes* species primarily with coarse sands. For example, free-swimming juvenile and adult Pacific sand lance off British Columbia aggregate over a mixture of coarse and very coarse sand (Haynes et al., 2007). Holland et al. (2005) determined that late-juvenile sand eel in the North Sea select subtidal burial habitats consisting of medium and coarse sands, or those with moderate levels of fine gravel, and avoid habitats characterized by coarse gravel, silt, and fine sand or sites with high or low levels of fine gravel.

Uniform grain sizes were used in the laboratory to simplify comparisons of preferred sediments and to better understand their link to potential structural and morphological adaptations for burial. The Pacific sand lance has been associated with mixed sediments in sub-

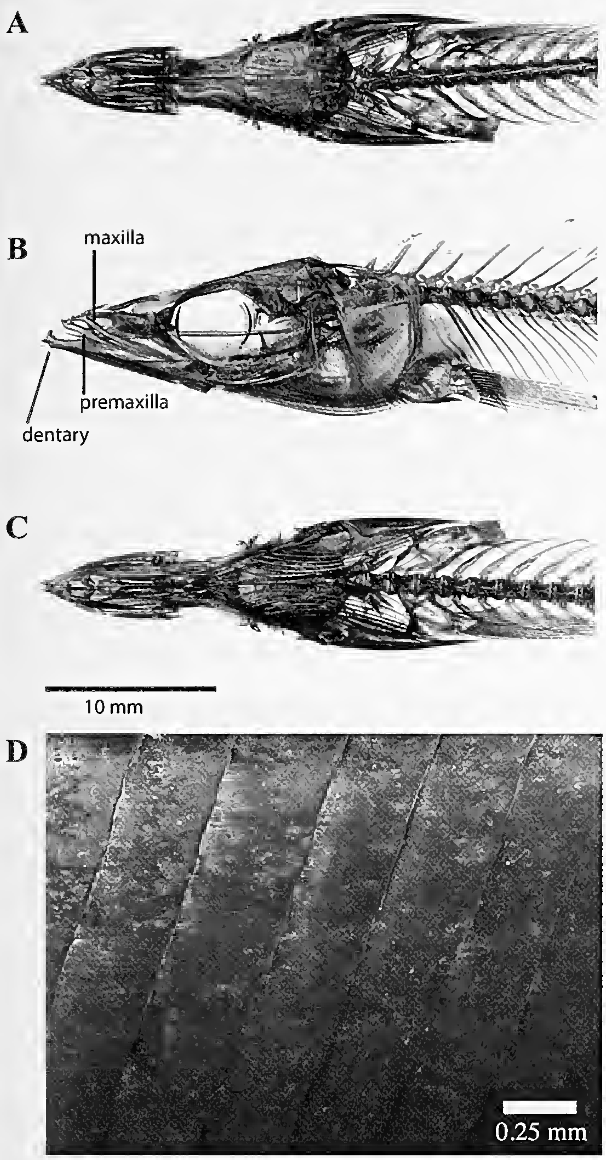

Figure 6

Images of the skull of a Pacific sand lance (*Ammodytes personatus*), (**A**) dorsal, (**B**) lateral, and (**C**) ventral, taken by CT scan, and (**D**) image of body scale pattern taken with a scanning electron microscope.

tidal (Haynes et al., 2007) and inner-continental-shelf (this study) regions, where uniform sediment types are uncommon; however, in both types of regions, coarse sands were dominant among heterogeneous sediment types. Furthermore, habitat associations of Pacific sand lance may indicate sediments that are used for foraging and for predator avoidance, in addition to those used for burial purposes (Hobson, 1986; Johnson et al., 2008; Robinson et al., 2013). Whereas swimming Pacific sand lance aggregate over a variety of substrate types as well as in open water, they consistently use habitats that are dominated by coarse sands for burial purposes.

Marine sand wave fields appear to serve as an important deepwater habitat refuge for Pacific sand lance. Sand wave fields are found on the continental shelf and inland waters in areas with a nearby source of sediment and a narrow range of high current velocities that remove the smaller clast sizes without carrying in larger gravels (Barrie et al., 2009). Their occurrence is therefore limited, but these seafloor features are more stable than intertidal and subtidal habitats of Pacific sand lance—habitats that are typically dynamic and often ephemeral (Parks et al., 2013). Our data indicated that preference for this comparatively rare substrate probably results from the typically high proportions of medium to coarse sand and consistent bottom currents that provide a steady flow of oxygen to unconsolidated substrates. More research incorporating a variety of grain sizes and seabed features is necessary to better determine the importance of sand wave fields as habitat of Pacific sand lance and to identify the characteristics that drive this association.

Potential burial habitat appears to be much broader than realized burial habitat for Pacific sand lance. It is well established that *Ammodytes* species prefer well-drained sediments (Robards and Piatt, 1999). Therefore, relatively small grain sizes that lack sufficient interstitial spaces for water flow are mainly avoided. In our laboratory experiments, most fish remained swimming when presented with uniform silt or fine sand substrates, and those fish that burrowed soon reoriented themselves so that their heads were exposed. This behavior also has been reported for the lesser sand eel (*A. tobianus*) under laboratory conditions. When subjected to decreasing sedimentary oxygen levels from normoxic to anoxic in fine sand, individual lesser sand eels moved closer to the sediment surface, eventually exposing their heads to facilitate respiration (Behrens et al., 2007). Pacific sand lance universally rejected silt when presented with other burial sediments (Pinto et al., 1984). The sand eel avoids sediments that contain >10% silt or clay, and variation in its abundance has been negatively associated with the amount of sedimentary silt (Wright et al., 2000). Grain sizes smaller than medium sand generally are too poorly oxygenated to enable protracted burial by the Pacific sand lances and other *Ammodytes* species; therefore, respiratory tolerances influence sediment choice and limit the realized burial habitat of species of *Ammodytes*.

An upper boundary of grain size for burial of Pacific sand lance is more difficult to establish but appears to fall within the size range of gravel. A positive association between length and grain size was reported for the sand eel (Holland et al., 2005) and is supported by the results of our experiments on burial capability of Pacific sand lance. Some possible explanations for this association are that larger subadult and adult specimens, compared with smaller juvenile specimens, 1) can more easily generate the force necessary to penetrate gravels, 2) have greater respiration demands and require more well-drained sediments, and 3) are less susceptible to abrasion. The largest grain size used in this study, very fine gravel, was not commonly selected; however, the Pacific sand lance is able to use uniform very fine gravel (this study) and a mixture of very fine-to-medium gravel for long-term burial (Pinto et al., 1984). The force required or damage incurred by burial in increasingly coarse sediment will outweigh the benefit of increased water flow at some tolerance point, above which larger grain sizes will be avoided. Coarse sand with pebbles and fine–medium gravel have been reported as burial habitat for *Ammodytes* species (Robards and Piatt, 1999; Holland et al., 2005), but sediments dominated by coarse gravel, pebble, or cobble may represent such a boundary. More research is needed to determine the largest grain size or sizes of sediment that Pacific sand lance can use as burial habitat and how this tolerance varies with life stage and oxygen level.

Although clear sediment preferences were established, there was substantial variability in results among experimental trials that appears to be related to behavior. Even in trials where grain preference was marked, there was considerable inter-trial variability in the total number of fish that buried themselves. Additionally, the relative proportion of buried fish was highly variable among trials involving increasing grain sizes from coarse sand–very fine gravel. Inter-trial variability was greatest between coarse sand and very coarse sand at night, possibly because it is more difficult for Pacific sand lance to distinguish between similar sediment types without visual cues. As reported by Pinto et al. (1984), Pacific sand lance did not burrow en masse. Instead, individuals buried themselves at different times and sometimes switched sediment types during a trial. Variability in burial preference among trials therefore seems to be related to the individual differences in behavior. This variability appears to be enhanced when sediment types of comparable preference are available.

Results of day and night experiments and field collections generally ran counter to the established diurnal burial pattern of *Ammodytes* species. *Ammodytes* species seek refuge in the seafloor at night to avoid predators, and they are typically active during the day (Robards and Piatt, 1999). However, when food is scarce (Winslade, 1974) or predators are active (Hobson, 1986), species of *Ammodtyes* also will burrow during the daytime. No food was provided to Pacific sand lance during trials, and the tank enclosure did not represent typical daytime feeding habitat, which is open water. A high proportion of Pacific sand lance may, therefore, have buried themselves during daylight hours to conserve energy or to reduce stress. Field collections indicated that substantially more Pacific sand lance were buried during the day than at night. This result may be an artifact of limited temporal and spatial sampling. Distribution of Pacific sand lance is known to be patchy (van der Kooij et al., 2008), and perhaps too few samples were collected to capture this spatial variability. There also may have been additional considerations, such as current flux or predator dynamics that influence the relative number of day and night burials. It also is possible that the diurnal pattern of habitat use that has been established for *Ammodytes* species is more complex or not directly attributable to Pacific sand lance. More research is needed to determine day and night use of deepwater habitats by Pacific sand lance.

Because of the importance of Pacific sand lance to regional trophic dynamics and the potential for anthropogenic disturbance of important burial grounds, determining and conserving preferred habitats of Pacific sand lance may be a future management consideration. There is no active fishery for Pacific sand lance in U.S. waters, and the main drivers of abundance of *Ammodytes* species are environmental conditions and density dependence during early life stages (Arnott et al., 2002). However, fishing activities (especially bottom trawling) can change substrate composition, primarily by decreasing complexity and fluidizing sediment, generally leading to a proliferation of smaller grains sizes (e.g., muds instead of sands) (Auster et al., 1996). Other anthropogenic disturbance (e.g., dredging and sediment mining) also may alter sediment composition, reduce the amount of preferred sediment types, or cause direct mortality to populations of Pacific sand lance (Eleftheriou and Robertson, 1992).

The field techniques described in our study can be used with data that have been previously collected, newly accumulated from seafloor imagery, or bottom-sampled in areas of interest to create and evaluate maps of potential habitat. Crucial habitats of Pacific sand lance can then be identified and conserved through establishment of marine protected areas or other no-take zones. This type of applied research is currently being conducted on populations of Pacific sand lance in British Columbia (Robinson et al., 2013) and has important considerations for American populations of Pacific sand lance off Washington and in the Gulf of Alaska.

Different geological characteristics explained the variable amount of potential preferred and suitable habitat among fishing grounds in southeast Alaska. Preferred habitat for Pacific sand lance was predicted to be most abundant at Edgecumbe, where sand surrounds a recent lava flow in deep water (>120 m; Greene et al., 2007a). Small pockets of sand and gravel, including sand waves, occur on Fairweather Ground, as do a majority of sandy sediments (Greene et al., 2007b). These sands and gravels have been eroded from the extensive sandstone on the Fairweather Ground, and they account for the greatest amount of predicted preferred and suitable habitat among locations. Some of the preferred habitat of Pacific sand lance at Fairweather Ground is associated with a sand wave field that has been formed by an underlying syncline (Greene et al., 2007b). Dense aggregations of Pacific sand lance have been observed on this feature (Greene[3]), lending support to our predictions of preferred habitat. Much of the seafloor at Cape Ommaney consists of either rock, a mixture of granite and sand, or large, unconsolidated

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[3] Greene, H. G. 2010. Personal commun. Friday Harbor Laboratories, Univ. Wash., Friday Harbor, WA 98250.

grain sizes (gravel–cobble). Therefore, preferred habitat was predicted to be scarce at Cape Ommaney.

Overall, preferred habitat of Pacific sand lance was limited on the fishing grounds we examined off southeast Alaska. This result is not surprising because rock-associated species (e.g., tiger rockfish [*Sebastes nigrocinctus*]; yelloweye rockfish [*Sebastes ruberrimus*]; lingcod [*Ophiodon elongates*]) are the main targets of regional fisheries (Greene et al., 2011). However, Robinson et al. (2013), using a habitat suitability model that incorporated several physical variables, indicated that only 6% of the study region in the Strait of Georgia was suitable habitat for Pacific sand lance. Our predictions, therefore, probably overestimate the amount of preferred and suitable habitat types on fishing grounds of southeast Alaska. The amount of preferred and suitable burial grounds would probably be reduced if more factors, such as depth and current speed, were considered in addition to grain size.

Our day and night experiments indicated that the Pacific sand lance does not rely exclusively on visual cues to assess habitat quality and that this species chooses sediments that are more difficult to penetrate in order to access preferred grain sizes. Some visual assessment of the substrate is supported by the equivalence of coarse and very coarse sand in night trials, but we have no explanation for the decrease in the variability in choice of sediment at night. Fish were often observed "nosing" the sediment before burrowing, and they may use tactile cues to determine grain size. We suppose that coarse sand provides a desired combination of ease of penetration and interstitial space that represents the best means for reducing the energetic costs of burrowing while maintaining access to oxygenated waters. This supposition was supported by our anecdotal observation that in medium sand and smaller grain sizes, fish often reoriented themselves after burrowing so that their entire head was exposed. Larger grain sizes may be too difficult to penetrate for subadult Pacific sand lance or may abrade the skin.

Burrowing in terrestrial environments usually is associated with elongation of the body, reduced eyes and limbs, and well-ossified skulls with fused elements (i.e., Summers and O'Reilly, 1997; Lee, 1998). Although the Pacific sand lance does have an elongate body, a small cross section, and fins that lie flat, it otherwise has little in common with typical burrowers. The Pacific sand lance has prominent, large eyes, its skull is complex, and no mineralization is evident in several areas, including the dorsal region caudal to the maxilla. The lower jaw is well mineralized, but no more so than in fish species that do not burrow. Nevertheless, individuals of this species are able to bury themselves rapidly and in a wide variety of sediments. They can burrow for several body lengths and spend considerable periods of their lives under the substrate. There is evidence that they do not fluidize the sand in advance of penetration—a behavior that would have explained what appears to be poorly adapted morphological features for burrowing (Gidmark et al., 2011).

We are left to remark upon the sole morphological oddity for which we can imagine a burrowing function. The scales of the Pacific sand lance, and those of all species of *Ammodytes*, are unusual with respect to other fishes in that they are fused into bands that run roughly dorsoventrally. These bands are of taxonomic interest, although no function has ever been ascribed to them (Orr et al., 2015). We propose that the spacing of the scale bands interacts with the grain size of the substrate to produce a reduced frictional interaction between the fish and the sand. Indeed, movement was observed to be considerably more rapid for subadult Pacific sand lance in saturated coarse sand than in other grain sizes. Our supposition should be testable with nanoscale accurate replicas of the Pacific sand lance and may be modeled with contact mechanics in an unusual low-force regime (Persson and Scaraggi, 2014). Because of the variation in scale spacing on the body in different *Ammodytes* species and the change in spacing as an individual grows to maturity, the implication of a friction reducing system is that habitat preference of sand lances should vary with spacing on inter- and intraspecific bases.

Other habitat characteristics (e.g., depth, current velocity, oxygen content, particle sorting) are important, but none is a more fundamental driver of distribution and abundance of Pacific sand lance than grain size. Considerable laboratory and field evidence indicates that coarse sand sediments and mixtures of sediment that are dominated by coarse sand are preferred burial habitats for Pacific sand lance in the subadult and adult life stages from intertidal depths to at least 80 m. Use of deepwater habitats, especially sand wave fields, are poorly understood but should be further evaluated because seafloor structure, current regimes, and other physical factors play an unknown role in habitat selection of Pacific sand lance. Determination of sediment preferences of the Pacific sand lance will be important for potential habitat-based management of this important forage species because trawling, dredging, and other anthropogenic disturbances may modify its preferred habitat. A link between form and function has been established for Pacific sand lance, in that this species appears to have a scale pattern that is adapted for rapid burial in coarse sand. Future research should focus on investigating the spacing of scale bands on the body for different sizes and species of *Ammodytes* and the role of such spacing in reducing friction during burial.

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Long-term site fidelity of endangered smalltooth sawfish (*Pristis pectinata*) from different mothers

Gregg R. Poulakis (contact author)[1, 2]
Philip W. Stevens[3]
Amy A. Timmers[1]
Christopher J. Stafford[3]
Demian D. Chapman[4]
Kevin A. Feldheim[5]
Michelle R. Heupel[6, 7]
Caitlin Curtis[3]

Email address for contact author: gregg.poulakis@MyFWC.com

[1] Fish and Wildlife Research Institute
Florida Fish and Wildlife Conservation Commission
Charlotte Harbor Field Laboratory
585 Prineville Street
Port Charlotte, Florida 33954

[2] Department of Marine and Environmental Systems
Florida Institute of Technology
150 West University Boulevard
Melbourne, Florida 32901

[3] Fish and Wildlife Research Institute
Florida Fish and Wildlife Conservation Commission
100 Eighth Avenue SE
St. Petersburg, Florida 33701

[4] Institute for Ocean Conservation Science
School of Marine and Atmospheric Science
Stony Brook University
Stony Brook, New York 11794

[5] Pritzker Laboratory for Molecular Systematics and Evolution
The Field Museum
1400 S. Lake Shore Drive
Chicago, Illinois 60605

[6] Australian Institute of Marine Science
1526 Cape Cleveland Road
Cape Cleveland, Queensland 4810, Australia

[7] Centre for Sustainable Tropical Fisheries and Aquaculture
College of Marine and Environmental Sciences
James Cook University
1 Angus Smith Drive
Townsville, Queensland 4811, Australia

Abstract—Understanding how endangered species use nursery habitats is vital for recovery planning. Research on the smalltooth sawfish (*Pristis pectinata*) has shown that areas of estuarine nurseries, called *hotspots*, are used consistently. The objectives of our study were 1) to determine whether 10 young-of-the-year smalltooth sawfish in an artificial, non-main-stem portion (i.e., a seawall canal system) of a hotspot were descended from one or different mothers and 2) to document long-term habitat use by these individuals. At least 4 mothers contributed to the group, which comprised siblings, half-siblings, and unrelated individuals. Young sawfish exhibited site fidelity to their capture location, spending 61% of their time there. Continuous residency lasted as long as 86 days, but these fish made small-scale diel (<1 river km) movements between the capture location (day) and the nearby main-river-stem portion of the hotspot (night). Larger-scale (5–7 river km) downriver and upriver relocations between the capture location and the river mouth, including 2 other known natural hotspots, occurred after a tropical storm. This research shows that 1) young-of-the-year from different mothers can have high site fidelity at specific locations within a nursery hotspot and 2) these hotspots can be important for young-of-the-year even when there is a drastic change in freshwater inflow.

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Ecological niche theory (since Grinnell, 1917) and habitat suitability modeling (more recently) have been used to evaluate how the fitness of individuals is linked to their environment (Hutchinson, 1957; Guisan and Zimmermann, 2000). This concept is often extrapolated to the population level in an effort to understand how groups of individuals use their environment. According to a review by Hirzel and Le Lay (2008), the presence of a species depends on 3 location characteristics: 1) the location is accessible, 2) the local environment allows the population to grow (Grinnellian niche, Grinnell, 1917), and 3) interactions with other species (e.g., competition) allow the species to persist (Eltonian niche, Elton, 1927). Further, within a general location at which a species occurs on a variety of spatial scales, smaller portions of the total area (e.g., niche envelopes, cold-core eddies, nursery hotspots) are often more heavily used for reasons related to factors such as food availability, optimal temperature, and reduced predation risk (Jones, 2002; Polovina et al., 2006; Estók, 2007). In teleosts and elasmobranchs, niche characteristics often reflect an ontogenetic shift because the importance of different biotic and abiotic factors changes with growth (e.g., Stevens et al., 2007; Grubbs, 2010). For example, reducing predation risk is likely to be more important during early life history stages, whereas access to mates becomes more important for adults (Grubbs, 2010).

For the smalltooth sawfish (*Pristis pectinata*), which is listed as endangered under the U.S. Endangered Species Act and as critically endangered on the International Union of Conservation of Nature and Natural Resources IUCN Red List of Threatened Species, an understanding of fine-scale patterns of habitat use in the occupied nursery areas in south and southwest Florida has become a priority in recent years for researchers and fishery managers (Fed. Register, 2009; NMFS[1]; Norton et al., 2012). For example, research data and encounter reports from the public of encountering this species indicate that pregnant females enter nurseries briefly, to give birth, and that juveniles occupy the tidal portions of rivers, estuaries, and coastal bays for approximately their first 3 years (to ~2.2 m in total length) (Seitz and Poulakis, 2002; Simpfendorfer et al., 2008b, 2010), and larger juveniles and adults are typically found in open-water, marine habitats (Poulakis and Seitz, 2004; Carlson et al., 2014; Waters et al., 2014). Research in the Charlotte Harbor estuarine system has led to important insights into the ecology of juvenile smalltooth sawfish: 1) nursery habitats include main-stem and non-main-stem riverine habitats, and these habitats are used differently throughout ontogeny (Simpfendorfer et al., 2011; Poulakis et al., 2013); 2) juveniles have affinities for specific abiotic conditions during their nursery residency (Poulakis et al., 2011); and 3) responses of juveniles to freshwater inflow-related changes can be delayed (Poulakis et al., 2013).

Multiple lines of evidence, such as higher catch rates and large numbers of acoustic position estimates (estimates of the location of individuals in the study area based on data recorded on acoustic receivers), indicate that juvenile smalltooth sawfish occur at specific locations within estuarine nurseries, areas that are referred to as *hotspots* (Poulakis et al., 2011, 2013). However, we know little about how these sites are selected, the extent of site fidelity, or what factors influence site fidelity. A nursery hotspot may simply result from a single female giving birth at or near the site, and the young remain there for extended periods. Alternatively, the young may originate from different females that give birth at or near the site or throughout the nursery, and the young converge on or remain at a hotspot because it offers a fitness advantage.

Our objective was to use genetic information and acoustic monitoring of individuals captured at a discrete location within a hotspot to test 4 hypotheses regarding their relatedness and movements: 1) related and transient: multiple smalltooth sawfish were found at the location because one female gave birth at or near that location and the young remained there for a brief time period before permanently dispersing more broadly in the estuary; 2) related and resident: multiple smalltooth sawfish were found at the location because one female gave birth at or near that location and the young remained there for an extended time period; 3) not related and transient: multiple smalltooth sawfish were found at the location because multiple females gave birth at or near that location and the young remained there for a brief time period before permanently dispersing more broadly in the estuary; and 4) not related and resident: multiple smalltooth sawfish were found at the location because multiple females gave birth at or near that location and the young remained there for an extended time period.

Materials and methods

Study area

One of the largest estuaries in subtropical Florida, the 56-km-long 700-km^2 Charlotte Harbor estuary, is a recognized nursery for the endangered smalltooth sawfish (Seitz and Poulakis, 2002; Poulakis et al., 2011). In 2009, the National Marine Fisheries Service designated most of this estuarine system as 1 of 2 critical habitat areas for juveniles of this species (NMFS, 2009; Norton et al., 2012). To date, most research on smalltooth sawfish has been conducted in the Caloosahatchee River, which is in the southern portion of the estuary, where most of the historical sightings of smalltooth sawfish have been recorded in the Charlotte Harbor system (Seitz and Poulakis, 2002; Poulakis et al., 2011, 2013; Poulakis et al.[2]). The Caloosahatchee River contains 4 of the 5 hotspots where catch rates and the number of acoustic position estimates of smalltooth sawfish have been highest (Poulakis et al., 2011, 2013; Poulakis et al.[2]). Paradoxically, this estuarine river, located adjacent to the cities of Cape Coral and Fort Myers, is one of the most highly altered, flow-managed estuarine waterways in the current range of this species. The fifth hotspot is in the lower Peace River, in the northern portion of the estuarine system, which has a natural freshwater flow regime.

Freshwater inflow into the Caloosahatchee River is regulated by water-control structures (e.g., Franklin Lock) that the South Florida Water Management District uses to manage water levels in Lake Okeechobee,

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[1] NMFS (National Marine Fisheries Service). 2009. Recovery plan for smalltooth sawfish (*Pristis pectinata*), 78 p. Prepared by the Smalltooth Sawfish Recovery Team for the National Marine Fisheries Service, Silver Spring, MD. [Available at website.]

[2] Poulakis, G. R., P. W. Stevens, R. D. Grubbs, D. D. Chapman, J. Gelsleichter, G. H. Burgess, T. R. Wiley, J. A. Olin, L. D. Hollensead, A. T. Fisk, et al. 2014. Smalltooth sawfish (*Pristis pectinata*) research and outreach: an interdisciplinary collaborative program. Final report to the National Marine Fisheries Service for Award NA10NMF4720032. FWRI File-code F2858-10-14-F. 214 p. [Available at website.]

the largest lake in Florida (Stoker, 1992; Chamberlain and Doering, 1998; Barnes, 2005). The anticipation of increased rainfall during the wet season (June–November) or of tropical cyclones approaching the region often prompts water managers to release freshwater from Lake Okeechobee to preclude flooding of surrounding residential and agricultural areas. These freshwater releases occur mainly during the wet season but can also occur during the rest of the year. In general, such changes in freshwater inflow are known to influence habitat use by fish that reside in the tidal portion of the river, including juvenile smalltooth sawfish (Heupel and Simpfendorfer, 2008; Simpfendorfer et al., 2011; Poulakis et al., 2013; Stevens et al., 2013).

Shoreline habitats near the mouth of the Caloosahatchee River, where this study occurred, are fragmented and range from natural shorelines dominated by red mangrove (*Rhizophora mangle*) to extensive canal systems lined with concrete seawalls. In general, the shallow (depths <1 m) areas dominated by red mangrove have been identified as important habitats for juvenile smalltooth sawfish throughout the current range of this species (NMFS, 2009; Simpfendorfer et al., 2010; Poulakis et al., 2011; Norton et al., 2012).

Field sampling, acoustic equipment, and study development

This study occurred near the mouth of the Caloosahatchee River during 2006 and 2007 as part of a larger, ongoing research project on smalltooth sawfish. Smalltooth sawfish were sampled primarily with 152-mm stretch-monofilament-mesh gill nets (detailed gear descriptions and sampling protocols are available in Poulakis et al., 2011). Gill nets were soaked for 1 h and were checked when fish of any type were seen in the net or every 0.5 h, whichever came first. Hook-and-line gear, with live or dead pink shrimp (*Farfantepenaeus duorarum*) as bait, also was used to capture smalltooth sawfish. Smalltooth sawfish were measured (stretch total length [STL] in millimeters), assessed for overall health, fin-clipped (free rear tip of second dorsal fin; see *Genetic analysis* section), and tagged (with rototags: 35×9 mm, Dalton ID Systems Ltd.[3], Henley on Thames, UK; with passive integrated transponders: 12 mm, 134.2 kHz, Super Tag II, Biomark Inc., Boise, ID; and with acoustic tags: 29 mm long, weight in water 2.9 g, 69 kHz, V9-2H-R04K, Vemco, Halifax, Nova Scotia, Canada).

All smalltooth sawfish captured during sampling were tagged with an acoustic tag. The tags were programmed to emit a unique acoustic sequence at 69 kHz on a random delay every 60–180 s. The delays were used to minimize signal collisions when more than one smalltooth sawfish were simultaneously within range of acoustic receivers and to maximize battery life (~8 months). In accordance with our endangered species research permit, tags were attached externally, by using a cable tie covered with an epoxy gel, to the flat half of the rototag. As with previous studies (e.g., Whitty et al., 2009), tags were applied to the thicker, anterior margin of the second dorsal fin first by using a leather punch to make a hole for the projecting half of the rototag and then by joining the 2 halves (see photograph in Poulakis, 2012).

Because the smalltooth sawfish is endangered, we distributed signs broadly across the region, encouraging the public to report their encounters with this species (for details about methods used for collecting these data, see Seitz and Poulakis, 2002). In 2006, multiple anglers reported encounters in a small, non-main-stem seawall canal system in the city of Cape Coral (i.e., the Cape Coral capture location) that is part of a larger hotspot near the mouth of the Caloosahatchee River that contains non-main-stem and main-stem habitats (Fig. 1; Poulakis et al.[2]). Subsequently, an effort was made to catch, tag with acoustic transmitters, and obtain tissue samples from the second dorsal fin for DNA from individuals at this location. Because no acoustic receivers had been deployed at the capture location before reports of encounters began, 8 VR2 Vemco single-frequency, omnidirectional data-logging acoustic receivers were deployed and maintained there between May 2006 and September 2007. In addition, 3 receivers were installed in Glover Bight, a non-main-stem embayment near the mouth of the Caloosahatchee River that had previously been identified as a hotspot (Fig. 1; Poulakis et al., 2011), to monitor use of this area by smalltooth sawfish. When combined with data from acoustic receivers already maintained in the main stem of this river to monitor movements of smalltooth sawfish and other species (see Fig. 1; Simpfendorfer et al., 2011), movement between these and other regions of the nursery could be monitored.

Acoustic receivers were placed such that they maximized coverage of the entire study area, including the relatively complex non-main-stem habitats of the ~1-km^2 Cape Coral capture location (referred to as *capture location* throughout; Fig. 1) and Glover Bight, and continuously recorded the date, time, and identity of all tagged smalltooth sawfish that swam within detection range. The maximum detection range of receivers was estimated as 800 m (mean=450 m; Collins et al., 2008). Data were downloaded from receivers and all biofouling was removed approximately every 2 months. A detailed examination of receiver and array performance (e.g., code-detection efficiency, code-rejection coefficients, noise quotients) in the study area can be found in Simpfendorfer et al. (2008a).

The 2 non-main-stem regions of the nursery, which are the focus of this article (i.e., Cape Coral capture location and Glover Bight), were chosen for detailed study because each had one connection to the main stem of the Caloosahatchee River (a single entry and exit point for juveniles; see Fig. 1). These locations allowed unequivocal assessment of the presence or absence of

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[3] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the Florida Fish and Wildlife Conservation Commission or the National Marine Fisheries Service, NOAA.

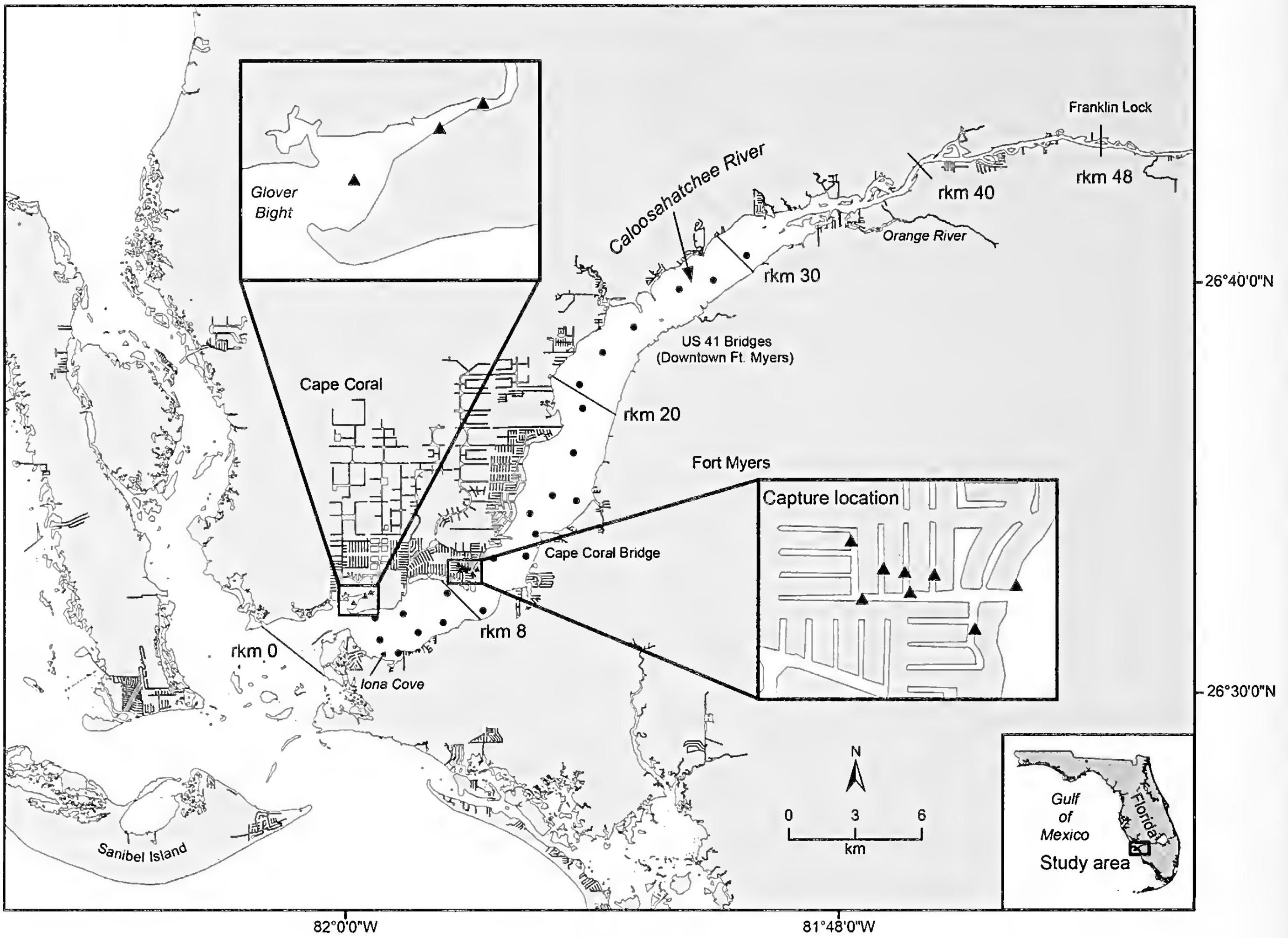

Figure 1

Non-main-stem acoustic receiver arrays (11 receivers, indicated by triangles within the enlargements) and their association with the main-river-stem receiver array (22 receivers, indicated by circles) in the Caloosahatchee River, Florida. Glover Bight and the area around the Cape Coral Bridge, including the Cape Coral capture location used in this study in 2006 and 2007, have been identified as hotspots for juvenile smalltooth sawfish (*Pristis pectinata*) on the basis of multiple years of scientific catch data, acoustic data, and encounter data from the public (Seitz and Poulakis, 2002; Poulakis et al., 2011; Poulakis et al.[2]). River kilometer (rkm) refers to the distance from the river mouth (at rkm 0).

tagged individuals. The capture location was lined entirely by concrete seawalls and had been dredged to a depth of 2–3 m. The substrate was primarily mud with some sand near the corners of the canals. Shorelines in Glover Bight, which includes a natural creek and a small marina, were lined almost entirely by red mangrove and contained a range of water depths from <1 m near shore to >3 m near the marina. The substrate was mud in the creek and in deeper areas (>2 m) and was sand (with the presence of oysters) at the creek mouth and near shore.

Genetic analysis

Ten juvenile smalltooth sawfish were caught at the capture location in the Caloosahatchee River between April and August 2006. The individuals were all <1500 mm STL, indicating that they were young-of-the-year (Simpfendorfer et al., 2008b; Scharer et al., 2012). Fin clippings were stored in 95% reagent-grade ethanol at room temperature. Genomic DNA was extracted by using a PureGene DNA extraction kit (Qiagen Inc., Valencia, CA). Six polymorphic microsatellite loci labeled with fluorescent markers (Feldheim et al., 2010; Table 1) were amplified with 2 multiplexed polymerase chain reactions (PCRs). Approximately 100 ng of template DNA was used in a 12.5-µL PCR containing 0.3125 U GoTaq DNA Polymerase (Promega Corp., Madison, WI), 0.8 µM of each primer, 200 µM of each dNTP, 2.5 mM $MgCl_2$, 0.5× bovine serum albumin (BSA), and 1× colorless GoTaq buffer. The PCR profile consisted of an initial denaturation at 94°C for 2 min, followed by 30 cycles at 94°C for 30 s, 53°C for 30 s, 72°C for 30 s, and a final extension step at 72°C for 5 min.

The resulting fragments were analyzed on an automated Applied Biosystems 3130 Genetic Analyzer

Table 1

Microsatellite loci, size of loci (in base pairs), number of alleles (K, K_T, K_C), and corresponding reference for smalltooth sawfish (*Pristis pectinata*) caught in 2006 in the Caloosahatchee River, Florida. K is the number of alleles per locus for all samples (n=10); K_T is the number of alleles per locus for the smalltooth sawfish containing the mitochondrial DNA control region haplotype T (n=7); K_C is the number of alleles per locus for the smalltooth sawfish containing the mitochondrial DNA control region haplotype C (n=3).

Locus	Size	K	K_T	K_C	Reference
Ppe69	153–173	4	3	2	K. Feldheim, unpubl. data
Ppe114	181–285	11	7	5	Feldheim et al., 2010
Ppe122	236–264	6	6	3	Feldheim et al., 2010
Ppe160	164–174	3	2	2	Feldheim et al., 2010
Ppe165	251–299	8	7	4	Feldheim et al., 2010
Ppe168	219–257	8	4	6	Feldheim et al., 2010

(Thermo Fisher Scientific Inc., Waltham, MA) with GeneScan ROX 500 Size Standard (Thermo Fisher Scientific Inc.). Fragment lengths were analyzed by using GeneMapper software, vers. 4.0 (Applied Biosystems, Thermo Fisher Scientific Inc.), and modified by hand when necessary. Loci were checked for Hardy-Weinberg equilibrium by using GENEPOP (Raymond and Rousset, 1995; Rousset, 2008). The prevalence of null alleles, large allele dropout, and scoring errors was estimated by using MICRO-CHECKER, vers. 2.2.3 (van Oosterhout et al., 2004).

To address our hypotheses, we used a combination of mitochondrial and microsatellite data to conservatively estimate the number of mothers that contributed to the group of smalltooth sawfish. Microsatellite data were analyzed separately within each of the mitochondrial lineages that were identified.

For mitochondrial-DNA sequencing analysis, we amplified a portion of the control region that had approximately 750 base pairs (bp), using the forward primer ProL2 (CTGCCCTTGGCTCCCAAAGC) (Pardini et al., 2001) and reverse primer CRR (ATGCAAATATTATGTCGAGGGTAG) (Phillips et al., 2011). Approximately 100 ng of DNA was used as template in a 50-µL PCR reaction containing 1.25 U GoTaq DNA Polymerase, 0.8 µM of each primer, 200 µM of each dNTP, 2.5 mM $MgCl_2$, 0.5× BSA, and 1× colorless GoTaq buffer. The PCR profile consisted of an initial denaturation at 94°C for 2 min, followed by 30 cycles at 94°C for 45 s, 52°C for 45 s, 72°C for 45 s, and a final extension step at 72°C for 5 min. After amplification, the PCR reaction was run for approximately 45 min at 90 amps on a 1.5% TAE agarose gel with ethidium bromide. PCR products were visualized, extracted from the gel, purified by using Stratagene Gel Cleaning spin columns (Agilent Technologies, Santa Clara, CA), and eluted in sterile water.

Purified PCR products were assessed for quantity and quality by using a NanoDrop 2000 microvolume spectrophotometer (Thermo Fisher Scientific Inc.). Approximately 200 ng of each PCR product was cycle-sequenced with ABI Prism BigDye Terminator sequencing mix (Applied Biosystems) by using the following reaction profile: 30 cycles at 95°C for 30 s, 55°C for 15 s, and 72°C for 4 min. Sequencing reactions were precipitated with ethanol and sodium acetate, dried for at least 30 min at 37°C, and resuspended in 23mL of Hi-Di formamide (Thermo Fisher Scientific Inc.). Samples were analyzed in both directions on an Applied Biosystems 3130xl DNA Analyzer (Thermo Fisher Scientific Inc.). Forward and reverse sequences were assembled into contiguous sequences by using Sequencher, vers. 4.9 (Gene Codes Corp., Ann Arbor, MI) and, when necessary, modified by hand. Primer sequences were identified and excised.

Microsatellite loci exhibited as many as 29 alleles per locus in >200 individuals surveyed in southwest Florida and showed high levels of heterozygosity (C. Curtis, unpubl. data). No evidence of null alleles, large allele dropout, or scoring errors was detected with MICRO-CHECKER among the 6 loci, although locus Ppe69 was found to be out of Hardy-Weinberg equilibrium. With 95% confidence limits and 1000 randomizations, ML-Relate software (Kalinowski et al., 2006) was used to obtain a maximum likelihood estimate of the coefficient of relatedness (r) among potential siblings to estimate the number of mothers represented in the samples.

Movement analysis

Analysis of acoustic data followed the method of Poulakis et al. (2013). The position of individuals along the Caloosahatchee River was estimated by using a mean-position algorithm (Simpfendorfer et al., 2002, 2008a). Each acoustic receiver was assigned a position along the centerline of the river on the basis of the distance of the receiver from the mouth of the river (the mouth of the river was assigned the position of river kilometer 0; the capture location was assigned the position of river kilometer 10.5). A geographic information system (GIS) layer of the centerline of the river was extracted from the U.S. Geological Survey National Hydrography Dataset (website) and divided into 0.2-km (0.1-nautical mile) segments. The mean position estimates of the location of smalltooth sawfish, averaged in 1-h increments, formed the basis of all subsequent analyses.

In general, a location designation (e.g., present at the capture location) was given only to smalltooth sawfish position estimates that exactly matched the river kilometer values assigned to each acoustic receiver. For example, to be counted as present at the non-main-stem Cape Coral capture location, data from a transmitter had to be recorded on at least one of the interior receivers for an entire hour (i.e., without main-stem detections). A smalltooth sawfish positioned at the entrance of the capture location would have yielded a mean river kilometer position estimate intermediate between the capture location and a nearby main-stem receiver and, therefore, would have been placed in the main stem. Receivers at the capture location and in Glover Bight were isolated from direct acoustic connection with nearby main-stem receivers to help with this determination.

We analyzed movement data for smalltooth sawfish that retained their acoustic tags for at least 25 days. Position estimates for individuals were used to calculate daily activity space (in river kilometers), residence time, and diel activity. As with previous studies (e.g., Simpfendorfer et al., 2011; Poulakis et al., 2013), daily activity space was calculated for each individual as the difference between the most upriver and most downriver position estimates for each day. Main-stem and non-main-stem activity (presence or absence) were calculated for each individual as the percentage of hourly position estimates that corresponded with each habitat. Diel activity was estimated by adding hourly position estimates that occurred during local day (0600–1859) and night (1900–0559) in the 2 habitats for each individual. A chi-square test was used to determine whether the observed day and night position estimates in the 2 habitats (pooling all individuals) differed from an even distribution. The South Florida Water Management District provided data on daily mean freshwater inflow from the Franklin Lock (located at river kilometer 48) for comparison with movement tracks of individual smalltooth sawfish.

Results

Genetics

Two mitochondrial haplotypes were identified among the 10 smalltooth sawfish from the capture location. The haplotypes differed by one C or T transition. The capture location contained 3 individuals with haplotype C and 7 individuals with haplotype T (Table 1), indicating that at least 2 mothers had contributed to the group of juveniles sampled because, in vertebrates, mitochondrial DNA is inherited from the mother.

To further refine the number of mothers that contributed to the group, microsatellite data from the 2 mitochondrial groups (C and T) were analyzed separately using ML-Relate software to estimate the relatedness of individuals within each of the 2 maternal lineages. Within the C group (3 individuals), 2 microsatellite loci (Ppe114 and Ppe168) had more than 4 alleles per locus (Table 1). In the absence of mutation, the maximum number of alleles that can be shared among full siblings at a single locus is 4. The presence of more than 4 alleles at more than 1 locus indicates that more than 2 parents contributed to the C group of juveniles because simultaneous, independent mutational events at more than 1 locus are highly unlikely. Within the T group (7 individuals), 3 loci (Ppe114, Ppe122, and Ppe165) contained more than 4 alleles per locus, and 2 of the loci had 7 alleles per locus (Table 1), indicating that more than 2 parents contributed to this group as well. An excess of alleles (i.e., >4) was not observed in loci Ppe69 and Ppe160 for either of the mitochondrial groups. This finding is not inconsistent with more than 2 parents contributing to the group because one or both contributing parents may have been homozygous for the alleles detected for these loci.

The values of *r* among the 10 unique smalltooth sawfish caught and monitored at the capture location indicate that at least 4 pairs of individuals in the T group had high *r* estimates (~0.5) and that those pairs were full-sibling dyads (Table 2). There was a potential half-sibling pair (*r*= ~0.25) in the C group, along with several unrelated individuals in each mitochondrial lineage. These estimates of relatedness indicate that at least 4 mothers contributed to the 10 individuals. These data support hypotheses 3 and 4 (not related and transient; not related and resident [see more on distinguishing between these hypotheses below]) and are inconsistent with hypotheses 1 and 2 (related and transient; related and resident).

Movements

Young-of-the-year individuals ranged from 775 to 1218 mm STL when they were tagged, and mean tag retention was 184 days (standard error [SE] 68.5; Table 3). Half of the 10 individuals were caught on hook and line, and half were caught in gill nets. Six individuals met the analysis criterion of at least 25 days of tag retention. The mean daily activity space of these individuals was 0.7 river km (SE 0.2).

We documented the presence of multiple smalltooth sawfish at the capture location at the same time; on average, individuals spent 61% of their time there. Up to 4 of the tagged individuals used the capture location at the same time (sawfish 2, 4–6; 1–14 August), and 2 individuals used it at the same time for 2.5 months (sawfish 2 and 4; 1 June–14 August) (Fig. 2). The individual with the longest tag retention (sawfish 4) resided at the capture location for long periods, ranging

Table 2

Pairwise coefficients of relatedness (*r*) among the 10 smalltooth sawfish (*Pristis pectinata*) caught in 2006 in the Caloosahatchee River, Florida. Pairwise comparisons are shown within each of the 2 mitochondrial DNA lineages (mtDNA haplotypes C and T).

Individual pair	*r*	mtDNA haplotype
FWC33: FWC36	0.2530	C
FWC33: FWC38	0	C
FWC36: FWC38	0	C
FWC26 : FWC27	0	T
FWC26: FWC28	0.0824	T
FWC26: FWC29	0.0830	T
FWC26: FWC31	0.0236	T
FWC26: FWC32	0.6096	T
FWC26: FWC37	0.5652	T
FWC27: FWC28	0.5985	T
FWC27: FWC29	0	T
FWC27: FWC31	0.1658	T
FWC27: FWC32	0	T
FWC27: FWC37	0	T
FWC28: FWC29	0	T
FWC28: FWC31	0	T
FWC28: FWC32	0	T
FWC28: FWC37	0.0824	T
FWC29: FWC31	0.0236	T
FWC29: FWC32	0.5459	T
FWC29: FWC37	0.5000	T
FWC31: FWC32	0.0263	T
FWC31: FWC37	0.0236	T
FWC32: FWC37	0	T

from a few days to as many as 86 consecutive days, without being detected on a main-stem receiver beyond the receiver located at the entrance of the canal system of the capture location. In addition, 3 smalltooth sawfish tagged with acoustic transmitters in the main-stem of the river moved into the capture location array, remaining there for as long as 14 consecutive days.

Tracks of the analyzed individuals showed that daily activity space was limited to a small portion of the study area when smalltooth sawfish were associated with the capture location, but they occasionally moved greater distances (Fig. 3). During September in 2006, after Tropical Storm Ernesto passed over the study area (29 August–1 September), prompting a freshwater release from the Franklin Lock that exceeded 500 m^3/s, 4 individuals (sawfish 3–6) moved downriver away from the capture location to at least river kilometer 5. At least 3 of these individuals (sawfish 4–6) returned to the capture location after the storm passed, and 1 individual remained in the vicinity of the capture location for at least 10 more months (sawfish 4). After the storm, sawfish 5 and 6 returned to the capture location periodically but continued to make excursions downriver even without large increases in freshwater flow. Sawfish 3 may have exited the river because of the effects of the storm, and sawfish 6 may have exited the river or shed its tag by the end of the year. These data, when combined with the genetic analyses, eliminate hypothesis 3 as a possibility and support hypothesis 4: young-of-the-year smalltooth sawfish that occurred at the capture location were 1) from at least 4 mothers and 2) long-term users of this location.

While they were associated with the capture location, all 4 young-of-the-year smalltooth sawfish that

Table 3

Summary of movement and habitat use by 6 smalltooth sawfish (*Pristis pectinata*) after they were tagged and monitored in 2006 and 2007 at the Cape Coral capture location in the Caloosahatchee River, Florida. Individuals are listed in order by date (month/day/year) of first detection from most recently tagged (top) to oldest. Residence time is the percentage of total hourly position estimates that occurred within the non-main-stem seawall canal capture location or in the main stem of the river. Sawfish 4 and 5 were monitored for ~1 year or more because they were recaptured and re-tagged. Four additional individuals were captured but shed their acoustic tags within a few days (their sizes were 800, 805, 842, 1200 mm stretch total length [STL]). Standard errors (SEs) of the mean are given in parentheses. The asterisk (*) indicates that although the individual was tagged at the capture location on 24 April 2006 the acoustic receivers were not deployed until 22 May 2006.

Smalltooth sawfish	STL (mm)	Sex	Date of first detection	Date of last detection	Days monitored	Mean (SE) daily activity space (km)	Residence time in canals (%)	Residence time in main stem habitats (%)
6	1097	F	08/01/06	12/29/06	150	1.2 (0.2)	28.1	71.9
5	1218	F	08/01/06	07/19/07	352	0.7 (0.1)	36.7	63.3
4	950	F	06/14/06	08/20/07	432	0.4 (0.0)	80.3	19.7
3	996	F	06/07/06	08/23/06	77	1.0 (0.3)	70.5	29.5
2	1045	M	06/07/06	08/15/06	69	0.9 (0.1)	51.6	48.4
1	775*	F	05/22/06	06/16/06	25	0.0 (0.0)	99.7	0.3
Mean (SE)	1013 (SE 61)				184 (SE 68.5)	0.7 (SE 0.2)	61.2 (SE 11.1)	38.8 (SE 11.1)

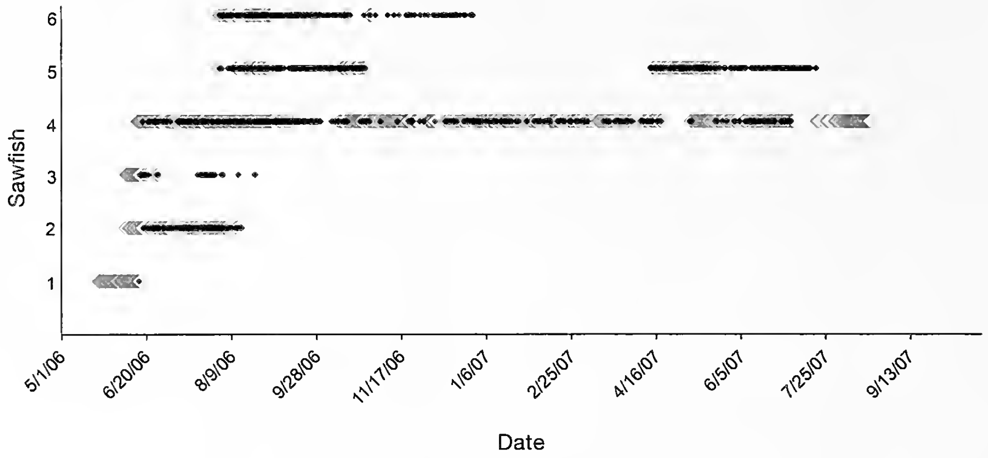

Figure 2

Monitoring histories and activity budgets of 6 smalltooth sawfish (*Pristis pectinata*) by date (month/day/year) in 2006 and 2007. Large gray diamonds indicate presence at the capture location in Cape Coral, Florida, and small black diamonds indicate presence in the main stem of the Caloosahatchee River. Individuals (sawfish 1–6) are listed chronologically from most recently tagged (top) to oldest in order to correspond with data in Table 3. See Table 3 for length, sex, dates of first and last detection, days monitored, mean daily activity space, and residence times of each individual.

carried tags for multiple months (sawfish 2, 4–6) periodically exhibited a diel behavioral pattern. They used the capture location throughout the day, but sometimes used the main stem of the river at night (Figs. 4 and 5). Juveniles typically left the capture location and entered the main stem of the river between 1700 and 2200, then left the main stem and returned to the capture location between 0100 and 0700. These movements occurred as many as 20 times in 1 month. Interestingly, the individual with the longest tag retention and most consistent association with the capture location (sawfish 4) exhibited this pattern the least.

The 3 young-of-the-year smalltooth sawfish with the longest tag retention (sawfish 4–6) used other known nursery hotspots when they left the capture location. One smalltooth sawfish (sawfish 5) exhibited the diel pattern described in the previous paragraph before the passage of Tropical Storm Ernesto and after the storm in another known nursery hotspot, Glover Bight, further downriver (Fig. 5). Before the storm, this individual exhibited the diel pattern associated with the capture location, sometimes not returning to it, but always passing the main-stem acoustic receiver near river kilometer 11.8 at night (Fig. 5A). After the storm, this juvenile moved to Glover Bight (river kilometer 3) and resumed a diel movement pattern there (Fig. 5B). It used a shallow, red-mangrove-lined creek during the day and the deeper, more open waters of red-mangrove-lined Glover Bight at night. In addition, the 3 smalltooth sawfish with the longest tag retention times used the Iona Cove hotspot (at ~river kilometer 5), located on the opposite side of the river from Glover Bight, for short 4- to 5-day periods and for as many as 35 days at a time (Fig. 3).

The total number of smalltooth sawfish position estimates at the capture location and in the main stem (pooling all individuals) differed significantly between day and night, indicating that a diel pattern had occurred (Table 4; $P<0.001$, $\chi^2=208$, df=1). Because the percentages of time spent at the capture location during the day and at night were nearly identical (49.6% day and 50.4% night), the differences detected in the chi-square test were attributed to diel differences in the main stem (33% day and 67% night); activity of smalltooth sawfish in the main stem of the river was greater at night than during the day.

Discussion

The concepts of niche theory and habitat suitability, which link the fitness of individuals to their environment (Hutchinson, 1957; Guisan and Zimmermann, 2000), are especially important to apply to endangered species because their populations are small and their fitness must be maximized to foster recovery. Smaller portions of the total area in which a species occurs (e.g., nursery hotspots) are often more heavily used than other areas for a variety of reasons, such as food availability, optimal temperature, and reduced predation risk (e.g., Jones, 2002; Polovina et al., 2006; Estók, 2007). For smalltooth sawfish, multiple lines of

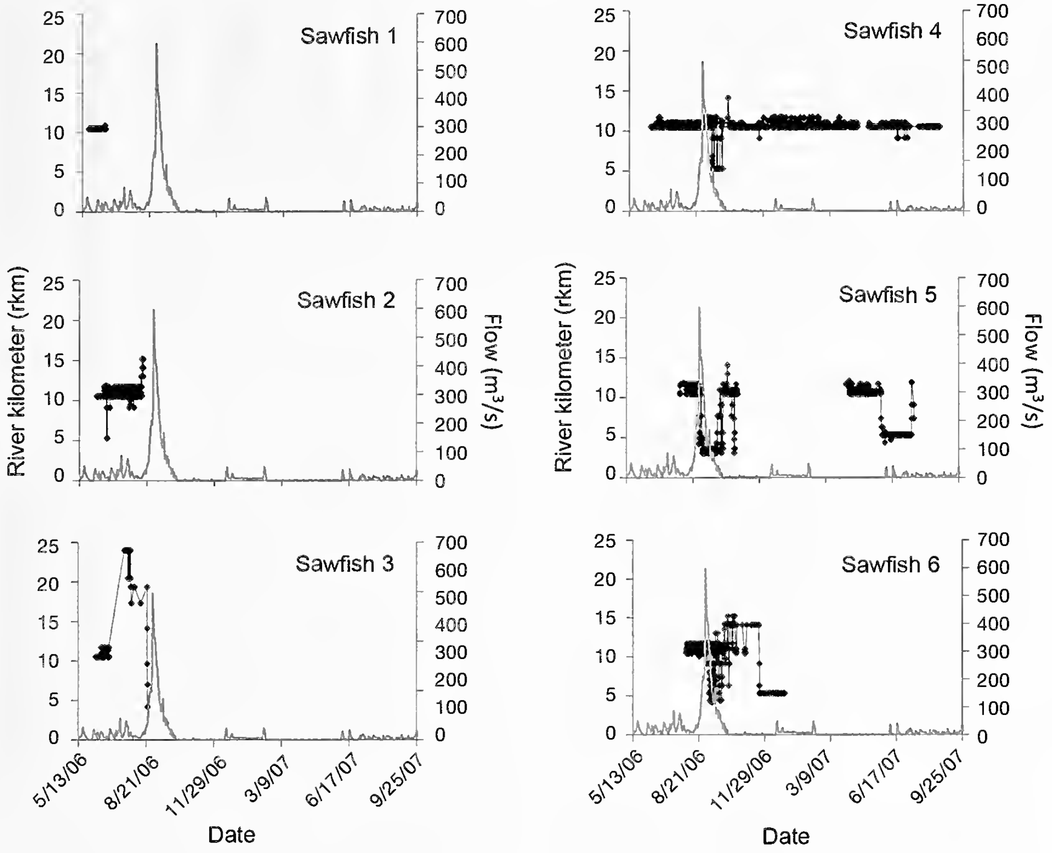

Figure 3

Movement tracks of individual smalltooth sawfish (*Pristis pectinata*) in 2006 and 2007 between the capture location in Cape Coral, Florida, at river kilometer 10.5 and the main stem of the Caloosahatchee River. Tracks are indicated by black lines and freshwater flow is indicated by gray lines by date (month/day/year). The number of each smalltooth sawfish (1–6) is given in the upper right of each graph (see Table 3 and Fig. 2 for more data on each individual). In general, smalltooth sawfish were present at the capture location throughout the day and sometimes made excursions to the main stem of the river at night (see Figs. 4 and 5). The large river discharge indicated in the graphs was related to the passage of Tropical Storm Ernesto in 2006 (29 August–1 September). Sawfish 4 and 5 lost their original transmitters, were later recaptured during routine sampling, and had new transmitters applied. River kilometer (rkm) refers to the distance from the river mouth (at rkm 0).

evidence indicate that such nursery hotspots, found throughout much of the species' current range, are consistently important for multiple cohorts of young fish for their first 3 years of life (Poulakis et al., 2011; Hollensead et al., 2016; this study). Similarly, in Australia, researchers have recognized localized capture sites for juvenile sawfishes, including largetooth sawfish (*P. pristis*), dwarf sawfish (*P. clavata*), and green sawfish (*P. zijsron*) (Morgan et al., 2011, 2015), indicating that nursery hotspots may also be important for other pristids. These nursery hotspots will likely be an emerging focus of research, management, and conservation.

The DNA and acoustic monitoring results presented here support the hypothesis that a group of young-of-the-year smalltooth sawfish from different mothers occurred in the vicinity of the Cape Coral capture location for extended periods because they had an affinity for that location (hypothesis 4), which is part of a larger hotspot (Poulakis et al.[2]). Acoustic tracking and monitoring data from multiple nurseries have shown that young-of-the-year smalltooth sawfish initially have small activity spaces, or home ranges, that increase in size as the fish grow (Simpfendorfer et al., 2010, 2011; Poulakis et al., 2013). Progeny from at least 4 mothers were born in the hotspot at or near the capture location and resided there for their first few weeks of life. The young could have been born elsewhere in the river and converged on the capture location, but, on the basis of previous work in the same river system that showed that the youngest individuals had the smallest

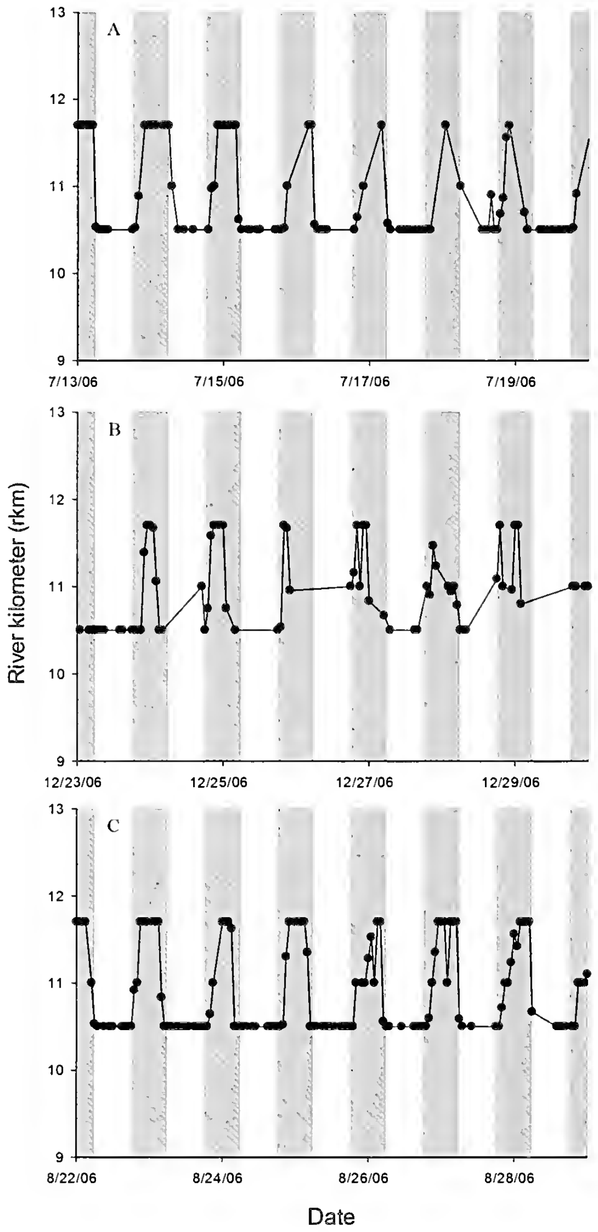

Figure 4

Diel movement patterns of 3 smalltooth sawfish (*Pristis pectinata*) in 2006 between the capture location (at river kilometer 10.5) in Cape Coral, Florida, and the main stem of the Caloosahatchee River by date (month/day/year): (**A**) sawfish 2 (1045 mm stretch total length [STL]), (**B**) sawfish 4 (950 mm STL), and (**C**) sawfish 6 (1097 mm STL). River kilometer (rkm) refers to the distance from the river mouth (at rkm 0). The gray bars indicate night. The acoustic receiver at river kilometer 11 was just outside the entrance to the capture location and the receiver at river kilometer 11.8 was in the main stem of the river near the Cape Coral Bridge (see Fig. 1). In general, these individuals remained within the seawall-lined canal system at the capture location during the day, left the capture location and moved into the main stem of the river at night between 1700 and 2200, and returned to the capture location between 0100 and 0700.

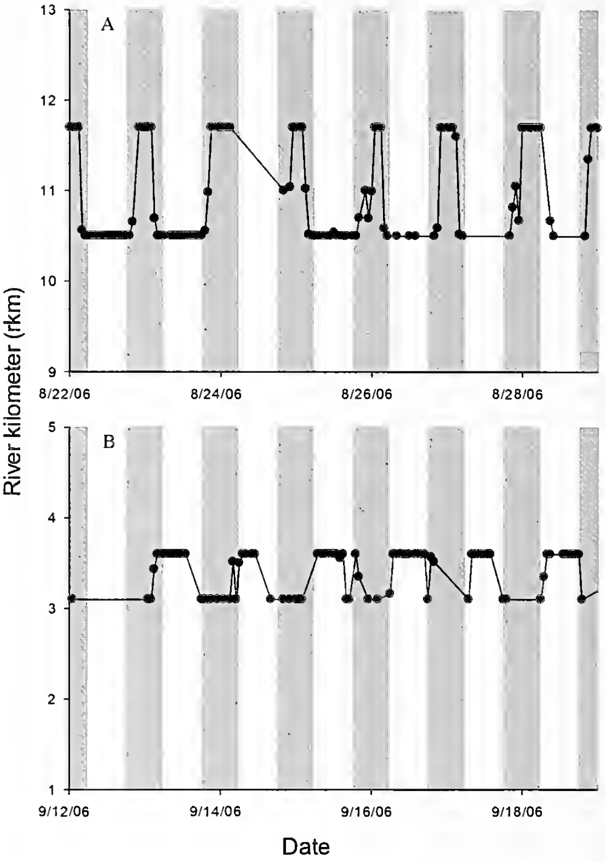

Figure 5

Diel movement patterns of a 1218-mm stretch-total-length smalltooth sawfish (*Pristis pectinata*) (sawfish 5) that occurred in 2006 at the capture location (at river kilometer 10.5) in Cape Coral, Florida, and at the Glover Bight hotspot by date (month/day/year). River kilometer (rkm) refers to the distance from the river mouth (at rkm 0). The gray bars indicate night. (**A**) Diel movement pattern between the capture location (day) and the main stem of the Caloosahatchee River (distance from the river mouth >10.5 river km; night) leading up to the passage of Tropical Storm Ernesto that occurred 29 August–1 September. (**B**) Diel movement pattern of the same individual after passage of Tropical Storm Ernesto. Between 13 and 19 September (the only portion shown for detail), this juvenile was located in the natural red-mangrove-lined creek portion of the Glover Bight hotspot (river kilometer 3.6) during the day and moved into the open water portion of Glover Bight at night (river kilometer 3.1) before eventually returning to the capture location (see Fig. 3 for a longer time series).

activity spaces, it seems unlikely (Simpfendorfer et al., 2011). The fact that some of these smalltooth sawfish had a high degree of site fidelity for their capture location, for months at a time, although their activity spaces were predicted to increase as the fish grew, is noteworthy.

Table 4

Diel activity of smalltooth sawfish (*Pristis pectinata*) monitored at the non-main-stem Cape Coral capture location and in the main stem of the Caloosahatchee River, Florida, in 2006 and 2007. The number of position estimates (all individuals pooled) was significantly greater at night in the main stem of the river ($P<0.001$, $\chi^2=208$, df=1). STL=stretch total length.

		Capture location activity		Main-stem activity	
Smalltooth sawfish	STL (mm)	Day	Night	Day	Night
6	1097	176	61	114	492
5	1218	361	249	489	565
4	950	1437	1892	277	538
3	996	100	80	22	53
2	1045	262	125	60	310
1	775	207	182	1	0
Total		2543	2589	963	1958

Movements of related species associated with small portions of their nurseries have been observed in other systems. Using a variety of data including acoustic tracking, Gruber et al. (1988) documented site fidelity by juvenile lemon shark (*Negaprion brevirostris*), as well as habitat partitioning by young-of-the-year, juvenile, and adult lemon shark on short (up to 8 days) and long temporal scales. The "nursery zone" of their study area in Bimini, Bahamas, was used by the smallest juveniles and was characterized by the shallowest water and mangrove-fringed shorelines, similar to the habitats used by smalltooth sawfish in their natural hotspots (Poulakis et al., 2011). Using acoustic monitoring, Heupel et al. (2003) documented movements of blacktip shark (*Carcharhinus limbatus*) away from and back to Terra Ceia Bay (a small bay connected to the Tampa Bay estuary in Florida)—movements that were associated with the passage of a tropical storm. They analyzed several environmental factors and attributed the response of the blacktip shark to changes in barometric and hydrostatic pressure. Collectively, these data are examples of patterns of general habitat-use and the types of environmental cues that influence habitat use by elasmobranchs in localized coastal nurseries (for review, see Simpfendorfer and Heupel, 2012).

Factors influencing the initial use of nursery hotspots by juvenile elasmobranchs could include selection of the area as a birth site by their mothers. Long-term interdisciplinary data have indicated that adult female lemon shark and blacktip shark are philopatric and, therefore, multiple cohorts of juveniles use localized nursery habitats (Feldheim et al., 2004; Hueter et al., 2005; DiBattista et al., 2008). Adult female smalltooth sawfish and largetooth sawfish also have been shown to be philopatric (Phillips et al., 2011; K. Feldheim, unpubl. data); therefore, they are returning to the same general nursery areas to give birth, findings that have implications for management, conservation, and recovery on a large spatial scale.

Upon arrival in the nurseries, pregnant female smalltooth sawfish may give birth to their young in the hotspots rather than more broadly in the river system. In general, these hotspots are close to shore (<100 m), shallow (<1 m) and near deeper (at least 2 m) water. Hotspots identified in previous studies are currently dominated by shorelines with natural red mangroves and shallow water, and these characteristics have been incorporated into the official critical habitat designation for juvenile smalltooth sawfish (Poulakis et al., 2011; Norton et al., 2012). Historical aerial photographs indicate that the capture location, which was the focus of our study, may have been dominated by shorelines with natural red mangrove and shallow water (<1 m) before canals were constructed. Whether this site represents a relict habitat, genetically imprinted into the species, or is in fact suitable habitat that provides a fitness advantage for individual smalltooth sawfish is of interest in the context of niche theory.

We document a close association of juvenile smalltooth sawfish with hotspots at a nursery-level spatial scale. Although the entire river was accessible, multiple individuals used the habitats associated with the capture location for months at a time. As in previous studies, however, large-scale movements were observed when juveniles moved downriver as freshwater flows associated with a tropical storm exceeded 500 m^3/s (Poulakis et al., 2013). That individuals returned to their *exact* capture location even with its artificial, seawall-canal habitats, after relatively large-scale movements of 5–7 river km away from the capture location, was somewhat unexpected. It indicates that factors supporting site fidelity (outside the period of the tropical storm), such as adequate food availability, optimal temperature, and reduced predation risk, were satisfied in these artificial habitats.

At a small spatial scale, a diel activity pattern was observed for multiple individuals at the capture location and for one individual after relocation to a downriver hotspot after a tropical storm. This observation indicates that the factors eliciting these diel behaviors were present at both places. For example, predation pressure may have caused the juveniles to reside in non-main-stem portions of both hotspots during the day, whereas insufficient food may have caused them to move into open-water habitats at night.

In contrast to detection of diel movements, we observed continuous use of the capture location. There are likely multiple explanations for these apparently contradictory behaviors. A portion of the population may favor refuge, continuously occupying habitats off the main stem of the river, such as those in the capture location canal system. The long periods of site fidelity documented at the capture location indicate that adequate food was available there, at least for some individuals. Another portion of the population may be driven to maximize growth and is more likely to venture into the main stem of the river, especially if intra- or interspecific competition occurs in the refuge habitat. Those individuals exhibiting a diel pattern may fall between these 2 behaviors. Trade-offs between food and refuge have been discussed thoroughly in the literature on juvenile fish and may help explain the varied movement patterns of juvenile smalltooth sawfish in relation to their nursery hotspots (e.g., for background, see Beck et al., 2001 and Heupel et al., 2007).

There is some support for the idea that predation influences habitat use by juvenile smalltooth sawfish, especially for the smallest individuals. For example, at the Glover Bight hotspot, neonate smalltooth sawfish have been observed among red mangrove prop roots during the day while a shark (species unknown) was feeding in the central portion of the creek (Poulakis et al., 2011; Fig. 1), providing anecdotal evidence that behavior of smalltooth sawfish is influenced by predator avoidance. Further support for the use of non-main-stem habitats as refuges from predation comes from research that has shown that juvenile bull shark (*Carcharhinus leucas*), the primary potential predator of juvenile smalltooth sawfish in most nurseries (Simpfendorfer et al., 2005), are more common in the deeper, open-water portions of the Caloosahatchee River (Heupel et al., 2010), which smalltooth sawfish have been observed to often avoid during the day. Juvenile blacktip sharks have exhibited what might also be predator-avoidance behaviors whereby they aggregate during the day and disperse at night (Heupel and Simpfendorfer, 2005).

Feeding is likely to have a major influence on habitat use of smalltooth sawfish because growth is fast in the nursery; smalltooth sawfish double in length during their first year of life (Simpfendorfer et al., 2008b; Scharer et al., 2012). Little is known about the diet of smalltooth sawfish beyond general characterizations and a few observations from field sampling, anglers, and necropsies to document food items, including pink shrimp (as bait) and fish, such as clupeids, carangids, mullet (*Mugil* spp.), pinfish (*Lagodon rhomboides*), and a stingray (*Dasyatis* sp.) (Bigelow and Schroeder, 1953; Poulakis et al., 2013). Traditional stomach-content analyses are not possible because the smalltooth sawfish is endangered; therefore, indirect methods such as analysis of stable isotopes (Fisk et al., 2002) have been employed, and such studies have indicated that fish make up the majority of the diet at all life stages (Poulakis et al.[2]).

Monitoring the distribution and abundance of potential prey fish may provide insights into habitat use by smalltooth sawfish. Also, given the distinct side-to-side movements of the rostrum that the smalltooth sawfish uses for feeding (Wueringer et al., 2012), research is underway that uses a novel indirect method, the attachment of acceleration data loggers (Whitney et al., 2012), to monitor potential feeding behavior and may help determine whether food acquisition or other factors (e.g., temperature; Schlaff et al., 2014) contribute to the large-scale relocation of individuals within the nursery or the smaller-scale diel movements observed in this study.

Evidence that movements of juvenile smalltooth sawfish in the nursery are cued, directly or indirectly, by changes in the volume of freshwater inflow (Poulakis et al., 2013) and evidence that juveniles move between nursery hotspots (this study) highlight the need for freshwater inflow management strategies that would minimize the need for these movements in flow-managed systems such as the Caloosahatchee River (Barnes, 2005). Although there appears to be little predation on smalltooth sawfish in the nursery (Poulakis et al., 2011), predation risk may increase during the time it takes for smalltooth sawfish to find suitable alternate habitats. Predation risk could also increase because predators, like the bull shark, tend to relocate toward the river mouth and co-occur with smalltooth sawfish when freshwater flow increases (Heupel and Simpfendorfer, 2008). As a result, freshwater inflow management strategies that mimic the environmental variability induced by the natural dry and wet seasons may maximize survivorship of smalltooth sawfish (see Poulakis et al., 2011; Simpfendorfer et al., 2011).

Our study was conducted in a highly altered nursery, but the hotspot concept introduced by Poulakis et al. (2011) appears to also apply to smalltooth sawfish in other nurseries. Poulakis et al.[2] showed that the number of hourly acoustic detections from tagged smalltooth sawfish in upper Charlotte Harbor was greatest in a 2-river-km portion of the Peace River associated with the only documented hotspot in this river (Poulakis et al., 2011). In 2010–2013, between river kilometers 8 and 10 of the Peace River, there were more than twice the number of hourly detections than there were in either of the 2-river-km sections above and below this location, indicating that for most of their monitoring periods, individuals from multiple cohorts remained in a small region of this large, relatively unaltered river. The high site fidelity to hotspots of juveniles observed in the Peace and Caloosahatchee rivers, including the hotspot with artificial habitats in our study, indicates that habitat fragmentation in the highly altered Caloosahatchee River may not be the only factor driving patterns of habitat use there. Therefore, growing evidence indicates that hotspots exist in nurseries throughout the range of smalltooth sawfish, including in regions to the south of the Charlotte Harbor estuarine system, such as the relatively pristine Ten Thousand Islands and Everglades National Park (Simpfendorfer et al., 2010; Hollensead et al., 2016; O'Donnell[4]). In these areas, catch rates, acoustic monitoring, and acoustic tracking have all shown high interannual occurrence in hotspot-like areas, such as Faka Union and Mud bays (Simpfendorfer et al., 2010; Hollensead et al., 2016).

Consistent, large-scale acoustic monitoring in these regions would test our hypothesis that hotspots exist in nurseries throughout the range of smalltooth sawfish and would help identify their boundaries. Further, multiple lines of evidence indicate that hotspots also exist for larger (>2.2 m STL) size classes of smalltooth sawfish, after they leave the nurseries, and the same techniques that have been applied in the nurseries are being applied in northwest Florida Bay and off southeast Florida to elucidate the spatial and temporal extent of these high-use areas by smalltooth sawfish (Poulakis and Seitz, 2004; Waters et al., 2014; Papastamatiou et al., 2015). Testing the hotspot concept in a variety of localities and for multiple life stages may help focus future research, management, and conservation efforts for the Pristidae and ultimately promote recovery of these endangered elasmobranchs.

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Evaluating the vulnerability of an atypical protogynous hermaphrodite to fishery exploitation: results from a population model for black sea bass (*Centropristis striata*)

Jessica Blaylock[1]
Gary R. Shepherd[2]

Email address for contact author: jessica.blaylock@noaa.gov

[1] Integrated Statistics
16 Sumner Street
Woods Hole, MA 02543
Present address: 3825 South Drive
Fort Worth, Texas 76109

[2] Northeast Fisheries Science Center
National Marine Fisheries Service, NOAA
166 Water Street
Woods Hole, Massachusetts 02543

Abstract—Fish species that are protogynous hermaphrodites generally have sex ratios skewed toward males at large sizes. These skewed sex ratios at length can make a stock more vulnerable to overexploitation, particularly in fisheries where minimum size is regulated, resulting in the removal of reproductively active males. We developed a length-based population model to test the vulnerability to exploitation of a population with an atypical protogynous life history, in particular the northern stock of black sea bass (*Centropristis striata*). Black sea bass north of Cape Hatteras, North Carolina, are unusual for protogynous species in that they may undergo prematurational transformation, remain female at large sizes, involve secondary males in spawning, and undertake seasonal migrations. The model was developed to examine the impact of participation by secondary males in population productivity, the influence of size at sex transformation, and the subsequent robustness of the population under exploitation, in comparison with equivalent gonochoristic and typical protogynous populations. Although the model does not capture all the dynamics of a population, such as density-dependent regulation of sex transformation, our results indicate that the northern stock of black sea bass may be more resilient in response to exploitation than would be expected if they were typical protogynous hermaphrodites.

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The capacity of a fish population to persist under exploitation is highly dependent on the life history of a species and its ability to effectively reproduce. In gonochoristic species, individuals remain the same sex throughout their lives, generally ensuring a constant presence of both males and females capable of reproduction, even under relatively strong exploitation. In contrast, species with a hermaphroditic life history (either protogynous or protandrous) might not have an adequate abundance of sexually mature individuals across sizes and ages (Provost and Jensen[1]). In protogynous species, a common pathway for sex change involves individuals initially maturing and reproducing as female and then changing sex to male as needed. The result is a sex ratio dominated by females at small sizes or young ages and males at large sizes or old ages (Warner, 1975; Allsop and West, 2004; Munday et al., 2006). It is typical in a protogynous species for the large, dominant male to monopolize spawning, although there is a wide range of behavioral roles for secondary males that may allow participation (Petersen, 1991). The resulting sex ratio, highly skewed toward males at large sizes, tends to make protogynous species vulnerable to size-selective fisheries, possibly resulting in a shortage of males—a shortage that limits the potential for egg fertilization (Huntsman and Schaaf, 1994; Heppell et al., 2006).

Characteristics of hermaphroditic species make them particularly challenging to assess and manage. Management of exploited fish stocks often is guided by the results of population dynamic models (Quinn and Deriso, 1999; Haddon, 2001; Brander, 2003), and basic models are predicated on certain assumptions about the life history of a species, including the ex-

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[1] Provost, M., and O. Jensen. 2013. Use of sex change considerations in stock assessments. *In* Proceedings from a workshop on modeling protogynous hermaphroditic fishes; Raleigh, NC, 29–30 August 2012 (G. Shepherd, K. Shertzer, J. Coakley, and M. Caldwell, eds.), p. 5–9. Mid-Atlantic Fishery Management Council, Dover, DE. [Available at website, accessed May 2015.]

pectation that both sexes will be equally vulnerable to exploitation. Along with other possible scenarios, such as differential spatial patterns by sex or significant sexual dimorphism, hermaphroditic life histories often violate this assumption, and unique analytical approaches may be warranted. Implications of a protogynous life history for stock assessments and management have been explored previously (Alonzo and Mangel, 2004; Heppell et al., 2006; Brooks et al., 2007; Alonzo et al., 2008). The conclusion from simulation models, as well as empirical observations, is that populations subjected to sex-specific exploitation as a consequence of having a hermaphroditic life history are at a higher risk of overexploitation than are gonochoristic species (Alonzo and Mangel, 2004; Heppell et al., 2006; Hamilton et al., 2007). Thus developing management measures for a protogynous species on the basis of the assumptions implicit for gonochoristic species could possibly result in overfishing.

The black sea bass (*Centropristis striata*) is a protogynous hermaphroditic species that is distributed from the Gulf of Maine to the Gulf of Mexico (Lavenda, 1949; Collette and Klein-MacPhee, 2002) and is managed as 3 unique stocks segregated by Cape Hatteras, North Carolina, and the Florida Keys (Bowen and Avise, 1990; Roy et al., 2012; McCartney et al., 2013). Each stock is subjected to commercial and recreational exploitation and is primarily caught in trawl gear and fish pots, as well as on hook-and-line gear in the coastal regions (Shepherd and Terceiro, 1994). Black sea bass in the South Atlantic and Gulf of Mexico are not migratory and are commonly associated with local structured habitat (Drohan et al., 2007). In contrast, black sea bass in the Middle Atlantic (i.e., the northern stock) undergo seasonal migrations of up to 300 km, moving from the continental shelf or slope edge in winter to the coastal waters in spring and summer (Musick and Mercer, 1977; Moser and Shepherd, 2009).

Life history characteristics vary between the 2 southern stocks and the northern stock. Spawning in the Middle Atlantic stock occurs within a relatively limited period compared with the spawning period of black sea bass in the southern stocks. In all 3 stocks, dominant males in spawning aggregations are characterized by a prominent nuccal hump and bright blue coloration around the hump and eyes (Lavenda, 1949); however, in the northern stock, additional males in ripe gonadal condition but lacking the secondary sex characteristics are also present in the spawning areas (NEFSC[2]). These features are typical of secondary males that may attempt to fertilize the eggs of females spawning with dominant males (Pitcher, 1993; Taborsky, 1994; Young et al., 2013). Empirical data collected during bottom trawl surveys of the Northeast Fisheries Science Center (NEFSC), National Marine Fisheries Service, indicate that about 30% of black sea bass at each size below 30 cm (all lengths in this manuscript refer to total length) were male and that a significant proportion (i.e., up to 45%) of black sea bass at sizes above 45 cm were female (NEFSC[2]). Additionally, growth in this stock appears to be similar for males and females. The characteristics of the black sea bass of the northern stock, such as a prematurational sex change, the presence of mature secondary males, and the relative abundance of large females (>45 cm), distinguish them from typical protogynous hermaphrodites.

The northern Atlantic population of black sea bass has been evaluated in stock assessments to support fishery management programs (NEFSC[2]). One of the main uncertainties cited in relation to the development of management measures is the implication of a protogynous life history. Specifically, there is concern that the removal of large males (>45 cm) would significantly affect the reproductive capability of the stock, thereby making it particularly vulnerable to exploitation. Implicit in that concern is that only the large males reproduce or, if not, the large females change sex to compensate for the reduced availability of large males.

Although methods for stock assessment of protogynous populations have been evaluated, in several studies, the implications of the possible contribution of secondary males to spawning in a population have not been explored fully. In particular, the response to exploitation of a stock with functional secondary males that result from a prematurational sex change has not been tested to determine whether the stock is more or less vulnerable to overexploitation than a gonochoristic or typical protogynous stock with similar population attributes. Our objective was to develop a population model based on the northern stock of black sea bass to evaluate the robustness of an atypical protogynous population in response to fishery exploitation. We do not intend to provide new methods for stock assessments of hermaphroditic species but rather to improve the understanding of the relationship between the life history of a species and its ability to withstand exploitation.

We developed a length-based population model to evaluate the impact of a range of exploitation intensities on population persistence and catch, under different theoretical life history scenarios. We considered an experimental atypical protogynous (AP) population with 3 scenarios: 1) no mature secondary males contribute to spawning (AP-0); 2) 50% of mature secondary males participate in spawning (AP-50); and 3) 100% of mature secondary males participate in spawning (AP-100). Note that our use of the term *secondary* simply implies that these males are not visibly dominant (i.e., secondary males lack the prominent nuccal hump and bright blue coloration). We did not make any assumptions about the behavior of secondary males except that some of them are able to spawn.

Results from simulations for the AP population were compared against those for equivalent gonochoristic (G) and typical protogynous (TP) populations,

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[2] NEFSC (Northeast Fisheries Science Center). 2012. 53rd Northeast regional stock assessment workshop (53rd SAW) assessment report. Northeast Fish. Sci. Cent. Ref. Doc. 12-05, 559 p. [Available at website, accessed May 2015.]

where spawning is monopolized by the dominant males. The model was structured to reflect black sea bass life history, and parameters were derived from empirical data when possible. Sex ratio characteristics for the AP population were based on the northern stock of black sea bass, and the TP population follows a biased sex ratio expected among protogynous species (Provost and Jensen[1]) with recruits being 100% female and all fish transitioning to male by the time they reach the maximum size in the model. We examined stock performance in the form of relative stock size, catch, spawning stock biomass (SSB), and number of recruits (R) under increasing levels of fishing-induced mortality [hereafter: fishing mortality] (F), and we ran sensitivity analyses to evaluate the effect of different capture probabilities, female-to-male transition rates, proportions of secondary versus dominant males, and recruitment scenarios, as well as the effect of possible spawning by secondary males in the TP population.

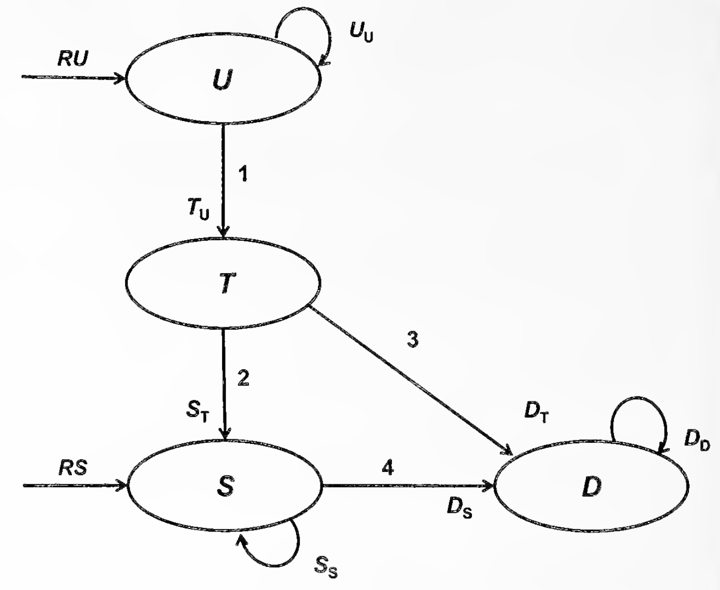

Figure 1

Diagram of possible trajectories through 4 different life stages of black sea bass (*Centropristis striata*) (female [U], transitional [T], secondary male [S], and dominant male [D]) in the length-based model developed to study the response to exploitation by gonochoristic, atypical protogynous, and typical protogynous populations. Recruits are either female (RU) or male (RS), and there are 4 types of transitions in which a fish can transform into a different life stage by the next time period: 1) a female can become a transitional, 2) a transitional can become a secondary male, 3) a transitional can become a dominant male, or 4) a secondary male can become a dominant male. Subscripts letters U, T, S, and D indicate the life stage at the previous time step. When available, parameter and other values used in the model came from data collected during bottom trawl surveys conducted by the NOAA Northeast Fisheries Science Center during 1984–2013.

Materials and methods

Model structure

A length-structured model was developed to compute the abundance at length of fish in 4 life stages (i) over time: females (i=U), transitionals (i=T), secondary males (i=S), and dominant males (i=D). The model follows a monthly time step where each time period (t) is equivalent to the corresponding year (y) and month (m) (i.e., t=month m in year y).

Recruits (≤11 cm) in the model are initially all female but some immediately undergo a change into male, according to population-specific recruit sex ratios. Female and male recruits can follow several potential trajectories over their lifetime (Fig. 1). Female recruits (RU) will either remain female over their entire life or become transitional-phase fish [transitionals] for a single time step followed by transformation into either secondary or dominant males. Male recruits (RS) enter the population as secondary males and either remain secondary males or become dominant males (without a transition phase). Therefore, at any time t, the abundance at length l of fish at a specific life stage is the sum of recruits of the given sex, of individuals entering the stage, and of individuals that were previously in that stage and survived:

$$U_{lt} = RU_{lt} + U_{Ult}, \tag{1}$$

$$T_{lt} = T_{Ult}, \tag{2}$$

$$S_{lt} = RS_{lt} + S_{Tlt} + S_{Slt}, \tag{3}$$

$$D_{lt} = D_{Tlt} + D_{Slt} + D_{Dlt}, \tag{4}$$

where the subscripts U, T, S, and D indicate life stage at the previous time step (for example, S_T refers to the portion of all secondary males at time t that were transitionals in the previous month). Total population abundance over all lengths, N_t, is then calculated as

$$N_t = \sum_{l=1}^{l_{max}} (U_{lt} + T_{lt} + S_{lt} + D_{lt}), \tag{5}$$

where l_{max} = the maximum length of a fish in the population.

From one time step to the next, processes affecting the population occur in the following order: mortality (including natural mortality [M] and F), transition, and growth. At each time step, SSB (calculated by using maturity-at-length and length-weight equations), recruitment, and catch are determined.

Natural mortality at time t (M_t) is assumed constant across stages, lengths, and months; therefore,

$$M_t = \frac{M_y}{12}, \qquad (6)$$

where M_y = total annual natural mortality for year y.

Fishing mortality for length l and time t is defined as

$$F_{lt} = \frac{F_y}{12}\delta_l, \qquad (7)$$

where F_y = total annual fishing mortality for year y; and
δ_l = the probability of capture (i.e., capture selectivity) at length l defined by a logistic 2-parameter model,

$$\delta_l = \left(1 + e^{\left(-\frac{l-\alpha}{\beta}\right)}\right)^{-1}, \qquad (8)$$

where α = the length of the fish with 50% probability of being retained (L_{50}); and
β = the slope of the selectivity curve δ_l.

It follows that catch in numbers for stage i at length l at time t is

$$C_{ilt} = \left(\frac{F_{lt}}{F_{lt} + M_t}\right) N_{ilt}\left(1 - e^{-(F_{lt}+M_t)}\right). \qquad (9)$$

In the protogynous population scenarios, we assumed transitions (Fig. 1) occur only after spawning in month 11 or 12, where a fish can transform from a female to a transitional (m=11), from a transitional to a secondary male (m=12), from a transitional directly to a dominant male (m=12), or from a secondary male to a dominant male (m=12). For simplicity, fish can be in the transitional stage only during one time step. The probability of an individual of stage i_1 and length l in month m becoming stage i_2 in the following month is represented by $\theta_{lm}^{i_1 i_2}$. Transition rates are all zero for the G population because no sex change occurs; the sex ratio for this scenario is fixed at 1:1.

Growth is assumed constant across stages and sexes but varies annually by quarter, (q). Growth occurs at the start of each quarter and is defined by a lower triangular matrix (G_q) with dimensions $l_{max} \times l_{max}$, in which each element $g_{L2,L1,q}$ represents the probability that a fish of length $L1$ at the start of quarter q will grow to length $L2$ by the start of the following quarter. Therefore, the abundance of individuals of stage i_2 at length $L2$ at time t, $N_{i_2 L2t}$, is calculated as the number of individuals of stage i_1 at length l (where $l \leq L2$) at time (t–1) that survived, that transitioned to stage i_2, and that grew to length $L2$, summed over all values of l:

$$N_{i_2 lt} = \sum_{l=1}^{L2}\left[N_{i_1 l(t-1)} e^{(-(M_t+F_{lt}))} \theta_{lm}^{i_1 i_2} g_{L2,l,m}\right]. \qquad (10)$$

Given the uncertainty resulting from defining SSB as female only (Brooks et al., 2007), recruitment levels are determined from male and female spawning stock as of September (m=9) and from recruits that entered the population in October (m=10). Maturity is specified by using sex-specific maturity ogives defined by a 4-parameter logistic equation, as follows:

$$O_{jl} = \frac{a_j - d_j}{1 + \left(\frac{l}{c_j}\right)^{b_j}} + d_j, \qquad (11)$$

where O_{jl} = the proportion of fish of sex j (where j=U for females, j=V for males) and length l that are mature;
a_j = the minimum asymptote parameter for sex j;
b_j = the slope parameter for sex j;
c_j = the inflection point (i.e., length at 50% maturity) parameter for sex j; and
d_j = the maximum asymptote parameter for sex j.

Spawning stock biomass is the sum of female and male spawning biomass (i.e., biomass of mature fish) for the G population and is the sum of female and dominant male spawning biomass for the TP scenario. For the AP population scenarios, SSB is the sum of female and dominant male spawning biomass, plus the spawning biomass of the given proportion (0%, 50%, or 100%) of secondary males.

The lack of an accepted stock-recruitment model for black sea bass, combined with the desire to include the SSB sex ratio as a factor influencing recruitment, led to the development of an approach that involves recruitment bins. With this approach, depending on the value of SSB and the sex ratio (in numbers) within the spawning stock, recruitment abundance for year y (R_y) was randomly drawn (on the basis of a random number seed) from 1 of 3 cumulative distribution functions (CDF) representing 3 productivity bins. Each CDF was created from a lognormal distribution generated by using a mean recruitment and standard deviation from empirical data.

As long as the proportion of females (*PropF*) and males (*PropM*) in the spawning stock remained above a sex ratio threshold (H_1) (i.e., as long as the sex ratio in the spawning stock was not overly skewed), recruitment was drawn according to the value of SSB in relation to 3 SSB breakpoints, B_{min}, B_1, and B_2, which delimit the productivity bins. Recruitment values associated with $B_{min} \leq$ SSB $< B_1$ represent the low productivity bin, recruitment values associated with $B_1 \leq$ SSB $< B_2$ represent the medium productivity bin, and recruitment values associated with SSB $\geq B_2$ represent the high productivity bin. If *PropF* or *PropM* dropped below H_1 but remained above a minimum sex ratio threshold (H_{min}), recruitment was drawn from the low productivity bin. There was no recruitment (i.e., R_y=0) when SSB $< B_{min}$ or if either *PropF* or *PropM* dropped below H_{min}. As mentioned above, recruits can enter the population either as females or secondary males. Respectively, if m=10, the abundance of female and secondary male recruits at length l at time t, RU_{lt} and RS_{lt}, are calculated as

Table 1

Values and descriptions of parameters used in the length-based population model developed to study the response to exploitation by gonochoristic, atypical protogynous, and typical protogynous populations of black sea bass (*Centropristis striata*).

Parameter	Value	Description
R_1	15,000,000	Initial number of recruits (i.e., initial population)
M_y (y=1–5)	0.01	Annual full natural mortality rate for year y, initial years
M_y (y=6–45)	0.40	Annual full natural mortality rate for year y
F_y (y=1–14)	0.00	Annual fishing mortality rate for year y, initial years
F_y (y>14)	{0.00, 0.10, …1.50}	Annual fishing mortality rate for year y
α	27.09	Length (cm) of 50% probability of retention by the gear (L_{50})
β	1.053	Slope of fishing selectivity curve δ_l
$B_{\min}$	690	Minimum spawning stock biomass (SSB; metric tons [t]) required for potential recruitment
B_1	2,955	SSB Breakpoint 1 (t)
B_2	7,388	SSB Breakpoint 2 (t)
$H_{\min}$	0.02	Minimum sex ratio threshold (proportion female or male in spawning stock)
H_1	0.10	Sex ratio threshold 1 (proportion female or male in spawning stock)
a_U	0.000	Minimum asymptote parameter of female maturity ogive O_{Ul}
b_U	6.569	Slope (steepness) parameter of female maturity ogive O_{Ul}
c_U	21.233	Inflection point parameter of female maturity ogive O_{Ul}
d_U	1.000	Maximum asymptote parameter of female maturity ogive O_{Ul}
a_V	0.000	Minimum asymptote parameter of male maturity ogive O_{Vl}
b_V	6.374	Slope (steepness) parameter of male maturity ogive O_{Vl}
c_V	18.506	Inflection point parameter of male maturity ogive O_{Vl}
d_V	1.000	Maximum asymptote parameter of male maturity ogive O_{Vl}
L_∞	56.7	Asymptotic maximum length (cm)
K	0.0585	Growth coefficient (per seasonal quarter)
q_0	1.888	Theoretical age (in seasonal quarter units) at length l=0 cm
C	0.026	Oscillation amplitude coefficient for seasonal growth model
s_1	−0.2794	Sine wave starting time coefficient (in seasonal quarter units) for the seasonal growth model
$v_{1U} = v_{1T}$	−10.912	Weight-at-length coefficient for female (U) and transitional (T) stages
$v_{2U} = v_{2T}$	2.9120	Weight-at-length exponent for female (U) and transitional (T) stages
$v_{1S} = v_{1D}$	−10.954	Weight-at-length coefficient for secondary (S) and dominant (D) male stages
$v_{2S} = v_{2D}$	2.9094	Weight-at-length exponent for secondary (S) and dominant (D) male stages

$$RU_{lt} = R_y p_l(1 - \rho_l), \tag{12}$$

$$RS_{lt} = R_y p_l \rho_l, \tag{13}$$

where p_l = the proportion of recruits at length l; and
ρ_l = the proportion of male recruits at length l.

If $m \neq 10$, then $RU_{lt} = RS_{lt} = 0$.

Finally, weight at length for a fish of stage i and length l (W_{il}) was modeled with the allometric relationship:

$$W_{il} = \exp\left(\ln(v_{1i}) + v_{2i}\ln(l)\right), \tag{14}$$

where v_{1i} = the weight-at-length coefficient for stage i;
v_{2i} = the weight-at-length exponent for stage i; and
v_{1i} and v_{2i} are constants under the assumption of isometric growth.

Parameter estimation and input values for models

All parameter and input values were estimated on the basis of empirical data from the northern stock of black sea bass (an exploited stock) where appropriate. Estimated model parameters specifying M, selectivity, maturity, and weight at length were identical for all experimental populations, as were growth, initial population size, and recruitment inputs (Table 1). Transition rates and size at transition were specific to each experimental population, resulting in differences in the proportion of male recruits and population sex ratio.

For the first 5 years, M_y was set to 0.01 to initialize the simulated populations and for subsequent years was held constant and equal to 0.4 (Shepherd[3]). Initially, F_y was 0.00, after which F_y was set to different levels as described later in the *Simulations and sensitivity analyses* section. Selectivity parameters were estimated from assessment results (Shepherd[3]). Maturity ogives for females and males were defined on the basis of maturity data from the NEFSC spring survey cruise (1984–2013; n=3285), and weight-at-length parameters were estimated by using results from the NEFSC bottom trawl surveys conducted in spring and fall, 1993–2013 (Shepherd[3]).

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[3] Shepherd, G. R. 2009. Black sea bass 2009 stock assessment update. Northeast Fish. Sci. Cent. Ref. Doc. 09-16, 30 p. [Available at website, accessed May 2015.]

Quarterly growth matrices were derived from average length-at-age data from the NEFSC spring (1984–2011, n=3026), fall (1983–2010, n=3381), and winter (1992–2007, n=3390) surveys, and from commercial catch data from a summer survey (2012, n=59). Quarterly average length at age from all seasonal quarters were initially fitted (nonlinear regression, SAS 9.4[4], SAS Institute Inc., Cary, NC) to a von Bertalanffy growth curve for length in quarter q,

$$L_q = L_\infty(1-e^{-K(q-q_0)}). \tag{15}$$

Data were then fitted to a seasonal growth model by using parameter values from the von Bertalanffy growth model as defined in Pitcher and MacDonald (1973):

$$L_q = L_\infty(1-e^{-K_1}), \tag{16}$$

where $$K_1 = C\sin\left(\frac{2\pi(q-s_1)}{52}\right)+K(q-q_0), \tag{17}$$

where C = the amplitude of the oscillation around the average (nonseasonal) growth curve; and
s_1 = the starting time for the sine wave.

Expected quarterly growth by length was calculated for each centimeter from the sine wave growth model and formed the basis for deriving the quarterly growth matrices, which also were calibrated to empirical length distributions by quarter. Growth was fastest during the summer (quarter 3) and almost negligible during winter (quarter 1)—findings that are consistent with those from another study (Able and Hales, 1997).

Initial recruitment in year 1 was 15 million individuals, a value scaled to produce catch at equilibrium equivalent to empirical estimates at corresponding F values (Shepherd[3]). The length distribution of recruits was based on the average length distribution (at age zero), from NEFSC fall surveys, for lengths 4–11 cm for 2009–2013 (n=214). The 3 recruitment productivity bins were structured to reflect the distribution of SSB and recruits in the most recent assessment of the northern stock of black sea bass (Shepherd[3]). We defined 3 overlapping lognormal distributions (1 for each bin) and randomly drew 500 values from each distribution to create a CDF for each productivity bin. The average (and standard deviation [SD]) of the 500 R values per bin were 6517.9 individuals (SD 3058.0) for the low recruitment bin, 13,912.1 individuals (SD 4057.0) for the medium recruitment bin, and 29,634.3 individuals (SD 9289.1) for the high recruitment bin. Sex ratio thresholds ($H_{\min}$ and H_1) were set to 0.02 and 0.10, respectively; these values were assumed reasonable because no empirical data were available.

Input data specifying the proportion of male recruits and the transitions between the 4 different stages (i.e., female–transitional–secondary–dominant) differed among the 3 experimental populations. For the G case, half of the recruits were males (ρ_l=0.5; Fig. 2A) for a population with only females and males (i.e., transition rate from female to transitional [θ^{UT}_{lm}] and transition rate from secondary to dominant male [θ^{SD}_{lm}] were equal to zero). For the protogynous populations, the sex ratio of recruits and transition rates were determined such that the sex ratios of the AP population would reflect empirical data for the northern stock of black sea bass from the NEFSC bottom trawl surveys (n=13,107) conducted in spring (1984–2013, n=4040), fall (1983–2013, n=5126), and winter (1992–2007, n=3941), and the sex ratio of the TP population would have a characteristic pattern with all female recruits and all fish transitioning to male by 65 cm (Fig. 2C).

Transition rates were identified with F set at 0.35 to approximate the F that was likely occurring when the empirical data were collected. In the AP population, 23% of recruits were male and θ^{UT}_{lm} were equal to zero for lengths below 15 cm and followed a linear relationship (with slope 0.0039 and intercept −0.0492) for lengths 15 to 65 cm. The TP population had zero male recruits, and θ^{UT}_{lm} were zero for lengths below 12 cm, approximated a linear trend with slope 0.0108 and intercept −0.0228 for lengths 12–43 cm, and were constant at 0.43 for lengths 44–65 cm. Because of a lack of data to inform the split into secondary and dominant males in the protogynous populations, we assumed that half of males were dominant at 60 cm and set transition rates accordingly. Transition rates from transitional to dominant male (θ^{TD}_{lm}) and from secondary to dominant male (θ^{SD}_{lm}) were held constant for all protogynous populations according to a linear relationship that was derived from the data, with slope 0.0109 and intercept −0.1522 for lengths 15 to 60 cm. A constant transition rate of 0.5 was applied for lengths greater than 60 cm.

Simulations and sensitivity analyses

For each experimental population, the model was run for 45 years with values of annual F ranging from 0.0 to 1.5 by increments of 0.1 (i.e., 16 constant F scenarios). Each model configuration was run 100 times, with random draws of recruitment values (R_y). Results from year 45 were assumed to be representative of the populations at equilibrium under each configuration. For each scenario, the average and 95% confidence interval (CI) for stock abundance, stock biomass, catch abundance, catch biomass, SSB, and R were calculated at each value of F. Stock results represent abundance on 1 May (m=5), and catch results represent the sum of catch over the entire year.

Additional runs were performed to evaluate the sensitivity of the model results to capture selectivity, rates of sex change by females, proportion of dominant males, sex ratio thresholds of the spawning stock, and inclusion of the TP secondary males in SSB. For selectivity, the AP-50 configuration was run with values of ±10% (24.38 cm and 29.8 cm) for parameter α (L_{50}) of the selectivity curve δ_l (Eq. 8). To assess the influence

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[4] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

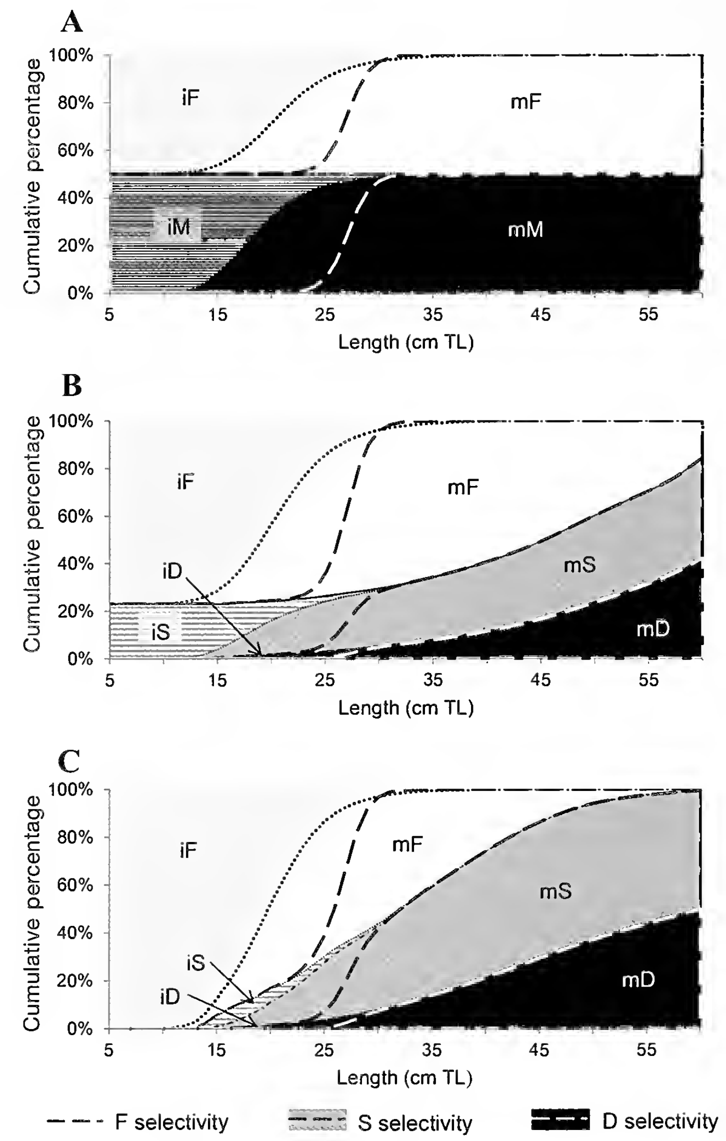

Figure 2

Cumulative percentages of life stages of black sea bass (*Centropristis striata*) by length (centimeters in total length [TL]) for (**A**) gonochoristic, (**B**) atypical protogynous, and (**C**) typical protogynous populations, which were simulated to study response to exploitation by using a length-based population model. Each shade of gray represents a different maturity state, immature (i) and mature (m), and life stage, including female (F), secondary male (S), dominant male (D), or simply male (M), in the gonochoristic case. Dashed lines represent fishing selectivity curves for each stage, as indicated in the key. Life stage characteristics were based on data collected, when available, from bottom trawl surveys conducted by the NOAA Northeast Fisheries Science Center during 1984–2013.

of female transition rates, the AP-50 and TP configurations were run with values of ±10% of $\theta_{\text{lm}}^{\text{UT}}$. We ran 2 sets of simulations on the protogynous populations to consider sensitivity of these populations to the proportion of males that were dominant, using transition rates that resulted in 25% and 75% males at 60 cm being dominant, respectively. Another set of sensitivity runs evaluated the effect of adding a third sex ratio threshold for the spawning stock (H_2). This additional threshold acts as a penalty for moderately skewed sex ratios in the spawning stock by directing R_y to be drawn from the medium productivity bin if *PropF* or *PropM* is between H_1 (where H_1=0.20) and H_2; all population simulations were rerun with H_2=0.40. Finally, the effect of spawning by secondary males in the TP population was explored with runs allowing participation in spawning by 50% and 100% of mature secondary males.

Results

Simulations

All simulation results showed a similar pattern at equilibrium (year 45) of decreasing average stock size (Fig. 3) with increasing F, and of catch increasing with F, reaching a maximum, and then decreasing to zero as harvesting pressure rose (Fig. 4). However, the specific performance of each experimental population differed. A general pattern existed where the results for G and TP populations bounded the results from the 3 AP populations, the size of which declined in an order consistent with the percentage of contributions to spawning made by secondary males. The performance of the AP-0 population was closest to the TP scenario; the AP-100 population generally did not differ from the G case, and the AP-50 population fell in between.

The size of the TP population at equilibrium remained well below levels reached by the G case at the same F, with stock size (abundance and biomass) never exceeding 81% of the size of the G population and remaining below 50% of the size of the G population for $F \geq 0.2$, before approaching zero at F=1.0 (Fig. 3). The equilibrium stock size of the AP-0 population started at almost the same size as that of the G population (94%) under no exploitation but dropped to levels similar to those of the TP population once F increased, even dropping below the size of the TP population for F=0.2 and F=0.3. The AP-50 equilibrium stock size was equivalent to that of the G population for low values of F but declined at a faster rate as F increased (Fig. 3). In contrast, the AP-100 stock size was almost identical to that of the G population for all values of F.

The difference in response of the AP populations stemmed from the increase in participation of secondary males in spawning—an increase that guarded against abrupt stock size decline under exploitation.

For example, at F=0.3, stock abundance for the AP-0 population was 31% of the abundance of the G population, the abundance of the AP-50 population was 76% of the abundance of the G population, and the AP-100 population was 98% of the abundance of the G population (Fig. 3A); the corresponding stock biomass results were 23%, 82%, and 100%, respectively (Fig. 3B). Stock abundance approached zero at F=1.1 for the AP-0 population and at F=1.4 for the AP-50 population, and stock abundance of the AP-100 population collapsed above F=1.5.

Contributions of secondary males to spawning altered the F associated with maximum catch. Catch for the G population was highest at F=0.3 before declining more than 65% by F=0.7 and approaching zero near F=1.5 (Fig. 4). Maximum yield in the TP configuration was reached at a lower F (0.2) with catch number equal to 42% of the maximum catch number for the G population (Fig. 4A) and with catch biomass equal to 45% of catch biomass of the G population (Fig. 4B). Catch and F at maximum catch in the AP scenarios increased with contributions of secondary males to spawning. The AP-0 population reached a maximum catch abundance of 34% of maximum G catch at F=0.1 (i.e., lower than the maximum of the TP population), and the AP-50 and AP-100 populations reached 81% and 99%, respectively, of maximum G catch at F=0.3 (Fig. 4A); corresponding catch biomass amounts for the 3 populations were 41%, 83%, and 100% (Fig. 4B).

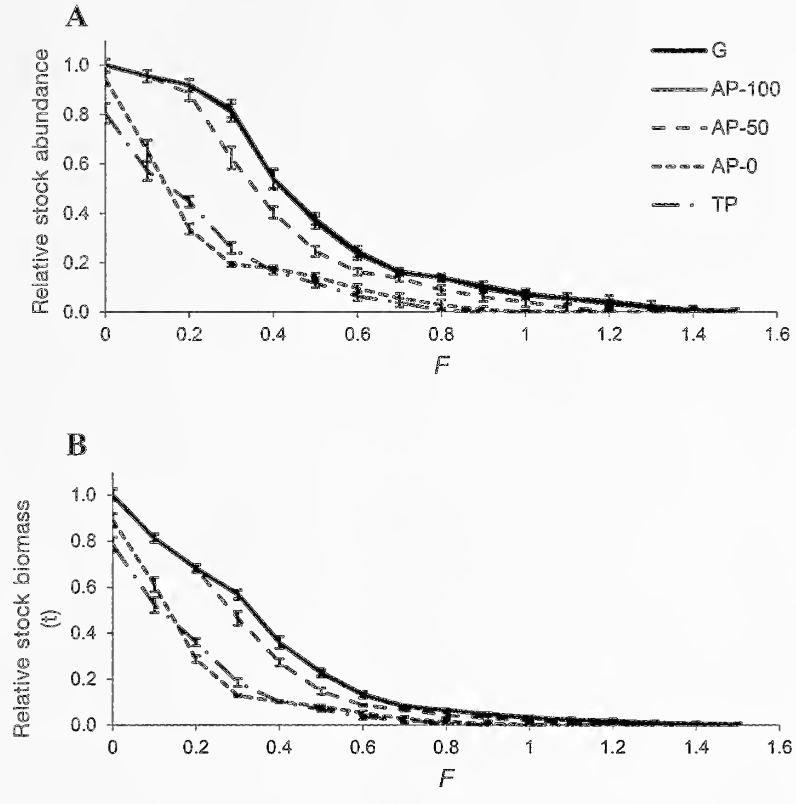

Figure 3

Equilibrium (year 45) (**A**) relative stock abundance and (**B**) relative stock biomass (measured in metric tons [t]) at values of fishing mortality (F) from 0.0 to 1.5 by 0.1 increments for 5 experimental populations of black sea bass (*Centropristis striata*): a gonochoristic (G) population (thick, solid line), atypical protogynous (AP) populations with 100% (AP-100; thin, solid line), 50% (AP-50; dashed line), and 0% (AP-0; dotted line) of mature secondary males contributing to spawning, and a typical protogynous population (TP; dashed-dotted line). Each line indicates the average stock size (from 100 runs performed at each value of F) in relation to the average stock size of the G population at F=0.0; vertical bars represent 95% confidence intervals. These results were obtained by using a length-based population model to study the response of these populations to exploitation.

Analysis of trends in SSB and R (averages and 95% CIs of the 100 simulation runs) versus F provided information in relation to recruitment and indicated which productivity bin each population was drawing from at equilibrium (year 45) at different values of F (Fig. 5). These results confirmed the pattern that, in general, results for the G and TP populations represented extremes in population productivity; the results of the 3 AP populations, however, occurred in a consistent order in between those extremes. Although all populations began in the high productivity recruitment bin (SSB$\geq B_2$) at F=0.0, the progression to the end of recruitment as F increased and SSB fell below $B_{\min}$ differed across populations. The G and AP-100 populations remained in the high productivity bin until F=0.3, at which point average recruitment declined to the medium and low productivity bins when F=0.4–0.8 and approached zero as F reached 1.5. The AP-50 population functioned under high productivity until F reached 0.2 before following a similar decline and approaching recruitment failure at F=1.2. The TP and AP-0 scenarios stayed in the high productivity bin only if there was zero exploitation and SSB and R declined more rapidly than they did for the other populations as F increased, reaching recruitment failure by F=0.9.

As seen with the results of stock size discussed previously, the results for SSB and R for the AP-0 population were below those of the TP population when F=0.2 and F=0.3. With increasing exploitation, females quickly constituted more than 90% of the spawning stock abundance for the AP-0 population (Fig. 2B), such that *PropM* fell between the sex ratio thresholds ($H_{\min}$=0.02 and H_1=0.10), causing recruitment to drop to the low productivity bin (Fig 5B). In contrast, the TP population had a higher proportion of males in the spawning stock (Fig. 2C); therefore, the spawning stock sex ratio did not become overly skewed and did not trigger lower recruitment.

Stock-recruitment curves indicate that the TP and AP-0 populations had a lower productivity relationship than that of the G, AP-100, and AP-50 populations (Fig. 6). A lower line indicated that R was drawn more often from the low recruitment bin because of an overly skewed spawning stock sex ratio (i.e., less than 10% males or less than 10% females).

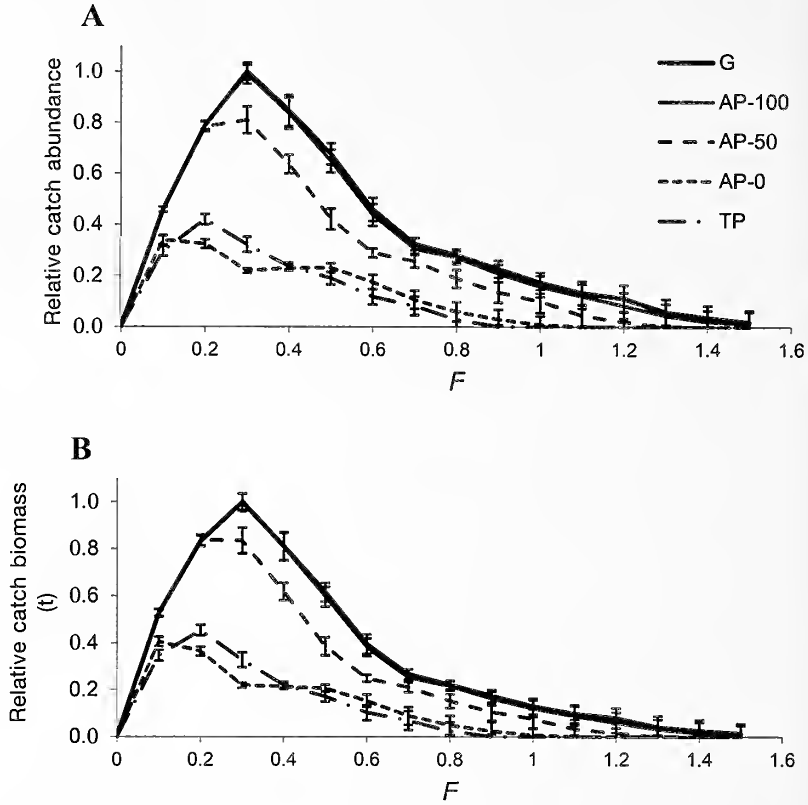

Figure 4

Equilibrium (year 45) (**A**) relative catch abundance and (**B**) relative catch biomass (measured in metric tons [t]) at values of fishing mortality (F) from 0.0 to 1.5 by 0.1 increments for 5 experimental populations of black sea bass (*Centropristis striata*): a gonochoristic (G) population (thick, solid line), atypical protogynous (AP) populations with 100% (AP-100; thin, solid line), 50% (AP-50; dashed line), and 0% (AP-0; dotted line) of mature secondary males contributing to spawning, and a typical protogynous population (TP; dashed-dotted line). Each line indicates the average catch size (from 100 runs performed at each value of F) in relation to the average catch size of the G population at F=0.0; vertical bars represent 95% confidence intervals. These results were obtained by using a length-based population model to study the response of these populations to exploitation.

Sensitivity analyses

The sensitivity analysis on capture selectivity for the AP-50 population produced the expected results. A 10% decrease in length at capture caused 1) lower stock size and earlier stock collapse (average decrease of 27% for stock abundance and 31% for stock biomass, with both reaching zero by F=1.1), 2) a higher (about 10% increase) maximum catch that occurred at a lower F (0.2) but was followed by lower catch numbers, on average 36% (46% for catch biomass) below the levels of the base AP-50 population, and 3) a general reduction in recruitment (46% decrease in R on average). On the other hand, a 10% increase in length at capture generated the opposite results: 1) stock size increased by about 53% (66% for stock biomass) and did not collapse by F=1.5; 2) maximum catch was close to the results from the base AP-50 population and still occurred at F=0.3, and catch numbers for values of F beyond where the maximum occurred were on average 55% (83% for catch biomass) higher than the values for the base AP-50 population; and 3) R increased by about 25%.

Sensitivity to changes in $\theta_{\mathrm{lm}}^{\mathrm{UT}}$ differed for the AP-50 and TP populations. All results indicate that stock performance for the AP-50 population had very low sensitivity to a 10% change in $\theta_{\mathrm{lm}}^{\mathrm{UT}}$, closely matching the results for the base AP-50 population. In contrast, the TP population was more sensitive to change in $\theta_{\mathrm{lm}}^{\mathrm{UT}}$. A 10% decrease in sex change transitions resulted in slightly higher stock sizes (7% increase on average for stock number and stock biomass) and higher catch (9% average increase in catch numbers and biomass), and a 10% increase in sex change transitions resulted in slightly lower stock sizes (8% decrease on average for stock number and stock biomass) and lower catch (9% average decrease in catch numbers and 8% average decrease for catch biomass); F at maximum catch remained the same as it was for the base AP-50 population. Finally, drawing from recruitment bins was not affected by a 10% change in the sex transition rates.

Modifying the proportion of dominant versus secondary males in the protogynous populations had a greater effect on the AP-0 and TP populations than on the AP-50 configuration, and it had no effect on the AP-100 scenario because all males already contributed to spawning in this population. For the AP-50 population, reducing the proportion of dominant males to 25% at 60 cm led to an average decrease of just over 6% for stock abundance and catch at F>0.2 and to average drops of 8% in SSB and of 5% in R. With transition

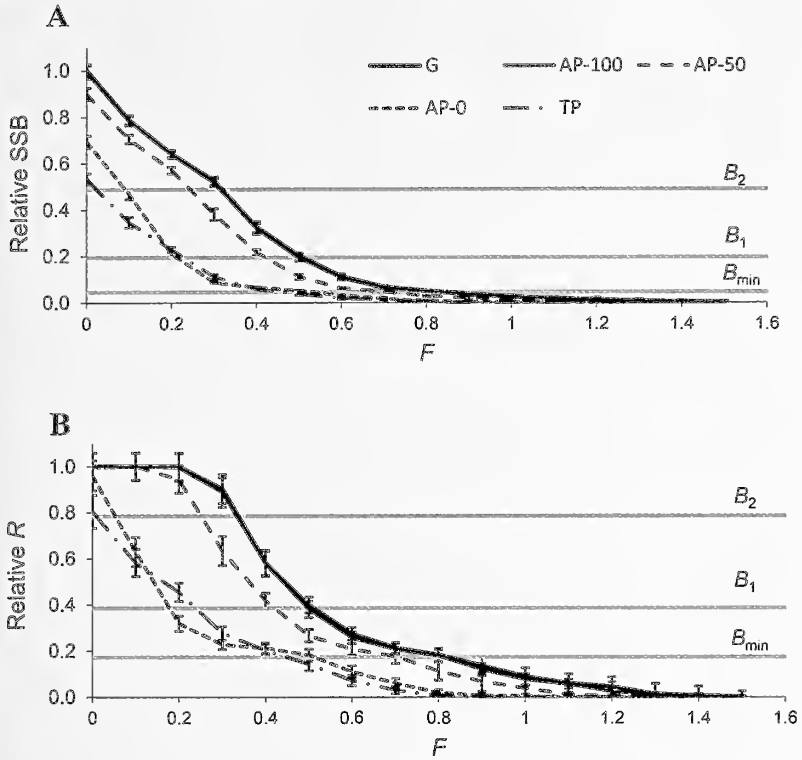

Figure 5

Equilibrium (year 45) (**A**) relative spawning stock biomass (SSB) and (**B**) relative number of recruits (R) at values of fishing mortality (F) from 0.0 to 1.5 by 0.1 increments for 5 experimental populations of black sea bass (*Centropristis striata*): a gonochoristic (G) population (thick, solid line), atypical protogynous (AP) populations with 100% (AP-100; thin, solid line), 50% (AP-50; dashed line), and 0% (AP-0; dotted line) of mature secondary males contributing to spawning, and a typical protogynous population (TP; dashed-dotted line). Each line indicates the average SSB or R (from 100 runs performed at each value of F) in relation to the average SSB or R of the G population at F=0.0; vertical bars represent 95% confidence intervals. The gray reference lines indicate the position of the SSB breakpoints (B_{min}, B_1, and B_2) and corresponding R delimiting the recruitment productivity bins. These results were obtained by using a length-based population model developed to study the response of these populations to exploitation.

rates set for 75% of males being dominant at 60 cm, the population experienced an average increase of 4% in stock abundance and catch and a rise in SSB and R of about 5%.

In contrast, the 2 populations with no secondary males contributing to spawning were more sensitive to a change in proportion of dominant males. With transition rates set for 25% of males being dominant at 60 cm, stock size and catch for the AP-0 population dropped by around 65% for F values below 0.3 and by just above 50% for F≥0.3. The TP population stock size and catch dropped by about 40% for F values below 0.3 and by close to 65% for F≥0.3. Average SSB for the AP-0 population decreased by 58%, and R declined by 52%; corresponding values for the TP configuration showed an average 62% decrease in SSB and 55% decrease in R. The increase in proportion of dominant males for the AP-0 population resulted in an increase in stock size and catch up to 70% at F values below 0.3 and in an increase of around 45% for F≥0.3. The increase for the TP population was just over 30% for F<0.3 and over 50% for F≥0.3. On average, SSB increased by 60% and R by 55% for both the AP-0 and TP populations. In general, the performance of the AP-0 and TP populations moved closer to that of the base G population with additional dominant males.

Sensitivity analyses that considered the effect of an alternative recruitment process indicated that most protogynous scenarios were sensitive to the addition of a third sex ratio threshold for the spawning stock, H_2, where recruitment was drawn from the medium productivity bin if *PropF* or *PropM* was between H_1=0.10 and H_2=0.40. Because sex ratio in the G population was fixed at 1:1, this population was not affected by any of the sex ratio thresholds. However, all the protogynous populations experienced a significant drop in recruitment with the addition of H_2, especially at lower F values (F≤0.7), where R decreased by an average of 32% for the AP-100 population, 28% for the AP-50 population, and 16% for the AP-0 and TP populations. At higher values of F, recruitment declined as well, but it did so less dramatically (from 0% to 19%) because recruitment was already low as a result of low SSB at those levels of exploitation. Recruitment for the protogynous scenarios was limited to the low and medium productivity bins, indicating that the spawning stock sex ratio of these populations was always skewed (Fig. 2), specifically that *PropM* was always less than H_2=0.4.

In parallel to the decrease in recruitment, all other measures of stock performance (SSB, stock size, and catch) decreased as well. In general, all results for the AP populations moved closer to the results for the TP population, while continuing to follow the order that the TP population was least productive, followed by the AP-0, AP-50, and AP-100 populations, as seen in the results from the runs of the base model.

In the final sensitivity analysis, the effect of spawning by secondary males in the TP population was considered. Allowing all secondary males to spawn brought the TP population performance close to the performance of the base G and AP-100 populations, although it was

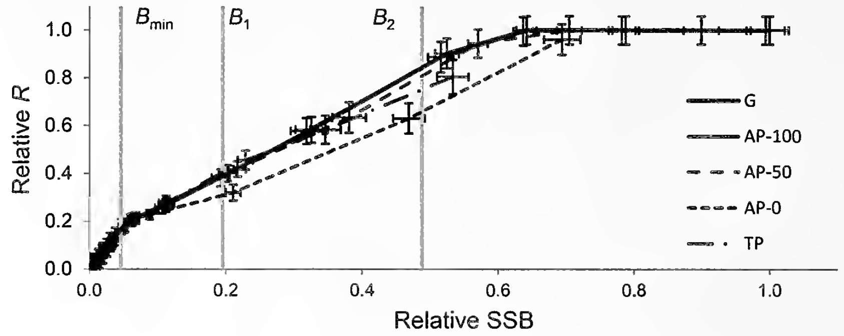

Figure 6

Equilibrium (year 45) relationship between stock and recruitment represented by the relative number of recruits (R) versus the relative spawning stock biomass (SSB) for 5 experimental populations of black sea bass (*Centropristis striata*): a gonochoristic (G) population (thick, solid line), atypical protogynous (AP) populations with 100% (AP-100; thin, solid line), 50% (AP-50; dashed line), and 0% (AP-0; dotted line) of mature secondary males contributing to spawning, and a typical protogynous population (TP; dashed-dotted line). Each line indicates the average R and SSB (from 100 runs performed at each value of F) in relation to the average R and SSB of the G population at F=0.0; vertical and horizontal bars represent 95% confidence intervals for relative R and relative SSB, respectively. The gray reference lines indicate the position of the SSB breakpoints (B_{min}, B_1, and B_2) that define the different recruitment productivity bins. These results were obtained by using a length-based population model to study the response of these populations to exploitation.

still slightly (about 3%) below it. Similarly, a 50% rate of spawning by secondary males in the TP population brought stock performance closer to that of the AP-50 population; however, levels for the TP population were 12–17% lower.

Discussion

We developed a length-based model to simulate the dynamics of a protogynous fish population and examined the resilience of an atypical protogynous population on the basis of the northern stock of black sea bass. The model allowed evaluation of the impact of exploitation on a population's resilience and productivity across a range of life history attributes. The life history of the northern stock of black sea bass is atypical for a protogynous species because the sex ratio at length is not as skewed toward females at small sizes (<30 cm) nor toward males at large sizes (>45 cm), as would be expected in a typical protogynous population. Previously published findings (Alonzo and Mangel, 2004; Heppell et al., 2006; Hamilton et al., 2007) indicate that protogynous stocks are more susceptible to overexploitation than are equivalent gonochoristic stocks. Our results support that conclusion, in particular for typical protogynous stocks, which have highly skewed sex ratios. However, we found that atypical protogynous life histories, for which a higher proportion of the small fish are males, may provide added resiliency to exploitation in comparison with typical protogynous species for which all small fish are females and males arise only as a result of subsequent sex change by females. The different transition rates that led to different proportions of females, secondary males, and dominant males in the 2 types of populations resulted in different SSB and SSB sex ratios and ultimately in higher recruitment for the AP population. Moreover, we found that the ability of the AP population to persist under exploitation increases as a function of contribution of secondary males to spawning.

The different response to exploitation by the G, AP, and TP populations can be explained by considering which individuals contribute to SSB because the composition of the spawning stock defines recruitment and ultimately population persistence. In the G case, we assumed that all mature males and females contributed to spawning, whereas in the protogynous cases, SSB was the sum of females and the subset of dominant males and had a range of possible contributions from secondary males for the AP populations. Size-selective exploitation primarily targeting large individuals decreased SSB, skewed protogynous population spawning stock sex ratios (Fig. 2, B and C), and made the populations more likely than the G population to experience decreased fertilization because of sperm-limitation. Within the protogynous scenarios, different sex ratios of mature fish also affected the size of SSB and thus

R, particularly if a substantial portion of the mature males were nonspawning secondary males. For example, even in the absence of fishing, the TP population was less productive than the other populations (Fig. 5) because of a smaller SSB that resulted in recruitment occasionally being drawn from the medium recruitment bin, whereas all other populations had SSB high enough to have *R* drawn consistently from the high productivity bin.

Our results indicate that as the contribution to spawning by secondary males in the AP population increased, SSB approached that of the G species and the population became more resilient to exploitation. The addition of secondary males to the spawning stock not only increased SSB but also shifted the sex ratio and lowered the risk to the population of being sperm-limited. In other words, the more secondary males participated in spawning, the more an AP population would perform as if it were gonochoristic because the spawning stock sex ratio approached 1:1. Consequently, the greater the contribution of secondary males, the more resilient the AP population was to fishing. Sensitivity analyses indicated that the same was true for the TP population; however, for equivalent scenarios (i.e., both having 50% or 100% of secondary males spawn) the AP population was still more resilient than the TP population, although the magnitude of the difference depended on the proportion of secondary males included in SSB.

The presence of male recruits in the AP population was also an important characteristic. It not only dampened the effect of size-selective exploitation by changing the sex ratios at length but also provided a constant supply of males to the population from a source other than postmaturational sex change. Although transition rates were held constant in our model, previous research (Huntsman and Schaaf, 1994; Alonzo and Mangel, 2004; Taylor, 2013) suggests that sex change is likely to be socially controlled and transition rates may increase in response to removal of males as a way to maintain stable sex ratios. Under severe exploitation, such compensatory sex change would be the only avenue for a typical protogynous population to avoid extremely low abundance of males. In contrast, an atypical protogynous life history, with males available beginning at recruitment, provided an additional buffer against overharvesting of dominant males. Our sensitivity analyses support this interpretation with results indicating that abundance and SSB in an AP configuration were more robust than abundance and SSB in the TP case in response to variations in the rate of sex change. In addition, the proportion of mature dominant males played a significant role because a higher fraction of dominant males brought sex ratios closer to a gonochoristic situation and rendered the population more resilient to fishing.

The life history characteristics of the AP population increased robustness in response to exploitation, and we expect they would also improve resistance to changes in capture selectivity. Results from sensitivity analyses on the selection pattern indicated that a reduction in size at selection had some influence on abundance for the AP-50 population. Because any changes to selectivity would apply across all scenarios, we would expect similar results in the other populations if we tested them. However, as seen with other factors, the TP population was generally more vulnerable to a drop in recruitment because of a decrease in SSB or an overly skewed SSB sex ratio; therefore, reducing the size at selectivity would likely affect this population more severely. The sensitivity tests were not performed in a multiplicative manner, and, as a result, it is possible that a response to changes to exploitation with changes to transition rates could alter the outcome. In addition, the effect of dome-shaped selectivity was not explored here because of the lack of empirical evidence supporting it, but the influence of this type of selectivity could be explored in the future.

The sex ratio at maturity, the presence of mature secondary males, and large mature females all worked toward making the AP population more stable than a population with a typical protogynous life history. Black sea bass north of Cape Hatteras undergo seasonal migrations to the edge of the continental shelf—movements that in some cases cover long distances (Musick and Mercer, 1977; Moser and Shepherd, 2009). Such behavior is generally not seen in tropical or subtropical species for which hermaphroditism is most common. The advantages of hermaphroditism evolved in natural systems where there is a selective advantage of size differences between sexes (Ghiselin, 1969; Warner, 1975; Kazancıoğlu and Alonzo, 2010).

The selective advantage gained by beginning life as female then switching to male as needed depends on feedback about the existing sex ratio within the reproductive community; too many large males would reduce any reproductive size advantage and only increase sperm competition (Petersen, 1991). However, in a migratory species adopting this life history, the feedback loop may be seasonally interrupted. If individual fish stray from the group with the sex ratio that elicited the sex change, the advantage of being a large male could be lost. In the northern stock of black sea bass, results from a tagging study indicate that the farther the migration, the lower the probability of returning to the original spawning aggregation (Moser and Shepherd, 2009). Consequently, the possibility of returning to a group with large males already present would reduce the advantage gained by switching sex before the migration. Under such conditions, gonochoristic traits may provide more reproductive stability than typical protogyny.

Although our model inputs were based on empirical data as much as possible, we had to make assumptions about several key population processes. The relationship between SSB, spawning stock sex ratio, and subsequent recruitment for black sea bass is poorly understood, and productivity might be highly influenced by environmental factors (Able and Hales, 1997). Given the absence of an accepted stock-recruitment model, we used a binned recruitment approach on the basis of empirical data, recognizing that the intent of our model was to evaluate the contrast in response between the population life histories rather than to estimate absolute abundances. We included spawning stock sex ratio as bounds within the stock-recruitment relationship for cases where the sex ratio became extremely skewed.

Even though our approach was rather rudimentary, it allowed us to highlight the importance of incorporating spawning stock sex ratio in addition to SSB in the determination of recruitment for protogynous stocks. If a small proportion of males can successfully fertilize all mature females, the population could remain fully productive even with a skewed sex ratio. In contrast, if the optimal fertilization sex ratio is close to 1:1, recruitment would drop if the spawning stock sex ratio became more skewed toward females, as seen in our sensitivity analyses, which showed that recruitment was sensitive to changes in the sex ratio thresholds for the spawning stock. These findings also emphasized the need for more information on spawning behavior of black sea bass and of protogynous hermaphrodite species in general. Specifically, data on male-to-female ratios within a spawning event would help to improve understanding of when a population might be susceptible to sperm limitation or egg limitation and to evaluate the effectiveness of using the combined male and female SSB for populations with skewed sex ratios. Finally, it is unknown to what degree compensatory recruitment may occur and to what extent such recruitment might alter population resilience under exploitation. Given the lack of data to inform this process, we chose not to include it in our model; however it could warrant further exploration.

Transition rates were determined to produce sex ratios at length closely resembling those of the northern stock of black sea bass, but actual transition rates, including their timing and the split between secondary and dominant males, are unknown. We assumed that sex change occurred quickly and that rates of transition from secondary to dominant male were the same for all protogynous populations because our main concerns were to investigate the implication of the atypical life history and the contribution of secondary males to spawning in the north. The duration of the transition made little difference in our model as long as it did not overlap with the spawning period; as long as all transitions were completed before SSB was calculated, there was little influence from this factor. However, it is possible that true transition probabilities differ significantly from what we assumed and that transition rates may differ between an atypical versus typical hermaphrodite population. Moreover, we did not incorporate density dependence into our model, although density-dependent transition rates (compensatory sex change) may have a significant effect on adjusting sex ratios under high fishing pressure (Ellis and Powers, 2012). Molloy et al. (2007) found that flexibility in size at sex change may make protogynous populations as resilient to fishing as gonochoristic populations; therefore, this feature should be included in future research, if possible.

We did not model social behavior, competition among males, or density-dependent changes in fertilization success for each life stage in part because of a lack of empirical data. The lack of secondary sex characteristics in secondary males would indicate that they are opportunistic spawners rather than direct competitors of dominant males. Although it is unlikely that males participating in spawning as secondary males would be as effective as the dominant males from a behavioral perspective, there is evidence that relative gonad size of secondary males may be larger than that of dominant males, resulting in increased likelihood of fertilization success (Knapp and Carlisle, 2011).

Finally, other possible factors, such as density- or size-dependent M, movement, or spatial patterns, were not incorporated into the model. We also assumed that growth was constant across sexes; however, if sexual dimorphic growth favoring males exists, one would expect the G and AP scenarios to become even more aligned.

Despite the limitations of the data and the model, the results of this research provide new insight into the response of protogynous species to exploitation. In particular, atypical sex ratios at length and possible contribution of secondary males to spawning have the potential to significantly increase the resilience of a protogynous stock to fishing. This study indicates that stocks exhibiting atypical protogynous characteristics, such as the northern stock of black sea bass, may be more resilient to exploitation than typical protogynous hermaphrodites characterized by dominant males monopolizing spawning opportunities. In addition, our results highlight the need for a better understanding of factors that govern key processes, such as sex change, secondary male spawning, and recruitment, in protogynous species in general and in black sea bass in particular.

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of *Fishery Bulletin*

Abstract—Age, growth, and monthly reproductive characteristics were determined for the orange-spotted grouper (*Epinephelus coioides*) from northern Oman. This species is characterized by a prevalence of females (1–11 years old), and males make up 6.5% of the total sample. Growth parameters indicate a typical pattern for groupers with a low growth coefficient (K=0.135). The trajectory of the von Bertalanffy growth function was almost linear with no evidence of asymptotic growth. Estimates of mortality revealed a low natural mortality of 0.14/year but a high fishing mortality of 0.59/year. More alarming was the high rate of exploitation (0.81/year), considered unsustainable for a slow-growing grouper. The population off southern Oman is diandric protogynous, and sex change takes place between 449 and 748 mm in total length (TL) or over a period of 4–8 years. The gonadosomatic index for females showed a short spawning season from March through May, although ~30% of females were ripe for 7 months of the year. Size and age at 50% maturity for females was estimated to be 580 mm TL and 4 years, respectively. We suggest that substantial changes in the management of this species will be vital in sustaining viable populations of orange-spotted grouper and other species of Epinephelidae within Oman.

Demographic profile of an overexploited serranid, the orange-spotted grouper (*Epinephelus coioides*), from northern Oman

Jennifer L. McIlwain[1,2]
Aisha Ambu-ali[1]
Nasr Al Jardani[1]
Andrew. R. Halford[3]
Hamed S. Al-Oufi[4]
David A. Feary (contact author)[5]

Email address for contact author: david.feary@nottingham.ac.uk

[1] Department of Marine Science and Fisheries
College of Agricultural and Marine Sciences
Sultan Qaboos University
P.O. Box 34, Al-Khod 123
Sultanate of Oman

[2] Department of Environment and Agriculture
Faculty of Science and Engineering
Curtin University
G.P.O Box U1987
Perth, Western Australia 6487, Australia

[3] Marine Science Program
Department of Parks and Wildlife
Dick Perry Avenue
Kensington, Western Australia 6151, Australia

[4] Ministry of Agriculture and Fisheries
P.O. Box 1700, Muscat 111
Sultanate of Oman

[5] School of Life Sciences
University of Nottingham
University Park
Nottingham NG7 2RD, United Kingdom

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The recent increase in demographic analyses of economically important reef fish stocks from the Indo-Pacific region has made a major contribution to our understanding of key population parameters (Ferreira and Russ, 1992; Newman et al., 1996; Russ et al., 1996; Adams et al., 2000; Grandcourt, 2002, 2005; Williams et al., 2003; Pears et al., 2006). What has emerged is increasing evidence that many exploited reef fish species have life history strategies of extended life spans, slow growth, and, in the absence of fishing, an accumulation of older individuals—strategies usually associated with species in temperate (10–25°C) and deep (>30 m) waters (Choat and Robertson, 2002; Pears et al., 2006; Wakefield et al., 2013, 2015). Stocks that have such traits present challenges for fisheries managers, especially in developing countries where life history information is rarely collected (Polunin et al., 1996), mostly because of a lack of funding. The presence of these traits may also explain why conventional fisheries management practices have failed, even for sectors that are regulated (Coleman et al., 2000; Sadovy de Mitcheson et al., 2013).

The epinepheline serranids, or groupers, form an important taxonomic group both from a commercial and ecological perspective (Sluka et al., 2001; Sadovy de Mitcheson et al., 2013). However, a suite of life history strategies and certain behavioral traits make most species of grouper susceptible to overfishing. Life-history strategies include slow growth and late-onset reproduction (Manooch, 1987; Pears et al., 2006), long life spans (Manooch, 1987; Bullock et al., 1992; Grandcourt, 2005; Wakefield et al., 2015), complex and variable reproductive patterns (Shapiro, 1987; Fennessy and Sadovy, 2002; Rhodes and Sadovy, 2002) including sex change, while traits include a propensity to aggregate during spawning (Colin, 1992; Sadovy et al., 1994; Samoilys and Squire, 1994; Robinson et al., 2015; Tuz-Sulub and Brulé 2015), and a high degree of site fidelity (Zeller and Russ, 1998; Starr et al., 2007; Luckhurst, 2008).

Frequently published low values of natural mortality (M) for grouper indicate that harvesting levels should be set at less than 10% of the total biomass (Walters and Pearse, 1996; Coleman et al., 2000). However, low rates of M coupled with commonly cited low rates of growth can also lead to "growth overfishing" with resultant truncated size-distributions as fish are unable to grow to their maximum sizes (e.g. Beverton and Holt 1957). It has also been suggested that populations in which sex change is predominately female to male, under a scenario of increased fishing mortality (F), lose reproductive capacity in 2 ways: through selective removal of larger, mostly male individuals that in turn cause sperm limitation (Coleman et al., 1996) and a decrease in the size of females that in turn reduces total fecundity (Sadovy, 1996; Adams et al., 2000; Sadovy de Mitcheson and Liu, 2008).

Hermaphroditism in groupers can be expressed in 1 of 2 ways: as *simultaneous,* when individuals are capable of reproducing as both male and female, or *sequential* (the most common mode), when sex change is made from female to male (protogyny) (Smith, 1965; Shapiro, 1987; DeMartini et al., 2011; Wakefield et al., 2015). Protogynous populations can be further characterized on the basis of the sexual pathway of male development. Monandric protogyny occurs when males develop only from females (secondary males) through a transitional phase, whereas diandric protogyny involves 2 pathways for male development: directly from juveniles (primary males) or through sex change from mature females (Reinboth, 1967). To date, most grouper investigated in detail and that belong to the genus *Epinephelus* have been confirmed to be monandric hermaphrodites (Sadovy et al., 1994; Brulé et al., 2000; Pears et al., 2006; DeMartini et al., 2011; Wakefield et al., 2015). However, increasing evidence indicates that some species are diandric hermaphrodites, such as the catface grouper (*Epinephelus andersoni*) (Fennessy and Sadovy, 2002), and Sadovy and Colin (1995) confirmed gonochorism, with the potential for protogynous sex change, in the Nassau grouper (*Epinephelus striatus*). Clearly, the genus shows sexual plasticity in reproductive development, which has a bearing on population persistence and fisheries management.

In Oman, grouper, or *hamoor*, are considered one of the most economically important groups of finfish in the artisanal fishery and are heavily targeted by fishermen with a variety of fishing gear (Siddeek et al., 1999). One species, the orange-spotted grouper (*Epinephelus coioides*) occurs primarily in northern Oman along the Musandam Peninsula, inhabiting deep rocky reefs to depths of 100 m (Randall, 1995). Fishermen that target this species use semicircular wire basket traps with, a radius of 2.5–3.5 m, and 35-mm wire-mesh. Traps are deployed either from fiberglass boats of 4–10 m in overall length (and an average of 28 traps were deployed per trip at an average water depth of 70 m) or from large wooden dhows <25 m overall length (and an average of 75 traps were deployed per trip at an average water depth of 150 m) (senior author, unpubl. data). To date, there are no restrictions on fishing effort for this fishery, and therefore no limits on the numbers or size of orange-spotted grouper landed by fishermen. The official fisheries statistics reveal that catches of grouper from Musandam have remained steady since 1993 at an average of 370 metric tons (t) per year (MAF[1]). This catch represents 10.5% of the total landings of grouper for Oman from 1993 to 2009 (MAF[1]). On the basis of the total landings of grouper in Musandam, the orange-spotted grouper is the thirdmost important species of grouper by numbers and secondmost important by weight (senior author, unpubl. data).

Despite the importance of orange-spotted grouper to both the southeast Asian aquacultural industry and the trade of live reef fish (Grandcourt, 2012), little demographic or reproductive information exists on wild stocks from throughout its geographic range (but see Grandcourt et al., 2005, 2009), with recent calls by the International Union for Conservation of Nature and Natural Resources for further work that would examine the demography and life history of this species (Cornish and Harmelin-Vivien, 2004). The earliest reproductive study produced some evidence of sex change through social control, but it did so for only a few individuals (n=6) kept in captivity (Quinitio et al., 1997). More recently, Grandcourt et al. (2005, 2009), focusing on populations within the southern Arabian Gulf, and Liu and Sadovy de Mitcheson (2009), through a controlled experiment of hatchery-reared juvenile orange-spotted grouper, confirmed diandric protogyny.

The lack of basic biological data on orange-spotted grouper from the Oman region, the recent anecdotal evidence of declines in landings, and concerns over the spread of the trade of live reef fish into the west Indian Ocean prompted the work described here to determine the demographic makeup of orange-spotted grouper in Oman and to characterize the sexuality and spawning times of this species.. Specifically, we set out to describe the age structure, growth parameters, and mortality schedules of the population from northern Oman. In light of the heavy fishing pressure on this species both in Oman and neighboring countries (Grandcourt et al., 2005, 2009), we also discuss potential management strategies that are appropriate for hermaphroditic populations.

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[1] MAF (Ministry of Agriculture and Fisheries). 2010. Fisheries statistics book 2009, 130 p. [Available from Omani Fisheries Statistic Department, Ministry of Agriculture and Fisheries, P.O. Box 467, Muscat, Sultanate of Oman.]

Materials and methods

Sampling

Between May 2004 and August 2005, 214 orange-spotted grouper were purchased at monthly intervals either directly from fishermen or agents at the fish landing site at Dibba, the only landing site on the east coast of the Musandam Peninsula that extends into the Strait of Hormuz at the mouth of the Arabian Gulf (Fig. 1). Sampling took place during the first week of each month. Fish were kept on ice until they were processed, and all fish were measured to the nearest millimeter in total length (TL) and weighed for total wet weight to the nearest gram. An additional 156 individual orange-spotted grouper were weighed and measured at the landing site and included in the length–weight relationship. Because of the difficulty in staging nonripe gonads of grouper macroscopically (Fennessy and Sadovy, 2002), all gonads, ovaries and testes, were removed and weighed to the nearest 0.01 g and preserved in 10% formalin solution for later histological preparation. Gutted whole weights (GWs) of fish were also taken. Sagittal otoliths were removed, cleaned in distilled water, and stored dry for subsequent age determination.

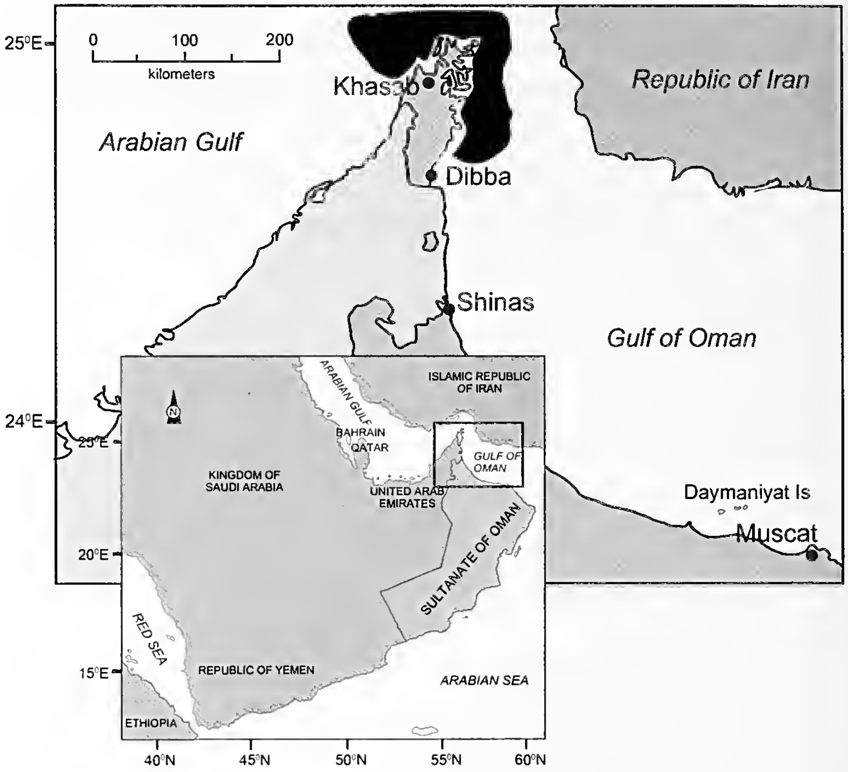

Figure 1

Map of the major landing site (Dibba) where samples of orange-spotted grouper (*Epinephelus coioides*) were collected between May 2004 and August 2005 for this study. The black area denotes the fishing grounds that support the fishery for orange-spotted grouper in northern Oman.

Otolith processing and validation of otolith age

For microscopic examination, one sagittal otolith was weighed (to the nearest 0.001 g), then set in Crystalbond[2] resin (Aremco Products Inc., Valley Cottage, NY) on the edge of a glass microscope slide. A modified grinding wheel, with P600 wet and dry grit paper, was used to grind the otolith as close to the nucleus as possible (Choat et al., 2003). The otolith half was then repositioned in the middle of the slide, with the polished side facedown, and again ground as close to the nucleus as possible. Sectioned otoliths were left on a hot plate at 60°C for 1–2 h until dark brown in color. The age of each individual was determined by counting the annual growth increments under a microscope, with transmitted light at 15–25× magnification. All otoliths were read twice by the senior author using the double-blind method (Russ et al., 1998). If the 2 readings disagreed by more than one increment, the otolith was read a third time. The otolith was eliminated from the analysis if the third reading differed as well. The date of May 1 was assigned as the nominal hatching date for each individual on the basis of the peak spawning period between April and May for orange-spotted grouper from Musandam (Grandcourt et al., 2005, 2009).

Either marginal zone or edge analysis was used to validate the annual deposition of opaque zones on the otoliths of orange-spotted grouper by inferring the time of year that the increments were formed (Radebe et al., 2002). The otolith margin was recorded as either opaque or translucent for 91% of individuals. To determine the time when deposition took place, the frequency of otoliths with opaque margins was plotted by month.

Determination of sex and maturity stages

After preservation, gonads were subject to standard histological preparations and examination to assign a sexual and maturity stage to individual fish (West, 1990) . A thin, transverse section was taken from the central region of one gonad lobe, dehydrated in a Shandon Citadell 1000 processor (Thermo Fisher Scientific Inc., Waltham, MA), embedded in wax, sectioned at 5–7 µm, mounted on a slide, and stained with haematoxylin and eosin stains. Each gonad was assigned to both a maturity stage (Sadovy and Colin, 1995; Adams, 2002; Fennessy and Sadovy, 2002; Pears et al., 2007) from

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[2] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the authors or the National Marine Fisheries Service, NOAA.

one of the following 8 classifications: 1) undetermined inactive female; 2) immature female; 3) mature, resting or inactive female; 4) mature, active female; 5) spent female; 6) transitional individual; 7) immature male; 8) mature, active male. Ovaries were classified by the presence of the most advanced oocyte, regardless of its abundance (West, 1990).

Maturity schedules for female fish were calculated by plotting the percentage of frequency of both mature, active and mature, inactive females by 3-mm size classes and age groups for the entire sampling period. Females whose spawning history could not be determined (undetermined, inactive females) were not included. Ideally, effective maturity estimates are preferred, and those estimates include only females that are sexually active during the spawning period (Pears et al., 2006). However, it was not possible to limit our estimates in this way because very few active females were sampled during the spawning months. A logistic curve was fitted to the data in the following form:

$$P = 1/(1 + \exp[-r(L - L_m)]), \qquad (1)$$

where r = the slope of the curve fitted to $\ln[(1-P)/P]$ versus TL;
P = the proportion of mature female fish; and
L_m = the mean length at sexual maturity.

The size (L_{50}) and age (t_{50}) at first maturity for females was estimated to be the intercept point at which 50% of individual fish were mature.

Seasonal reproductive patterns for female fish were calculated by plotting estimates based on the gonadosomatic index (GSI) and the frequency of reproductive stages by month. Because males were sampled rarely, the GSI was calculated only for female fish with the following equation:

$$GSI = GonW \,/\, GW \times 100, \qquad (2)$$

where $GonW$ = the gonad wet weight (in grams).

To determine the frequency of reproductive stages each month, only mature, active (ripening, ripe, and running ripe) and mature, inactive (resting) females were included. The relationship between gonad weight and total length and age was explored to determine whether there was a disproportionate increase in gonad weight above size and age at first maturity, as seen in other epinepheline groupers (Pears et al., 2006).

Data analysis

The relationship between TL and GW was estimated for 371 fish by using linear regression analysis. To linearize the power curve that best described this relationship ($GW=aTL^b$), both variables were transformed by using the natural logorithm of x, $\log_e x$. The line of best fit for the linear relationship was described by

$$\log_e GW = \log_e a + \log_e TL. \qquad (3)$$

Because of the low numbers of male fish, the von Bertalanffy growth function (VBGF) was fitted to length-at-age data for all sexes combined, by calculating a nonlinear least-squares regression of TL on age:

$$L_t = L_\infty (1 - e^{-K(t - t_0)}), \qquad (4)$$

where L_t = TL at age t;
L_∞ = mean asymptotic TL;
K = the growth coefficient; and
t_0 = the age at which theoretical TL is zero.

The age-based catch curve technique, estimated for all sexes combined, was used to determine the annual instantaneous rate of total mortality (Z). The natural logarithm of the number of fish between each age class was then plotted by age, and the annual instantaneous rate of Z was then taken as the slope of the line of best fit. The first 4 age classes (320–650 mm TL) were not included in this analysis because they represented fish not fully vulnerable to the fishing gear. Calculations of M were made with the equation of Pauly (1980), which incorporates water temperature and the VBGF parameters of L_∞ and K. A second method for estimating M, developed by Hoenig (1983), also was applied by using the general equation

$$\log_e Z = 1.46 - 1.01\,(\log_e t_{max}), \qquad (5)$$

where Z is analogous to M in an unexploited population.

The mean annual water temperature for Musandam is 26.8°C (Wilson[3]). The instantaneous mortality rate (F) was estimated by subtracting the estimate of M from Z estimated as the slope in the descending limb of the age-based catch curve ($F=Z-M$). An estimate of the rate of exploitation (E) was calculated as $E=F/Z$:

$$\ln(M) = 0.55 - 1.61\ln(L) + 1.44\ln(Linf) + \ln(K). \qquad (6)$$

To characterize the state of the fishery, the following F-based biological reference points were calculated: 0.5 M for a precautionary target (F_{opt}) and 0.67 M for the mortality limit (F_{limit}).

Results

Age determination and validation

Annuli (opaque zones that appeared darker than the adjacent translucent ones) were counted on sectioned sagittal otoliths of orange-spotted grouper. Among the opaque zones, the first one was more difficult to define because it was usually much wider than the remainder. Of the 214 sections, 206 were read successfully, with ages of 1–15 years. The presence of an opaque zone at the margin of otolith (identified by edge analysis) indicated that annuli had a strong seasonality in deposition. More than 80% of all fish sampled from September through December had opaque margins. A lag of 3 months was observed between the peak in seawater

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[3] Wilson, S. 2011. Unpubl. data. Five Oceans Environmenal Services, Way 3021, Al Qurm, Muscat, Sultanate of Oman

Table 1

Number, size frequency (total length [TL] in millimeters), and mean gutted whole weight (GW), with standard errors (SEs), of samples of orange-spotted grouper (*Epinephelus coioides*) used in this study. Samples were collected in northern Oman between May 2004 and August 2005. *n*=number of fish sampled.

Sex	*n*	TL $_{\text{min–max}}$	Mean TL (SE)	GW $_{\text{min–max}}$	Mean GW (SE)
Female	175	281–900	589.58 (7.21)	250–9436	2680.37 (102.06)
Transitional	11	449–748	646.00 (24.60)	1101–5156	3539.27 (1452.26)
Male	13	443–1005	617.54 (44.88)	1008–18906	3866.15 (327.79)

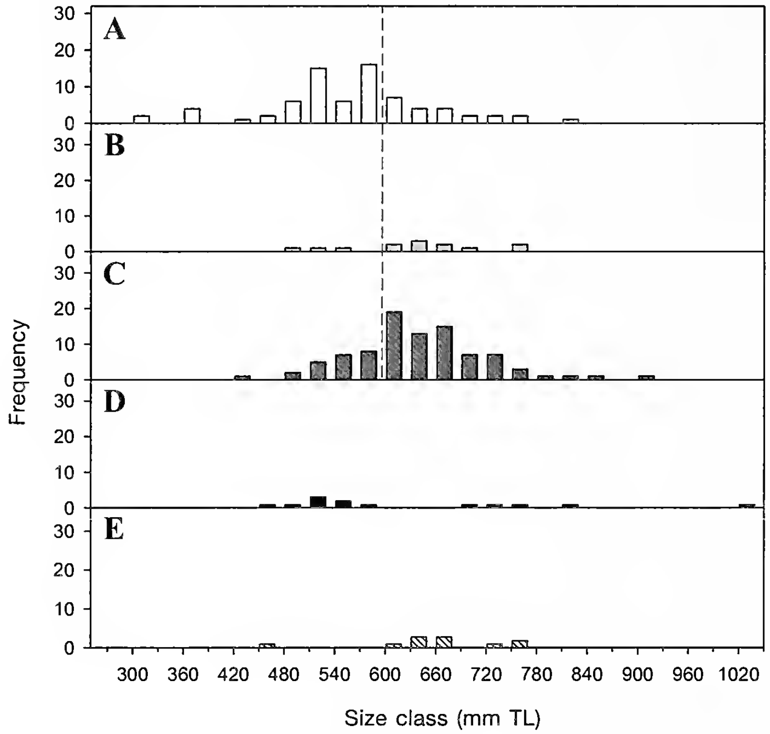

Figure 2

Relationship between size class (total length in millimeters) and frequency of (**A**) immature female (*n*=74), (**B**) undetermined maturity-stage female (*n*=17), (**C**) mature female (*n*=84), (**D**) male (*n*=13) , and (**E**) transitional phase (*n*=11) orange-spotted grouper (*Epinephelus coioides*) within samples collected in northern Oman between May 2004 and August 2005. The dashed line indicates size at maturity.

temperature in June and the formation of the opaque zone at the otolith edge in September.

Size and age distribution

The relationship between TL and GW for both sexes combined was best described by the equation

$$GW = 5.27^{-6} \times (\mathrm{TL}^{3.129}), \qquad (7)$$

where the sample size was 356 and the coefficient of determination (r^2) was 0.97 ($P<0.001$).

Females dominated the sample (n=178, 86.5 %) and ranged in size from 281 to 900 mm TL and in age from 1 to 11 years (average: 589.6 mm TL [standard error (SE) 7.21]) (Table 1; Fig. 2, A–C; Fig. 3, A–C). More than half the females (n=77) sampled, however, were classified as undetermined, inactive because it was not possible to establish their spawning history. Males composed only 6.5% of the sampled population, but they were found throughout the size and age classes (Figs. 2D and 3D). The smallest male was 443 mm TL and 2 years old, and the largest male was 1005 mm TL and 15 years old (average:596.4 mm TL[SE 65.40]) (Table 1). The male-to-female ratio was 1:14. The size and age at sex change was described by a relatively narrow size range (449–748 mm TL) and age range (4–8 years) for transitional individuals (Figs. 2E and 3E).

Growth models and mortality estimates

Because of the small number of males that were sampled, the VBGF curve was fitted to all fish, regardless of sex. The plot of size at age revealed a trend in the growth trajectory that was almost linear and had no apparent asymptote (Fig. 4A). Estimates of the VBGF parameters were as follows: L_∞=927.97 mm, K=0.135, t_0= −2.33. There was a curvilinear relationship between otolith weight and age, best described by the following equation:

$$Age = 0.101 + -0.0023(OW) + 0.0017(OW)^2, \qquad (8)$$

where OW = otolith weight (to the nearest 0.001 g) (r^2=0.555, n=204) (Fig. 4B).

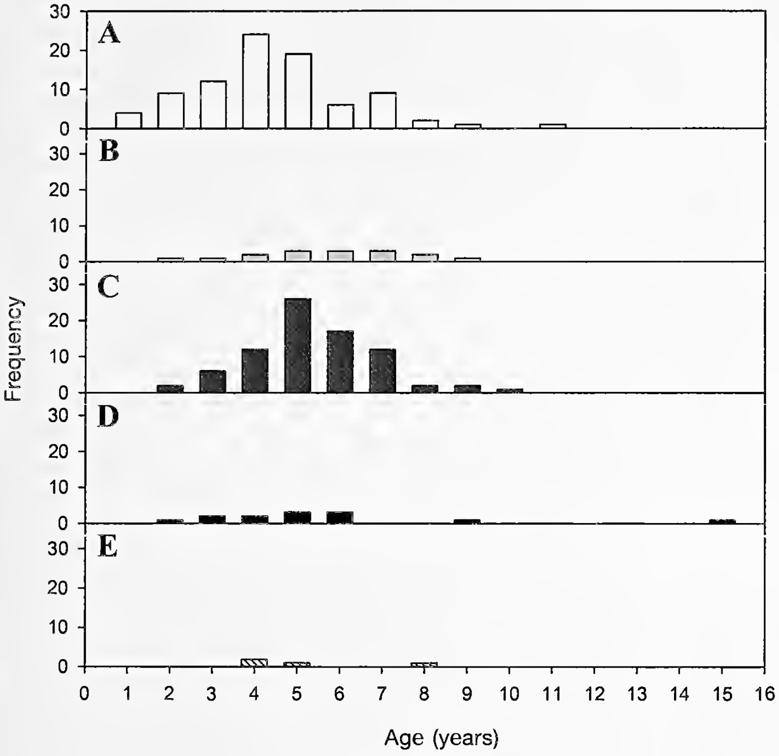

Figure 3

Relationship between age (years) and frequency of (**A**) immature female (n=71), (**B**) undetermined female (n=16), (**C**) mature female (n=80), (**D**) male (n=13) , and (**E**) transitional phase (n=11) orange-spotted grouper (*Epinephelus coioides*) within samples collected in northern Oman between May 2004 and August 2005.

The instantaneous rate of mortality (Z), estimated with the age-based catch curve, was 0.722/year (Fig. 4C; Table 2). The estimate of M determined from Pauly's (1980) equation was 0.14/year, which is comparable to the estimate derived from Hoenig's equation (Hoenig, 1983). Although the estimates of F from the 2 methods were similar (0.65/year and 0.59/year, respectively), both values were much greater than the F_{opt} and F_{limit} biological reference points. The estimate for E was 0.81/year.

Description of sex and maturation stages

Females As with most species of *Epinephelus* described to date, the gonads were fused posteriorly, bilobed, and unequal in size (Sadovy and Colin, 1995; Fennessy and Sadovy, 2002). Ovaries of immature females (n=71) were small, had a thin gonad wall, and lamellae were dominated by gonia and previtellogenic oocytes in the primary growth stage (Fig 5B). Precursory dorsal sperm sinuses were present in 29% of these individuals. Undetermined, inactive females (n=17) were included as a secondary classification for females for which reproductive history could not be determined. For undetermined, inactive females, gonads were packed with gonia and previtellogenic, primary growth oocytes similar to those found in immature females, and no spermatogenic material was present. The tunica (gonad wall) for these individuals also resembled that of immature individuals. Precursory dorsal sperm sinuses were present in 38% of undetermined, inactive females (Fennessy and Sadovy, 2002).

The ovaries of mature inactive females (n=27), were larger in diameter than the ovaries of immature females, and they were dominated by previtellogenic oocytes at the cortical alveolar stage, the most advanced stage of oocytes (Fig. 5B). Evidence of recent spawning as a female was provided by the presence of a thick gonad wall and intralamellar muscle bundles in 26% of mature, inactive females caught during November and December. Mature, active females (n=57) contained oocytes in all stages of development (Fig. 5C). Those females in active vitellogenesis (ripening and ripe) were dominated by oocytes in the cortical alveolus and yolk globule stages, respectively. One spent female was observed (Fig. 5D).

Transitional individuals In gonads of all transitional individuals (n=11), evidence of prior female spawning was found. That evidence included a thick ovarian wall or the presence of intralamellar muscle bundles (scarring) and the occasional presence of brown bodies. Seven individuals were classified as early transitional fish because they contained crypts of primary and secondary spermatocytes or spermatids and oocytes in either the cortical alveoli stage or yolk globule stage (Fig. 6A). There was no evidence of degeneration of either male or female germ cells. In the 4 individuals classified as late-stage transitional fish, degenerating or atretic oocytes in the presence of maturing testicular tissue at differing stages of development were observed (Fig. 6B). The late-stage transitional fish were sampled within the perceived spawning season (March–May). Spermatozoa had not filled the dorsal sperm sinuses of any transitional individual.

Males The gonads of immature males (n=11) were small and compact and while contained the basic structure of an immature female, also had crypts of primary and secondary spermatocytes and spermatids (Fig. 6C). The gonia of 1 individual appeared to be degenerating. There was a relationship between fish size and total area of spermatogenic tissue, with larger fish containing more spermatogenic crypts scattered

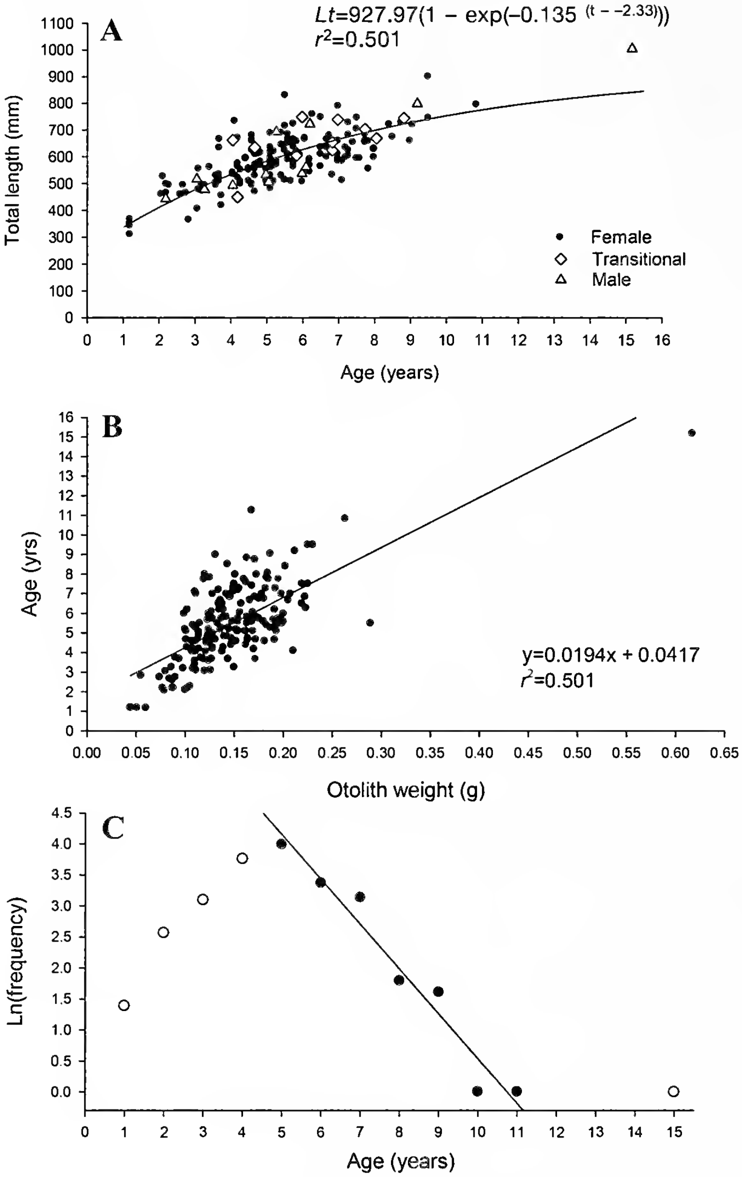

Figure 4

Plots (**A**) of size at age, (**B**) of otolith weight against age, and (**C**) of age against otolith weight for orange-spotted grouper (*Epinephelus coioides*) collected in northern Oman between May 2004 and August 2005.

among the previtellogenic oocytes. Dorsal sperm sinuses were present in 4 immature males. Two of these males were primary males with small gonads and no sign of prior female spawning.

In mature active males (n=2), the testes were dominated by spermatogenic tissue and the the sperm sinuses were filled with spermatozoa (Fig. 6D). Primary and secondary spermatocytes and spermatids were also present in both fish. There was no evidence of previtellogenic oocytes in these testes. No spent males were sampled.

Seasonal reproduction

The GSI for orange-spotted grouper indicated a relatively protracted spawning season that peaks between March and May. A comparison of values from the GSI with data on seawater temperature revealed that the onset of the spawning season in March occurred at the same time that temperatures rose above 26°C. The plotting of the frequency of mature, active and mature, inactive females by month revealed the timing of reproductive output. During the 3-month peak spawning period, more than 90% of the sample was composed of mature, active individuals (ripening and ripe), and only one mature, inactive RS female found. In addition, ripe females made up more than 30% of each of the monthly samples for 7 months of the year, indicating a protracted spawning season beyond the one described by the GSI.

Size and age at maturation

Plots of age and size frequency for females during the full spawning season (April–June) showed a large proportion of immature or undetermined inactive females. By contrast, the numbers of mature inactive females within this period were low. The size and age at 50% maturity for female orange-spotted grouper sampled throughout the year were estimated to be 580 mm TL and 4 years, respectively.

Discussion

The fishery for catch of orange-spotted grouper in northern Oman comprised primarily young, mostly immature females with a maximum age of 11 years. Males were rare and made up only 6.5% of the sample. We found that this species is slow growing (had a low K), and produces a VBGF curve best described as linear (with no apparent asymptote). This latter result, coupled with the maximum age (15 years) that is lower than the age of orange-spotted grouper in other locales, indicates that the observed age structure is heavily truncated as a result of overfishing. The histological evidence, based on representative fish from all size classes and samples throughout the year, indicates that the population of orange-spotted grouper from northern Oman is diandric protogynous—a finding that is similar to that from reports on this species within the Arabian Gulf (Grandcourt et al., 2005, 2009). Furthermore, we found through the presence of primary males (smaller males than those at first reproduction) that the devel-

Table 2

Estimates of mortality for orange-spotted grouper (*Epinephelus coioides*) calculated for this study and by Grandcourt (2005) by using methods of Pauly (1980) and Hoenig (1983). Also included are the precautionary biological reference points, precautionary target (F_{opt}) and limit (F_{limit}), which are based on Hoenig's estimate of natural mortality (M) and exploitation rate.

Parameter (value/year)	This study	Grandcourt (2005)
Total mortality—(from the catch curve)	0.72	0.97
Natural mortality (Hoenig)	0.08	0.19
Natural mortality (Pauly)	0.14	–
Fishing mortality (Hoenig)	0.65	0.78
Fishing mortality (Pauly)	0.59	–
Exploitation rate	0.81	0.80
$F_{opt} = 0.5\ M$ (Hoenig)	0.04	0.10
$F_{limit} = 0.67\ M$ (Hoenig)	0.05	0.13

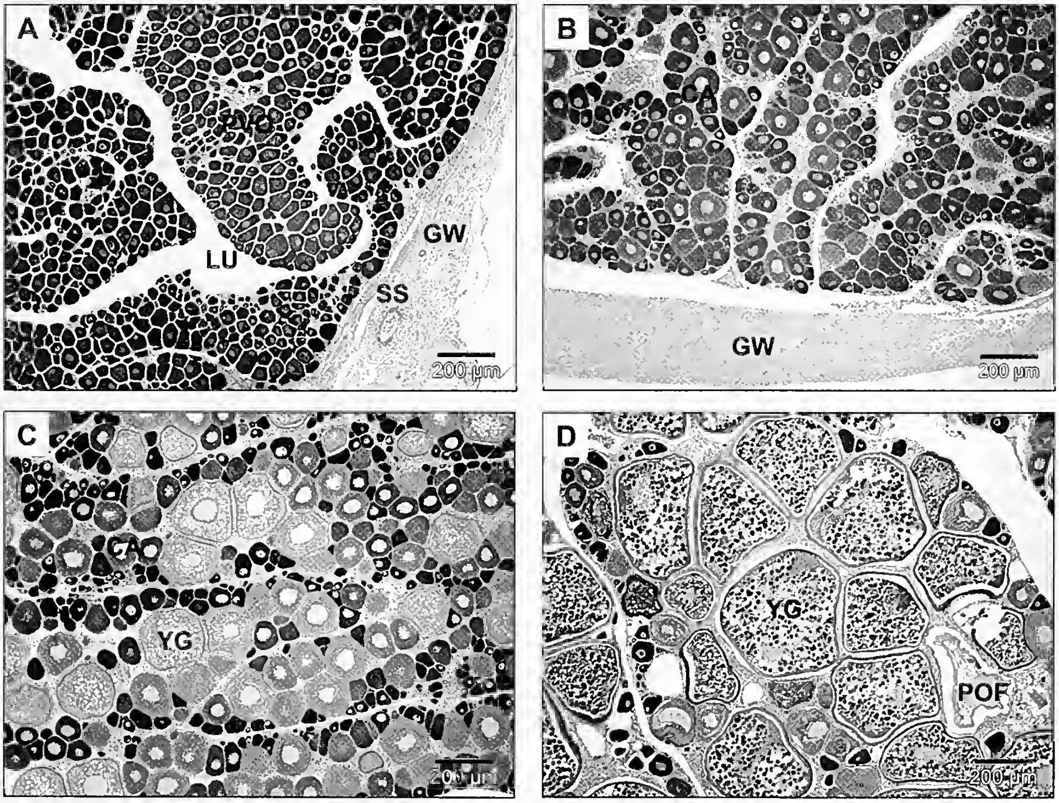

Figure 5

Photomicrographs of histological sections taken from gonads of orange-spotted grouper (*Epinephelus coioides*) collected in northern Oman between May 2004 and August 2005: (**A**) immature female gonad, with previtellogenic oocyte (PVO), gonad wall (GW), lumen (LU), and sperm sinus (SS); (**B**) mature, inactive female gonad, with CA and GW; (**C**) mature active (ripe) female gonad, with CA and yolk globule stage oocyte (YG); and (**D**) mature, active (spent) female gonad, with YG and postovulatory follicle (POF).

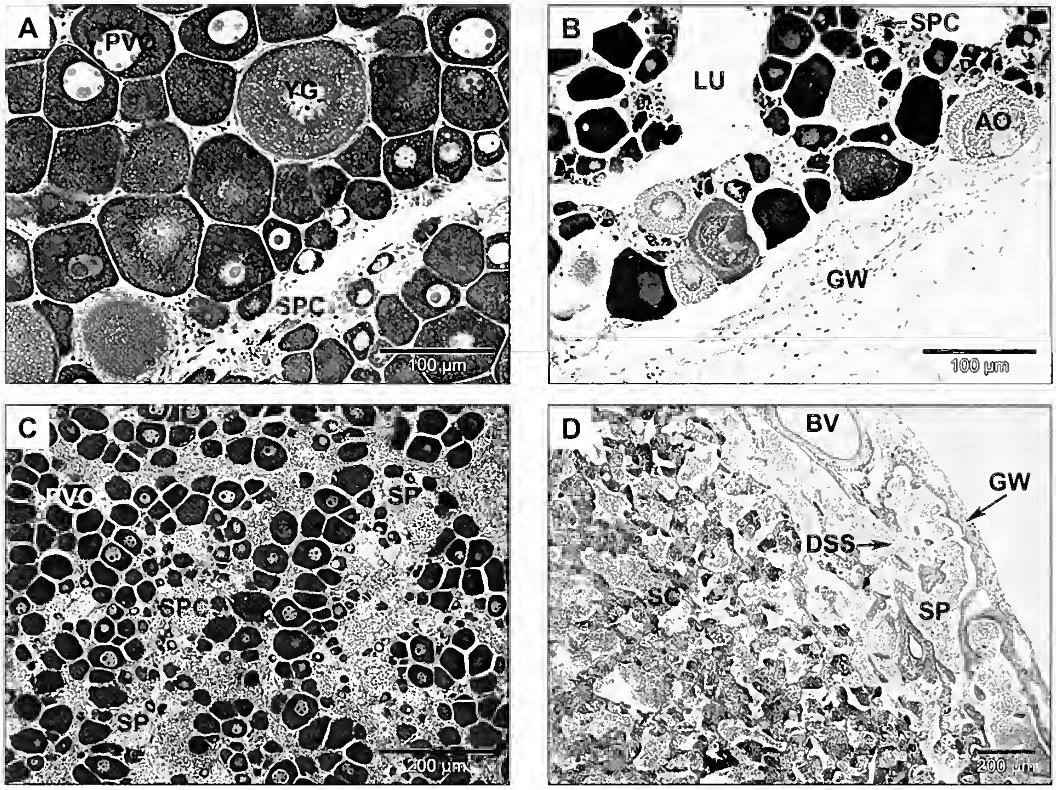

Figure 6

Photomicrographs of histological sections taken from gonads of orange-spotted grouper (*Epinephelus coioides*) collected in northern Oman between May 2004 and August 2005: (**A**) early-stage transitional gonad, with previtellogenic oocyte (PVO), yolk globule-stage oocyte (YG), and spermatogenic crypt (SPC); (**B**) late-stage transitional gonad, with atretic oocyte (AO), lumen (LU), gonad wall (GW), and SPC; (**C**) immature male gonad, with PVO, SPC, spermatids or spermatozoa (SP); and (**D**) mature (ripe) male gonad, with dorsal sperm sinus (DSS), spermatocytes (SC), SP, gonad wall (GW), and blood vessel (BV).

opmental pathway of male orange-spotted grouper from Oman is diandric and occurs in one of 2 ways, directly from juveniles or by sex change from fully functioning mature females (Rhodes and Sadovy, 2002).

Demography and fishing impacts

The demographic characteristics of orange-spotted grouper from Oman were very similar to the characteristics observed in 2 other growth studies of this species conducted in the region (Kuwait: Mathews and Samuel, 1987; Abu Dhabi: Grandcourt, 2005). All the growth parameters, L_∞ (93.07 cm TL; 97.9 cm TL), K (0.1655; 0.14), and t_0 (–0.39; –1.5), for fish in Kuwait and Abu Dhabi were almost identical to the parameters in our results. However, the most significant and perhaps most worrying differences within the Oman population of orange-spotted grouper are the values for the two demographic parameters that are often cited as indicators of overfishing: a reduced maximum age and a truncated age structure. The maximum ages for orange-spotted grouper from the Abu Dhabi study and from our Oman study were 12 and 15 years, significantly lower than the maximum age of 22 years reported for fish from Kuwait during the 1980s (Mathews and Samuel, 1987). Similarly, nearly 23% of the samples from Kuwait (averaged over 4 years of sampling) represented fish older than 10 years, compared with 0.5% of the samples from Abu Dhabi (Grandcourt et al., 2005, Grandcourt, 2012) and 2% of the samples from Oman.

Although it is still not clear how sex change is controlled in orange-spotted grouper, the large size range over which sex change occurs indicates behavioral control, which acts independently of the social group size (Shapiro, 1987). It has been shown that if sex change is controlled by behavior, the removal of the larger, older males through fishing results in rapid sex change by a large female in a group (Huntsman and Schaaf, 1994). Such a compensatory effect means that sex ratios are not necessarily affected by fishing; however, the size and age at which sex change occurs is reduced significantly (Adams et al., 2000). For socially mediated sex change to occur, female assessment of either the size (size-ratio assessment) or sex (sex-ratio assessment) of the group is made. In the latter scenario, when males are rare, the numbers of fish undergoing transition increases (Coleman et al., 1996).

Although male orange-spotted grouper were uncommon in the population in northern Oman, there was no evidence that the numbers of transitional fish had increased, and therefore the compensatory mechanism has largely been overridden (Levin and Grimes, 2001). Dramatic reductions in male numbers have previously been documented for gag (*Mycteroperca microlepis*) and scamp (*Mycteroperca phenax*) in the Gulf of Mexico, with dire consequences for those populations (Coleman et al., 1996). We argue that the smaller numbers of older fish and secondary males in our sample are not attributed to fishing selectivity (e.g., gear type or depth). Sampling was rigorous and representative of the fishing fleet, which included large wood dhows (>10 m length overall) and small fibreglass boats (4–10 m length overall). Unpublished creel surveys of the Musandam region reveal that dhows are landing the largest individuals because they fish for longer periods in waters up to 300 m (senior author, unpubl. data). Wire-mesh traps, the most common gear type for this fishery, have an average opening size of 100 cm (Al Masoori et al., 2004), big enough to allow the largest orange-spotted grouper to enter and to ensure that they are vulnerable to being caught by the gear type.

Grouper populations, whose male biomass is eroded through selective fishing, face a significant loss of reproductive output. Although there are no baseline data on the sex ratio of orange-spotted grouper, the males composed 6.5% of the sampled population, similar to results of study on the Atlantic coast of the United states of male gag, whose numbers declined from 19.6% to 1.9% over 12 years (McGovern et al., 1998). A loss of males can seriously affect female reproductive success to the point where some groups of females with limited access to males remain reproductively inactive (Coleman et al., 1996). Limited contact between the sexes also can destabilize social hierarchies (Levin and Grimes, 2001) and, therefore, the overall functioning of the aggregation (Coleman et al., 1996). Historical aggregations of individuals that return each year to spawn in a particular place can be reduced substantially in density if newly recruited fish have no older, experienced fish from which to learn the migration route (Coleman et al., 1996). Future biological monitoring of the population in Oman should include an increased sample size during the spawning season that would allow for detection of nonspawning females. Likewise, a reduction in the male-to-female sex ratio could be a useful indicator of stock recovery after the introduction of regulations, and male biomass can be used as a proxy for reproductive potential (Levin and Grimes, 2001).

Spawning season

The peak of the spawning season for the Oman population of orange-spotted grouper occurred over a 3-month period from March through May, and there was evidence of protracted spawning across the majority of the year. This spawning period contrasts not only with the spawning periods of populations of orange-spotted grouper in the Arabian Gulf but also with those of a range of other species in the gulf in which spawning periods are much more constrained when compared with the spawning periods of conspecific populations outside the gulf. Populations of the pink ear emperor (*Lethrinus lentjan*), spangled emperor (*L. nebulosus*), and black spot snapper (*Lutjanus fulviflamma*) in the Arabian Gulf all spawn for 2–4 months between April and July (Grandcourt et al., 2006a, 2006b), whereas conspecific populations (pink ear emperor and spangled emperor) within the Indo-Pacific, Indian Ocean, and Red Sea will spawn for the whole year or between August and March (Carpenter and Allen, 1989; Kailola et al., 1993). Such constrained spawning would be expected to be associated with seasonal extremes in water temperature; all spawning of gulf species occurs as water temperatures increase into summer, and spawning ceases during the hottest summer months (August and September).

Implications for the regional and global management of groupers

There is no evidence to indicate that the structure of stocks of orange-spotted grouper within Oman waters at the time of this writing (2015) is any different from the structure we have presented here (data collected in 2005). The high rate of F and high rate of E, considered unsustainable for slow growing grouper, are similar to the rates reported for species of grouper worldwide (Sadovy de Mitcheson et al., 2013).

We argue that immediate management of populations of orange-spotted grouper and of species of grouper throughout this region is warranted. However, the management options are limited for the populations of this species (and the majority of species of Epinephelidae) within Oman and the wider Indian Ocean simply because these species predominantly inhabit deep water (Heemstra and Randall, 1993; senior author, unpubl. data). For this group of species, implementing minimum size limits and quotas for catch would be impractical, not only because barotrauma experienced by fish upon release causes a significant rate of mortality but also because this fishery includes a number of different serranid species (senior author, unpubl. data). One management option that has been shown to be useful in a range of regions around the globe is area closure, and we argue that area closures may be an effective management option for this species and species of Epinephelidae because this approach has the potential not only to reduce overall fishing effort but also to protect key benthic habitats (Anderson et al., 2014).

Closure of the fishery within Oman at certain times of the year, specifically those times associated with the peak spawning period (March–May) may be useful, and the introduction and increased support of no-take zones or marine protected area that encompass critical habitat and aggregation or spawning sites of grouper will be vital for the persistence of viable populations of orange-spotted grouper and other species Epinephelidae within Oman.

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Environmental conditions of 2 river drainages into the northern Gulf of Mexico during successful hatching of Alabama shad (*Alosa alabamae*)

Paul F. Mickle[1]
Jacob F. Schaefer (contact author)[1]
Susan B. Adams[2]
Brian R. Kreiser[1]
William T. Slack[3]

Email address for contact author: jake.schaefer@usm.edu

[1] Department of Biological Sciences
The University of Southern Mississippi
118 College Drive, #5018
Hattiesburg, Mississippi 39406-5018

[2] Southern Research Station
U.S. Forest Service
U.S. Department of Agriculture
1000 Front Street
Oxford, Mississippi 38655

[3] Waterways Experiment Station EE-A
Engineer Research and Development Center
U.S. Army Corp of Engineers
3909 Halls Ferry Road
Vicksburg, Mississippi 39180-6199

Abstract—In recent years, the Alabama shad (*Alosa alabamae*) has experienced dramatic declines and extirpations from portions of its native range. Habitat degradation and barriers to migration are considered contributing factors to contraction in the distributional range this species. To identify conditions during successful spawning, river temperatures and discharges in 2 drainages of the northern Gulf of Mexico (the Apalachicola and Pascagoula rivers) were characterized during successful hatching "windows." Sampling during 2005–2009 yielded 400 juvenile Alabama shad of which 261 were aged from counts of rings on sagittal otoliths. Results from logistic regression revealed that successful spawning coincided with increases in temperature within a specific range (9.4–21.5°C) and with an average drainage-dependent discharge volume (625.6 m^3/s in the Apalachicola River and >400.7 m^3/s in the Pascagoula River). Timing of successful hatching windows differed between drainages but not between years within each drainage. Documenting and identifying the river conditions during successful reproduction provide important information on how to manage rivers to aid in the recovery of this species of conservation concern.

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Migratory species invest significant resources in moving through a variety of disparate habitats to reach spawning sites (Gross, 1987; Roff, 1988). For fish species, these costs can be substantial (i.e., osmoregulation in diadromous fishes, or movement through suboptimal habitat that increases exposure to predators, disease, or anthropogenic disturbances), and spawning migrations require energetic demands associated with gonad maturation (Leggett and Whitney, 1972; Hodgson and Quinn, 2002). These costs also are linked inextricably to a number of species life history traits (e.g., body size, fecundity, and age of maturation) that have co-evolved with migration to maximize individual fitness (Gross, 1987; Kinnison et al., 2001). Despite the evolution of complex behaviors, physiology, and morphology, many migratory species show substantial flexibility in the timing of migration and the routes taken (Berthold, 2001; Alerstam et al., 2003).

River conditions that are possible cues for migration in fish species include discharge, flow velocity, temperature, suspended sediment, pH, conductivity, and dissolved oxygen (McLean et al., 1982; Quinn and Adams, 1996; Hewitt, 2003). These parameters are susceptible to rapid changes during spring rains when river discharge increases dramatically, possibly ultimately influencing the potential for successful recruitment (Maurice et al., 1987). Flow and temperature are correlated with oxygen levels and egg development time and ultimately with hatching success (Mann, 1996). After hatching, temperatures, nutrient levels, and turbidity are expected also to influence growth and survivorship. Therefore, individuals spawned at different

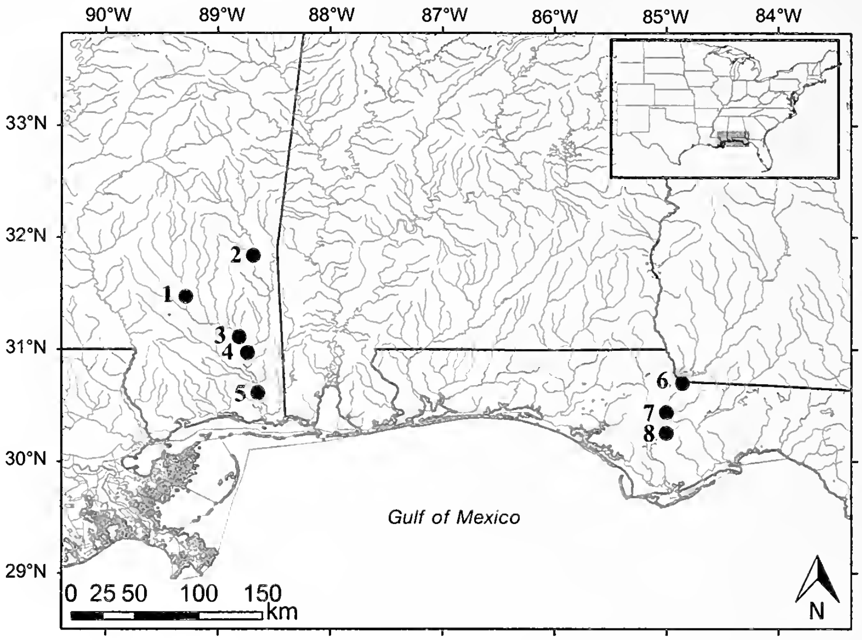

Figure 1

Map of the 2 river drainages sampled for Alabama shad (*Alosa alabamae*) during 2005–2009. Five sites were sampled in the Pascagoula River basin in Mississippi: 1) Eastabuchie; 2) Shubuta; 3) McLain; 4) Merrill; and 5) Wade. Three sites were sampled in the Apalachicola River basin in Florida: 6) Woodruff; 7) Wewahitchka; and 8) Blountstown.

times (early versus late) in a season may have different growth and mortality rates (Limburg, 1996).

Fish otoliths provide a detailed history of the daily and annual growth of an individual and can provide a useful tool for retrospective assessment of the growth rates of early juveniles (Parsons and Peters, 1989; Geffen, 1992). Daily growth rings on otoliths consist of alternating calcium- and protein-rich layers (Geffen, 1992; Armstrong et al., 2004). Diel feeding cycles lead to variable growth, resulting in alternating opaque and translucent rings in each 24-h period. Unlike other skeletal elements, otoliths do not undergo bone remodeling that would potentially resorb layers (Simkiss, 1974). Therefore, the daily deposition of otolith rings provides a method for determining age (in days) in species of bony fish.

Alabama shad (*Alosa alabamae*) is an anadromous fish that ascends rivers in the Mississippi River basin and northern Gulf of Mexico to spawn during spring months (February–May) of each year (Mettee and O'Neil, 2003). A special conservation status has been conferred on Alabama shad by several states, including Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, and Missouri (Meadows et al., 2006). Population-level genetic data indicate a high degree of site fidelity within some populations of Alabama shad (Bowen, 2005). As a result, differences in spawning timing and recruitment due to genetic drift or local adaptation are possible.

Until recently, much of the basic biology for Alabama shad had been inferred from research on the American shad (*A. sapidissima*) in northern Atlantic basins. The American shad is fairly well studied; published works address fecundity, spawning, feeding behavior, and even restoration (Olney and McBride, 2003; Walter and Olney, 2003). Much less is known about the ecology of Alabama shad (Mickle et al., 2010). The goals of this study were to improve our understanding of river conditions during successful hatching of Alabama shad and compare these conditions among river drainages. Our objectives were 1) to assess differences in successful hatching timing of Alabama shad between 2 basins, 2) to evaluate and compare river conditions during successful hatching between the basins, and 3) to assess interannual variability in both hatch timing and river conditions during successful hatching. For this study, we defined successful hatching as hatching that resulted in larval survival to the juvenile stage. Once the timing of successful hatching was determined (from daily age data), we were able to compare river condition and hatch timing for the 2 rivers and sampling years—findings that would help to identify the necessary conditions for recruitment of Alabama shad.

Materials and methods

Hatch timing and river conditions during hatching were compared between 2 rivers in the central Gulf of Mexico (Fig. 1). The Apalachicola River is a large river in the Florida panhandle and contains the largest extant population of Alabama shad (Mettee and O'Neil, 2003). This river has a dam on the mainstem (Jim Woodruff Lock and Dam, at river kilometer 171), and Alabama shad spawn below the dam, although some pass through a lock system that is opened twice a day (Laurence and Yerger, 1967; Ely et al., 2008). The Pascagoula River, located in Mississippi, is the last large, free-flowing river in the contiguous United States (Dynesius and Nilsson, 1994). No spawning grounds of Alabama shad have been documented for the Pascagoula River, although juveniles are consistently present within this river system (Mickle et al., 2010).

To investigate drainage-level differences in successful hatch timing, juveniles were collected from the Pascagoula River basin in Mississippi during 2005–2009 and from the Apalachicola River basin in Florida during 2007–2008 and then aged by counting daily otolith rings. Samples were taken during June and October in the Apalachicola River basin and in June through October in the Pascagoula River basin, excluding September and October 2005 after Hurricane Katrina. The year 2007 was the only year during the sampling period that was categorized as a severe drought year by the Palmer Drought Severity Index for the region (U.S. Drought Monitor, website). The other years (2005, 2006, 2008, and 2009) were characterized as low water, but not drought, years.

Fish were collected with a SR-14EB[1] electrofishing boat (Smith-Root Inc., Vancouver, WA) operated at 5000 W and 16 A with pulses-per-second ranging from 7.5 to 120. Electrofishing effort typically occurred for 1200 s at each site and was focused on the habitat types of sand bar, open channel, and bank. Alabama shad were tagged individually and placed in 95% ethanol. During low-water periods, some sites (typically shallow sand bars) were not accessible by the electrofishing boat. Because Alabama shad undergo ontogenetic shifts in habitat use (Mickle et al., 2010), these sites were seined and occasionally a cast net was used to ensure individuals of all ages were sampled throughout the sampling period. Cast nets had diameters of 1.52–2.43 m and a bar mesh of 1.59 cm. Seines were 3.0–3.7 m wide by 1.8–2.4 m deep and had a 0.3-cm-wide mesh.

Age was estimated in days by counting daily rings on the sagittal otoliths (Secor et al., 1992). One otolith per individual fish was removed and mounted on a slide with Crystalbond mounting adhesive (Ted Pella. Inc., Redding, CA). Otoliths were mounted with the primordia facing down and sanded by hand with sequentially finer grit-size paper, as necessary, to expose the rings. Daily rings were counted with a Wild Heerbrugg compound microscope (Leica Microsystems Inc., Buffalo Grove, IL). Magnification ranged from 290× to 1080× depending on the diameter (0.25–0.50 mm) of the otolith. Otolith images were taken with a SPOT Insight Color digital camera (Diagnostic Instruments Inc., Sterling Heights, MI) and by using SPOT Advanced software, vers. 3.3 (Diagnostic Instruments Inc.), and were enhanced by using Image-Pro Express, vers. 4.0.1 (Media Cybernetics Inc., Rockville, MD).

Age rings on each otolith were counted 3 times by the same person during separate sessions, and counts were averaged over the 3 observations. Of the otoliths read, 5% were randomly selected for independent validation by another reader and compared with the range of original readings. The otoliths of older fish (>250 days, all from individuals >100 mm in total length [TL] captured later in the year) were thick and brittle, making sanding and accurate reading of daily rings difficult. These older fish were removed from our analysis. As suggested by Geffen (1992), fish age was determined by adding 10 days to each daily ring count to compensate for the posthatching yolk stage that precedes daily ring formation in certain species. The hatch date for each individual was determined by counting back the age of the fish from its collection date. The successful hatching period (average time from the start of hatching to the end of hatching [hereafter "hatch window"]) for each drainage and year was defined as the period between the earliest and latest hatching date for all Alabama shad younger than 250 days captured within a given drainage in a given year.

To evaluate river conditions during the successful hatching time, mean daily river discharge and temperatures were analyzed for the period January–July of each year. For the Pascagoula River basin, river data were collected from the U.S. Geological Survey flow-gauging station (02479000) at Merrill (at river kilometer 137; Fig. 1). For the Apalachicola River basin, the data were collected from a U.S. Geological Survey gauging station (02358000) and a Florida Department of Environmental Protection station at Jim Woodruff Dam (at river kilometer 171; Fig. 1). All missing data (usually 1 or 2 daily mean values per data lapse) were compensated with mean datum from before and after the lapse period (<2% of all data).

Overall difference in the timing of successful hatching between rivers was assessed as the modal difference between rivers and with years pooled. To test for significance of this value, we used a randomization procedure to build a null distribution of expected modal differences given the observed hatching times. For 10,000 permutations, 50 observations were randomly drawn (without replacement) for 2 groups (representing the 2 rivers) and a modal difference calculated (by using the sample function in R statistical software, vers. 3.2.3 [R Core Team, 2015]). The significance of the observed modal difference between rivers was assessed by comparison with the distribution of permuted values.

We used logistic regression to assess our ability to predict hatching periods from river conditions. In each of the 7 river-year combinations, we divided the first 182 d into 26 7-d periods. For each period, we calculated standardized (z-score) mean temperature and

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[1] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

Table 1

Summary table of successful hatch windows for Alabama shad (*Alosa alabamae*) and river conditions during those windows in 2005–2009 in the Pascagoula River basin, Mississippi, and in 2007–2008 in the Apalachicola River basin, Florida (only data from within hatch windows are summarized). Minimums and maximums are for daily means during hatch windows.

Year	Number of Alabama shad collected	Julian days of hatching	Hatch window duration (d)	Mean temperature (°C)	Mean discharge rate (m^3/s)	Minimum temperature (°C)	Maximum temperature (°C)	Minimum discharge rate (m^3/s)	Maximum disharge rate (m^3/s)
Pascagoula River									
2005	85	32–58	27	13.1	747.8	12.8	15.8	255.1	1322.4
2006	79	38–73	36	14.9	436.5	10.2	16.5	187.7	914.6
2007	49	38–65	28	14.2	187.9	10.8	21.5	130.5	294.5
2008	57	32–79	48	15.2	422.2	10.7	21.0	166.2	852.3
2009	34	27–54	28	13.9	209.2	11.0	16.1	115.2	535.2
Grand Mean	60.8	33.4–65.8	33.4	14.3	400.7	11.1	18.18	170.9	783.8
Apalachicola River									
2007	52	6–64	58	13.3	592.3	9.8	18.4	385.1	974.1
2008	44	10–67	58	14.5	658.4	9.4	18.8	285.9	1602.7
Grand Mean	48.0	8.0–65.5	58.0	13.9	625.6	9.6	18.60	335.5	1288.4

discharge (m^3/s) and the change in temperature and discharge (difference in standardized temperature and flow from the previous period). Each period was scored as if it were part of the hatch window if it overlapped by ≥1 d with the established hatch window for that river and year. The logistic regression model (developed by using the glm function in R) predicted hatching from the mean and change in temperature and flow conditions and with year nested within river as factors.

Results

Over the 5 years of this study, 400 juvenile Alabama shad were collected: 304 from the Pascagoula River basin (85, 79, 49, 57, 34 individuals in each year during the period 2005–2009, respectively) and 96 individuals from the Apalachicola River basin (52 and 44 in 2007 and 2008, respectively). Although it was sometimes difficult to find otoliths clear enough to reveal a continuous sequence of rings, daily ring counts were completed successfully on otoliths from 208 Alabama shad from the Pascagoula River basin and from 53 Alabama shad from the Apalachicola River basin. Variability between the repeated counts of rings for individuals was low. Repeated counts typically differed by less than 5 rings; maximum disparity of 18 occurred in 1 otolith and the individual with maximum disparity was removed from analyses. Validation of the ring count was confirmed by the second reader whose counts fell within the range of the original 3 readings for each otolith. In all readable otoliths, the interior rings (around the primordium) remained clear; however, some fish collected later in the year had otoliths that were too thick to be aged accurately.

Daily age validation was conducted by comparing natural, date-specific markers with specific river conditions (MacLellan and Saunders, 1995). We compared the number of rings during low growth periods (high-water events) with the number of days of high water events. This identification of events also allowed date-specific markers to be used for a comparison of ages by year and river. A 1:1 ratio of incremental count versus age was observed—a relationship that is consistent with results from other clupeid species for which daily otolith rings have been validated (Geffen, 1982; Campana et al., 1987; Moksness and Wespestad, 1989).

In the Pascagoula River basin, successful hatch windows began from late January to early February (Julian days 32, 38, 35, 32, and 27 in each year in the period 2005–2009, respectively) and ended from late February to late March (Julian days 58, 73, 65, 79, and 55) (Table 1, Figs. 2–5). For the Apalachicola River basin, the successful hatch windows began in early January (Julian days 6 and 9 in 2007 and 2008, respectively) and ended in early February (Julian days 64 and 67) (Table 1). Median hatching days (midway within each window) in the Pascagoula River basin occurred from early to late February (Julian days 45, 55.5, 50, 55.5, and 41 for the years in the period 2005–2009, respectively) compared with early to mid-February in the Apalachicola River basin (Julian days 35 and 38 for 2007 and 2008). Length of time of a successful hatch window was shorter in the Pascagoula River basin; an average window length was 30.8 d (SD 10.8), compared with 58 d (SD 0.0) in the Apalachicola River basin (Table 1, Figs. 2 and 3). All successful hatches occurred in the first 12 weeks of each year in both river basins. When all years were pooled, the modal hatching time

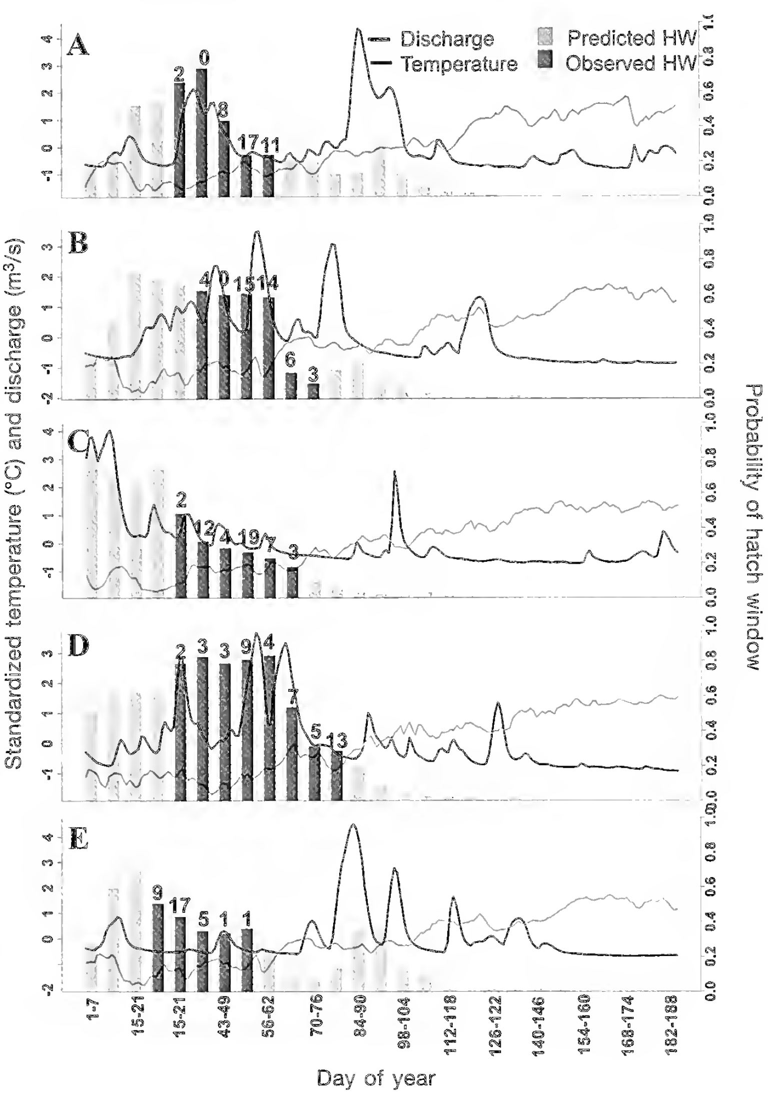

Figure 2

Standardized (z-score) discharge (black line) and temperature (gray line) values for the Pascagoula River basin in Mississippi during (**A**) 2005; (**B**) 2006; (**C**) 2007; (**D**) 2008; and (**E**) 2009. Bars represent logistic regression results for the probability of a time period being in the hatch window (HW) (Table 1). Dark gray bars represent periods of the observed hatch windows. Numbers above bars represent the total number of individuals aged back to that 7-d period.

was 33 days later in the Pascagoula River basin (day 52) than in the Apalachicola River basin (day 19). This difference was greater than 99.3% of the permuted values ($P<0.003$).

The logistic regression allowed us to correctly classify 86% of 7-d periods (with the use of a 50% probability cutoff) as within or outside hatch windows. Mean temperature and discharge were significant contributors to the model and hatch windows occurred during periods of lower temperatures and higher discharges (Table 2). Neither change in temperature, nor change in discharge, were significant factors, but river as a factor was marginally significant. Averaging across years, grand mean temperature during hatching was 14.3°C in the Pascagoula River and 13.9°C in the Apalachicola River, with average rates of discharge during hatching of 400.7 m³/s and 625.4 m³/s, respectively (Table 1). In general, predicted and observed hatch windows were similar in the Apalachicola River, whereas observed hatching in the Pascagoula tended to be 2–3 weeks later than that predicted by the logistic regression.

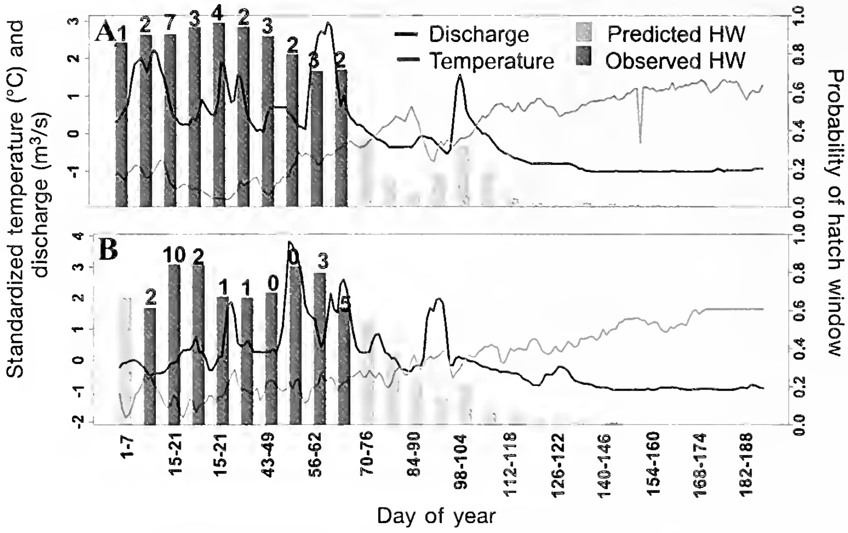

Figure 3

Standardized (z-score) discharge (black line) and temperature (gray line) values for the Apalachicola River basin in Florida in (**A**) 2007 and (**B**) 2008. Bars represent logistic regression results for the probability of a time period being in the hatch window (HW) (Table 1). Dark gray bars represent periods of the observed hatch windows. Numbers above bars represent the total number of individuals aged back to that 7-d period.

Discussion

Hatch windows for Alabama shad were consistently longer and earlier in the Apalachicola River basin than in the Pascagoula River basin. There are 3 possible explanations for these observed differences: 1) the cues, or timing of cues, that trigger spawning runs or acts of spawning differ between these river basins; 2) spawning runs and hatching take place at the same time, but in the years sampled, differences in recruit survival gave the appearance of significantly different hatch windows; or 3) the fish populations are different in the river basins, and they respond differently to cues. Previous work on population genetics of Alabama shad has indicated some drainage-level fidelity that would allow for interpopulation variability in the timing of spawning runs (Bowen, 2005; Bowen et al., 2008). Other, unmeasured variables may also play key roles as migration or spawning cues. It should be noted that a lower sample size in the Apalachicola River basin (2 versus 5 years sampled) could have contributed to differences. However, annual variation in timing of hatch windows within the drainages was clearly less than the variation in timing of hatch windows across drainages.

The distribution of successful hatching dates from each drainage and year indicates that prolonged or multiple spawning events occurred (Figs. 2 and 3). The overall lengths of the windows for successful hatching from the Apalachicola River basin were comparable with lengths of hatch window documented for American shad on the St. Lawrence River, between Canada and the United States (Maltais et al., 2010). In general, in our study, hatch windows of Alabama shad coincided with spring floods that had above-average flows and low, but increasing water temperatures. Spawning migrations of American shad are also linked to river temperature (Leggett and Whitney, 1972; Quinn and Adams, 1996). The Apalachicola River is impounded, and the effects of that control have the potential to artificially influence temperature and flow. The buffering of these parameters by the impoundment would be expected to prolong spawning windows—an outcome that would be consistent with our logistic regression results, which indicated that longer periods of lower temperatures were associated with successful spawning.

In contrast to our findings, previous studies by Laurence and Yerger (1967) and Mills (1972) have reported that spawning temperatures fall in a range of 19–22°C. However, Ely et al. (2008) found adult Alabama shad present on the spawning grounds when temperatures were similar (13.1–15.2°C) to temperatures found in other studies (Laurence and Yerger, 1967; Mills, 1972) during the hatch windows that we detected. We found that mean daily water temperatures of 19–22 C did not occur until late March and April, after 96% of all documented successful hatching was complete.

Mean temperature and discharge were the most important variables for predicting hatch windows. With these variables, hatch windows were found to be similar in the 2 basins, but, in general, observed hatch windows were later than predicted in the Pascagoula River

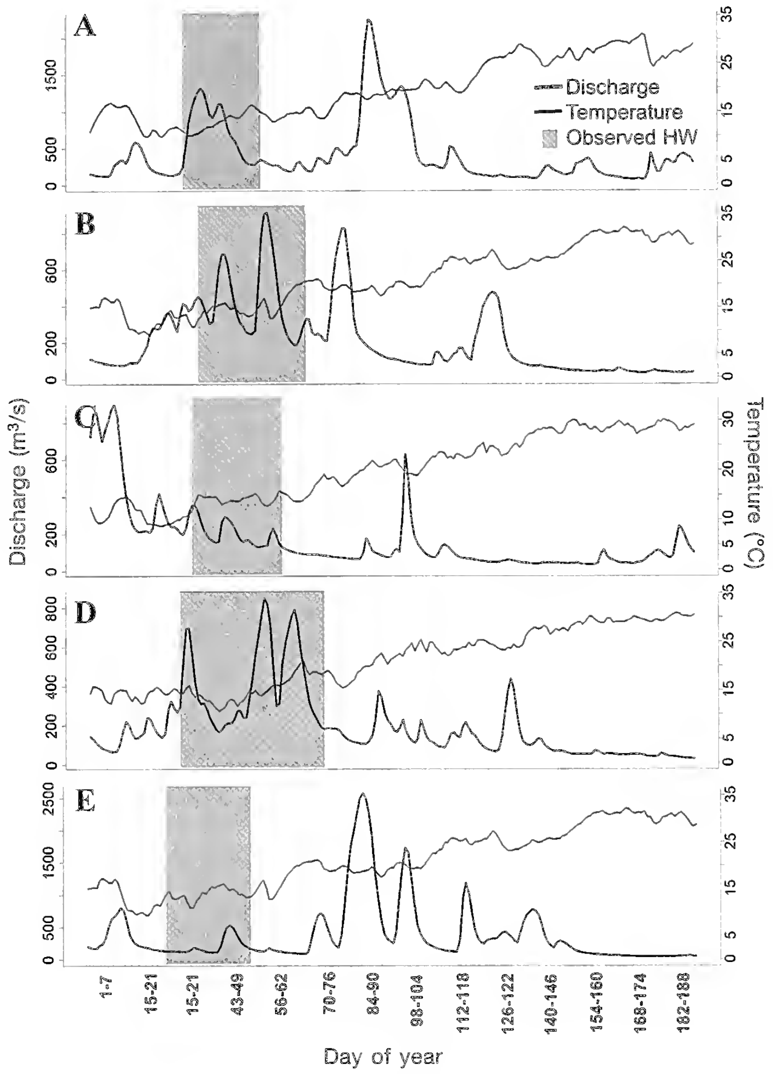

Figure 4

Raw discharge (black line) and temperature (gray line) values for the Pascagoula River basin, Mississippi, in (**A**) 2005; (**B**) 2006; (**C**) 2007; (**D**) 2008; and (**E**) 2009. Shaded areas represent the observed hatch windows (HWs)

basin. This result indicates that temperature and discharge conditions in the Pascagoula River basin may be suitable when there is either limited spawning or recruitment earlier in the year. It should be noted that we cannot rule out the possibility that spawning in one or both rivers occurs over longer periods and that the differences in successful hatch timing among drainages reflect differences only in the time of successful recruitment. It is also possible that other cohorts of Alabama shad were present but not collected, or represented in the analysis; we deem this possibility unlikely given the extensive sampling in the drainages and the absence of outliers to the observed hatch windows. Size data (Mickle et al., 2010) also are consistent with size of fish when hatching occurs in early spring (no smaller individuals [<55 mm TL] were sampled later in the year than July) and growth occurs through the summer and early fall. Therefore, drainage differences were more likely due to other unmeasured variables that might have contributed to the reduced hatch window or successful recruitment in the Pascagoula River basin.

The data regarding differences in successful hatch windows provide valuable information but do not allow for definitive conclusions on the mechanisms at work. Additional spatial and temporal data are needed to elucidate these mechanisms. Unfortunately, the Alabama shad is a species in decline and sample sizes are likely to be too small, or populations judged too imper-

Table 2

Results from the logistic regression in predicting hatch windows from mean and change (Δ) in standardized temperature and discharge. Significant predictors (chi-squared statistic) are shown in bold font. df=degrees of freedom.

	df	Estimate	Standard error	χ^2	*P*
Discharge	**1**	**0.576**	**0.260**	**4.91**	**0.027**
Δ Discharge	1	0.121	0.261	0.64	0.645
Temperature	**1**	**−2.490**	**0.463**	**51.89**	**<0.001**
Δ Temperature	1	−1.207	0.734	2.76	0.097
River	1	−1.109	0.815	0.972	0.061
River*Year	5			1.53	0.909

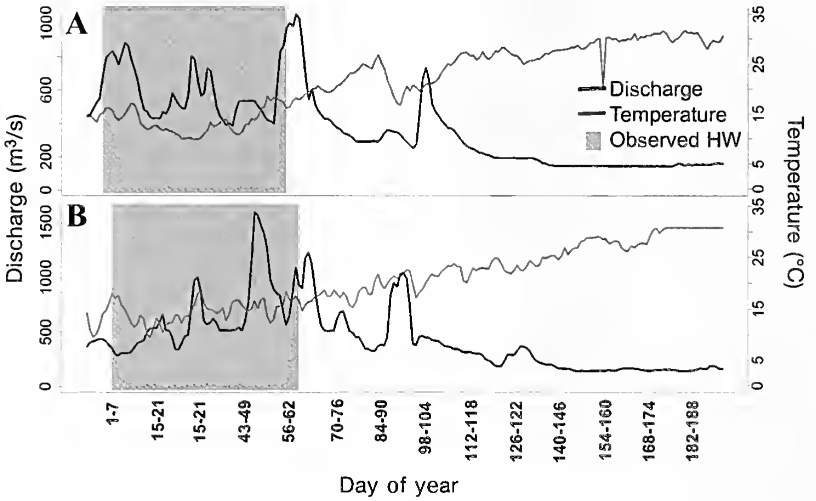

Figure 5

Raw discharge (black line) and temperature (gray line) values for the Apalachicola River basin, Florida, in (**A**) 2007 and (**B**) 2008. Shaded areas represent the observed hatch windows (HWs).

iled, to allow for intensive adult sampling for migration analyses. The initial experimental design of this study included a comparison of samples from inland drainages in Arkansas and Missouri, where spring increases in water temperature would lag behind those in Gulf of Mexico drainages. However, 3 years of sampling during August within these systems, at sites where fish had been collected in earlier years, yielded no juvenile fish.

Within impounded water systems, flow regimes may be managed through water releases to mimic natural cues and allow populations to complete their spawning runs. Castro-Santos and Letcher (2010) found that flow-regulated systems altered spawning success and migration timing of American shad both upstream and downstream. Other riverine species have also been affected adversely by altered flow regimes. Manipulated flows physically altered habitats, resulting in decreased diversity of fish species (Freeman et al., 2001). Altering systems in which Alabama shad reproduce may add stressors to migrating juveniles and adults. Unfortunately, the drainages along the coast of the northern Gulf of Mexico are also areas that have been rapidly developed over the past 75 years and that development may ultimately lead to further extirpations of this species (Turner, 1990).

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  Using the recruitment model, estimates of age-1 recruitment were determined. [Estimates were not using the recruitment model.]

  Correct:
  Using the recruitment model, we determined age-1 estimates of recruitment. [The participle now modifies the word *we,* i.e., those who were using the model.]

  Incorrect:
  Based on the collected data, we concluded that the mortality rate for these fish had increased. [We were not based on the collected data.]

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- Notate probability with a capital, italic *P*.
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