Title

Reproductive Biology and Evolution of Epinephelid and Serranid Fishes (Perciformes, Epinephelidae, Serranidae)

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Reproductive biology and evolution of epinephelid and serranid fishes (Perciformes, Epinephelidae, Serranidae)

A dissertation submitted in partial satisfaction of the
requirements for the degree Doctor of Philosophy

in

Marine Biology

by

Brad Edward Erisman

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Chair

University of California, San Diego

2008

This work is dedicated to the person I admire most – my wife, Kristin,
whose endless love and positive spirit inspire me to be a better person.

…and to Nacho the dog.

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This project would never have happened without the mentorship of Larry Allen and the assistance of Matthew Craig. Larry Allen introduced me to the Gulf of California in the fall of 1999 and inspired me to focus my research efforts on the biology of fishes from this region. Matthew Craig took me on a field expedition to Loreto in the summer of 2003, and during that trip, we collected an incredible amount of data that has since shaped my entire dissertation.

Many people assisted me in the field and in the laboratory and most are named at the end of each chapter. However, this list is likely incomplete, and I offer my apologies to those whom I have omitted. Special thanks to all my fellow labmates for their help and support for the past five years: Matthew Craig, John Hyde, Hsiu-Chin

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The text of Chapter 1 has been published under the same title, “Spawning patterns in the leopard grouper, *Mycteroperca rosacea*, in comparison with other aggregating groupers”, in Marine Biology (2007) 151:1849—1861. I was the primary author and Michele Buckhorn and Philip Hastings were co-authors of this work.

Chapter 3 has been published in Environmental Biology of Fishes (2008) 82: 23—33, as a paper under the same title, "Evidence of gonochorism in a grouper, *Mycteroperca rosacea*, from the Gulf of California, Mexico". I was the lead author and Jorge Rosales-Casián and Philip Hastings were co-authors of this manuscript. The text in Chapter 6 has been submitted to Proceedings of the National Academy of Sciences under the same title "Breakdown of the size-advantage: evolutionary changes in mating behavior influence the loss of sex change in groupers (Teleostei: Epinephelidae)". I am the primary author and Philip Hastings is the co-author of this work.

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Erisman BE, Hastings PA (In review) Breakdown of the size-advantage: evolutionary changes in mating behavior influence the loss of sex change in groupers. Proceedings of the National Academy of Sciences.

Erisman BE, Konotchick T, Blum S (In review) Observations of spawning in the Leather Bass, *Dermatolepis dermatolepis*, at Cocos Island, Costa Rica. Pacific Science.

Erisman BE, Rosales-Casián J, Hastings PA (2008) Evidence of gonochorism in a grouper, *Mycteroperca rosacea*, from the Gulf of California, Mexico. Environmental Biology of Fishes 82:23—33.

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ABSTRACT OF THE DISSERTATION

Reproductive biology and evolution of epinephelid and serranid fishes (Perciformes, Epinephelidae, Serranidae)

by

Brad Edward Erisman

Doctor of Philosophy in Marine Biology

University of California, San Diego, 2008

Philip A. Hastings, Chair

Teleost fishes exhibit an unrivaled diversity of reproductive patterns compared to other vertebrates, and the groupers (Epinephelidae) and seabasses (Serranidae) represent an extreme example of this diversity, because significant variations in sexual pattern, mating behavior, and reproductive anatomy occur among species in both families. For this research, I investigate these three aspects of the reproductive biology in five species of groupers from the eastern Pacific. I then combine my data with published results on the reproductive biology of other groupers and seabasses to describe taxonomic differences in these aspects and reconstruct their evolutionary history. Finally, I use a comparative phylogenetic approach to evaluate the evolutionary relationship between mating system characteristics (i.e., mating group structure and sperm competition) and sexual pattern. More specifically, I test whether evolutionary shifts in sexual pattern and, in particular the loss of sex change, were influenced by changes from paired to group spawning and associated increases in sperm competition among males. These analyses allow me to determine whether predictions of sex-allocation theory regarding the influence of mating behavior on sex allocation are congruent with fine-scale, evolutionary patterns of sex change within a resolved phylogeny.

My results indicate that characteristics of the sexual pattern, mating behavior, and reproductive anatomy in both families are exceptionally labile. Evolutionary transformations in these features do not strictly follow taxonomic lines and remarkable changes have evolved in closely related species and genera. The most parsimonious phylogenetic reconstructions of the evolutionary history of sexual patterns indicate that protogynous hermaphroditism is the ancestral character state in both the Epinephelidae and the Serranidae, and other forms of hermaphroditism evolved secondarily from this state. Tests of correlated evolution showed that evolutionary transformations in sexual pattern from protogyny to gonochorism are correlated with transformations in mating group structure from paired to group spawning, and sperm competition is significantly higher in gonochoric species than protogynous species. These results suggest that the loss of sex change was influenced by changes from paired to group spawning and associated increases in sperm competition among males. More importantly, this research provides phylogenetic support for predictions of the size-advantage model and empirical evidence for the influence of sperm competition on sex allocation in animals.

CHAPTER 1

Spawning patterns in the leopard grouper, *Mycteroperca rosacea*, in comparison with other aggregating groupers.

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RESEARCH ARTICLE

Spawning patterns in the leopard grouper, *Mycteroperca rosacea*, in comparison with other aggregating groupers

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Philip A. Hastings

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Abstract We documented the spawning patterns of the leopard grouper, *Mycteroperca rosacea*, from April to June 2005 in the central Gulf of California, Mexico to draw comparisons with other aggregate-spawning groupers and provide information useful for management of their fishery. Adults formed spawning aggregations of 150 to >700 individuals at specific sites, and spawning occurred daily at these sites from late April through early June. Courtship occurred throughout the day, but spawning was restricted to the evening hours. Adults spawned in groups of 6–40 fish, and pair-spawning was not observed. The group-spawning behavior of adults and the gonosomatic indices of mature males (maximum = 7.2%) suggest that sperm competition was present. The site-specificity of leopard grouper spawning aggregations and diel spawning period were typical of most aggregating groupers, and the size and structure of these aggregations was similar to other species in the genus *Mycteroperca*. Leopard grouper behavior patterns were unusual in that spawning aggregations persisted for extended periods, spawning was not synchronized with the lunar cycle, and adults aggregated during non-spawning periods. The extensive duration and site-specificity of spawning aggregations and the propensity of *M. rosacea* to form aggregations year-round increases the vulnerability of the species to overfishing. Policies that limit harvest from these aggregations are needed to improve the management of leopard grouper fisheries in the Gulf of California.

Introduction

Epinepheline serranid fishes (groupers) exhibit a remarkable diversity of reproductive patterns (Sadovy 1996); however, several trends have been identified among species that form spawning aggregations (reviewed by Sadovy et al. 1994a; Domeier and Colin 1997). Fish migrate considerable distances and form spawning aggregations at specific sites (Aguilar-Perera and Aguilar-Dávila 1996; Zeller 1998). All or most spawning activity happens within these aggregations, where it happens in the evening near dusk and is synchronized with the lunar cycle (Smith 1972; Thresher 1984; Colin 1992). Spawning aggregations are seasonal and persist only for several days to 2 weeks before the fish disperse (Shapiro 1987; Levin and Grimes 2002; Rhodes and Sadovy 2002). During non-reproductive periods, fish are usually solitary and do not aggregate (Sadovy and Eklund 1999; Domeier and Colin 1997). Exceptions to these trends are rare but do exist. For instance, *Epinephelus guttatus* lives in overlapping home ranges during non-reproductive periods and spawning is not lunar-related near its northern range limit (Burnett-Herkes 1975; Sadovy et al. 1994a). All medium and large-sized groupers (maximum TL of ≥50 cm) aggregate to spawn, but this behavior is uncommon among smaller species (Sadovy 1996).

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Other characteristics of aggregating groupers are more variable. Spawning aggregations of some species are patchily distributed over broad physical areas, whereas others are highly localized and discrete (Colin 1992; Coleman et al. 1996). Mating systems also differ among groupers. Spawning occurs in pairs within single male, multiple female clusters in *E. guttatus* and several *Cephalopholis* species, and this behavior coupled with the small size of ripe testes (≤2% of body wt.) suggests that sperm competition is absent (Sadovy et al. 1994a; Donald 1995; Chan and Sadovy 2002). Conversely, *E. striatus* spawns in the aggregates of female led, multiple male groups, and sperm competition is evident from this behavior and the relatively large size of testes (≥10% of body wt: Sadovy and Colin 1995).

The leopard grouper, *Mycteroperca rosacea*, is one of several groupers that form spawning aggregations in the Gulf of California, Mexico. There is little published data on their reproductive biology, but existing studies indicate that *M. rosacea* behavior patterns are different from that of other aggregating groupers. Sala et al. (2003) observed courtship during both new and full moons within aggregations of hundreds of individuals and theorized that temporal spawning patterns of this species may be unrelated to the lunar cycle. Also, leopard groupers are social during non-reproductive periods and aggregate in considerable numbers to feed on schools of small fishes and mysids (Hobson 1965; Parrish 1992: Sala et al. 2003). Finally, leopard groupers are gonochoric (Erisman et al. 2007); individuals function as either males or females, and post-maturational sex change does not occur. Gonochorism has been reported only in one other grouper species (*Epinephelus striatus*: Sadovy and Colin 1995). For these reasons, the behavior and spawning patterns of leopard groupers warrant further research and may provide a better understanding of the diversity of reproductive patterns among groupers and related fishes.

The leopard grouper is also of interest because of its ecological and economic importance. It is the most abundant grouper of the central and lower regions of the Gulf and the most heavily targeted grouper by commercial, recreational, and artisanal fisheries (Ramírez and Rodríguez 1990; Sala et al. 2003). Increased fishing efforts on leopard groupers over the past several decades are correlated with significant declines in annual landings and sizes of landed fish (Sala et al. 2004), and these declines are attributed to intense fishing pressure on both spawning and feeding aggregations. Consequently, the World Conservation Union (IUCN) currently lists *M. rosacea* as a "Threatened" species (http://www.iucnredlist.org). Information on the spawning patterns of leopard groupers is needed to determine which management policies are necessary to promote population recovery and create a more sustainable fishery.

The purpose of this study was to describe the reproductive patterns of *M. rosacea* in comparison with other aggregate-spawning groupers, including details on: (1) the mating system, (2) sperm competition, (3) site-specificity and duration of spawning aggregations, (4) and spawning periodicity.

Materials and methods

Field observations

To document the behavior patterns of adult *M. rosacea*, observations using SCUBA were conducted daily at 24 sites near Loreto, Baja California Sur, Mexico (26°00′N, 111°21′W) from 12 April to 12 June 2005 (Fig. 1). Observations of spawning behavior were made primarily at two sites, Punta Lobos (20–23 April, 6–9 May, and 23–26 May 2005) and Punta Coyote (weekly from 18 April to 13 May and 28 May to 12 June, daily from 14 to 22 May 2005). These sites were chosen because they are known spawning aggregation sites for *M. rosacea* and are heavily exploited by commercial and recreational fishers (R. Murillo and F. Arcas, personal communication). Observations were conducted at 22 additional sites (e.g. Isla Danzante, Isla Coronado) to compare behaviors and abundances of adult leopard groupers between aggregation and non-aggregation sites.

Dives were made throughout the day, but most were carried out during the late afternoon and evening

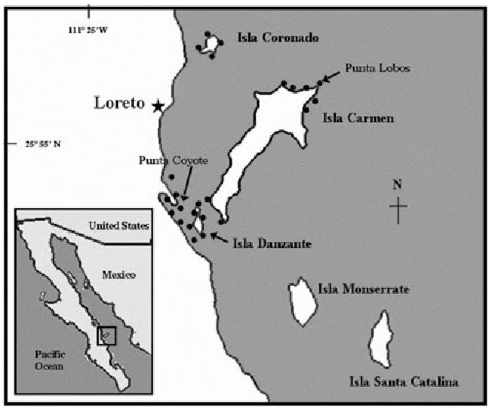


Fig. 1 Map of Loreto, BCS, Mexico, showing dive sites (*filled circle*) and location on the Baja peninsula. The majority of dives were conducted at Punta Lobos and Punta Coyote

(1,600–2,100 h). Dives ranged from 30 to 120 min in duration and 3 to 30 m in depth. Visibility fluctuated between 3–15 m and water temperatures varied from 16 to 23.5°C. Aggregation sizes were estimated at each site by counting the number of adults present while swimming parallel to the reef. Consistently poor visibility and strong currents precluded the use of transects or other quantitative methods for estimating aggregation size and fish density. Observations focused on documenting details of courtship, spawning, and aggressive behaviors. Data were recorded with pencil on plastic slates or by underwater digital video cameras. Frame by frame analyses of video were used to analyze behaviors in greater detail. The sex of individuals was determined by a combination of behaviors, color patterns, and body shape, and verified by histological analyses of fish collected by BE during dives made at Punta Lobos and Punta Coyote (see below).

Terminology and descriptions of behaviors followed those reported for the serranid *Paralabrax clathratus* (Erisman and Allen 2006). Behaviors included: following, courtship chase, mobbing, female darting, spawning rush, rubbing, bumping, lateral display, gaping, nipping, dorsal flare, aggressive chase, and spiraling. Other behaviors were described previously by Sala et al. (2003) for *M. rosacea*, and they included: *head shake* = a male shook the anterior portion of his body and head repeatedly upon approach to a female, and: *burst rise* = a male swam rapidly from the reef in an upward vertical direction for several m and then swam rapidly back down to the reef.

Collection and preparation of samples

Standard length (to the nearest mm) and weight (to the nearest g) data were collected daily from leopard groupers caught at or near Punta Lobos by recreational fishers (hook and line; $n = 203$) from 14 April to 10 June 2005. All fish were sampled in the early afternoon (1,200–1,400 h) on the day of capture. Additional specimens were collected by speargun at Punta Lobos ($n = 39$; 22–23 April, 6–9 May, 23–25 May 2005) and Punta Coyote ($n = 6$; 16–17 May 2005). Sampling was opportunistic, as data were gathered for all *M. rosacea* provided by fishers or collected by BE. Gonads were removed from the body cavity and preserved in a 10% formalin-seawater solution. The gonads of 75 females and 100 males were weighed to the nearest 0.1 g prior to preservation, and gonosomatic indices [GSI = (gonad weight/body weight) × 100] were calculated for these fish. The relationship between gonad weight, GSI, and standard length (SL) were analyzed by Spearman rank-correlation.

The sex and developmental class of all collected individuals ($n = 248$) were assessed by macroscopic and microscopic evaluations of gonads (Table 1). Criteria used in the classification of gonad class were adapted from Moe (1969), Sadovy et al. (1994a), and Rhodes and Sadovy (2002). Stages of gametogenesis followed the definitions of Moe (1969) and Nagahama (1983). Standard histological techniques were used for microscopic evaluations. Pieces of gonad tissue were embedded in paraffin, sectioned at 6 μm, mounted, stained with Mayer's hemotoxylin-eosin, and observed under a compound microscope (Humason 1972). Tissue was taken from the central portion of one lobe and sectioned transversely in larger gonads, while smaller gonads were sectioned longitudinally in their entirety. Immature individuals were collected (see Tables 1, 2) but were excluded from all subsequent analyses.

Mating system and sperm competition

Behavioral observations were used to describe the mating system (i.e., group vs. pair-spawning) of *M. rosacea*. The number of fish that released gametes during spawning events could not be determined from observations. Therefore, the intensity of sperm competition was inferred by the number of male participants in observed spawns and the GSI of mature males (M2—M4; $n = 100$) from sample collections. In broadcast-spawning fishes, GSI is indicative of male investment in gamete production, which in turn is correlated with the intensity of sperm competition (Warner 1997; Petersen and Warner 1998; Taborsky 1998).

Spawning periodicity

Methods used to estimate spawning periodicity and determine if spawning was synchronized with the lunar cycle included observations of spawning behavior in the field and temporal changes in the proportion of spawning females. Collected females with evidence of imminent or recent spawning (F4–F6) were classified as spawning females, and these data were organized weekly by lunar phase (first quarter, full, last quarter, new) and pooled by lunar phase only. The relationship between lunar phase and the proportion of spawning females (vs. non-spawning, mature) was tested using a Pearson χ^2 goodness of fit test. Collected females with ovaries that contained both hydrated oocytes and post-ovulatory follicles (F5) were used as evidence of daily spawning (Hunter et al. 1985; Hunter et al. 1986).

Analyses of daily and weekly fluctuations in GSI of mature females (F2–F6; $n = 75$) provided additional

Table 1 Macroscopic and microscopic descriptions of gonadal development classes in male and female leopard grouper, *Mycteroperca rosacea*

Gonadal development class	Macroscopic	Microscopic
Ovaries		
F1 (immature)	Gonad small and thin; clear, pink or cream in color: oocytes indiscernible; indistinguishable from M1 males	Thin gonad wall; tightly packed primary growth state oocytes (chromatin nucleolar, perinucleolar) and oogonia; no evidence of previous spawning
F2 (mature, inactive)	Gonad small but rounded; gray in color with thickened gonad wall; oocytes small or indiscernible; often indistinguishable from M1 males	Thick gonad wall; primary growth stage and cortical alveolar oocytes abundant; previous spawning indicated by the presence of distinct muscle bundles or vitellogenic oocytes in late stages of atresia
F3 (mature, active)	Gonad large; cream in color with transparent gonad wall; large vitellogenic oocytes visible and tightly packed throughout ovary	Thin gonad wall; large vitellogenic oocytes abundant; primary growth stage oocytes present in variable abundance; hydrated oocytes and post-ovulatory follicles absent; no large scale atresia
F4 (mature, ripe)	Gonad large with clear, hydrated oocytes; eggs released with application of light abdominal pressure	All oocyte developmental stages present with the exception of post-ovulatory follicles; hydrated oocytes few to abundant; atretic vitellogenic oocytes may be present
F5 (mature, ripe and post-spawn)	Gonad resembles F4 or F6 females	All oocytes developmental stages present, including both hydrated ooctyes and post-ovulatory follicles
F6 (post-spawn)	Gonad flaccid with visible capillaries; visible ooctyes less abundant than in F3 or F4 females; hydrated oocytes absent	Thick gonad wall; most oocyte developmental stages present with the exception of hydrated oocytes; post-ovulatory follicles and muscle bundles present; atretic vitellogenic oocytes present and often abundant; intralamellar debris and cytoplasmic strands present
Testes		
M1 (immature or inactive)	Gonad small and thin; indistinguishable from F1 and F2 females	Stroma present in variable amounts; early proliferation of spermatocytes evident; sperm sinuses and seminiferous lobules may be present; previtellogenic oocytes may be present and variable in abundance
M2 (mature, active)	Gonad larger and more rounded than M1 males; grayish in appearance; some milt may be visible	Most stages of sperm development present; spermatozoa abundant within seminiferous lobules and sperm sinuses
M3 (mature, ripe)	Gonad large and white copious amounts of visible milt; milt release possible with light abdominal pressure	Seminiferous lobules enlarged, coalesced and filled with mature spermatozoa; sperm sinuses large and full of mature spermatozoa; early stages of spermatogenesis rare or absent
M4 (post-spawn)	Gonad flaccid with visible blood; milt release may still occur with abdominal pressure	Thick gonad wall; seminiferous lobules are separated and smaller than in M3 males; lobules and sperm sinuses mostly empty and contain very little or no mature spermatozoa; stromal tissue and spermatogonia present

Table 2 Number of sampled fish arranged by gonadal development class and month for the leopard grouper, *Mycteroperca rosacea*

Month	Females						Males				Total
	F1	F2	F3	F4	F5	F6	M1	M2	M3	M4	
April	3	0	13	6	2	1	1	7	17	0	50
May	3	2	21	24	19	9	1	6	52	16	153
June	1	1	3	6	5	1	2	6	15	5	45
Total	7	3	37	36	26	11	4	19	84	21	248

evidence of spawning periodicity. Variations in the daily mean GSI of females were examined by a Lomb-Scargle periodogram (Press et al. 2002). The average weekly GSI of females grouped by lunar phase was also calculated, and differences were tested using the Kruskal–Wallis test. Pairwise comparisons of weekly GSI were analyzed by Dunn's Multiple Comparison Test.

Results

Spawning aggregations

Spawning aggregations were identified at Punta Lobos and Punta Coyote (Fig. 1). The aggregation at Punta Lobos contained 600–700 adult leopard groupers scattered over a distance of several hundred meters along the northwest side of the point. These fish were abundant from the bottom to the surface and from the reef-sand interface (20 m) to the subtidal zone (1–2 m). At Punta Coyote, the aggregation consisted of 150–200 fish spread out along the southernmost portion of the point. Individuals were most common in the open water at depths of 8–15 m, and 2–10 m offshore of the reef. Adult leopard groupers were absent or present in low numbers (<15 fish) at all other sites visited from April through June 2005. Spawning was not observed outside these spawning aggregation sites, although putative courtship was observed once at Isla Coronado on 13 May 2005 between a female and two males.

Identification of sexes and color patterns

Females and males differed in their behavior and body shape. Females were less active than males and many had enlarged abdomens full of ripe eggs. Gravid females with enlarged abdomens also led spawning groups and spawning rushes. When not engaged in courtship with males, females rested on or just above the reef, or they hovered in the water column. Males lacked the enlarged abdomens of spawning females, as all fish collected by BE with this feature ($n = 10$) were females. Males swam more vigorously and were more curious of divers than females. This behavior was most intense in the late afternoon and evening, when males swam rapidly in all directions to court females, join spawning groups, or engage in brief aggressive interactions with other males. Unlike females, males often performed Burst Rise and Head Shake behaviors during courtship.

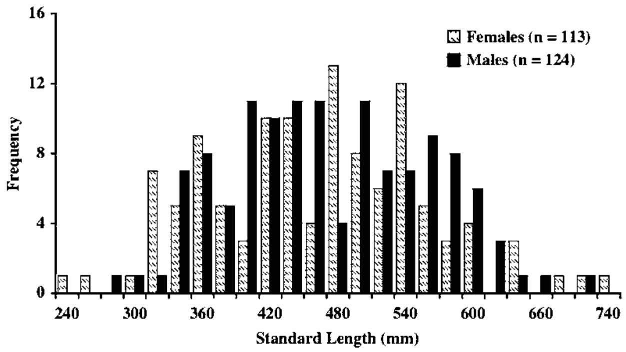
The color patterns of males and females were usually similar during courtship and spawning events, but sex-related differences in color were observed in a few individuals. For both sexes, the posterior half and ventral portions of the body were pale, the anterior portions of the upper body and head consisted of large black blotches surrounded by light, conspicuous rings, and the caudal fins were completely black (see Sala et al. 2003 for pictures of color patterns in *M. rosacea*). Of six fish with these color patterns collected by BE, four were male and two were female. However, some females were dark gray or black during spawning events, and the blotches on their heads and bodies were less distinct. Two dark colored fish leading spawning groups were collected by BE, and both were females. Both male and female golden phase leopard groupers (Thomson et al. 2000) were present in spawning aggregations and participated in spawning events. Of seven golden phase fish collected by BE, three were male and four were female.

Sample collections

Mature females (F2–F6; $n = 113$) ranged in size from 245 to 744 mm SL and weight from 355 to 8,800 g (Fig. 2). Mature males (M2–M4; $n = 124$) ranged in length from 288 to 708 mm SL and weight from 475 to 6,850 g. The mean length (±SD) of females (461.76 ± 95.82 mm SL) and males (470.22 ± 87.71 mm SL) were similar (unpaired t test, $t = 0.709$, $P = 0.479$). The mean weight (±SD) of females (2461.95 ± 1513.30 g) and males (2505.52 ± 1281.90 g) were also similar (unpaired t test, $t = 0.209$, $P = 0.835$).

GSI of mature females ($n = 75$) ranged from 0.39 to 11.51%, and the mean GSI (±SD) of mature females was 4.37 ± 2.18%. Gonad weight increased with standard length in mature females (Fig. 3a, Spearman rank-correlation, $r_s = 0.645$, $P < 0.001$), but GSI did not change with standard length (Fig. 3b, Spearman rank-correlation, $r_s = 0.009$, $P = 0.942$). GSI of mature males ($n = 100$) ranged from 0.20 to 7.21%, and the mean GSI of mature males was 2.34 ± 1.48%. Gonad weight increased with standard length in mature males (Fig. 4a, Spearman rank-correlation, $r_s = 0.391$,

Fig. 2 *Mycteroperca rosacea.* Size-frequency distributions of collected females and males arranged by 20 mm SL size classes



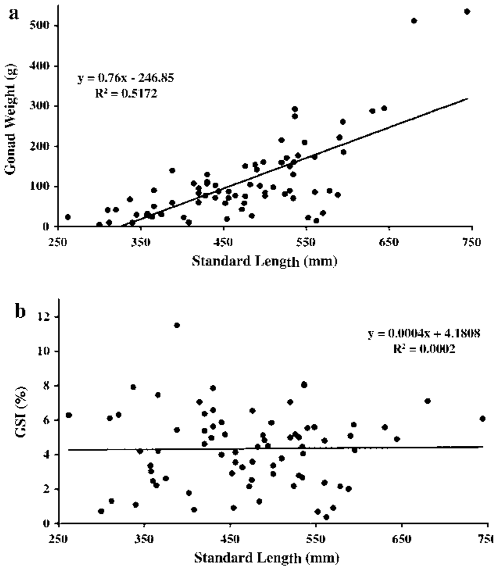


Fig. 3 *Mycteroperca rosacea.* Gonadal investment and body size relationships in 75 mature females. **a** Gonad weight (g) versus standard length (mm). **b** Gonosomatic index (GSI%) versus standard length (mm)

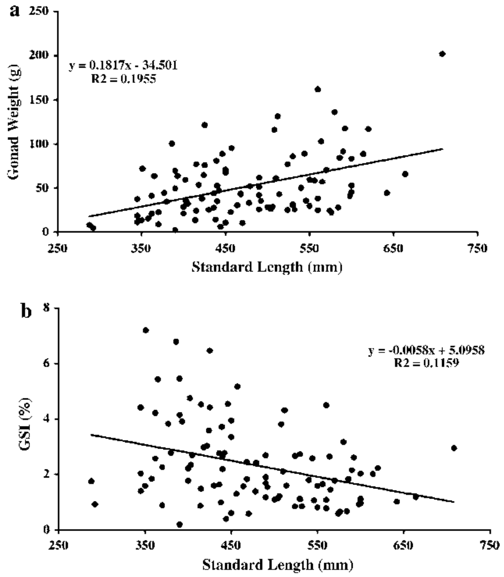


Fig. 4 *Mycteroperca rosacea.* Gonadal investment and body size relationships in 100 mature males. **a** Gonad weight (g) versus standard length (mm). **b** Gonosomatic index (GSI%) versus standard length (mm)

$P < 0.001$), and GSI decreased with standard length (Fig. 4b, Spearman rank-correlation, $r_s = -0.339$, $P < 0.001$).

Mating system

Thirty-two spawning rushes were observed during the study period (Table 3). Spawning rushes occurred in groups of 6–40 fish (mean 17.50, SD 10.86). Spawning groups at Punta Lobos were larger than at Punta Coyote (unpaired t test, $t = 4.398$, $P < 0.001$). Paired, sneak, or other types of spawning were not observed at either site. Spawning groups led by large females (>50 cm) contained more fish than those led by small females at Punta Lobos (Table 3, unpaired t test, $t = 7.704$, $P < 0.001$) and Punta Coyote (unpaired t test, $t = 2.931$, $P = 0.017$).

Courtship behaviors such as Rubbing, Bumping, Lateral Display, and Following occurred throughout the day, but behaviors associated with imminent spawning (chasing, mobbing, spawning rush) were limited to the late afternoon and evening hours (1,645–2,020 h). Spawning began approximately two hours before sunset (1,755–1,815 h), peaked at sunset (1,950–2,010 h), and

Table 3 Abundances of fish in spawning groups by site and size of the lead female for the leopard grouper, *Mycteroperca rosacea*

Site	Lead female	Observations	Range (# fish)	Mean (# fish)	SD
Punta Lobos	All females	21	8–40	21.56	11.31
	Large (>50 cm)	12	20–40	29.58	7.87
	Small (<50 cm)	9	8–15	10.78	2.68
Punta Coyote	All females	11	6–20	9.82	3.31
	Large	6	8–20	11.83	2.48
	Small	5	6–10	7.40	2.51
Pooled	All females	32	6–40	17.50	10.86
	Large	18	8–40	23.67	10.77
	Small	14	6–15	9.57	3.03

continued until dark when adults settled on the reef. Courtship events that ended with spawning lasted from 15 s to several minutes.

Spawning groups formed when several males visited females and performed various combinations of lateral display, rubbing, and bumping. These behaviors led to following and chasing involving one lead female and several males. When females leading chasing events decreased swimming speed, mobbing occurred. Mobbing was often interrupted when the lead female made an abrupt turn and darted away from the group (female darting). Chasing and then mobbing followed, as males continued to pursue the lead female. A spawning rush began when the female slowed down, turned upwards towards the surface, and rose slowly upwards. Males responded immediately by mobbing and forming a tight "ball" around the female with their vents facing her. Spawning groups often increased in size at this time, as additional males (and possibly females) joined. Led by the female, the entire group performed a spawning rush, a rapid vertical burst swim and release of gametes, then separated quickly in all directions (Fig. 5).

Fig. 5 *Mycteroperca rosacea*. Illustration showing the typical orientation of adult fish during a spawning rush. The lead female is *dark gray* with an enlarged abdomen. Surrounding individuals are males

Spawning rushes varied in direction, location, and behavior. Most involved a rapid vertical ascent of the group for 1–4 m; however, horizontal rushes of similar distances were also observed. Gamete release usually occurred 3–8 m below the surface, although spawning adults were observed breaching the surface at Punta Lobos during the evening of 25 May 2005. Groups of smaller adults often spawned near the surface above the shallow fore-reef, whereas larger adults usually spawned above the reef slope or off the reef entirely. There was anecdotal evidence of assortative mating. At both sites, large males (>50 cm) courted and spawned only with females of equal or larger sizes, but smaller males courted and spawned with all females.

Brief aggressive encounters among males were observed infrequently throughout the day and consisted of one or more of the following behaviors: lateral display, dorsal flare, gaping, nipping, bumping, aggressive chase, burst rise, and spiraling. Males did not defend discrete territories or guard females during courtship and spawning.

Spawning periodicity

Courtship and spawning were observed at Punta Lobos on 6–9 and 23–26 May 2005 and at Punta Coyote on 15–22 May, 29–30 May, and 8–9 June 2005. Seventy-three females (65% of mature females) with microscopic evidence of imminent or recent (F4–F6) spawning were collected daily from 18 April to 8 June 2005 (Fig. 6), and 53 of these females had ovaries with visibly hydrated oocytes (Fig. 7). Twenty-six females (23% of mature females; 35% of spawning females) with

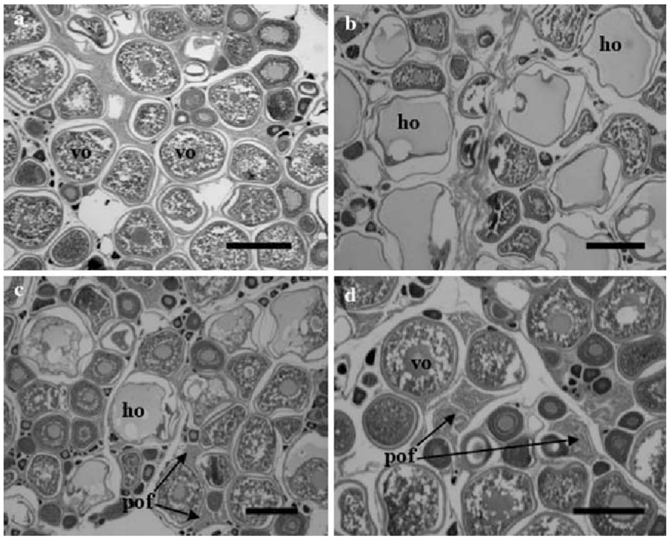


Fig. 6 *Mycteroperca rosacea*. Transverse sections of female gonads. **a** Mature, active (F3) female; **b** mature, ripe (F4) female; **c** mature, ripe and post-spawn (F5) female; **d** post-spawn (F6) female. *ho* hydrated oocytes; *pof* post-ovulatory follicles; *vo* vitellogenic oocytes. *Scale bar* 0.5 mm

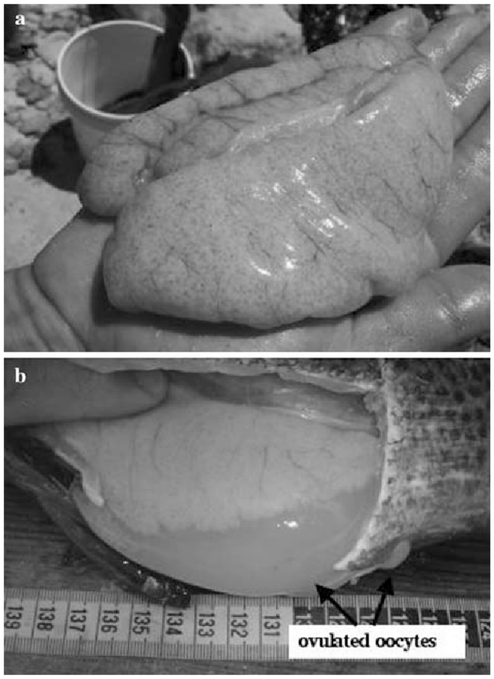


Fig. 7 *Mycteroperca rosacea*. Macroscopic evidence of imminent spawning in the ovaries of mature females. **a** Ovaries dissected from the body cavity of a mature, ripe female with visibly hydrated oocytes. **b** Close-up view of the ovary of a mature, ripe female still intact in the body cavity but with the abdominal wall removed. The ventral portion of the ovary is filled with clear, ovulated oocytes, and small portions of oocytes are leaking from the urogenital pore

evidence of daily spawning (F5) were collected weekly from 27 April to 7 June 2005. The monthly frequency of F5 females increased from April to June (Table 2; April = 9%, May = 25%, June = 31%).

During the first lunar quarter of April, only 20% of mature females were actively spawning (Fig. 8). The proportion of spawning females increased significantly from the first lunar quarter to the full moon in April (Pearson $\chi^2 = 5.000$, $df = 1$, $P = 0.025$). For the rest of the collection period, the weekly proportion of spawning females ranged from 50 to 88%, and differences were not significant (Pearson $\chi^2 = 6.751$, $df = 6$, $P = 0.344$). When these data were pooled by lunar phase only, there were no significant differences in the proportion of spawning females among lunar phases (Pearson $\chi^2 = 3.536$, $df = 3$, $P = 0.316$).

Weekly mean female GSI increased steadily from the first quarter moon in April (3.44%) to the new moon in May (6.07%) and decreased through the first quarter moon in May (4.17%, Fig. 8). A second peak in mean female GSI occurred during the last quarter moon at the end of May (5.19%) but was followed by a decline. Differences in weekly female GSI from April to June were significant (Kruskal–Wallis, $H = 15.050$, $df = 7$, $P = 0.035$), but all pairwise comparisons of female GSI over this period were non-significant (Dunn's Multiple Comparison, $P > 0.05$). Female GSI data pooled by lunar phase only showed no significant differences (Kruskal–Wallis, $H = 5.159$, $P = 0.171$). Changes in the daily mean GSI of mature females from April to June were not significant (Lomb-Scargle periodogram, $n = 27$, $\alpha = 0.277$).

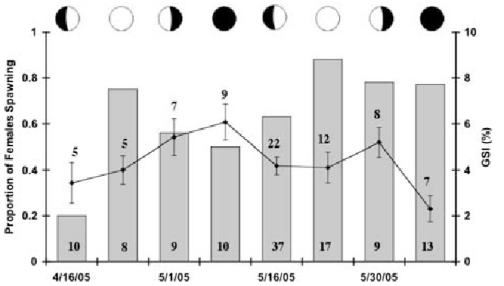


Fig. 8 *Mycteroperca rosacea*. Temporal changes in spawning activity of mature females collected from April through June 2005. *Bars* indicate the weekly proportion of collected females with evidence of imminent or recent spawning (F4–F6), and numbers at *bottom* of *bars* indicate sample sizes. *Line* indicates weekly mean (±SD) gonosomatic indices (*GSI*%) of females, and numbers above *error bars* indicate sample sizes

Discussion

Mating system and sperm competition

Leopard groupers in the Loreto area exhibited a group-spawning mating system, and there was no evidence of pair-spawning or alternative mating behaviors such as sneaking, territoriality, or female defense (Taborsky 1994; Gross 1996). Spawning groups consisted of a lead female and five to forty surrounding individuals, most of which were presumed to be males. Overall, the mating system of this species is similar to that of the Nassau grouper, *E. striatus*, and the kelp bass, *P. clathratus* (Colin 1992; Erisman and Allen 2006); all three species spawn in groups within larger aggregations and are non-territorial. Conversely, *M. rosacea* behaved differently than many other aggregating groupers including closely related species such as *M. tigris*, *M. phenax*, and *M. microlepis*, where males are territorial and fish spawn in pairs (Gilmore and Jones 1992; Coleman et al. 1996; Sadovy et al. 1994b; Samoilys and Squire 1994; Zabala et al. 1997).

In reef fishes, male mating behavior often varies with local population density. At very high densities, the reproductive success of territorial males may be reduced substantially by competition with other males, because territorial males must spend more time in defense and have less time available for mating (Warner and Hoffman 1980a, b; Warner 1982, 1984). As a consequence, territorial behavior is rare in fishes that spawn in large groups (Warner 1982; Erisman and Allen 2006). The behavioral patterns of male leopard groupers were similar to those of other group-spawning fishes; aggressive interactions among males were infrequent and short in duration, and males did not guard females or defend territories from other males. For leopard groupers, occasional aggression may reinforce dominance hierarchies among rival males and determine the arrangement of males around a spawning female (Hutchings et al. 1999; Erisman and Allen 2006), with dominant males gaining primary access to females during spawning. By occupying the closest position to the female, dominant males may fertilize more eggs and realize a higher reproductive success than peripheral males (Warner and Harlan 1982; Petersen and Warner 1998).

The large number of male leopard groupers in spawning groups indicates that sperm competition was present, and GSI of mature males was consistent with predictions of sperm competition theory. In general, testes are larger and GSI values are higher in group-spawning fishes characterized by sperm competition than in pair-spawning fishes where sperm competition is absent or low (van den Berghe and Warner 1989; Taborsky 1994; Warner 1997). In fact, male GSI may reach values of 7–12% in serranid fishes that spawn in groups (Sadovy and Colin 1995; Erisman and Allen 2006). Accordingly, testes sizes and GSI of male leopard groupers were similar to other group-spawning serranids (up to 7.2%) and considerably higher than closely related species that spawn in pairs. For example, *Mycteroperca microlepis*, *M. phenax*, and *M. tigris* all pair-spawn, have small testes, and maximum male GSI values range from 0.6 to 3.0% (Coleman et al. 1996; Sadovy et al. 1994b; Brulé et al. 2003).

The decline in male leopard grouper GSI with body size is a pattern typically associated with fishes that exhibit multiple male reproductive strategies (Choat and Robertson 1975; Robertson and Warner 1978; Petersen and Warner 1998). In these fishes, small males group or sneak spawn and overcome sperm competition by investing in gamete production. Conversely, large males pair-spawn, and they invest in territorial defense and female attraction rather than gamete production (Taborsky 1994, 1998; Warner 1997). We observed no differences in mating strategies with size in male leopard groupers, as both small and large males spawned in groups. However, it is possible that multiple mating strategies do exist, with pair-spawning and territorial behavior by large males occurring outside aggregations. Sadovy and Colin (1995) found small testes in a ripe male *E. striatus* taken from outside a spawning aggregation, and they offered a similar explanation. The low abundance of adult leopard groupers observed outside spawning aggregations suggests that territorial mating behavior would be a successful reproductive strategy for males at these sites.

Spawning aggregation characteristics

Spawning aggregations are an important life history trait for *M. rosacea*, because much or all of their reproductive activity occurs within these aggregations. Several features of leopard grouper spawning aggregations were analogous to those of other aggregating groupers. First, aggregations were site-specific, as adults were abundant only at certain locations and were rare at all other visited sites in the Loreto area. Next, there was evidence of migration to spawning aggregations. During non-spawning periods (summer, fall, winter), adult leopard groupers were common at most visited sites in Loreto (BE and MB, unpublished data), which suggests that some fish travel significant distances to reach spawning aggregation sites. The diel spawning period of *Mycteroperca rosacea* was also consistent with other groupers in that it occurred in the evening near dusk (Thresher 1984; Samoilys and Squire 1994; Sadovy 1996; Levin and Grimes 2002). The structure of leopard grouper aggregations was similar to that of *M. phenax* and *M. microlepis*, in that aggregations consisted of tens to hundreds of individuals spread out over a large area (Coleman et al. 1996).

Mycteroperca rosacea spawning aggregations in Loreto were unusual in that they persisted for extended periods, and this is supported by behavioral observations and data acquired from fishers. Fish were aggregated at Punta Lobos during all our observations, and fishers collected large numbers of adults daily from that site from early April through June. Thus, the aggregation at Punta Lobos likely formed sometime in April and remained intact for the entire spawning season. Also, the aggregation at Punta Coyote persisted for over 3 weeks and likely longer. This pattern is dissimilar to most grouper spawning aggregations, which are typically shorter in duration (Shapiro 1987; Sadovy 1996; Rhodes and Sadovy 2002).

The short duration of most grouper spawning aggregations coincides with spawning activity that follows a lunar or semi-lunar rhythm (Thresher 1984; Samoilys and Squire 1994; Rhodes and Sadovy 2002; Lee et al. 2002). Our results indicate that spawning in leopard groupers was not lunar-related, but rather it occurred daily from late April through early June. First, courtship and spawning behavior were observed during most dives at Punta Lobos and Punta Coyote in May and June. Next, spawning females (F4–F6) were collected daily and their occurrence was not synchronized with the lunar cycle. In fact, 35% of spawning females, were capable of spawning on consecutive days (i.e. daily spawning in F5 females), and these females were collected weekly throughout the study period. Finally, female GSI patterns were also inconsistent with a lunar spawning periodicity. Fishes that spawn in concert with the lunar cycle typically show drastic changes in female GSI during specific lunar phases when spawning activity occurs (Hoque et al. 1999; Lee et al. 2002; Rhodes and Sadovy 2002; Takemura et al. 2004). Female GSI did fluctuate daily and weekly, but these fluctuations were relatively small and did not occur in rhythm with the lunar cycle.

Factors that influence the temporal spawning patterns of *Mycteroperca rosacea* were not investigated, and we can only speculate as to why spawning did not follow the lunar cycle. For aggregating groupers that occur in low densities or are solitary during non-reproductive periods, lunar-related spawning may enhance reproductive output via increased mate encounter rates and synchronized gonadal maturation (Colin et al. 1987; Takemura et al. 2004). Leopard groupers may not require these cues to form spawning aggregations, because adults aggregate throughout the year.

The seasonality of phyto- and zooplankton blooms strongly influences the reproductive timing of fishes that that live in highly productive environments. Spawning is timed so that larvae are exposed to peak food densities, which results in increased growth and survival of larvae (Hjort 1914; Cushing 1975; Platt et al. 2003). There is evidence that the reproductive timing of leopard groupers is influenced by seasonal patterns in productivity. The Gulf of California is an area characterized by exceptionally high rates of primary productivity (Zeitzschel 1969), and plankton concentrations reach maximum levels in the central Gulf during the spring spawning season of *M. rosacea* (Kahru et al. 2004). During our observations at Punta Lobos and Punta Coyote, visibility was often poor due to high densities of phytoplankton and large swarms of larval fishes and invertebrates in the water column. The timing of large plankton blooms likely influences the reproductive patterns of other reef fishes in the Gulf, as several species engaged in courtship behavior at Punta Lobos and Punta Coyote during the study period (*Lutjanus argentiventris*, *Bodianus diplotaenia*, *Kyphosus elegans*, *Sphoeroides annulatus*, *Epinephelus labriformis*).

Non-spawning aggregations

Aggregating groupers are often solitary during the non-spawning times of the year (Domeier and Colin 1997; Sadovy and Eklund 1999); however, the leopard grouper does not follow this pattern. Adults occur in small to large groups throughout the year, and fish are rarely solitary (B.E. Erisman, personal observation).

Non-spawning aggregations usually contain fewer fish than spawning aggregations, although feeding aggregations may be quite large. Feeding aggregations of *Mycteroperca rosacea* may include more than one hundred individuals and form in areas characterized by high densities of small, schooling fishes such as *Harengula thrissina* and *Cetengraulis mysticetus* (Hobson 1965; Parrish 1992). The temporal and spatial dynamics of leopard grouper feeding aggregations have not been studied but are necessary to fully understand their social system and to compare with spawning aggregations. Both feeding and spawning aggregations of *M. rosacea* have been observed at Punta Coyote (Parrish 1992; B.E. Erisman, personal observation), which suggests that they may be present at specific sites for extended periods and use such sites for multiple purposes.

Management considerations

The extensive duration and site-specificity of spawning aggregations and the propensity to form feeding aggregations year-round make leopard groupers particularly vulnerable to fishing pressure. The effects of fishing on the population structure and reproductive activities are well documented for many reef fishes that form spawning aggregations, including several species of *Mycteroperca* in the western Atlantic. Such effects include disruptions of spawning activity, decreases in egg production, shifts in sex ratios and population structure, reductions in the sizes and ages at first maturity, and the local extirpation of aggregation sites (Coleman et al. 1996; McGovern et al. 1998; Harris et al. 2002; Levin and Grimes 2002; Matos-Caraballo et al. 2006; Sadovy and Domeier 2005). In turn, these changes may reduce population growth rates and slow recovery following exploitation (Hutchings and Reynolds 2004). Overfishing of aggregations also influences ecosystem stability by causing major shifts in fish community structure (Pauly et al. 1998; Sala et al. 2004).

Overfishing of leopard grouper spawning and feeding aggregations has resulted in substantial declines in landings and CPUE of this species by regional fisheries in the last decade (Sala et al. 2004), and fisheries management policies have done little to change this trend (Hernandez and Kempton 2003). Current regulations on *Mycteroperca rosacea* fisheries in the Gulf of California do not set catch quotas for commercial fishers, specify daily catch limits for recreational fishers that are unreasonably high, and regulations are not enforced (Sala et al. 2004). Conservation and management policies that protect *M. rosacea* spawning and feeding aggregations and limit harvest are needed to create a more sustainable fishery and stimulate population recovery. Potentially effective strategies include seasonal bans on fishing and trading during spawning, inclusion of spawning and feeding aggregation sites into no-take marine reserves, fishing quotas and gear restrictions for commercial fishers, and reduced daily catch limits for recreational fishers (Sala et al. 2002; Roberts et al. 2001; Colin et al. 2003; Nemeth 2005; Sadovy and Domeier 2005). Of course, any attempts to improve management of leopard grouper fisheries would require continuous monitoring of populations and the fishery, as well as strict enforcement of regulations (Sala et al. 2003; Sadovy and Domeier 2005).

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CHAPTER 2

Observations of spawning in the Leather Bass, *Dermatolepis dermatolepis* (Teleostei: Epinephelidae), at Cocos Island, Costa Rica.

Abstract

Spawning behavior in the Leather Bass, *Dermatolepis dermatolepis*, was observed from a manned submersible at a seamount at Cocos Island, Costa Rica on 19 days between November 2006 and February 2007. Spawning occurred in subgroups of 10–32 individuals that formed within larger aggregations of 50–70 individuals. The diel spawning period and courtship behaviors observed were similar to descriptions of other grouper spawning aggregations; however, spawning aggregations of Leather Bass were somewhat unusual in that aggregating and spawning behaviors were not restricted to specific periods of the lunar cycle. Spawning aggregations are a critical component of Leather Bass reproduction and should be considered in any conservation measures for this species.

Introduction

Reef fishes exhibit a remarkable diversity of reproductive behaviors (Thresher 1984), but our understanding of this diversity is relatively poor in comparison to that of the behavioral patterns of terrestrial animals (Robertson 1991, Owens 2006). Moreover, most information on the spawning behavior of reef fishes stems from observations on and experimental manipulations of small species, and much less is known about the reproductive patterns of large, predatory species (Domeier and Colin 1997, Levin and Grimes 2002). Detailed knowledge of spawning in large reef fishes could help to identify the relative importance of the proximate mechanisms and ecological parameters that have shaped the evolution of reproductive modes in fishes and other animals. Moreover, such knowledge may supply new insights into our understanding of sexual selection, mate choice, social evolution, life history adaptation, and other governing principles of behavioral ecology.

Information on spawning behavior is also vital for the creation of effective conservation strategies for reef fishes exploited by commercial and recreational fisheries. Fishing practices tend to reduce the reproductive output of adult populations via the disruption of spawning activities and alterations in population structure, which in turn, causes declines in population growth rates (Sadovy and Vincent 1998, Rowe and Hutchings 2003). Therefore, incorporation of mating system aspects (e.g., courtship patterns, sex ratios, temporal patterns, spawning habitats) into fisheries management will serve to determine conservation risks posed by different fishing practices and gear types and predict patterns of recovery from various management policies. Knowledge of reproductive behavior has contributed greatly to policies that have stimulated recovery of overfished stocks in certain regions, including the design of no-take reserves and timing of seasonal fishery closures in parts of the Caribbean and western Pacific (Nemeth 2005, Sadovy and Domeier 2005, Helfman 2007).

The Leather Bass, *Dermatolepis dermatolepis* (Teleostei: Epinephelidae) (Figure 1) is a medium-sized (to 1 m total length) diurnal predator of small fishes and benthic crustaceans (Montgomery 1975, Heemstra and Randall 1993). Found on rocky and coral reef habitats throughout the tropical eastern Pacific, it has been recorded from southern California to Ecuador, including most oceanic islands of the region (Heemstra and Randall 1993, Allen and Robertson 1994). Leather Bass are very abundant at Cocos Island, Costa Rica, where adults are present on most reefs from 6 to 40 m and are often observed in groups (Garrison 2000).

Leather Bass are reported to spawn on rocky reefs in aggregations of fifty or more individuals (Morris et al. 2000, Aburto and Hull In press), but little is known about the behavioral, spatial, or temporal aspects of reproduction. Spawning was first recorded in July 1995 in the film "Secrets of the Ocean Realm: Creatures of Darkness" (Howard Hall productions, ©MMVII Questar, Inc.) at Manuelita Island, a small island located a few hundred meters off the northern coast of Cocos Island, Costa Rica. Aburto and Hull (In press) also documented Spawning aggregations of Leather Bass in February 2006 at San Benedicto Island, in the Revillagigedo Archipelago, Mexico. Spawning was not observed, but reproductive activity was inferred from the enlarged abdomens, bright coloration, and heightened social activity of individuals within aggregations. Here we describe the behavior of a Leather Bass spawning aggregation at Everest seamount, Cocos Island, Costa Rica.

Many reef fishes aggregate at specific sites, seasons, and lunar phases for the purposes of spawning (Domeier and Colin 1997). Due to their predictability in both time and space, spawning aggregations are particularly vulnerable to fishing (Claydon 2004, Sadovy and Domeier 2005). Consequently, control of fishing pressure on aggregations is necessary for management (Coleman et al. 1999, Colin et al. 2003, Graham 2002). Since Leather Bass aggregate to spawn, knowledge of their spawning patterns would improve management and conservation actions for this species.

Materials and Methods

All observations were conducted from DeepSee, a three-person, one-atmosphere submersible with an acrylic viewing sphere. Spawning was first observed by T. Konotchick from 1610 to 1630 on 23 November 2006 (3 days after new moon) at a seamount located ca. 1 km northeast of Isla Manuelita at Cocos Island, Costa Rica (05º33’ N; 87º03’ W). The base of the seamount lies 100 m below the surface, and it rises up to a peak at 50 m depth. The site is characterized by rocky substrate covered with coralline algae and small corals, and it houses a rich diversity of fish and invertebrate fauna.

We made subsequent observations at Everest from 27 November 2006 to 24 February 2007. A total of 27 submarine dives were made on 19 different days (Table 1**).** Dive durations ranged from 30–150 min. However, observations of Leather Bass were brief (ca. 5–10 min) and were made during routine ascents from surveys of deeper reefs. Therefore quantitative estimates of spawning were not measured; only the presence or absence of courtship and spawning activity were recorded. Visual estimates of the total lengths (TL) of individuals in the aggregations were also recorded during some observations.

Videos of behaviors were recorded with a digital camera from inside the submersible during observations made on 23 November 2006. Still frames captured from videos were used to estimate the size of the aggregation as well as the number of participants for two spawning rushes. Descriptions of courtship and spawning behaviors (i.e., lateral display, crowding, spawning rush) followed descriptions of Domeier and Colin (1997) and Erisman et al. (2007) for other groupers.

Results

At 1610 h on 23 November 2006, an aggregation of ca. 70 adult Leather Bass was observed hovering just above the top of the seamount at depths of 48 to 50 m (Figure 2). Most individuals were oriented into the prevailing current. A few individuals made sporadic movements around the aggregation and engaged in brief interactions (bumping, lateral displays) with conspecifics. The fish ranged in estimated size from 50 to 80 cm total length (TL) and exhibited similar color patterns. Their bodies were light grey and covered with numerous, small white blotches. Faint grey bars were present on some individuals and were most prominent along the dorsal portion of the body. The fins were dark grey to black with narrow, bright yellow margins. Color change was observed in one individual during an intraspecific interaction. Upon approach of a nearby fish, the individual erected its dorsal fin, and the faint grey bars along its body darkened and became more conspicuous.

At 1624 h, intraspecific activity increased and the entire aggregation rose slowly into open water, 3–5 m above the seamount or 45–47 m in depth. Three minutes later at 1627 h, a pair of individuals positioned side-by-side and touching along their flanks performed a rapid, vertical spawning rush out from the top of the aggregation for 3 m. These two fish were followed closely by a subgroup of 20–25 fish that separated from the larger aggregation immediately as the pair initiated their rush. During the spawning rush, members of the subgroup swam in close proximity to one another, often making physical contact with surrounding individuals (crowding behavior). At the apex of the ascent, which occurred at a depth of ca. 42 m, the two lead fish separated and swam down towards the main aggregation. The pursuing subgroup followed the same swimming pattern as the lead fish, separating at the apex point and swimming back down to the main aggregation. This 4 s event was considered a ‘false spawn’, because no gamete release was observed.

Spawning was observed at 1628 h within a subgroup of 32 individuals that formed in the open water 3 m above the seamount. The subgroup first consisted of a lead individual, likely a gravid female, with an enlarged abdomen that was ‘crowded’ by seven surrounding individuals. The subgroup swam rapidly in a horizontal direction for 2 m, turned upwards, and performed a vertical spawning rush of 3 m (Figure 3) to a depth of ca. 40 m. As this event developed, ca. 25 other fish separated from the main aggregation and joined the subgroup in the spawning rush. At the apex of the spawning rush, the lead individuals released gametes, separated rapidly and swam back down towards the top of the seamount. The remaining members of the subgroup followed the pathway of the lead individuals, swimming rapidly through the apex point before separating from the subgroup and swimming down towards the reef. A cloud of gametes formed at the apex point of the spawning rush and grew in size as more and more individuals from the subgroup passed through it. The spawning event was 6 s in duration.

We documented spawning on 10 of 27 dives conducted from 23 November 2006 to 24 February 2007 (Table 1). During the study period, aggregations of 50 or more adult leather bass were present throughout the day on all dives, and courtship behavior was observed on all dives that occurred from 1300 h or later. Spawning events were observed on all dives that were performed from 1600 to 1830 h, and they occurred at depths of 40–50 m. The progression of behaviors during courtship was similar to those described above (Figure 4), and spawning events included 10–32 fish. Individuals in the aggregations ranged in total length from 40 to 90 cm.

Discussion

The behaviors observed in Leather Bass at Everest were similar to those captured in the film “Secrets of the Ocean Realm: Creatures of Darkness” (Howard Hall productions, ©MMVII Questar, Inc.) at Manuelita Island in 1995. In most observed spawnings, the mating sequence was characterized by subgroup formation and crowding behavior followed by a brief vertical spawning rush, gamete release, and the rapid separation of participants. The habitat, size and seasonality of the Leather Bass spawning aggregation at Cocos were consistent with descriptions by Aburto and Hull (In press) at the Revillagigedo Islands, Mexico. In that study, spawning aggregations formed in February over seamounts and consisted of 30–54 individuals.

We observed spawning at Cocos Island from November through February, whereas spawning was filmed in July at Manuelita Island in 1995. This suggests that the spawning season for Leather Bass at Cocos varies from year to year or is prolonged. Also, we observed the aggregation throughout the lunar cycle at Cocos, whereas aggregations were observed only during the new moon at the Revillagigedo Islands (Aburto and Hull In press). Since estimates of spawning frequency at Everest were not measured, it is uncertain whether spawning activity peaked during certain lunar phases and thus followed a rhythm. However, our observations demonstrate that aggregating and spawning persisted for long periods and were not restricted to certain lunar phases. Spawning during specific lunar phases is common among groupers that form spawning aggregations (Thresher 1984, Colin 1992, Sadovy 1996), but non-lunar spawning patterns have been reported for a few species (Hereu et al. 2006, Erisman et al. 2007).

Leather Bass are not important components of the commercial fisheries in most mainland regions of the eastern Pacific but are primary targets of artisanal and recreational fisheries in certain offshore island areas (Heemstra and Randall 1993, Ruttenberg 2001). The status of fish stocks and the impacts of fishing on their populations are poorly understood, yet a recent evaluation by the International Union for the Conservation of Nature (IUCN) concluded that the species was one of ‘Least Concern’ (Sadovy 2007) and a previous assessment by Morris et al. (2000) concluded that Leather Bass were not under any immediate threat of extinction. However, a study on artisanal fisheries at the Galápagos Islands suggested that Leather Bass populations are highly sensitive to fishing. Ruttenberg (2001) sampled communities of fishes at several heavily fished and lightly fished sites throughout the central islands of the archipelago and found that Leather Bass were completely eliminated in areas where fishing pressure was intense.

The sensitivity of Leather Bass populations to fishing is likely related to their spawning aggregations. Many groupers aggregate to spawn at very specific sites and times of the year, and commercial, artisanal, and recreational fisheries heavily exploit these aggregations (Levin and Grimes 2002, Colin et al. 2003). Heavy fishing pressure has serious, negative effects on the population structure and reproductive activity of aggregating groupers (Colin 1992, Coleman et al. 1996, Vincent and Sadovy 1998), and widespread overfishing on spawning aggregations has been responsible for dramatic population declines in many areas of the world (Claydon 2004, Sadovy and Domeier 2005). Although more information on Leather Bass spawning behavior is needed for effective management, it is clear that any conservation measure for this species should include protection for its spawning aggregations. This includes seasonal or area closures of seamounts and rocky reef promontories where aggregations have been located, since these areas are critical spawning habitats.

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Table 2.1. Timing and lunar phase of observations on the reproductive behavior of Leather Bass, *Dermatolepis dermatolepis* at Cocos Island, Costa Rica from November 2006 to February 2007. dafm = days after full moon; dbfm = days before full moon. A = aggregating behavior, C = courtship behavior, S = spawning.

Date	Moon Phase	Dive Interval	Behavior
23-Nov-06	12 dbfm	0900-1030	A
		1130-1230	A
		1530-1715	A, C, S
27-Nov-06	8 dbfm	1230-1400	A, C
6-Dec-06	1 dafm	1500-1630	A, C, S
8-Dec-06	3 dafm	0845-1015	A
10-Dec-06	5 dafm	0900-0930	A
11-Dec-07	6 dafm	0900-1030	A
15-Jan-07	12 dafm	1645-1800	A, C, S
16-Jan-07	13 dafm	0900-1000	A
		1130-1230	A
		1530-1700	A, C, S
17-Jan-07	14 dafm	0830-1100	A
23-Jan-07	10 dbfm	1530-1730	A, C, S
31-Jan-07	1 dbfm	1100-1300	A, C
		1600-1715	A, C, S
4-Feb-07	2 dafm	1630-1830	A, C, S
5-Feb-07	3 dafm	1530-1745	A, C, S
7-Feb-07	5 dafm	0845-1015	A
		1200-1330	A, C
		1615-1745	A, C, S
11-Feb-07	9 dafm	0830-1000	A
12-Feb-07	10 dafm	0845-1015	A
21-Feb-07	10 dbfm	1430-1600	A, C, S
23-Feb-07	8 dbfm	0945-1115	A
24-Feb-07	7 dbfm	1015-1115	A
		1415-1530	A, C

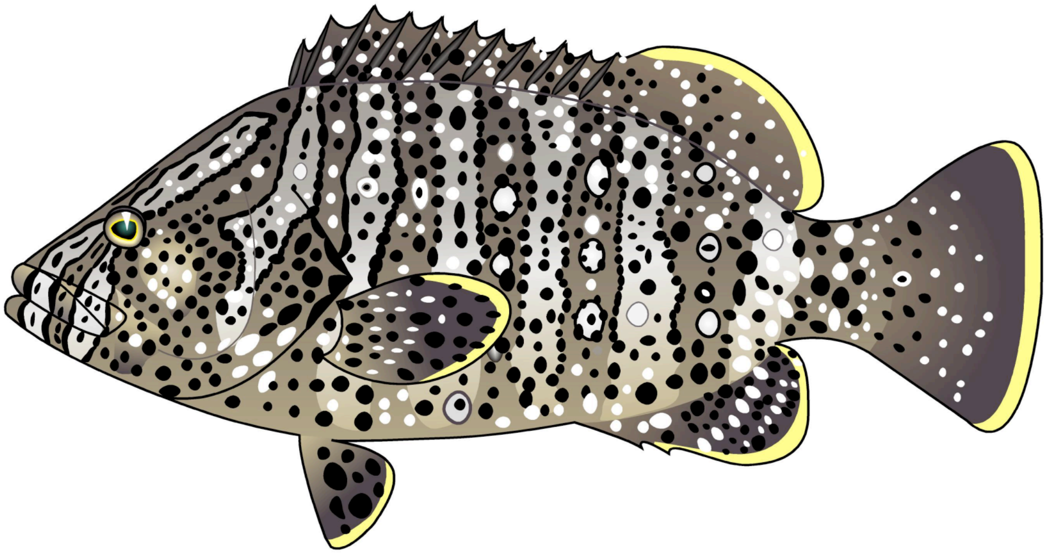

Fig. 2.1. The Leather bass, *Dermatolepis dermatolepis*.

Fig. 2.2. Spawning aggregation of the Leather Bass, *Dermatolepis dermatolepis*, at Cocos Island, Costa Rica.

Fig. 2.3. Spawning rush of Leather Bass, *Dermatolepis dermatolepis*, at Cocos Island, Costa Rica.

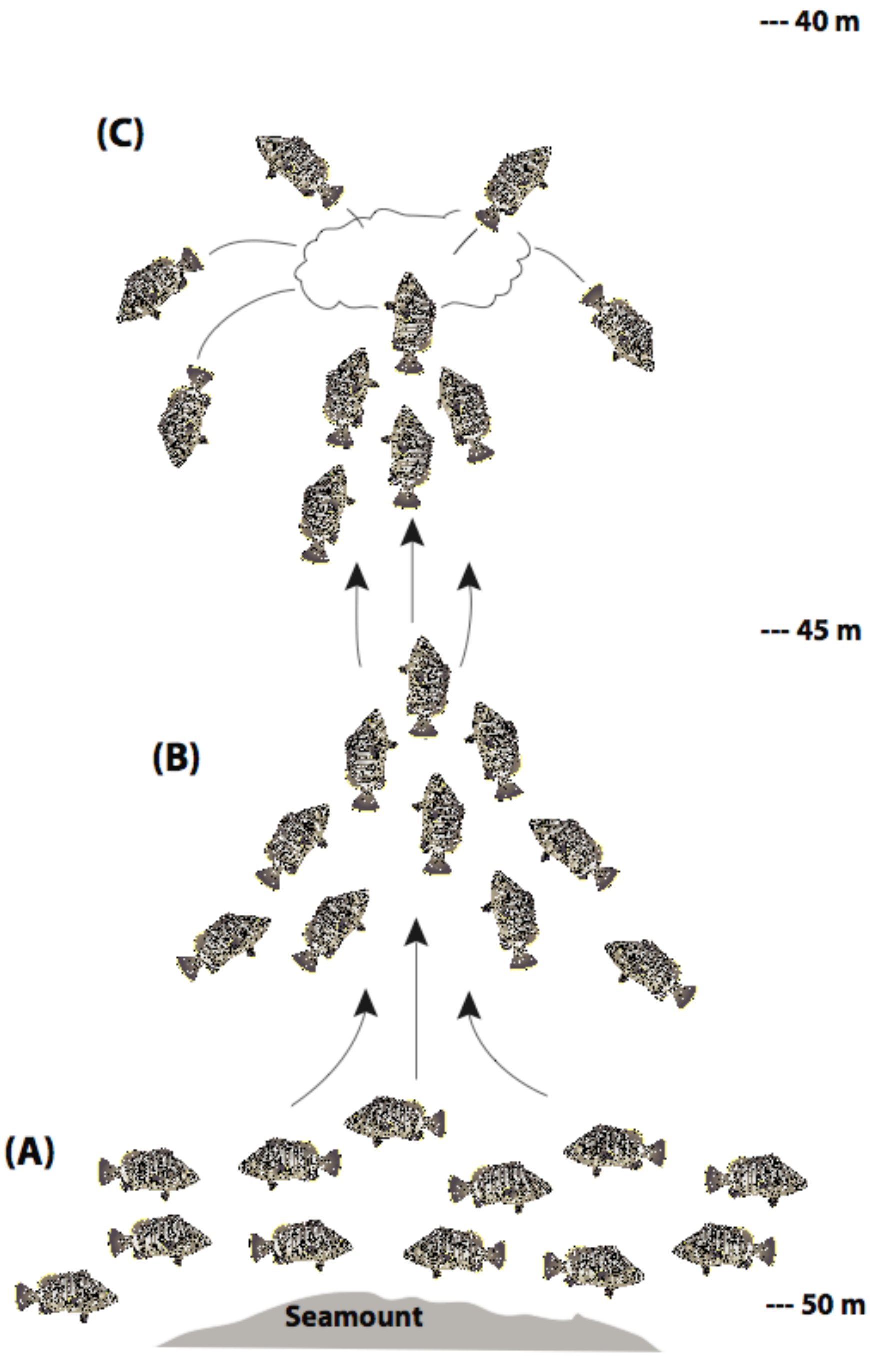

Fig. 2.4. Ethogram of a spawning event of Leather Bass, *Dermatolepis dermatolepis*, at Cocos Island, Costa Rica. (A) The aggregation forms above the top of the seamount, (B) a subgroup of several individuals separate from the main aggregation and swim into open water, (C) the subgroup performs a vertical spawning rush, release their gametes and separate, and fish return to the main aggregation.

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CHAPTER 3

Evidence of gonochorism in a grouper, *Mycteroperca rosacea*, from the Gulf of California, Mexico

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Evidence of gonochorism in a grouper, *Mycteroperca rosacea*, from the Gulf of California, Mexico

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Abstract The sexual pattern and sexual development of the leopard grouper, *Mycteroperca rosacea*, were investigated from 483 specimens collected from the Gulf of California, Mexico. Histological and population data indicated a gonochoric sexual pattern. Some juveniles passed through an immature bisexual phase of gonadal development, but no evidence of post-maturational sex change was found. The immature bisexual phase is believed to be associated only with male development. The size distribution and size at sexual maturity were similar for both males and females. In accordance with predictions of the size-advantage model, the gonochoric sexual pattern of *M. rosacea* is likely influenced by its group-spawning mating system.

Keywords *Mycteroperca rosacea*; Groupers · Sexual pattern · Gonochorism

Introduction

Hermaphroditism is common among teleost fishes and protogyny, the sequential change of sex from female to male, is the predominant sexual pattern among the epinepheline serranid fishes (groupers). Studies by van Oordt (1933), Reinboth (1962, 1968), Smith (1959, 1965), and others characterized these fishes as monandric protogynous hermaphrodites, where all males were believed to develop from sex-changed females. However, more recent investigations have shown that sexuality among the groupers is more complex and diverse. Several species are diandric; males develop directly from juveniles (primary males) as well as from females that change sex (secondary males) (Siau 1994; Ferreira 1995; Chan and Sadovy 2002; Fennessy and Sadovy 2002). Male to female (protandry) and bi-directional sex change have not been reported in wild populations but have been experimentally induced in a few species (Tanaka et al. 1990; Liu and Sadovy 2004a). Only one species of grouper, *Epinephelus striatus*, has been diagnosed as functionally gonochoric, although sex change may occur under certain conditions (Sadovy and Colin 1995).

Information on the sexual pattern of groupers is necessary for proper management of their fisheries, because protogynous fishes are more sensitive to certain size-selective fishing patterns and differ significantly in their response to most management plans compared to gonochoric fishes (Huntsman and

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Schaaf 1994; Alonzo and Mangel 2004; Alonzo and Mangel 2005; Heppell et al. 2006; Young et al. 2006). For protogynous fishes, policies that favor the taking of small individuals target females, while those that favor the taking of large individuals target males (Shapiro 1987; Sadovy 1996; Levin and Grimes 2002). The selective removal of individuals from either sex may disrupt operational sex ratios or alter important processes related to spawning, which could result in significant decreases in reproduction and population size (Russ 1991; Coleman et al. 1996; Vincent and Sadovy 1998; Armsworth 2001; Rowe and Hutchings 2003: Alonzo and Mangel 2004; Alonzo and Mangel 2005).

Determination of sexual pattern among species is also important for understanding the evolution of reproductive strategies in groupers. Systematists have long debated the usefulness of both sexual pattern and gonad morphology in resolving phylogenetic relationships in serranid fishes (Smith 1965; Gosline 1966; Smith and Atz 1969; Bortone 1977; Baldwin and Johnson 1993). A recent phylogeny by Smith and Craig (2007) concluded that the Serranidae is polyphyletic, with groupers (Epinephelinae) distantly separated from the Anthiinae and Serraninae. In addition, Craig and Hastings (2007) presented the first comprehensive molecular phylogeny of the Epinephelinae, providing a framework to compare life history patterns and examine the reproductive evolution ^fishes (Erisman et al. 2007).

The leopard grouper, *Mycteroperca rosacea*, occurs on shallow rocky-reef habitats from the southwest coast of Baja California to southern Jalisco, Mexico, and is abundant throughout the Gulf of California (Thomson et al. 2000). Leopard groupers are slow-growing, long-lived fishes that reach 1 m TL and 21 years in age (Díaz-Uribe et al. 2001). Adults form spawning aggregations of hundreds of individuals from March to June in the Gulf of California, with spawning occurring earlier in southern locations (Sala et al. 2003; Erisman et al. 2007). Spawning takes place in the evening within groups of 6–40 individuals, and reproductive activity is not correlated with the lunar cycle (Erisman et al. 2007).

Leopard groupers are the most heavily targeted grouper by commercial, artisanal, and recreational fisheries in the Gulf of California (Ramírez and Rodriguez 1990; Sala et al. 2003). Due to increases in fishing pressure and significant declines in fisheries landings and population abundances over the past few decades (Sala et al. 2004), the World Conservation Union (IUCN)[1] currently lists *M. rosacea* as 'Threatened'. Attempts at improving management of leopard grouper fisheries have suffered from a lack of information on their reproductive biology, including details of their sexual development and sexual pattern. The purpose of this study was to determine the sexual pattern and possible modes of sexual development of *M. rosacea* using information on their gonad morphology, population structure, and mating system.

Materials and methods

The study was conducted at two sites (Bahia de Los Angeles and Loreto) in the Gulf of California from June 1998 to June 2005. Data were gathered on 462 fish collected by hook and line, speargun, or from recreational fishers, and 21 specimens from the Marine Vertebrate Collection at the Scripps Institution of Oceanography (SIO) in La Jolla, CA, USA (Table 1). Standard length (to the nearest mm) was recorded for each fish, and gonads were removed from the body cavity and preserved in a 10% formalin–seawater solution.

Sex and reproductive classification were assigned mainly from microscopic evaluations of gonads and were based on the relative proportions of the most developed stages of germ cells. The size and shape of gonads assisted in the distinction between sexually mature and immature individuals. Classes of ovarian and testicular development were adapted from Sadovy and Colin (1995) and Fennessy and Sadovy (2002), and stages of gametogenesis followed the definitions of Moe (1969) and Wallace and Selman (1981).

Standard histological techniques were used for microscopic evaluations. Pieces of gonad tissue were embedded in paraffin, sectioned at 6 μm, mounted, stained with Mayer's haemotoxylin–eosin, and observed under a compound microscope (Humason 1972). Tissue was taken from the central, posterior, and anterior portions of one lobe and sectioned

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[1] (www.iucnredlist.org)

Table 1 Summary of *M. rosacea* specimens, organized by collection month and gonadal development class (F1 = immature female; F2 = mature, inactive female; F3 = mature, active or ripe female; F4 = mature, post-spawning female; B = immature bisexual; M1 = immature male; M2 = mature, inactive male; M3 = mature, active or ripe male; M4 = mature, post spawning male

Month	F1	F2	F3	F4	B	M1	M2	M3	M4	Total
Feb	4	6	0	0	0	0	4	0	0	14
Mar	2	17	6	0	0	1	17	7	0	50
Apr	5	1	21	1	1	0	0	24	0	53
May	3	2	66	8	0	1	0	60	16	156
Jun	3	1	19	2	0	0	3	24	6	58
Jul	4	23	10	8	2	3	2	16	2	70
Aug	9	2	0	0	1	0	0	0	0	12
Sep	15	19	0	0	2	2	10	0	1	49
Oct	6	8	0	0	1	2	4	0	0	21
Total	51	79	122	19	7	9	40	131	25	483

transversely in larger gonads, while smaller gonads were sectioned longitudinally in their entirety.

Diagnosis of sexual pattern was based primarily on histological evidence and followed the criteria outlined by Sadovy and Shapiro (1987), later refined for serranid fishes by Sadovy and Domeier (2005). The identification of fish undergoing sexual transition from female to male (i.e. transitionals) indicates protogyny, whereas the lack of such evidence suggests gonochorism. Transitionals are identified by the presence of degenerating vitellogenic stage oocytes or atretic follicles together with spermatogenic tissue (Sadovy and Shapiro 1987). The concomitant presence of thickened muscle bundles along with atretic oocytes (either previtellogenic or vitellogenic) may indicate prior female function (Shapiro et al. 1993) and, when present together with spermatogenic tissue, is evidence of sex change in some groupers (Fennessy and Sadovy 2002; Rhodes and Sadovy 2002). In order to validate the use of muscles bundles as an indication of prior spawning as a female, we tracked the monthly occurrence of this feature in mature, inactive female *M. rosacea.*

Size-frequency distributions by sex and details of the mating system were used as additional evidence of the sexual pattern (Warner 1984; Warner 1988; Sadovy and Shapiro 1987; Sadovy and Colin 1995). Other types of morphological evidence of sex change used in studies on wrasses and other hermaphroditic fishes (atretic bodies, peripheral sperm sinuses, ovarian lumen, female-biased sex ratios) were not used in this study due to their unreliability in diagnosing the sexuality of serranids (reviewed by Liu and Sadovy 2004a; Sadovy and Domeier 2005).

For both sexes, the size at sexual maturation was estimated from the minimum size of collected individuals classified as mature and from the smallest size class, grouped by 20 mm classes, that 50% of individuals were mature. A logistic curve was fitted to the length at maturity data to determine size at 50% maturity. Pathways of sexual development were based on the size ranges of gonadal development classes.

Results

General gonad morphology was similar to that described for other epinepheline serranids (Smith 1965; Moe 1969; Sadovy and Colin 1995; Fennessy and Sadovy 2002). The gonads were bilobed, elongated, and often unequal in size. The lobes were separated anteriorly, fused posteriorly near the urogenital opening, and connected to the dorsal surface of the body cavity by mesenteries. Macroscopically, male and female gonads were indistinguishable unless ripe, because both were rounded in transverse section and were similar in color. However, the testes of ripe males were triangular in transverse section, whitish in appearance, and produced milt when pressure was applied. Ripe females had enlarged, yellowish, rounded ovaries with visible oocytes. All gonads contained a central lumen surrounded by lamellae that projected medially from the gonadal walls.

Gonadal development classes

Immature female (n = 51, Fig. 1a)

Ovaries were small and compact, and the gonadal wall was thin. Lamellae contained only oogonia and closely packed primary growth stage (chromatin nucleolar, perinucleolar) oocytes.

Mature, inactive female (n = 79, Fig. 1b)

The transverse diameter and length of ovaries were larger compared to immature females. Primary growth and cortical alveolar oocytes were present. Evidence of prior spawning activity was apparent from the presence of muscle bundles, a thickened gonadal wall, or vitellogenic oocytes in late stages of atresia. Muscle bundles were a reliable indicator of previous spawning as a female, because they were common in female fish collected shortly after the spawning season (July–September) and steadily decreased in their incidence over subsequent months.

Mature, active or ripe female (n = 122, Fig. 1c)

Ovaries were enlarged and all oocyte stages were present, but they were dominated by primary growth, cortical alveolar, and vitellogenic oocyte stages. Hydrated oocytes, post-ovulatory follicles, and atretic vitellogenic oocytes were common in ripe females.

Mature, post-spawning female (n = 19, Fig. 1d)

Ovaries were dominated by vitellogenic oocytes in early and advanced stages of atresia. Primary growth oocytes were common and both cortical alveolar oocytes and late stage post-ovulatory follicles were occasionally present. The gonadal wall was thickened, and the gonad had a disrupted appearance with intralamellar debris and cytoplasmic strands.

Immature bisexual (n = 7, Fig. 2a)

Gonads were small and compact, and the gonadal wall was thin. Gonadal lamellae consisted mostly of primary growth oocytes and scattered areas of undeveloped spermatogenic tissue composed of gonia and cysts of early stage spermatocytes. Ovarian and testicular tissues were not physically separated, as spermatogenic tissue was present throughout the lamellae and intermixed with oocytes. These gonads showed no morphological evidence of prior sexual maturation as a male or female.

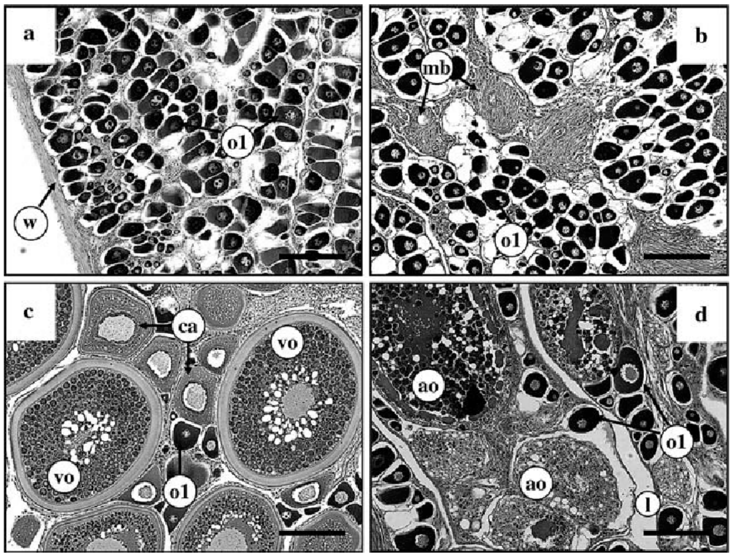

Fig. 1 Histological sections of immature and mature female *M. rosacea* gonads. (**a**) Immature female, 192 mm SL; (**b**) mature, inactive female, 402 mm SL; (**c**) mature, active female, 470 mm SL; (**d**) mature, post-spawning female, 495 mm SL. Scale bar = 200 µm. ao, atretic vitellogenic oocyte; ca, cortical alveoli oocyte; mb, muscle bundle; o1, primary growth oocyte; vo, vitellogenic oocyte; w, gonad wall

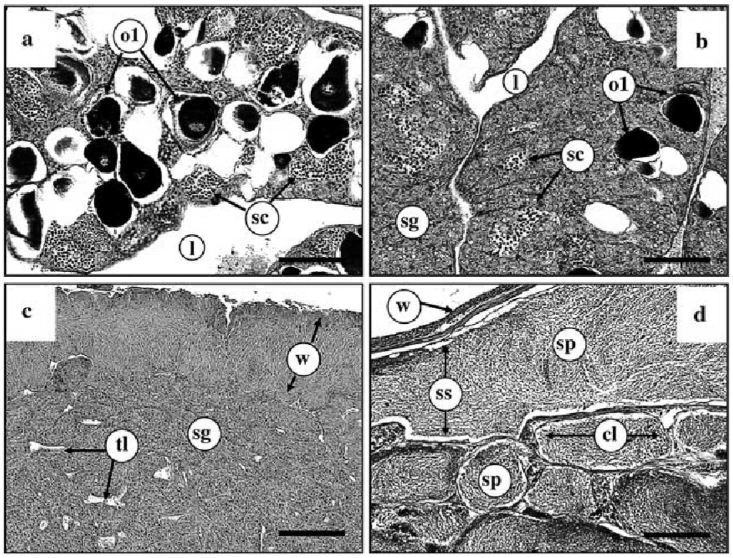

Fig. 2 Histological sections of immature bisexual, immature male and mature male *M. rosacea* gonads. (**a**) Immature bisexual, 200 mm SL; (**b**) immature male, 235 mm SL; (**c**) mature, inactive male, 481 mm SL; (**d**) mature, ripe male, 560 mm SL; (**e**) mature, post-spawning male, 520 mm SL. Scale bar = 200 μm. cl, coalesced lobule; l, gonadal lumen; o1, primary growth oocyte; rs, residual sperm; sc, cyst of spermatocytes; sg, spermatogonia; sp, spermatozoa; ss, sperm sinus; tl, testicular lobule, w, gonad wall

Immature male (n = 9, Fig. 2b)

Testes were small, compact, and translucent, and were dominated by cysts of spermatogonia and small, empty lobules. Scattered cysts of early stage spermatocytes were sometimes present. Primary growth stage oocytes were present in small numbers and were scattered amongst spermatogenic tissue in 6 of 9 immature males. Immature males could be distinguished from immature bisexuals by the relative amounts of female tissue found in histological sections of the gonads. Primary growth oocytes were few or absent in immature males but encompassed most of the gonadal tissue among inactive bisexuals.

Mature, inactive male (n = 40, Fig. 2c)

Transverse diameter and length of testes were large compared to immature males. Testes were dominated by large areas of spermatogonia, scattered cysts of spermatocytes, and small, empty lobules. Sperm sinuses were present but constricted. Small amounts of spermatozoa were sometimes present in sperm sinuses or scattered within lobules.

Mature, active or ripe male (n = 131, Fig. 2d)

Testes were enlarged and dominated by later stages of spermatogenesis. Spermatozoa were present in large amounts within the lobules and sperm sinuses, and early stage spermatocytes were relatively less abundant. Testes of ripe males contained lobules that were enlarged, coalesced, and full of sperm.

Mature, post-spawning male (n = 25, Fig. 2e)

Testes had a thickened gonadal wall and consisted mostly of lobules and sperm sinuses that were partially filled with sperm. Lobules were well separated and smaller compared to a mature, active or ripe male. Stromal tissue and spermatogonia were also present throughout the lamellae.

Size-frequency distributions; size at sexual maturity

Immature females occupied the smallest size classes (range = 90–315 mm SL), followed by immature bisexuals (range = 165–254 mm SL) and immature males (range = 202–310 mm SL) (Fig. 3). The mean size of immature females (206.5 mm SL) was significantly smaller than that of immature males (253.6 mm SL; 2-sample t-test, $t = -3.475$, $p = 0.002$). The mean size of immature bisexual individuals (209.0 mm SL) was significantly smaller than immature males (2-sample t-test, $t = -2.729$, $p = 0.017$), mature females (2-sample t-test, $t = -15.779$, $p < 0.001$) and mature males (2-sample t-test, $t = -16.678$, $p < 0.001$). Size distributions of

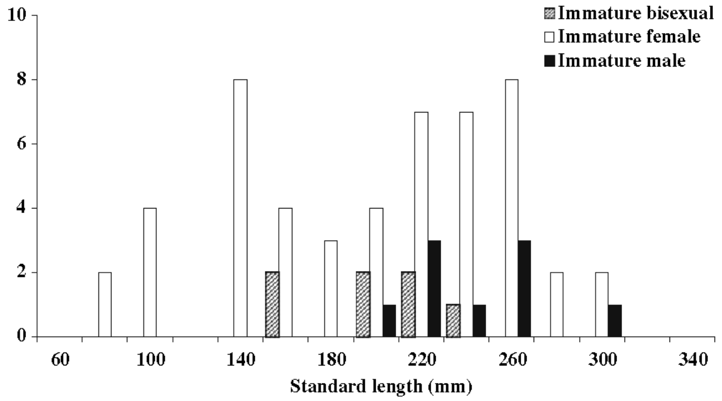

Fig. 3 Size-frequency distributions of immature female ($n = 51$), immature male ($n = 9$), and immature bisexual ($n = 7$) *M. rosacea*

mature females (215–744 mm SL) and mature males (223–708 mm SL) were similar (Fig. 4), and the mean sizes of mature females (432.7 mm SL) and mature males (446.1 mm SL) were not significantly different (2-sample t-test, $t = -1.586$, $p = 0.137$). The minimum size of maturity was 215 mm SL for females and 223 mm SL for males. The size at 50% maturity was 270 mm SL for females and 230 mm SL for males (Fig. 5). All females and males were mature by 340 mm SL.

Discussion

Sexual pattern

No histological evidence of adult sex change was found, suggesting that *M. rosacea* follows a gonochoric sexual pattern. The identification of transitional individuals is often difficult, because the timing of sex change in protogynous groupers happens at different times of the year depending on the species (Shapiro 1987; Ferreira 1995). It often occurs soon after the end of the spawning season (Smith 1965; Siau 1994; Chan and Sadovy 2002). The size at which sex change occurs also varies among species and locations (Shapiro 1987; Sadovy 1996). Finally, the proportion of transitionals collected during studies of sexual patterns on groupers tends to be low (i.e. <5%), despite large sample sizes (Smith 1965; Siau 1994; Mackie 2000; Fennessy and Sadovy 2002). Therefore, determination of the sexual pattern of groupers requires examinations of many individuals from all size classes of juveniles and adults that are collected at different times of the year. For this study, we examined many

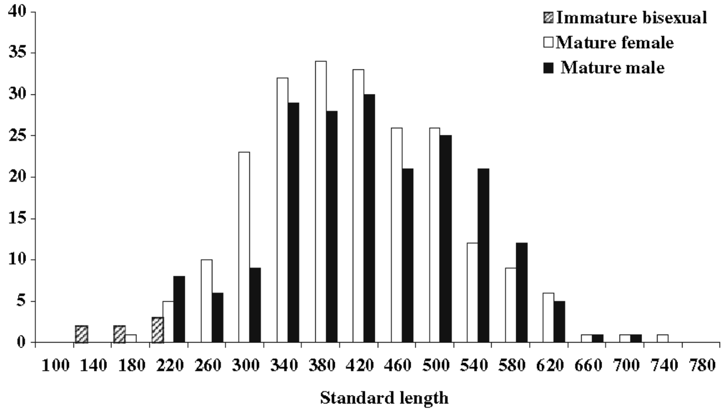

Fig. 4 Size-frequency distributions of immature bisexual ($n = 7$), mature female ($n = 220$), and mature male ($n = 196$) *M. rosacea*

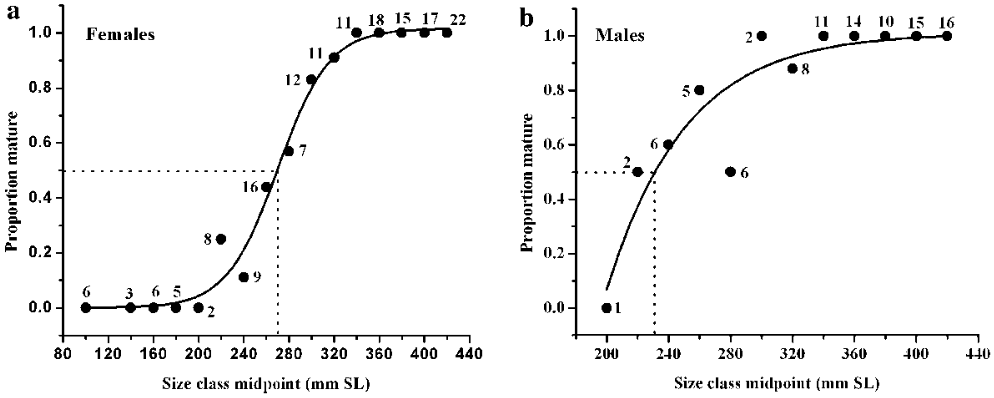

Fig. 5 Proportion of mature female (**a**) and male (**b**) *M. rosacea* by 20 mm SL size classes. Numbers beside circles indicate sample sizes. Dotted line indicates length at 50% maturity. Also shown is a logistic curve fitted to the data

juveniles and adults during both reproductive and non-reproductive periods of the year and found no indication of adult sex change.

A small number of gonads contained both male and female tissues but showed no definitive evidence of prior function as either sex. Specifically, they did not contain a thickened gonadal membrane, muscle bundles, or germ cells in advanced stages of gametogenesis. Also, these gonads were found in fishes that were smaller than the smallest mature females and mature males. As a result, they were classified as immature bisexuals. A juvenile (non-functional) bisexual phase of development occurs in both gonochoric and hermaphroditic fishes, including many species of groupers, and is therefore not indicative of sex change (D'Ancona 1949; Reinboth 1962; Atz 1964; Yamamoto 1969). For example, both males and females develop from the bisexual juvenile phase in the gonochoric grouper, *Epinephelus striatus* (Sadovy and Colin 1995) and the diandric grouper, *Cephalopholis boenak* (Chan and Sadovy 2002; Liu and Sadovy 2004b).

The overlapping size distributions and similar mean sizes of adult males and adult females support a gonochoric sexual pattern in *M. rosacea*. Because males develop from sex-changed females, size-distributions of protogynous fishes are often bimodal, with females occupying smaller size classes and males occupying larger size classes (Warner 1975a; Moyer and Nakazono 1978; Sadovy and Shapiro 1987). Conversely, the size distributions of females and males overlap broadly in most gonochoric fishes. On its own, population structure is not a reliable indicator of sexual pattern, because bimodal size and age distributions are found in gonochoric species that exhibit size dimorphism (Clarke 1983; Sadovy 1986) or differential rates of growth, maturation, or mortality by sex (Clarke 1970; Sadovy and Shapiro 1987). Also, bimodality in protogynous populations can be obscured by the inclusion of juveniles (i.e. immature females and immature bisexuals) and primary males (for diandric species) in size and age distributions (Sadovy and Shapiro 1987; Fennessy and Sadovy 2002). For this study, only adults were used to compare the size distributions by sex, and the complete overlap in the size ranges of male and female *M. rosacea* were consistent with the gonochoric sexual pattern indicated by the histological data.

Like many other marine fishes, sex change in groupers is influenced by social factors (Mackie 2003; Liu and Sadovy 2004a), and the size advantage model (Ghiselin 1969; Warner1975b; Muñoz and Warner 2003) provides the most widely accepted evolutionary explanation for the adaptive significance of sex change in animals. The model predicts that protogyny should occur in species or populations where large males are able to monopolize matings with females and exclude smaller males (Warner 1975b; Warner 1984). The protogynous sexual patterns of most groupers fit these predictions, as they are characterized by polygynous, haremic mating systems, where large males defend discrete territories and pair-spawn with females (Goeden 1978; Colin et al. 1987; Gilmore and Jones 1992; Donaldson 1995; Mackie 2000).

As the number of males in a population increases, the reproductive success of larger, territorial males should decrease and selection for sex change should decrease as well (Warner and Hoffman 1980; Warner 1984). Gonochorism should occur in fishes with group-spawning mating systems, where mate or spawning site monopolization by large males does not occur, all males have access to females, and sperm competition is intense (Warner 1982; Muñoz and Warner 2003; Erisman and Allen 2006). In accordance with these predictions, the only previously known gonochoric grouper, *E. striatus*, spawns in groups of 3–25 fish and is non-territorial (Colin 1992; Sadovy and Colin 1995).

The mating system of *M. rosacea* is congruent with the histological and population data in support of gonochorism and fits expectations of the size advantage model. Adults spawn in large groups, and pair-spawning has not been observed (Erisman et al. 2007). The high density of and intense sperm competition among males competing for access to females offers little opportunity for large males to monopolize matings with females, and aggressive interactions among males during courtship are rare (i.e. males are non-territorial). Since all males have access to females, large males do not accrue disproportionately high mating success in comparison to smaller males, and sex change is therefore not adaptive.

Pathways of sexual development (Fig. 6)

The size distributions of gonadal classes provided evidence of the sexual developmental pathways of *M. rosacea*. Immature females occupied the smallest size classes and were smaller than immature bisexuals and immature males, indicating that gonadal development in juveniles begins with an ovarian phase. This finding is not surprising, since the gonadal development of many fishes, regardless of their sexual pattern, involves passage through an early ovarian phase prior to definitive differentiation as female or male (Yamamoto 1969; Takahashi and Shimizu 1983; Shapiro 1992: Liu and Sadovy 2004a).

At larger sizes, some individuals of *M. rosacea* pass through an immature bisexual phase. Immature bisexuals were smaller than immature males, and scattered primary-growth oocytes were found in most immature males. This suggests that all males develop from the bisexual juvenile phase. It is not certain if all juvenile leopard groupers pass through an immature bisexual phase prior to differentiation as a male or female, or if the phase is only associated with the male developmental pathway; both scenarios have been reported in groupers (Sadovy and Colin 1995; Fennessy and Sadovy 2002; Liu and Sadovy 2004b). Immature females may pass through an immature bisexual phase, re-develop as immature females at larger body size, and eventually mature as adult females. However, the presence of immature females in all size classes of juveniles and the absence of residual male tissue in large immature females and mature females suggests that some, or possibly all,

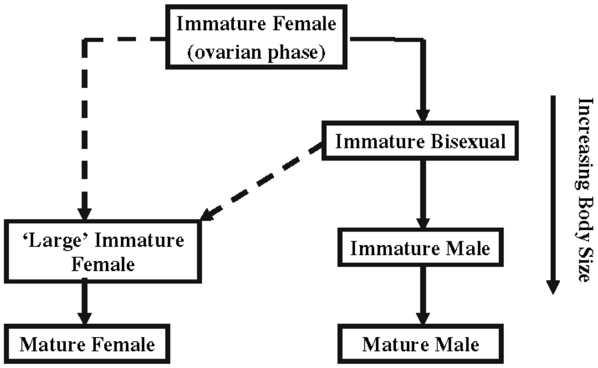

Fig. 6 Proposed pathways of sexual development in *M. rosacea*. Solid arrows represent known developmental pathways, and dashed arrows represent uncertainties in juvenile development

mature females develop directly from immature females and do not pass through a bisexual phase.

Conclusions

This study shows that leopard groupers are functional gonochores. Although some individuals pass through a bisexual juvenile phase prior to sexual maturation, adult sex change does not occur. Prior to this study, gonochorism had been reported only in one other grouper (*E. striatus*; Sadovy and Colin 1995). It is possible that gonochorism occurs in other groupers, and the lack of identification of this sexual pattern is in part due to a poor understanding of their complex sexual developmental pathways. Confusion over the difference between immature bisexuals and transitionals has led to the misdiagnoses of some species as hermaphroditic. Moreover, many studies have contributed to the belief that all groupers are hermaphrodites (see Heemstra and Randall 1993 and references found therein), and only recently have we begun to realize that grouper sexuality is considerably more varied and complex. Re-examination of groupers previously diagnosed as protogynous hermphrodites and future investigations on unstudied species will likely reveal that gonochorism is more common among the epinepheline serranids.

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Chapter III has been published in Environmental Biology of Fishes (2008) 82: 23—33, as a paper under the same title, “Evidence of gonochorism in a grouper, *Mycteroperca rosacea*, from the Gulf of California, Mexico”. I was the lead author and Jorge Rosales-Casián and Philip Hastings were co-authors of this manuscript.

CHAPTER 4

Reproductive biology of three small groupers (Teleostei: Epinephelidae) from Mexico

Introduction

The groupers (Teleostei: Epinephelidae: Epinephelinae) comprise about 160 species of carnivorous fishes that occur in all subtropical and tropical seas and hold great economic and ecological importance. Larger species are important to both commercial and recreational fisheries, accounting for the major portion of multi-million dollar live-fish markets in Asia and constituting the most valuable commercial fishes in fresh-fish markets throughout the tropics (Cesar et al. 2000; Morris et al. 2000; Sadovy 2007). In addition, smaller species and juveniles of larger species are highly prized by the aquarium trade (Heemstra and Randall 1993; Morris et al. 2000). Most species occupy the upper trophic levels of their local ecosystems and thus play a significant role in organizing community structure. As top predators in marine ecosystems, they regulate the abundance and variability of lower trophic levels organisms (top-down control), increase biodiversity and community stability, and may buffer against the ecological effects of climate change (Frank et al. 2005; Halpern et al. 2006; Sala 2006).

As with most other predatory fishes, grouper populations have been depleted by the overfishing of adult stocks, destruction of both juvenile and adult habitats, ineffective management plans for their fisheries, or the lack of any management policies at all (Coleman et al. 2000; Levin and Grimes 2002; Myers and Worm 2003). A recent analysis conducted by the International Union for the Conservation of Nature (IUCN) concluded that 20% of groupers are threatened by extinction and another 19% are categorized as “nearly threatened”, and these numbers are expected to rise due to increasing demands by consumers and unregulated harvests of stocks by commercial and recreational fisheries (Sadovy 2007).

At least 13 species of grouper occur along the Pacific coast of Mexico, and commercial, recreational, or subsistence fisheries target all of them (Heemstra and Randall 1993; Aburto et al. 2008). Increased fishing effort over the past three decades is correlated with significant declines in stock sizes and fishery landings of groupers in several regions (Sala et al. 2004; Saenz-Arroyo et al. 2005; Aburto et al. 2008), and current efforts to conserve remaining populations and create sustainable fisheries are hindered by an absence of biological information for most species (Aburto et al. 2008). Data on life-history parameters such as size and age of sexual maturation, annual growth rates, average lifespan, and spawning seasonality are sparse but are necessary to determine the vulnerability of species to exploitation and to create even the simplest management regulations such as minimum size restrictions or daily catch limits (Jennings et al. 1998; Young et al. 2006).

Understanding factors related to reproduction and growth are particularly important for the management and conservation of groupers. Due to slow growth rates, restricted spawning periods, sporadic annual recruitment patterns, and relatively great age and size at sexual maturity, many grouper species cannot sustain high levels of fishing pressure and decline rapidly when they are harvested commercially (Jennings et al. 1998; Coleman et al. 2000; Sadovy 2001; Levin and Grimes 2002). Many are protogynous hermaphrodites (i.e., change sex from female to male), and fishes that follow this reproductive strategy are more sensitive to size-selective fishing strategies than non-hermaphroditic fishes (Alonzo and Mangel 2004, 2005; Young et al. 2006). Most fisheries remove the largest individuals of a species, and heavy fishing of protogynous species often leads to a marked decline in the abundance of males and large, highly-fecund females in a population (Coleman et al. 1996; Beets and Friedlander 1999). Such size-selective mortality may drastically alter the population structure and reduce reproductive output through decreases in sperm production, fecundity, lifespan, mate encounter rates, size or age at first maturity, and the timing of sex change (Vincent and Sadovy 1998; Platten 2002; Hamilton et al. 2007).

For the present study, we investigated the reproductive biology of three species of small groupers from Mexico: *Alphestes immaculatus, Cephalopholis panamensis, and Epinephelus labriformis*. Our specific goals were to: (1) determine the sexual pattern of each species, (2) estimate the size and age of sexual maturity and sex change (if hermaphroditic), (3) determine population size and age structure, and (4) collect information on social structure and mating behavior. These three species were chosen for two reasons. First, they are abundant mesocarnivorous predators on rocky and coral reefs throughout the Tropical Eastern Pacific (TEP) and thus have significant ecological importance to the region. Second, their economic value in commercial fisheries in Mexico has increased in recent years due to population declines of larger grouper species from overfishing (Sala et al. 2004: Aburto et al. 2008).

Materials and Methods

Collections of specimens

Fish were collected from three locations (Loreto, Mulege, Puerto Vallarta) in Mexico from September 2001 to October 2007 (Table 1). Most were captured by hook and line or by pole-spear, but 47 specimens of *Alphestes immaculatus* were obtained from the Marine Vertebrates Collection at the Scripps Institution of Oceanography in La Jolla, California, USA. Standard length (to the nearest mm) and body weight (to the nearest g) were recorded for each fish. Gonads were removed, weighed to the nearest 0.1 g, and preserved in a 10% formalin-seawater solution.

Age determination

For age analyses, sagittal otoliths from 128 *Alphestes immaculatus* and 107 *Cephalopholis panamensis* were removed, and the right one was mounted on a small wood block with cyanoacrylate. Otoliths were cut with Buehler low speed isomet saw equipped with a diamond wafer blade (series 15 hc diamond) to obtain a 0.8 mm transverse section through the focus of the structure. Sections were then polished with 3M lapping film (360-A), cleaned, submerged in water and observed against a black background by reflected light under a dissecting microscope. Methods of age determination followed those by Craig et al. (1999) for *C. panamensis* and *E. labriformis*. Two different observers recorded the number of alternating opaque and translucent zones for each section. Each observer performed two counts of each section at different times, and if these counts differed, a third count was made. The counts of the two observers were then compared, and any that differed by more than one zone were removed from further analysis.

Reproductive development and population structure

Sex and reproductive classification were assigned from microscopic evaluations of gonads and were based primarily on the presence and relative abundance of different germ-cell stages (Table 2). The size and shape of gonads assisted in the distinction between sexually mature and immature individuals, and the presence of thickened muscle bundles in ovaries was used to distinguish mature females that were reproductively dormant (F2) from immature females (F1) that had never spawned (Shapiro et al. 1993; Erisman et al. 2007). Classes of ovarian and testicular development were adapted from Mackie (2000) and Erisman et al. (2007), and stages of gametogenesis followed the definitions of Moe (1969) and Wallace and Selman (1981).

For histological examinations, pieces of gonads were embedded in paraffin, sectioned at 6 µm, mounted on slides, stained with Mayer's haemotoxylin-eosin, and observed under a compound microscope (Humason 1972). Tissue was taken from the central, posterior, and anterior portions of one lobe and sectioned transversely in larger gonads, but smaller gonads were sectioned longitudinally in their entirety.

Diagnosis of sexual pattern was based primarily on histological evidence and followed criteria outlined for fishes in general by Sadovy and Shapiro (1987) and for serranid fishes in particular by Sadovy and Liu (2008). Fish undergoing sexual transition from female to male (i.e., transitionals) were identified by the presence of degenerating, vitellogenic oocytes or atretic follicles coincident with proliferating spermatogenic tissue (Table 2). Size and age-frequency distributions by sex, population sex ratios, and aspects of social or mating systems were used as additional evidence in diagnosing sexual pattern (Sadovy and Shapiro 1987; Warner 1984; Erisman et al. 2007).

For both sexes, the size at sexual maturity was estimated from the minimum size of collected individuals classified as mature and from the smallest size class, grouped by 10 mm classes, in which 50% of individuals were mature. Pathways of sexual development were based on the relative size ranges of gonadal development classes. For sex-changing species, the size at sex change was estimated from the size ranges of transitional fish and the size range in which females overlapped with males (Shapiro1984). Variations in size at sex change were also estimated from two ratios defined by Shapiro (1987): (1) size range of transitional fish divided by maximum size of fish in samples, and (2) range of overlap in size of males and females divided by maximum size of fish in samples.

Differences in mean length of maturity classes (i.e., mature males vs. mature females) of *E. labriformis* were analyzed by a one-way analysis of variance (ANOVA), and pair-wise comparisons (post-hoc) of mean length were made by a Tukey-Kramer Multiple Comparisons Test. Length and age data for *A. immaculatus* and *C. panamensis* did not conform to the assumptions of parametric statistics.

Therefore, differences in median length and age among maturity classes for *A. immaculatus* and *C. panamensis* were analyzed by a Kruskal-Wallis Test, and pair-wise comparisons of median length and age were made by a Dunn's Multiple Comparisons Test.

Estimates of gamete production and sperm competition

Gonads from 112 *A. immaculatus* (67 females, 45 males) and 101 *C. panamensis* (67 females, 34 males) were weighed to the nearest 0.1g prior to preservation, and gonosomatic indices [GSI = (gonad weight/body weight) x 100] were calculated for these fish. All gonads used for these analyses were verified histologically as reproductively active (F4 or M4). The relationship between gonad weight, GSI, and standard length was analyzed by linear correlation in order to discern patterns of gamete production in adults and sperm competition in males. In broadcast spawning fishes, GSI is indicative of adult investment in gamete production and is used to infer the intensity of sperm competition in males (Warner 1997; Petersen and Warner 1998).

Courtship and social behavior

We performed 94 focal-animal observations of adult *C. panamensis* in Loreto from July to August 2004 and from August to September 2006 in order to: (1) compare intraspecific and interspecific behavioral patterns by sex and time of day, and (2) describe courtship behaviors. Observations were made throughout the day (0600 –

2100 h) by SCUBA or while snorkeling, and data were recorded with pencil on plastic slates or by an underwater digital video camera. Dives ranged from 30 to 120 min and 3 to 20 m in depth. Individuals were chosen at random and were followed for 10 min periods. Observations of individuals were terminated before the 10 min limit if we lost sight of an individual for more than 10 s, and these data were then standardized to 10 min periods.

The sex of individuals involved in intraspecific interactions could be determined by color pattern, as determined during collections of individuals (see Results) while SCUBA diving in Loreto in 2003. Identification of specific behaviors for *C. panamensis* followed those described for other species of *Cephalopholis* (Donaldson 1989; Shpigel and Fishelson 1989; Donaldson1995a,b). The timing and duration of behavior bouts were recorded for all observations, the sex of participants was recorded for intraspecific interactions, and participating species were recorded for interspecific interactions. The proportion of time spent for each behavior type and the frequency of behavior types was standardized to 10-min periods. Observations were conducted throughout the day (i.e., 0700 – 2100 h) but were grouped into two time periods (before and after 1600 hrs; see Donaldson 1995b).

Data on social structures of all three species were collected in Loreto and Mulege during the months of April to November from 2004 to 2007. Observations were made with SCUBA or while snorkeling on the surface. We estimated social group size by following one individual for ca. 5 to 10 min and counting the number of individuals encountered.

Results

Gonad morphology and development

The gross morphology of gonads was the same for all three species. Gonads were bilobed and fused posteriorly near the urogenital opening, and they were suspended in the body cavity by a network of mesenteries. All gonads contained a central lumen surrounded by lamellae that projected medially from the gonadal walls. Both male and female germinal tissues developed within the lamellae (called lobules in males).

Gonadal development stages in all three species were also similar. Immature female gonads were small and thin, and they were composed almost entirely of primary growth stage oocytes (i.e., previtellogenic oocytes) packed tightly within rows of lamellae (Figure 1). These gonads contained no evidence of prior spawning activity, because they were small in transverse diameter and thickened muscle bundles were absent. The gonads of mature, inactive females were also dominated by primary growth stage oocytes. However, these gonads were larger in length, volume, and transverse diameter than immature females and contained evidence of prior spawning as evidenced by the presence of thickened muscle bundles and enlarged blood vessels within lamellae. Gonads of maturing females contained oocytes in the cortical alveolar stage. Previtellogenic oocytes were also abundant, sometimes along with a small number of early-stage vitellogenic oocytes. Female gonads that were reproductively active were filled with early to late stage vitellogenic oocytes tightly packed throughout the gonad, although primary growth stage oocytes were also present and scattered along the edges of lamellae. When spawning was imminent, the ripe ovaries contained oocytes in the migratory nucleus or hydrated stages, and post-ovulatory follicles were present in some individuals. Vitellogenic oocytes in early to late stages of atresia were also common in both active and ripe ovaries. The ovaries of females that had recently spawned (post-spawning females) or had ceased spawning (regressing females) were filled with vitellogenic oocytes in early to late stages of atresia as well as large amounts of intralamellar debris and cytoplasmic strands. Muscle bundles and enlarged blood vessels were present in the ovaries of some regressing females.

The testes of immature males were small and thin in appearance, and the transverse diameter was also small. Lobules were composed mainly of spermatogenic cysts with early stage germ cells (i.e., spermatogonia, spermatocytes; Figure 2). Peripheral sperm sinuses were present in some individuals but were not fully developed and were not completely filled with spermatids or spermatozoa. The testes of mature, inactive males appeared similar but could be distinguished from those of immature males by their relatively large transverse diameter, the presence of fully developed (but constricted) sperm sinuses, and small amounts of residual spermatozoa within lobules or sperm sinuses. Their lobules were filled with cysts of sperm cells at all stages of development but were dominated by spermatocytes and spermatogonia. The gonads of developing males showed signs of early maturation. Both early and late stages of gametogenesis were present in lobules, spermatozoa were present in some but not all peripheral sperm sinuses, and a small proportion of lobules were coalesced and filled with mature spermatozoa. In active and ripe males, the majority of lobules were coalesced and filled with sperm, and the peripheral sperm sinuses were expanded and also completely full of sperm. In contrast to developing males, early stages of gametogenesis were not present. In regressing males, the lobules and sperms sinuses were only partially full of mature sperm, large amounts of connective and stromal tissue were present throughout the gonad, and early stage germ cells were also present.

Gonads classified as immature or inactive bisexual were found in *Alphestes immaculatus* and *Cephalopholis panamensis* (Figure 3). These gonads contained nearly equal amounts of male and female tissues and showed no clear evidence of function for either sex or evidence that sex change was occurring. Therefore, these gonads were not classified as female, male, or transitional. However, functional sex change was detected in the gonads of three *C. panamensis* and one *Epinephelus labriformis*. Transitional gonads contained both widespread atresia of vitellogenic oocytes (demonstrating prior female function) and proliferating spermatogenic crypts and sperm sinuses (demonstrating development towards male function; Figure 3). In addition to individuals classified as bisexual or transitional, the gonads of both males and females often contained germinal tissues of the opposite sex (Table 3). In all three species, spermatogenic crypts were scattered within the lamellae of immature and mature females, and small numbers of previtellogenic oocytes were present within lobules of testes of mature males.

Population structure

Alphestes immaculatus — Differences in average size among maturity classes were significant (Table 4). Bisexuals and immature females occupied the smallest size classes, and they were similar in median length and size range (Table 5, Figure 4). Both immature females and bisexuals were significantly smaller than mature females and mature males (Tables 4 and 5, Figure 4). The size ranges of mature females and mature males overlapped, but females attained larger sizes and had a greater median length than males (Tables 4 and 5, Figure 4). The adult population was female-biased with a sample sex ratio of 1.92 to 1. The minimum size at sexual maturity was 120 mm SL for females and 130 mm SL for males, and all females and males were sexually mature by 130 mm SL (Figure 5).

Differences in median age among maturity classes were significant, but pair-wise comparisons between classes were not significant (Table 4). The age of a single bisexual individual was estimated to be less than one year (year class 0). Age ranges for mature females (0 to 8 yrs) and mature males (1 to 9 yrs) were similar, and the age frequency distribution of males and females was unimodal (Table 5, Figure 6).

Cephalopholis panamensis — Differences in the average sizes among maturity classes were significant (Table 6). Immature females occupied the smallest size classes and were smaller than bisexuals, transitionals, mature females, and mature males (Tables 5 and 6, Figure 4). The size range of mature females overlapped partially with males, but the maximum and median sizes of females were smaller than males (Tables 5 and 6, Figure 4). Bisexuals and transitionals were similar in length, and their median and size ranges were similar to both mature males and mature females (Tables 5 and 6, Figure 4). The sex ratio of adults was 2.74 females per male. The minimum size at sexual maturity was 140 mm SL for females and 200 mm SL for males, whereas the size at 50% maturity was 170 mm SL for females and 200 mm SL for males (Figure 5). All females and males were sexually mature by 220 mm SL. Immature females ranged in age from 1 to 3 years, and they were younger than both mature females and mature males (Tables 5 and 6, Figure 7). Mature females ranged from 1 to 11 years in age and were younger than mature males (Tables 5 and 6, Figure 7), which ranged from 3 to 16 years of age. Two bisexual individuals were 3 to 4 years of age, and one transitional individual was 6 years of age (Tables 5 and 6, Figure 7). The size range for sex change was 195 to 260 mm SL from transitionals and 200 to 287 mm SL from the size overlap of males and females, which equates to 59 to 88% of maximum body size.

Epinephelus labriformis — Differences in the average length of maturity classes were significant (Table 7). Immature females occupied the smallest size classes and were smaller than both mature females and mature males (Tables 5 and 7, Figure 4). Mature females overlapped in size range with mature males, but mature males attained a larger mean and maximum length than mature females (Tables 5 and 7, Figure 4). The one transitional individual was measured at 300 mm SL. The minimum size at sexual maturity was 150 mm SL for females and 230 mm SL for males, and the length at 50% maturity was 170 mm SL for female and 230 mm SL for males (Figure 5). The sex ratio of the adult population was 2.32 females to male. The size range for sex change was 230 to 346 mm SL based on the overlap of females and males, and this equates to sex change occurring between 54 to 82 % of maximum body length.

Spawning seasonality, gamete production, and sperm competition

Reproductively active female and male *Alphestes immaculatus* were collected from April to October at Mulege and Loreto (Table 1). Female GSI ranged from 0.7 to 8.1%, and mean female GSI was 3.28%. Male GSI ranged from 0.33 to 2.18 %, and mean male GSI was 0.96%. Female gonad weight increase with standard length (Figure 8: $n = 67$, $r^2 = 0.40$, $p < 0.001$), but the relationship between female GSI and body length was not significant ($n =67$, $r^2 = 0.056$, $p = 0.054$). Male gonad weight increased with body length (Figure 9: $n=45$, $r^2 = 0.22$, $p < 0.001$), but male GSI did not change with body length ($n = 45$, $r^2 < 0.01$, $p = 0.096$).

Active adult females of *Cephalopholis panamensis* were collected from May to September at Loreto, and active or ripe males were collected from April to November (Table 1). Female GSI ranged from 0.31 to 7.24% and averaged 2.01%. Male GSI ranged from 0.04 to 1.02% and averaged 0.26%. Female gonad weight was correlated with body length (Figure 10: n = 67, $r^2 = 0.22$, $p < 0.001$), but female GSI showed no relationship to body length (n = 67, $r^2 < 0.01$, p = 0.54). For males, neither gonad weight (Figure 11: n = 34, $r^2 = 0.11$, p = 0.62) nor GSI were correlated with body length (n = 34, $r^2 < 0.01$, p = 0.73).

Reproductively active adult *Epinephelus labriformis* were collected from April to October at Loreto and in January at Puerto Vallarta (Table 1).

Courtship behavior and social structure

Courtship in *C. panamensis* was observed on three dives in July 2004, where it occurred between 1855 to 1930 h and ranged from 35 to 140 s in duration. Courtship was also observed on 13 September 2006 at 1725 h and lasted for 45 s. During courtship, the male was a pale grey color, and the female was black, with high-contrast white bars on her head and body (Figure 12).

Courtship events observed on all three dates were similar and consisted of a combination of one or more of the following behaviors: Dance, Parallel Swim, Follow, Rise, Parallel Rest, Shake, and Bump. When a male approached a female, the female would rise up ca. 0.5 m above the reef, oriented in a head-up posture. She would then shake her head and body back and forth at a rapid pace (Dance). The male would then swim up alongside and parallel to the female and perform the same behavior. The two would then swim side by side along the reef for several seconds (Parallel Swim), or the male would follow close behind the female as she swam (Follow). While swimming, the female would change directions several times, and the male would respond by making contact with the opercular area or the abdomen of the female with his snout (Bump). During two courtship events, Swimming events were followed by Parallel Rise, where the pair rose up slowly into the water column for 1—2 m while maintaining body contact and oriented parallel. Next, the pair would separate and swim away from each other, or they would settle down onto the reef next to each other and facing the same direction (Parallel Rest). Once both settled onto the reef, the male would shake his head rapidly for 1-2 s, while remaining next to the female (Shake). After one or more Shake behaviors, the female responded by either swimming up into the water column and performing Dance behavior or swam away from the male. The release of gametes was not observed during any courtship events. Courtship behavior was not observed in *A. immaculatus* and *E. labriformis*.

Social group sizes of adult *C. panamensis* ranged from 3 to 7 individuals. Males patrolled territories of 100 to 400 m^2, and females occupied smaller territories within those of males. Indidividuals of *A. immaculatus* were usually observed as isolated individuals at Loreto, but a loose aggregation of ca. 50 adults was observed at one patch reef in Mulege on 10 November 2007. Social groups of *E. labriformis* consisted of 2 to 6 fish at reefs in Loreto.

Intraspecific and interspecific behavior patterns in C. panamensis

When solitary, males and females were similar in color pattern (Figure 12). However, both males and females of *C.panamensis* showed distinct color patterns during social interactions with conspecifics. During interactions with males or females, females were dark black with large, high-contrast white bars on their heads and bodies. In males, the ventral portions of the body and head were the same color as when solitary, but the dorsal portions of the body and head were pale and the stripes were either absent or faded.

For males, the frequency and proportion of time spent engaged in intraspecific interactions with females was higher in the evening than in the daytime (Table 8). The same was true for the total frequency and proportion of time engaged in intraspecific interactions with conspecifics of either sex (i.e., intraspecific pooled data). Neither interactions with other male *C. panamensis* nor interspecific interactions changed in frequency or proportion between daytime and evening time periods. Females showed patterns similar to those of males, because the frequency and proportion of time spent involved in interactions with other females or individuals of either sex (pooled data) were higher in the evening period than the daytime period (Table 8). However, the frequency and proportion of time females spent on intraspecific interactions with males or on interspecific interactions did not change in frequency or proportion between the daytime and evening periods.

The frequency of intraspecific interactions was higher in females than males during the evening and when data were pooled by time period, although this difference was not statistically significant (Table 9). However, females spent a significantly larger proportion of time engaged in intraspecific interactions (After 1600 h, pooled) during the evening period than did males. When data were pooled for time period, females spent a larger proportion of time involved in interactions with other females than males did with females.

The frequency and proportion of time spent involved in interspecific interactions was significantly higher for males than females in the daytime, evening, and when data were pooled for time (Tables 9 and 10). Small adult *Mycteroperca rosacea* (Serranidae) and *Bodianus diplotaenia* (Labridae) were the two most common species involved in interspecific interactions.

Discussion

We found clear evidence of protogynous hermaphroditism in *Cephalopholis panamensis* and *Epinephelus labriformis*. Transitional gonads were found in both species, and these contained atretic vitellogenic oocytes coexistant with proliferating spermatogenic crypts and sperm sinuses. The presence of a gonad undergoing sexual transition is the strongest evidence of sex change in fishes, and this characteristic has been used to diagnose sex change in other groupers (Moe 1969; Sadovy and Shapiro 1987; Mackie 2006). Male and female bisexual gonads were common in *C. panamensis*, in a size range similar to that of transitional individuals. It is possible that these fish were also undergoing sex change, although this could not be confirmed. Bisexual gonads occur in both gonochoric and protogynous groupers, and so they cannot be used to determine sexual pattern (Liu and Sadovy 2004; Erisman et al. 2007a).

The population structure of *C. panamensis* and *E. labriformis* provided further evidence of protogyny. Both species had bimodal size and age distributions, in which males were larger and older than females, and adult populations had female-biased sex ratios. These patterns are common in protogynous fishes, where males develop from sex-changed females, and social groups often consist of a large male and a group of females (Warner 1988; Munday et al. 2006). Differences in mortality rates, growth rates, and habitat preferences between the sexes may also produce bimodal distributions or female-biased sex ratios, and thus do not provide unequivocal evidence of hermaphroditism (Sadovy and Shapiro 1987; Devlin and Nagahama 2002). However, when present in combination with histological evidence of sex change, these characteristics do support this diagnosis.

The mating system and male testes sizes of *C. panamensis* were also consistent with a protogynous sexual pattern. Large males courted individual females and engaged in aggressive behavior with surrounding males. Also, males invested little energy in sperm production; testes weighed less than 1% of total body weight. Both aspects are common in protogynous reef fishes, where males attempt to maximize their mating success through defense of females rather than through sperm competition with other males (Muñoz and Warner 2003; Erisman 2008).

We found no conclusive evidence of sex change in either direction for *Alphestes immaculatus*. Females attained larger sizes than males, indicating that adults may follow a protandrous hermaphroditic sexual pattern. However, sex ratios were biased towards females and the age distributions of male and females were similar.

Bisexual individuals were present in the population but showed no evidence of prior function as adults of either sex. Such fish are likely bisexual juveniles, a stage that occurs during the sexual development of many fishes, and it shows no relationship to sexual pattern, since it occurs in both gonochoric and hermaphroditic species (Sadovy and Liu 2008).

The spawning season in all three species is relatively long, as in smaller groupers in general (Sadovy 1996). In the Gulf of California, the spawning seasons of several larger groupers, such as *Mycteroperca rosacea* and *M. prionura*, also occur during the spring and summer months (Erisman et al. 2007b; Sala et al. 2003).

All three species mature at relatively small sizes, and some *A. immaculatus* and *C. panamensis* become sexually mature during the first year. This characteristic would ameliorate problems associated with recruitment overfishing in many grouper fisheries in the Gulf of California (Aburto et al. 2008), since most fish will spawn at least once prior to capture. However, these three species share several life-history traits with other groupers that would make them vulnerable to fishing. Results from this study indicate that *A. immaculatus* and *C. panamensis* grow slowly, and Craig et al. (1999) found similar results for *C. panamensis* and *E. labriformis*. *A. immaculatus* live up to 9 years or more, *C. panamensis* may live in excess of 15 years, and *E. labriformis* live for up to 29 years (this study, Craig et al. 1999). Although they have prolonged spawning seasons, female GSI values for two of the three species are relatively low compared with other groupers, indicating that egg production is limited (Sadovy 1996). Data on annual recruitment patterns have not been collected for any of these species but are necessary to determine population turnover rates. However, a study in the Red Sea found that populations of small groupers did not recover three years after experimental removal, which suggests that population recovery in these fishes may be slow (Shpigel and Fishelson 1991a). The combination of these factors suggests that, like other groupers, these three species may be sensitive to fishing pressure and recovery slowly following periods of heavy exploitation (Jennings et al 1999).

The protogynous sexual pattern and population structure of *C. panamensis* and *E. labriformis* may also put them at risk to overexploitation. In both species, males are larger and less abundant than females. Since traditional fisheries tend to remove the largest fishes, this practice would select for the removal of males and may lead to problems of sperm limitation if removed males are not replaced by sex-changed females (Vincent and Sadovy 1998). In commercial grouper fisheries in the Caribbean, large declines in fisheries landings were attributed to the overfishing of males, which caused major shifts in population sex ratios and decreases in reproductive activity (Nemeth 2005; Ault et al. 2006).

Courtship and social behaviors observed in *C. panamensis* were similar to those described in other members of the genus. *C. boenak*, *C. spiloparaea*, *C. argus*, and *C. urodeta* also engage in paired courtship during the afternoon and evening, and mating groups consist of single male, multiple female groups (Donaldson 1989; 1995a). In addition, behaviors such as Dance, Parallel Swim, Follow, Rise, Parallel Rest, Shake, and Bump have all been observed in these species (Donaldson 1995).

Similarly, Shpigel and Fishelson (1991b) found that *C. miniata* and *C. argus* occurred in haremic groups consisting of a dominant male that patrolled fixed territories and multiple females that occupied smaller territories within those of the dominant males.

Intraspecific interactions for both males and females were more common in the evening than earlier in the day. Donaldson (1995b) reported similar results for *C. spiloparaea*, in which intraspecific interactions were most frequent during the evening pre-courtship period. In both species, adults may assess their place within the social hierarchy through a series of brief interactions with other members of the social group. Dominance hierarchies are common in protogynous reef fishes (Warner 1984; Ross 1990). Males attempt to maximize their reproductive success by defending females from other males and suppressing sex change of females through dominance behavior (Robertson 1972). Likewise, participation in aggressive encounters with conspecifics is common in females, because dominant females often change sex after the death of the dominant male. By doing so, these females may increase their future reproductive success by gaining access to all females in the social group and control of resources within the male's territory (Warner and Swearer 1991).

Adult *C. panamensis* showed sex-related differences in intraspecific and interspecific behavior patterns. Females spent a greater proportion of time involved in intraspecific interactions, whereas males expended more effort engaged in interspecific interactions. *C. spiloparea* are dissimilar in this regard, as adult males spend greater effort in both intraspecific and interspecific interactions. These discrepancies may result from sex-related differences in territory size and abundance.

Males are more likely to encounter potential competitors for resources, because they defend much larger territories than do females. Since females are more abundant and occupy small territories within those of males, they are more likely to encounter conspecifics while patrolling territories than males.

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Table 4.1. Summary of collected specimens, organized by species, collection date, and gonad development class. B = immature or inactive bisexual; F1 = immature female; F2 = mature, inactive female; F3 = mature, developing female; F4 = mature, active or ripe female; F5 = mature, post-spawning or regressing female; M1 = immature male; M2 = mature, inactive male; M3 = mature, developing male; M4 = mature, active or ripe male; M5 = mature, post-spawning or regressing male; T = transitional.

Alphestes immaculatus

		Gonad Development Class												
Collection Date	Location	B	F1	F2	F3	F4	F5	M1	M2	M3	M4	M5	T	Total
April 2005	Loreto		1	1		1					4			7
May 2005	Loreto					7					5			12
July 1965	Mulege	11	3	1		20	1				11			47
July 2003	Mulege					49	1				17			67
July 2004	Loreto					4	2				3			9
August 2006	Loreto					3					2			5
September 2006	Loreto			1							4			5
October 2007	Mulege	1		3		20	9				14	5		52
November 2006	Loreto						2							2
	Total	12	4	6	0	104	15	0	0	0	60	5	0	**206**

Cephalopholis panamensis

		Gonad Development Class												
Collection Date	Location	B	F1	F2	F3	F4	F5	M1	M2	M3	M4	M5	T	Total
April 2005	Loreto		4	12				1		2				19
May 2005	Loreto		6	24	3	3				7	4		1	48
June 2005	Loreto			4					2					6
July 2003	Loreto	2	18	8	8	48	2			1	20		2	109
July 2004	Loreto		1		1	10					10			22
August 2006	Loreto		1	6		3	4				2	1		17
September 2001	Loreto			1		3					3	1		8
September 2006	Loreto		14	7		6	5				2	2		36
November 2006	Loreto			1				1			1			3
	Total	2	44	63	12	73	11	2	2	10	42	4	3	**268**

Epinephelus labriformis

		Gonad Development Class												
Collection Date	Location	B	F1	F2	F3	F4	F5	M1	M2	M3	M4	M5	T	Total
January 2003	Puerto Vallarta					27	2				22			51
April 2005	Loreto		1	3		2					1			7
May 2005	Loreto		1	3	2	3				1				10
June 2005	Loreto		2	1		6	2				1	1		13
July 2003	Loreto					4					1			5
July 2004	Loreto		1	1	1	3								6
September 2006	Loreto		4											4
October 2007	Loreto		2	1		4					1		1	9
	Total		11	9	3	49	4	0	0	1	26	1	1	**105**

Table 4.2. Microscopic descriptions of gonad development classes based on histological investigations of gonads.

Gonad Development Class	Microscopic Description
B (Immature or Inactive Bisexual)	Transverse diameter of gonad is small and gonadal wall is thin. Lamellae are filled with primary growth stage oocytes and cysts of spermatogonia and spermatocytes. There is no morphological evidence of prior sexual maturation as a female (muscle bundles) or as a male (sperm sinuses, spermatids or spermatozoa).
F1 (Immature Female)	Transverse diameter of gonad is small and Gonadal wall is thin. Lamellae are narrow and filled with oogonia and primary growth stage (chromatin nucleolar, perinucleolar) oocytes, usually in orderly rows. No evidence of prior spawning. Spermatogenic crypts may be present in low numbers.
F2 (Mature, Inactive Female)	Transverse diameter of ovary is larger and gonadal wall is thicker than immature females. Lamellae filled with oogonia and primary growth stage oocytes. Evidence of prior spawning activity is apparent by the presence of muscle bundles and enlarged blood vessels. Spermatogenic crypts may be present in low numbers.
F3 (Mature, Developing Female)	Gonadal wall is thin, and lamellae are filled with primary growth stage oocytes and cortical alveolar oocytes. Early stage vitellogenic oocytes may be present in low numbers. Atretic oocytes of either stage are sometimes present. Spermatogenic crypts may be present in low numbers (CP).
F4 (Mature, Active or Ripe Female)	Transverse diameter of ovary is enlarged and gonadal wall is thin. Lamellae are filled with later stage vitellogenic oocytes, but oocytes of all stages are present. The presence of hydrated or migratory nucleus stage oocytes indicates that spawning is imminent, and post-ovulatory follicles signify a recent spawning event. Spermatogenic crypts may be present in low numbers.
F5 (Mature, Post-spawning or Regressing Female)	Gonadal wall is thickened, and the gonad has a disrupted appearance and contains intralamellar debris and cytoplasmic strands. Lamellae are loosely filled with vitellogenic oocytes in early to late stages of atresia, primary growth stage oocytes are present, and cortical alveolar are sometimes present in low amounts. Muscle bundles and enlarged blood vessels are present in varied amounts. Spermatogenic crypts may be present in low numbers (AI, CP).

Table 4.2. Continued.

Gonad Development Class	Microscopic Description
M1 (Immature Male)	Transverse diameter of testis is small, and gonadal wall is thick. Lobules are filled with cysts of spermatogonia and spermatocytes. Sperm sinuses are sometimes present are not fully developed and do not contain mature sperm (spermatids or spermatozoa). Primary growth stage oocytes may be present in low numbers (CP).
M2 (Mature, Inactive Male)	Transverse diameter of testis is large compared with immature male testis and gonadal wall is thin. Lobules are filled with cysts of sperm at all stages of development but are dominated by spermatogonia and spermatocytes. Sperm sinuses are fully developed but are constricted. Small amounts of residual spermatozoa are sometimes present in peripheral sperm sinuses or in lobules.
M3 (Mature, Developing Male)	Early (spermatogonia, spermatocytes) and late (spermatids, spermatozoa) growth stages of sperm are present in relatively equal amounts. Peripheral sperm sinuses are not completely enlarged or full of sperm, and not all lobules have coalesced and filled with sperm. Primary growth stage oocytes may be present in low numbers (CP).
M4 (Mature, Active or Ripe Male)	Testis is dominated by late stages of spermatogenesis (spermatids and spermatozoa). Spermatozoa are present in large amounts within the lobules and peripheral sperm sinuses, and early stages of sperm are not abundant or are absent. Lobules in ripe males are enlarged, coalesced and completely full of mature sperm. Primary growth stage oocytes may be present in low numbers.
M5 (Mature, Post-spawning or Regressing Male)	Lobules are only partially full of sperm and are more separated and smaller compared with ripe males. Peripheral sperm sinuses are enlarged or constricted and are not completely full of mature sperm. Stromal tissue, connective tissue, and spermatogonia are present throughout the gonad.
T (Transitional)	Gonadal wall is thickened, tissues appear disorganized. Gonad shows widespread atresia of vitellogenic oocytes and proliferation of early stage sperm (spermatogonia, spermatocytes) throughout the gonad. Muscle bundles, enlarged blood vessels, and connective tissue may be present.

Table 4.3. Percentage of bisexual gonads in collections, organized by species and gonad development class. Numbers inside parentheses indicate sample size of bisexual gonads for each gonad development class.

	Species		
Gonad Development Class	*A. immaculatus*	*C. panamensis*	*E. labriformis*
F1	25 (1)	14 (6)	18 (2)
F2	17 (1)	6 (4)	22 (2)
F3		8 (1)	0 (0)
F4	3 (3)	2 (1)	10 (5)
F5	20 (3)	9 (1)	0 (0)
M1		50 (1)	
M2		0 (0)	
M3		50 (5)	0 (0)
M4	2 (1)	17 (7)	15 (4)
M5	0 (0)	0 (0)	0 (0)
B	100 (12)	100 (2)	
T		100 (3)	100 (1)
Total % Bisexuals	10 (n =21)	12 (n=31)	13 (n=14)

Table 4.4. Summary of results of size and age analyses for *Alphestes immaculatus*. B = bisexual; IF = immature female; MF = mature female; IMM = immature male; MM = mature male; T = transitional.

Kruskal-Wallis Test of Median Size by Maturity Class

Development Class	n	Sum of Ranks	Mean of Ranks
IMF	4	53	13.25
MF	125	15288	122.3
MM	65	5873	90.34
B	12	107	8.92
		KW = 54.99	$p < 0.001$

Dunn's Multiple Comparisons Test of Median Size by Maturity Class

Comparison	Mean Rank Difference	P value
IMF vs. MF	-109.05	$p < 0.01$
IMF vs. MM	-77.11	$p < 0.01$
IMF vs. B	-4.33	$p > 0.05$
MF vs. MM	31.95	$p < 0.01$
MF vs. B	-113.39	$p < 0.001$
MM vs. B	-81.44	$p < 0.001$

Kruskal-Wallis Test of Median Age by Maturity Class

Development Class	n	Sum of Ranks	Mean of Ranks
MF	89	5518.5	62.01
MM	38	2734.5	71.96
B	1	3	3
		KW = 4.85	$p = 0.089$

Dunn's Multiple Comparisons Test of Median Age by Maturity Class

Comparison	Mean Rank Difference	P value
MF vs. MM	-9.955	$p > 0.05$
MF vs. B	-59.006	$p > 0.05$
MM vs. B	-68.961	$p > 0.05$

Table 4.5. Descriptive statistics related to size and age of collected specimens, organized by species and maturity class.

Alphestes immaculatus

Development Class	B	IF	MF	MM
Number	12	4	125	65
Mean Length (mm SL)	108	111	176	162
SE	2.75	10.66	2.58	1.95
Length Range (mm SL)	94 - 121	89 - 140	120 - 279	110 - 233
Mean Age (years)	0	NA	2.85	3.21
SE	NA	NA	0.21	0.28
Age Range	0	NA	0-9	1-8

Cephalopholis panamensis

Development Class	B	IF	MF	IMM	MM	T
Number	2	44	159	2	58	3
Mean Length (mm SL)	235	175	217	258	263	233
SE	5	2.98	2.27	27.5	3.74	19.65
Length Range (mm SL)	230 - 240	133 - 215	145-287	230-285	200-328	195 - 260
Mean Age (years)	3.5	1.75	3.66	NA	8.04	6
SE	0.5	0.21	0.25	NA	0.75	NA
Age Range (years)	3-4	1-3	1-11	NA	3-16	NA

Epinephelus labriformis

Development Class	IF	MF	MM	T
Number	11	65	28	1
Mean Length (mm SL)	202	251	303	300
SE	6.9	5.47	8.56	NA
Length Range (mm SL)	159-237	155-346	230-423	NA

Table 4.6. Summary of results of size and age analyses for *Cephalopholis panamensis*.

Kruskal-Wallis Test of Median Size by Maturity Class

Development Class	n	Sum of Ranks	Mean of Ranks
IMF	44	1832.5	41.648
MF	159	20378	128.16
MM	58	12472	215.03
B	2	338	169
T	3	491	163.67
		KW = 129.57	$p < 0.001$

Dunn's Multiple Comparisons Test of Median Size by Maturity Class

Comparison	Mean Rank Difference	P value
IMF vs. MF	-86.52	$p < 0.001$
IMF vs. MM	-173.38	$p < 0.001$
IMF vs. B	127.35	$p < 0.01$
IMF vs. T	-122.02	$p < 0.05$
MF vs. MM	-86.86	$p < 0.001$
MF vs. B	40.84	$p > 0.05$
MF vs. T	-35.51	$p > 0.05$
MM vs. B	-46.03	$p > 0.05$
MM vs. T	51.36	$p > 0.05$
B vs. T	5.33	$p > 0.05$

Kruskal-Wallis Test of Median Age by Maturity Class

Development Class	n	Sum of Ranks	Mean of Ranks
IMF	16	336	21
MF	65	3232.5	49.73
MM	23	2104	87.67
B	2	105.5	52.75
		KW = 48.50	$p < 0.001$

Dunn's Multiple Comparisons Test of Median Age by Maturity Class

Comparison	Mean Rank Difference	P value
IMF vs. MF	-28.731	$p < 0.01$
IMF vs. MM	-66.67	$p < 0.001$
IMF vs. B	-31.75	$p > 0.05$
MF vs. MM	-37.94	$p < 0.001$
MF vs. B	-3.02	$p > 0.05$
MM vs. B	34.917	$p > 0.05$

Table 4.7. Summary of results of size analyses for *Epinephelus labriformis*.

One-way Analysis of Variance (ANOVA) of Mean Size by Maturity Class

Source of Variation	d.f.	Sum of Squares	Mean Square
Treatments	2	94622	47311
Residuals	100	184457	1844.6
Total	102	279079	

F = 25.65 $p < 0.001$

Tukey-Kramer Multiple Comparisons Test of Mean Size by Maturity Class

Comparison	Mean Difference	q	P value
IMF vs. MF	-48.14	4.86	$p < 0.01$
IMF vs MM	-100.86	9.33	$p < 0.001$
MF vs. MM	52.73	7.66	$p < 0.001$

Table 4.8. Comparison of behavior patterns of male and female *Cephalopholis panamensis* by behavior type and time period. Differences in means between time periods were analyzed with Mann-Whitney U tests. Numbers inside parentheses indicate sample sizes for each time period.

Male Behaviors by Frequency (Bouts / 10 min)

Behavior	**Day**	**SD**	**Evening**	**SD**	**U**	**p**
	(n = 15)		(n = 23)			
Intraspecific w/ female	1.08	1.49	5.54	7.37	100.00	0.031*
Intraspecific w/ male	0.20	0.52	0.34	0.70	158.00	0.662
Intraspecific (pooled)	1.28	1.71	5.88	7.38	93.00	0.018*
Interspecific	0.98	1.02	1.32	1.23	145.00	0.419

Male Behaviors by Proportion of time (time spent / total time)

Behavior	**Day**	**SD**	**Evening**	**SD**	**U**	**p**
	(n = 15)		(n = 23)			
Intraspecific w/ female	0.02	0.03	0.09	0.12	99.00	0.029*
Intraspecific w/ male	0.01	0.03	0.03	0.07	157.00	0.640
Intraspecific (pooled)	0.03	0.04	0.12	0.13	87.00	0.011*
Interspecific	0.06	0.18	0.02	0.03	155.50	0.621

Female Behaviors by Frequency (Bouts / 10 min)

Behavior	**Day**	**SD**	**Evening**	**SD**	**U**	**p**
	(n = 24)		(n = 32)			
Intraspecific w/ female	1.31	1.32	6.34	4.95	104.00	< 0.001*
Intraspecific w/ male	0.38	0.68	1.42	1.95	281.50	0.085
Intraspecific (pooled)	1.69	1.46	7.75	6.12	85.00	< 0.001*
Interspecific	0.24	0.58	0.12	0.54	344.00	0.488

Female Behaviors by Proportion of time (time spent / total time)

Behavior	**Day**	**SD**	**Evening**	**SD**	**U**	**p**
	(n = 24)		(n = 32)			
Intraspecific w/ female	0.02	0.03	0.17	0.15	81.00	< 0.001*
Intraspecific w/ male	0.01	0.02	0.03	0.07	315.00	0.247
Intraspecific (pooled)	0.03	0.04	0.20	0.15	63.50	< 0.001*
Interspecific	0.01	0.05	0.01	0.03	345.00	0.499

Table 4.9. Comparison of behavior patterns between male and female *Cephalopholis panamensis* by behavior type. Differences in means between the sexes were analyzed with Mann-Whitney U tests. Numbers inside parentheses indicate sample sizes for each sex.

Behavior Frequencies (Bouts / 10 min)

Behavior	Female	SD	Male	SD	U	p
Before 16:00 hrs	(n = 24)		(n = 15)			
Intraspecific w/ female	1.31	1.32	1.08	1.49	159.50	0.56
Intraspecific w/ male	0.38	0.68	0.20	0.52	159.00	0.54
Intraspecific (pooled)	1.69	1.46	1.28	1.71	147.50	0.35
Interspecific	0.24	0.58	0.98	1.02	101.00	0.02*
After 16:00 hrs	(n = 32)		(n =23)			
Intraspecific w/ female	6.34	4.95	5.54	7.37	281.00	0.14
Intraspecific w/ male	1.42	1.95	0.34	0.70	263.00	0.07
Intraspecific (pooled)	7.75	6.12	5.88	7.38	259.00	0.06
Interspecific	0.12	0.54	1.32	1.23	134.00	< 0.001*
Pooled	(n = 56)		(n = 38)			
Intraspecific w/ female	4.18	4.57	3.78	6.16	872.00	0.14
Intraspecific w/ male	0.97	1.61	0.29	0.63	847.00	0.09
Intraspecific (pooled)	5.15	5.58	4.06	6.22	818.00	0.06
Interspecific	0.17	0.55	1.19	1.15	470.50	< 0.001*

Behavior Proportions (time spent / total time)

Behavior	Female	SD	Male	SD	U	p
Before 16:00 hrs	(n = 24)		(n = 15)			
Intraspecific w/ female	0.02	0.03	0.02	0.03	167.00	0.72
Intraspecific w/ male	0.01	0.02	0.01	0.03	165.00	0.67
Intraspecific (pooled)	0.03	0.04	0.03	0.04	156.00	0.50
Interspecific	0.01	0.05	0.06	0.18	97.00	0.02*
After 16:00 hrs	(n = 32)		(n = 23)			
Intraspecific w/ female	0.17	0.15	0.09	0.12	230.00	0.02*
Intraspecific w/ male	0.03	0.07	0.03	0.07	315.00	0.36
Intraspecific (pooled)	0.20	0.15	0.12	0.13	254.00	0.05*
Interspecific	0.01	0.03	0.02	0.03	139.00	< 0.001*
Pooled	(n = 56)		(n = 38)			
Intraspecific w/ female	0.10	0.13	0.07	0.10	812.00	0.05*
Intraspecific w/ male	0.02	0.05	0.02	0.06	940.50	0.33
Intraspecific (pooled)	0.125	0.143	0.088	0.114	850.0	0.100
Interspecific	0.008	0.039	0.035	0.117	470.5	< 0.001*

Table 4.10. Summary of interspecific interactions observed for *Cephalopholis panamensis* by species and family, sex of focal *C. panamensis* individual, and time period.

Family **Species**	**Female**		**Male**	
	Day	**Evening**	**Day**	**Evening**
Serranidae				
Mycteroperca rosacea			8	7
Epinephelus labriformis		1		1
Paranthias colonus			1	
Labridae				
Bodianus diplotaenia			1	8
Pomacentridae				
Abudefduf troschelii			1	
Stegastes rectifraenum	2			1
Muraenidae				
Gymnothorax castaneus	1	1		
Gymnothorax dovii	1			
Muraena lentiginosa	1		1	
Lutjanidae				
Lutjanus argentiventris				2
Scaridae				
Scarus ghobban				1
Pomacanthidae				
Holocanthus passer				1
Total	5	2	12	21

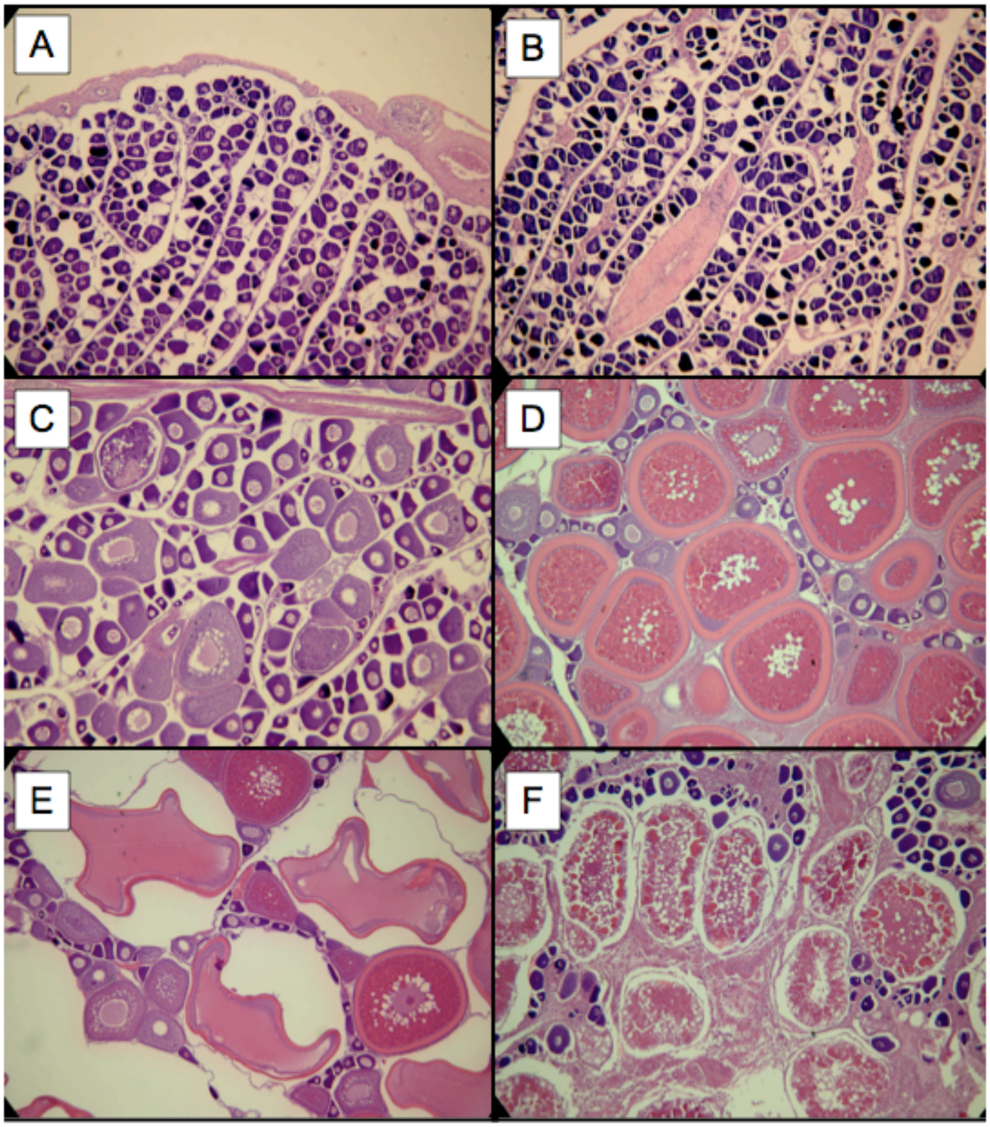


Fig. 4.1. Female gonad development classes. **A.** immature female (F1), **B.** mature inactive female (F2), **C.** developing female (F3), **D**. mature active female, **E.** mature active female with hydrated oocytes (F4), **F.** mature regressing female (F5).

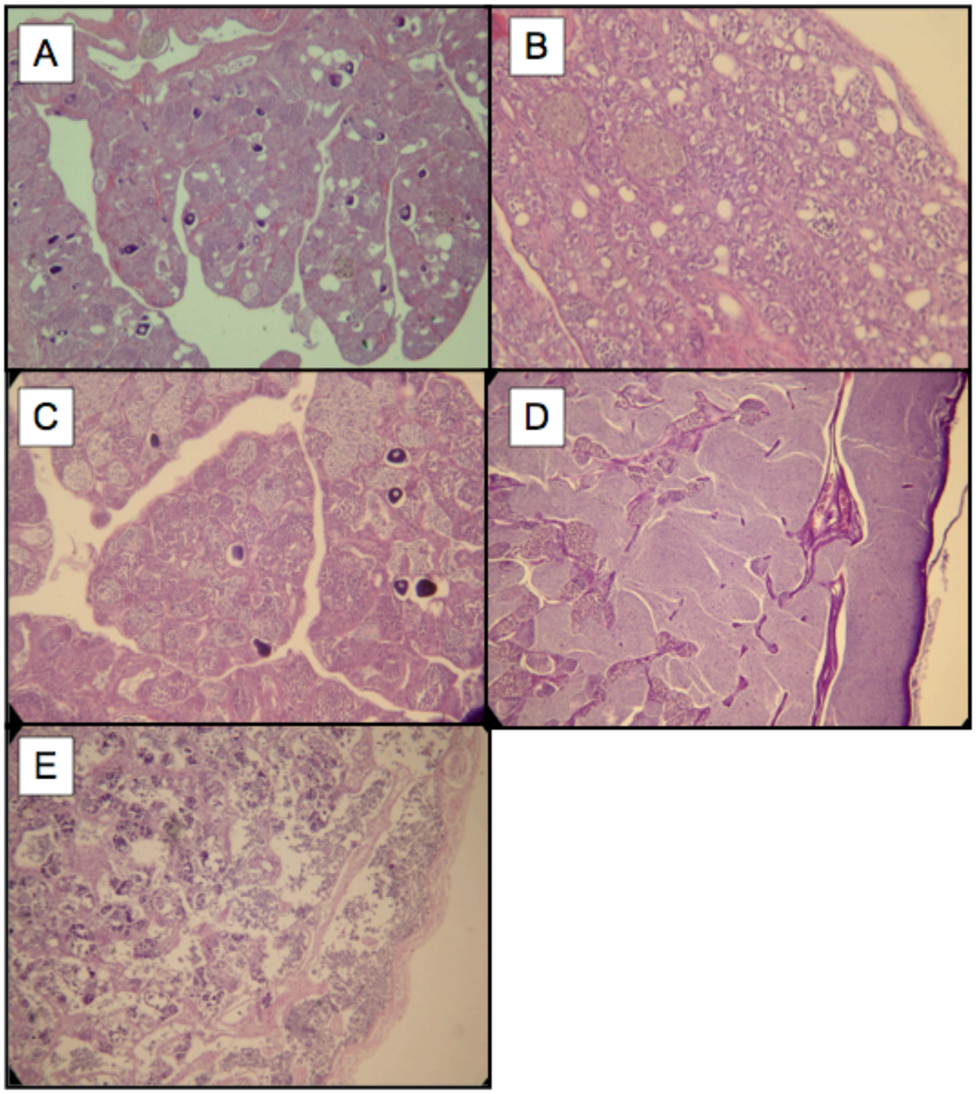


Fig. 4.2. Male gonad development classes. **A.** immature male (M1), **B.** mature inactive male (M2), **C.** developing male (M3), **D**. mature active male, **E.** mature regressing male (M5).

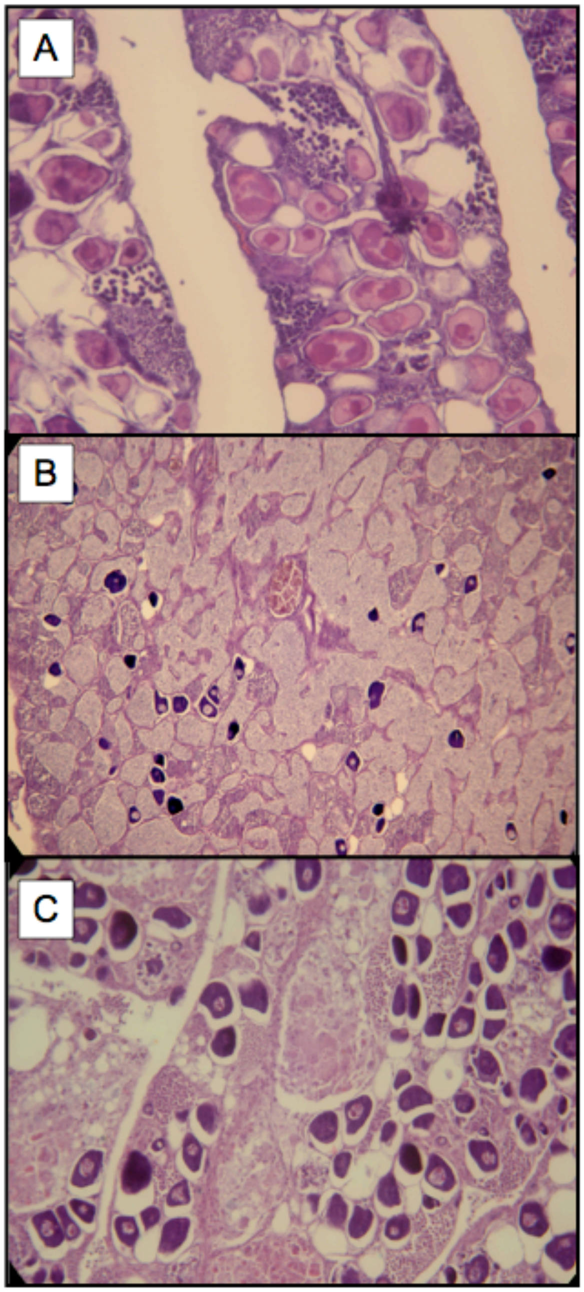


Fig. 4.3. Bisexual and transitional gonads. **A.** juvenile bisexual, **B.** mature male bisexual, **C.** transitional.

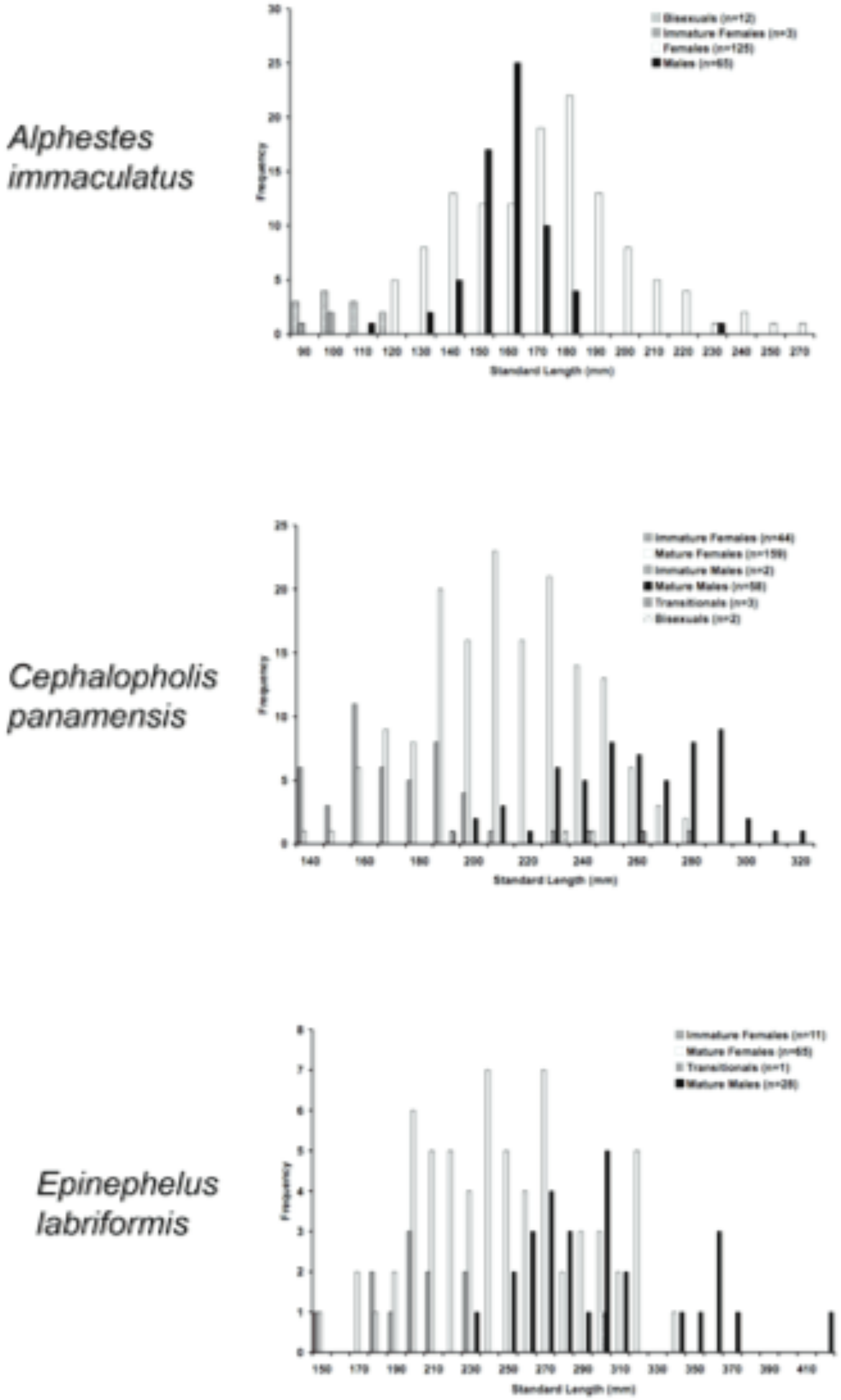
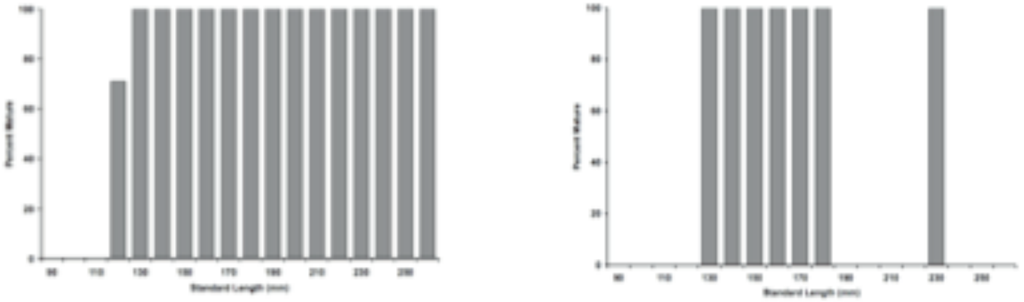
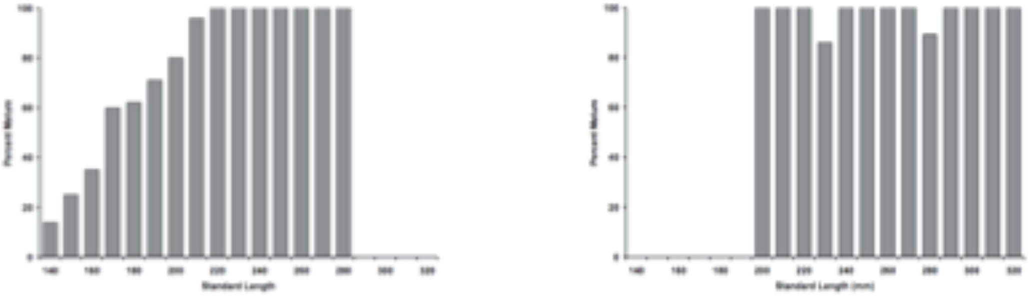


Fig. 4.4. Size-frequency distributions of *Alphestes immaculatus Cephalopholis panamensis*, and *Epinephelus labriformis*.

Alphestes immaculatus



Cephalopholis panamensis



Epinephelus labriformis

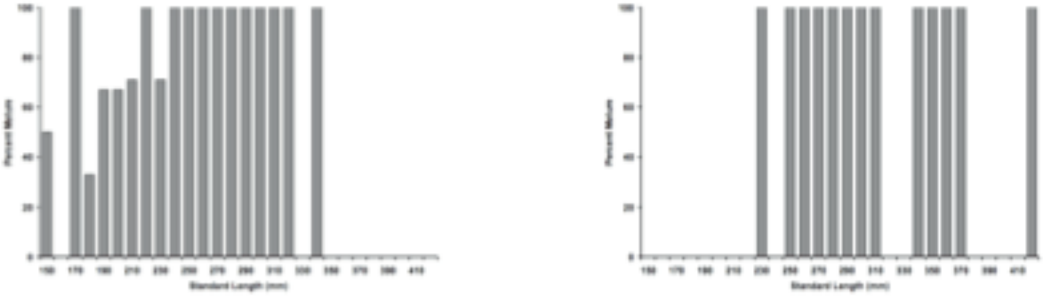


Fig. 4.5. Percentage of mature females (left column) and males (right column) of *Alphestes immaculatus*, *Cephalopholis panamensis*, and *Epinephelus labriformis*. Data are organized by standard length.

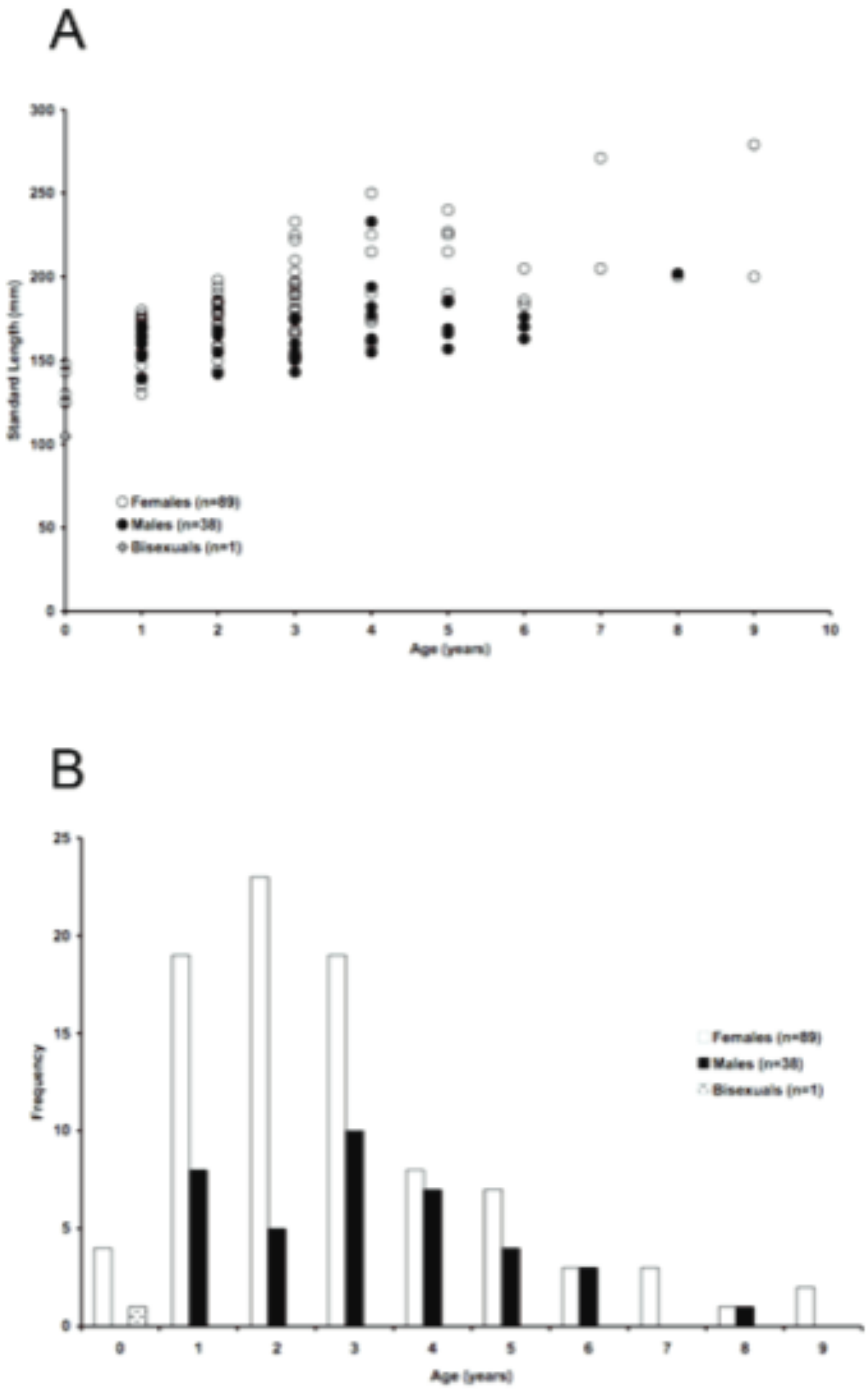


Fig. 4.6. Age distribution graphs of *Alphestes immaculatus*. **A.** Scatter plot of ages of individuals by standard length and sex, **B.** age frequency distribution by sex.

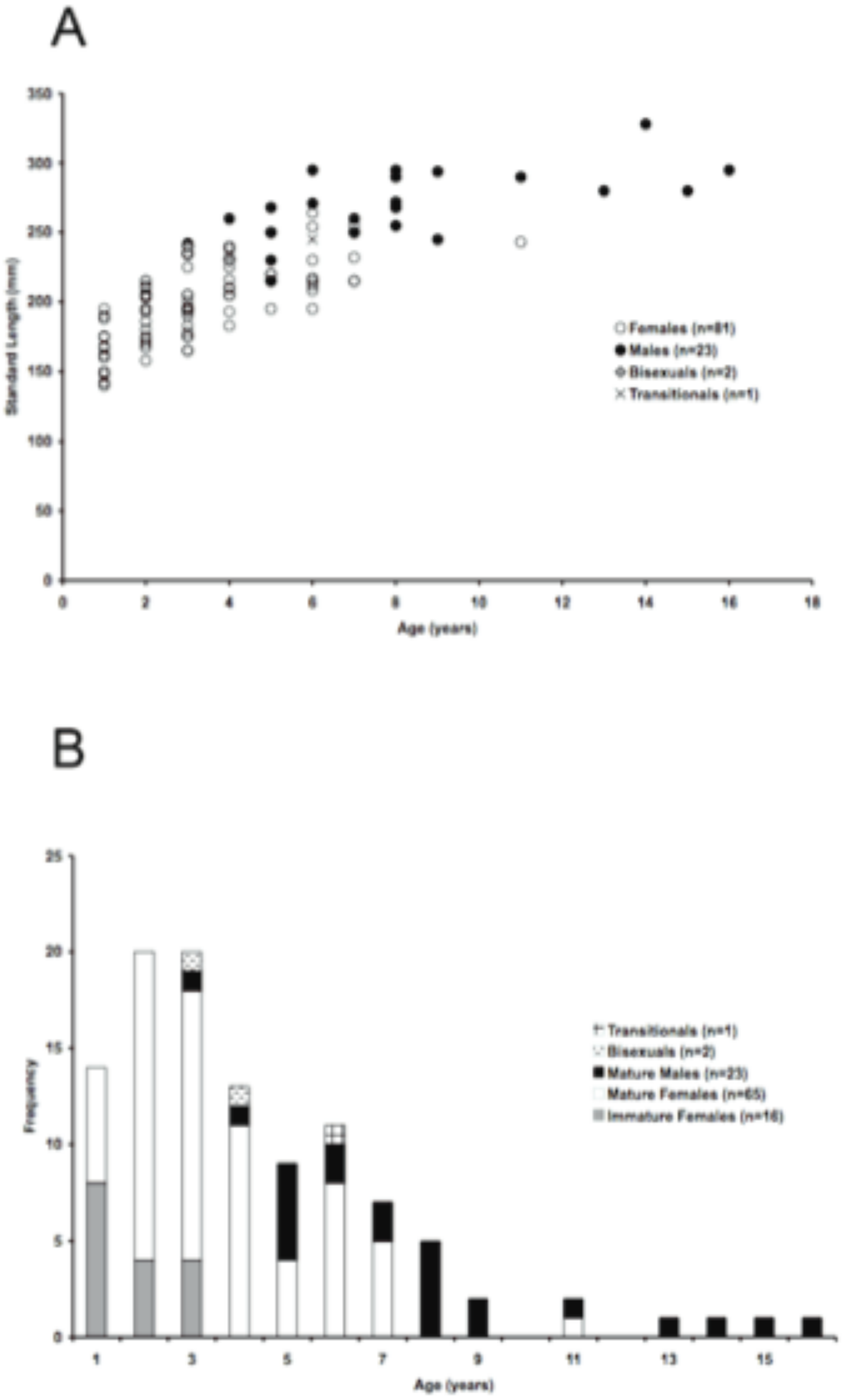


Fig. 4.7. Age distribution graphs of *Cephalopholis panamensis*. **A.** Scatter plot of ages of individuals by standard length and sex, **B.** age-frequency distribution by sex.

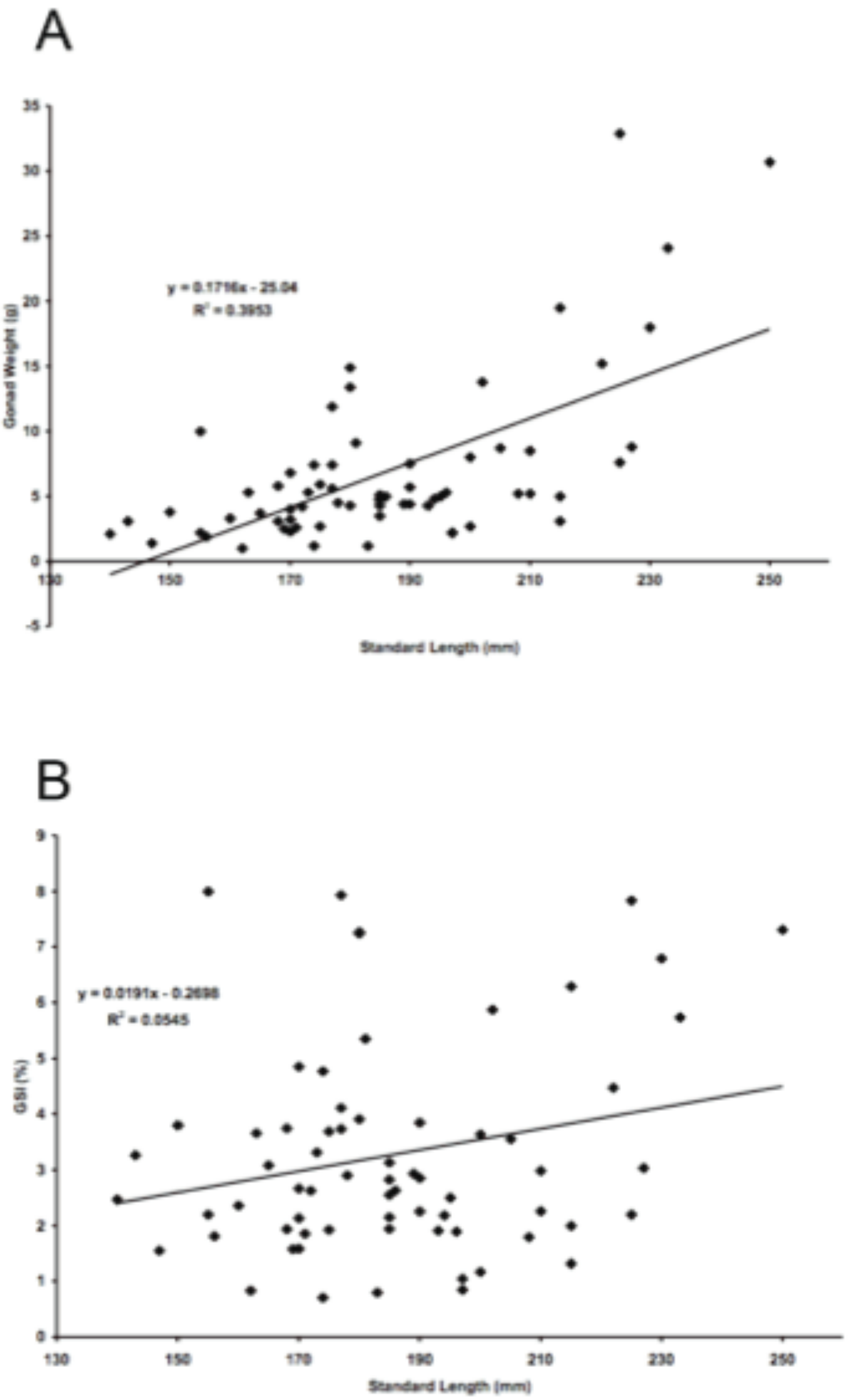


Fig. 4.8. Gonadal investment and body size relationships in reproductively active female *Alphestes immaculatus*. **A.** Gonad weight (g) versus standard length (mm), **B.** Gonosomatic index (GSI %) versus standard length (mm).

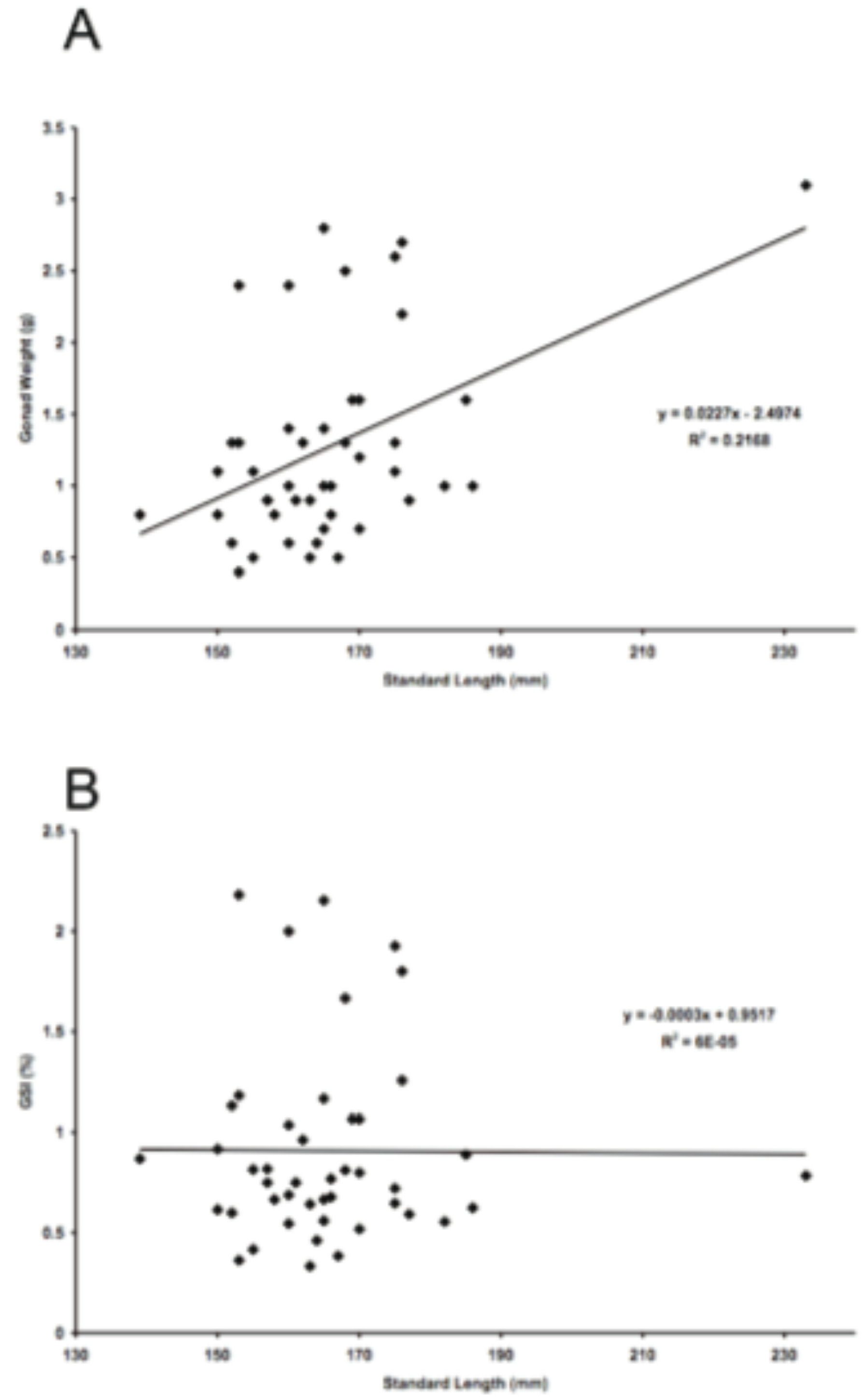


Fig. 4.9. Gonadal investment and body size relationships in reproductively active male *Alphestes immaculatus*. **A.** Gonad weight (g) versus standard length (mm), **B.** Gonosomatic index (GSI %) versus standard length (mm).

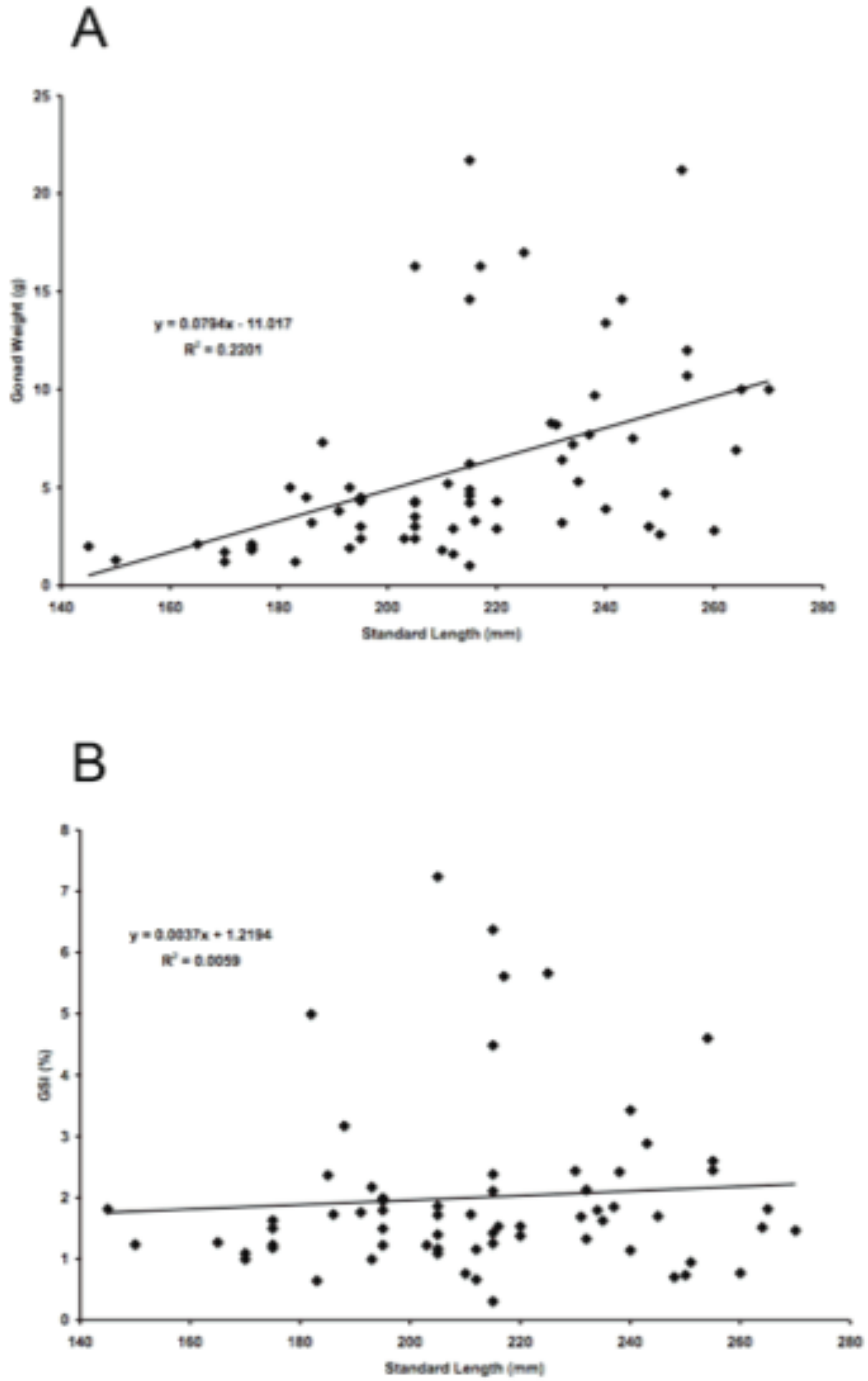


Fig. 4.10. Gonadal investment and body size relationships in reproductively active female *Cephalopholis panamensis*. **A.** Gonad weight (g) versus standard length (mm), **B.** Gonosomatic index (GSI %) versus standard length (mm).

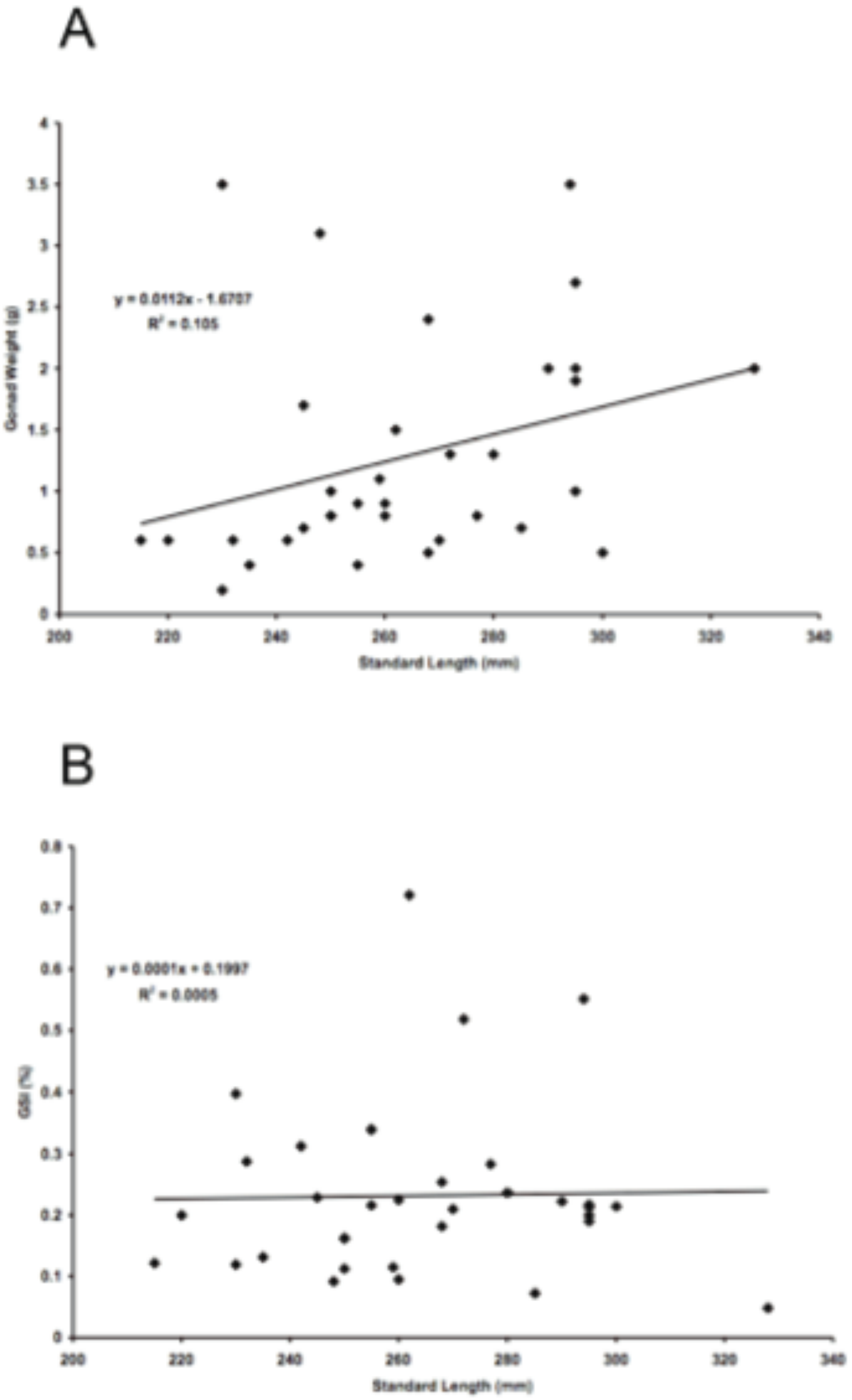


Fig. 4.11. Gonadal investment and body size relationships in reproductively active male *Cephalopholis panamensis*. **A.** Gonad weight (g) versus standard length (mm), **B.** Gonosomatic index (GSI %) versus standard length (mm).

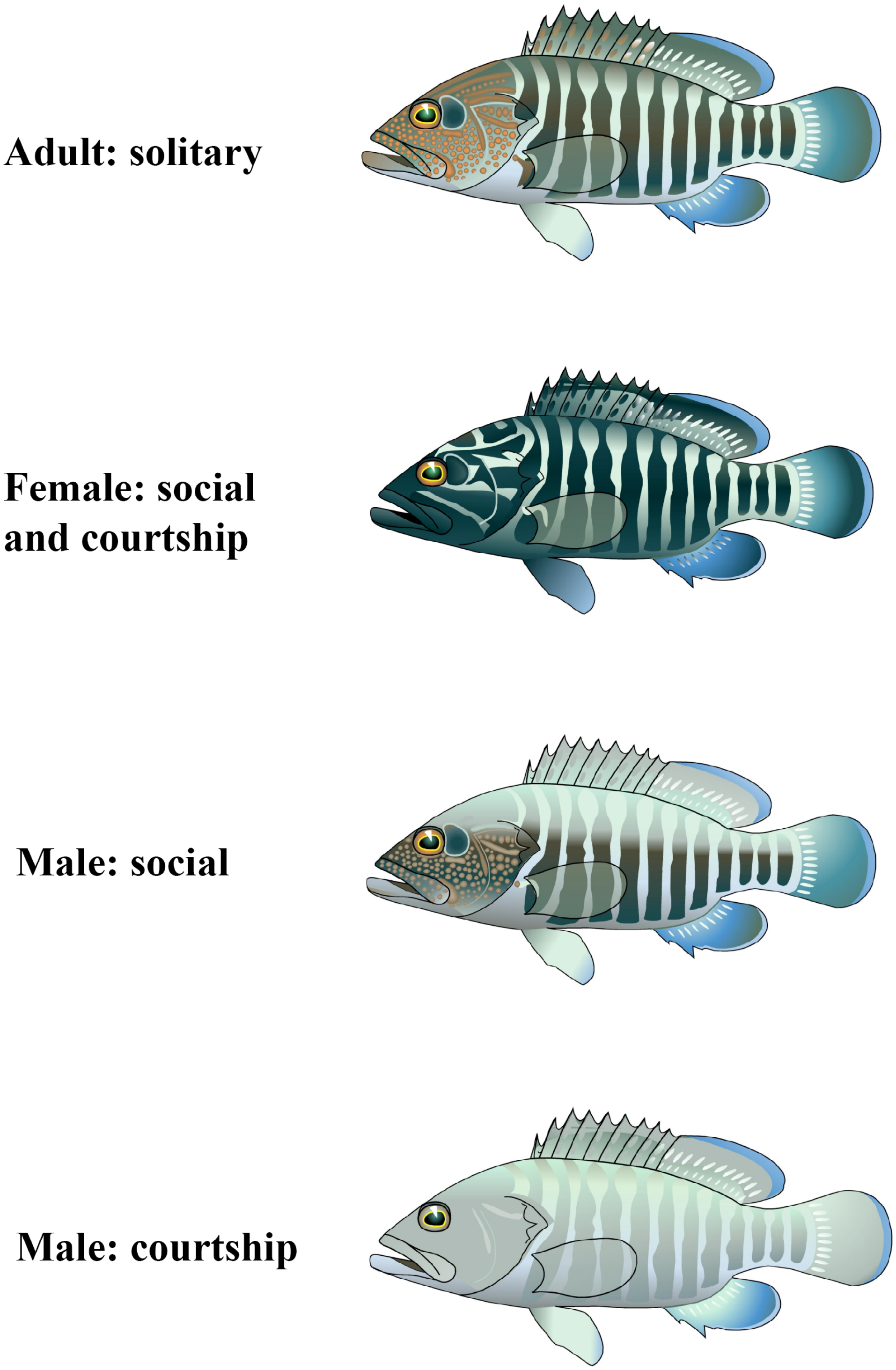


Fig. 4.12. Color patterns of adult *Cephalopholis panamensis*, organized by circumstances under which they are observed.

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CHAPTER 5

Evolution of reproductive anatomy and sexual pattern in epinephelid and serranid fishes (Perciformes, Epinephelidae, Serranidae)

Introduction

The reproductive patterns of teleost fishes are incredibly diverse compared to other vertebrates. Functional hermaphroditism is confirmed in 27 families from seven orders, reported in an additional 21 families from 6 orders, and takes on several forms including gynogenesis, parthenogenesis, protogyny, protandry, simultaneous hermaphroditism, and bi-directional sex change (Sadovy and Liu 2008). There are also substantial variations in the morphology and structural organization of gonads, such as the arrangement of male and female tissues, patterns of gonadal development, and the presence and forms of accessory reproductive structures (Bortone 1977b; Sadovy and Shapiro 1987; Cole 1990; Parenti and Grier 2004; Koya and Munoz 2007). Large-scale phylogenetic analyses of sex determining mechanisms and comparisons of gonad morphology and sexual development indicate that hermaphroditism has evolved several times independently in this group (Smith 1975; Ross 1990; Mank and Avise 2006; Mank et al. 2006; Sadovy and Liu 2008).

Serranid and epinephelid fishes represent an extreme example of teleost reproductive diversity, because significant variations in both sexual pattern and gonad morphology occur among species within both families. Although a few species are gonochoric (separate sexes), most exhibit some form of hermaphroditism (Shapiro 1987; Sadovy and Domeier 2005; Sadovy and Liu 2008). Many are simultaneous hermaphrodites, with individuals functioning as both males and females throughout their lifetime (D’Ancona 1949; Smith and Atz 1969; Bortone 1977a; Fischer and Petersen 1987). Others are protogynous; a form of sequential hermaphroditism in which some or all individuals in a population reproduce first as females and change sex to function as males (Reinboth 1964; Hastings 1981; Wenner et al. 1986; Shapiro 1987). At least two species of serranids exhibit a combination of simultaneous and protogynous hermaphroditism: most fish function as simultaneous hermaphrodites but larger, older individuals absorb their ovarian tissue and function only as males (Hastings and Petersen 1986; Petersen and Fischer 1986). Only protandry (male to female sex change) and bi-directional sex change have not been reported in the wild, although they have been socially or hormonally induced in captivity (Debas et al 1989; Tanaka et al. 1990; Liu and Sadovy 2004a).

Species within both families also differ with respect to the structural organization of gonads, and in particular, the arrangement of male and female tissues. Testicular tissue develops within discrete bands or islets on the posteroventral portion of each gonadal lobe in most serranids (Atz 1964; Smith 1965; D'Ancona 1949; Sadovy and Domeier 2005) but also arises along the edges of the germinal epithelium in a few species (Suzuki et al. 1978; Jones 1980; Webb and Kingford 1992). Male and female germinal tissues are not distinctly separated in most epinephelids but rather are intermixed throughout the gonad (Smith 1965; Sadovy and Liu 2008). However, spermatogenic tissue does originate in specific portions of the gonads in some epinephelids, such as near the dorsal blood vessel, along the ventral junction of the lamellae and allamellar region of the gonad, or within a single median lobe that lies within the dorsal wall of the oviduct (Smith and Atz 1969; Ferreira 1995; Fennessy and Sadovy 2002; Adams 2003; Mackie 2003).

Aspects of gonad morphology have been used often as diagnostic characters to determine phylogenetic relationships among species and genera of serranids (e.g., Okada 1965a,b; Smith 1965; Gosline 1966; Smith and Atz 1969; Smith 1973; Bortone 1977b; Touart and Bortone 1980; Hastings and Petersen 1986), because it was assumed that morphological characters were evolutionarily conservative with respect to other reproductive traits such as sexual pattern and mating behavior, and some have used reproductive anatomy characters to postulate evolutionary pathways in sexual pattern in these fishes (e.g., Smith 1965; Reinboth 1964; Smith and Atz 1969). However, recent studies have demonstrated that aspects of gonadal structure and sexual development in the Serranidae and Epinephelidae are more diverse and complex than previously thought (e.g., Webb and Kingsford 1992; Fennessy and Sadovy 2002; Adams 2003; Erisman et al. 2007), and this diversity may not follow the phylogenetic relationships proposed by many authors (Kendall 1977; Johnson 1983; Meisler 1987; Baldwin and Johnson 1993; Pondella et al. 2003). Moreover, Smith and Craig (2007) concluded that the Serranidae (sensu Nelson 2006) are polyphyletic, with groupers and related fishes (Epinephelidae) distantly separated from anthiines and serranines, which together form a monophyletic Serranidae. These redefined families include approximately 160 (Epinephelidae) and 245 (Serranidae) species. This and other phylogenetic hypotheses provide a new framework in which to reexamine the reproductive evolution of serranid and epinephelid fishes, including patterns of diversification in gonad structure and sexuality among various species and genera within both families.

In this study, I review published literature on the reproductive biology of serranid and epinephelid fishes and attempt to: (1) describe variations in reproductive anatomy and sexual pattern in a common format, (2) reconstruct the evolutionary history of both characters, and (3) compare these results to past hypotheses regarding the evolution of reproductive characters in theses fishes and their use in discerning phylogenetic relationships.

Materials and Methods

Sources of Data

I found published data for sexual pattern or gonad morphology in 50 species of serranids and 57 species of epinephelids (Table 1). Sexual pattern was divided into four categories: protogynous hermaphroditism, gonochorism, unconfirmed gonochorism, simultaneous hermaphroditism, and protogynoid hermaphroditism. Those species for which there is histological evidence or other evidence (i.e., bi-modal size distributions, female-biased sex ratios) of post-maturational sex change from adult female to adult male were classified as protogynous hermaphrodites (Sadovy and Shapiro 1987; Sadovy and Domeier 2005). For protogynous species, no distinction was made between monandric and diandric species, because this distinction is made infrequently in the literature. I categorized a species as a gonochore if histological analyses were performed and gonochorism was reported. A species was categorized as an unconfirmed gonochore if either gonochorism was reported and histological evidence was incomplete, or if histological and population demographic analyses found no evidence of post-maturational sex change. A species was classified as a simultaneous hermaphrodite if histological analyses indicate that individuals function concurrently as males and females throughout their reproductive life span. Two species in the genus *Serranus*, *S. psittacinus* and *S. baldwini* were scored classified as protogynoid hermaphrodites (Harrington 1971), because adults function first as simultaneous hermaphrodites and later absorb their ovarian tissue to function solely as males (Hastings and Petersen 1986; Petersen and Fischer 1986).

Gonad morphology types were classified according to the arrangement of male and female tissues, or more specifically, the location of initial spermatogenic tissue development within the gonads of females or juveniles. The types are summarized in Table 2 and were divided as follows: ventral bands, ventral bands and germinal epithelium, germinal epithelium, ventral germinal epithelium, dorsal germinal epithelium, and central lobe.

Phylogenetic Analyses

For this study, I treated the anthiine and serranine fishes as a family (Serranidae) distinct from the Epinephelidae, because their close relationship has been called into question by phylogenetic hypotheses proposed by Smith and Craig (2007). Phylogenetic trees of included epinephelid taxa reflected results from the maximum-likelihood analyses of the species relationships presented by Craig and Hastings (2007). Phylogenetic trees used for the serranid fishes were built from relationships presented by Meisler (1978), Pondella et al. (2003), and Baldwin and Johnson (1993). Two serranid (*Bullisichthys caribbeus*, *Epinephelides armatus*) and two epinephelid species (*Epinephelus summana, Epinephelus sexfasciatus*) were excluded from all phylogenetic analyses, because information on their systematic relationships are not available. In addition, species for which data on gonad morphology or sexual pattern were lacking were excluded from analyses of those characters (i.e., pruned from trees). I used data from a total of 48 species of serranids and 55 species of epinephelids for the phylogenetic analyses. Data from 43 serranid species and 46 epinephelid species were used to reconstruct the evolutionary history of gonad morphology in each family, and 48 serranids and 55 epinephelids were used to make phylogenetic reconstructions of sexual pattern evolution.

I used the parsimony analysis from MacClade version 4 (Maddison and Maddison 2000) to reconstruct the evolution of both characters in both families. For the epinephelid phylogeny, evolutionary transformations of both characters were reconstructed using both ACCTRAN and DELTRAN resolving options in order to compare scenarios related to the origination and reversal of character states within the phylogeny. The ACCTRAN option chooses character state assignments that accelerate changes toward the root of the tree, maximizing early gains and subsequent reversals. Conversely, the DELTRAN option forces changes toward the tips of the tree and maximizes parallel changes or convergence (Maddison and Maddison 2000). The serranid phylogeny contained numerous polytomies in the anthiine lineage, which precluded the use of ACCTRAN and DELTRAN resolving options. Polytomies in the serranid phylogeny were interpreted as "soft polytomies", which means that they did not indicate a rapid speciation event of several species (i.e., hard polytomies), but rather they reflect unresolved species relationships. Analyses in MacClade produce different reconstructions based on the designation of polytomies as "soft" or "hard", and I used a soft polytomy resolving option in phylogenetic reconstructions of the Serranidae.

Results

I. Reproductive anatomy

Gross morphology of gonads

The gonads in serranids and epinephelids are bilobate, hollow sacs (often asymmetrical) that fuse posteriorly to form a common oviduct. They lie below and posterior to the swim bladder and extend antero-dorsally in the body cavity. The urinary bladder lies on the dorsal surface of the ductal region of the gonads. The gonadal lobes are surrounded by connective tissue, lined with layers of smooth muscle, and supported by mesenteries. Female gonads are usually tubular and rounded in cross-section, whereas those of males are often flattened longitudinally and triangular in cross-section.

The structural organization of the gonads is the same in juveniles, females, and males of most species. The internal structure of the gonad consists of lamellae, folded layers of germinal epithelium that project into a membrane-lined central lumen from the dorsal and lateral walls. The germinal epithelium is composed of both germinal and somatic cells. There are no lamellae on the ventral regions of the lumen in the ductal region, which is continuous with the alamellar oviduct. Two species of anthiines have a structural organization different from that of other serranids and epinephelids. Testes of *Epinephelides armatus* and *Hypoplectrodes huntii* are solid and do not contain a lumen (Jones 1980; Moore et al. 2007).

In females, oocytes develop inside lamellae and are shed into the lumen at ovulation. They then course posteriorly through the alamellar region and oviduct and exit the body at the ovopore, which consists of loose collageneous tissue posterior to the anus but anterior to the genital papilla. In some serranids and epinephelids, eggs pass through a post-ovarian sinus (see *Accessory Gonad structures*), which differs in structure among species, prior to entering the oviduct (Smith and Atz 1969; Touart and Bortone 1980; Hastings 1981).

Germinal tissues in males as in females are arranged in lamellae, which are called lobules in testes (Grier 1993). Lobules extend from the lateral and dorsal gonadal walls into the gonadal lumen. Spermatogenic tissue develops within small crypts that occur throughout the length of a lobule, and individual sperm within a crypt are at the same stage of development. This type of testicular organization was referred to as “acinus” by Smith (1965) and was later categorized as the “unrestricted lobular” testis type by Grier (1993). Mature spermatozoa are transferred to peripheral sperm sinuses from a large network of seminiferous tubules that form through the rupture and joining of crypts. These sinuses may be extensive and surround both the lamellar and alamellar areas of the gonad. Spermatozoa drain peripherally and posteriorly from these sinuses, collect in the sperm duct (vas deferens) and exit the body through the urogenital papilla. Unlike in females, the gonadal lumen and oviduct are not used for sperm transport. Testes in the anthiines *Epinephelides armatus* and *Hypoplectrodes huntii* (and likely *H. maccullochi*) are unusual in that peripheral sperm sinuses are absent and mature sperm drain in sinuses that develop in the center of the gonads (Jones 1980; Webb and Kingsford 1992; Moore et al. 2007).

Arrangement of male and female tissues

Serranidae. In most anthiines, testicular tissue is inactive and restricted to bands or islets on the posteroventral portions of each gonadal lobe in juveniles and females (ventral band type, Table 2, Figure 1a; Reinboth 1963, 1964, 1967b; Atz 1964; Smith 1965; Okada 1965a,b; Fischer 1981; Hastings 1981; Coleman 1983). Thus, the testicular tissue is discretely separated from, and does not intermix with, ovarian tissue. This type of gonadal organization was referred to as “territorial hermaphroditism” by D’Ancona (1949, 1952), and as “undelimited type I” by Sadovy and Shapiro (1987). However, developing oocytes have been reported within the testicular bands in *Sacura margaritacea* and *Pseudanthias elongatus* (Reinboth 1963; Okada 1965a,b).

Similar to anthiines, most serranines follow the undelimited type I of gonadal organization. In *Centropristis*, *Diplectrum*, *Hypoplectrus*, *Paralabrax*, *Serraniculus*, and *Serranus*, testicular tissue is well separated from ovarian tissue and is restricted to bands that lie along the ventrolateral wall of the posterior portion of each gonadal lobe (ventral band type, Table 2, Figure 1a; van Oordt 1929; Dantchakoff 1936; D'Ancona 1949; Reinboth 1962; Atz 1964; Smith 1965; Hastings 1973; Bortone 1977). For example, spermatogenic tissue in *Paralabrax* arises ventrally within the gonad at the two points of junction between the alamellar gonad wall and the lamellae, and they run as bands laterally and medially along the gonad (Hastings 1989; Sadovy and Domeier 2005). Similarly, testicular tissue in *Centropristis* arises peripherally in the posteroventral section of the gonad along the epithelium of the oviduct (Lavenda 1949; Wenner et al. 1986). In most cases, male and female tissues come into close contact with one another but do not intermix. However, the occasional presence of scattered oocytes within testicular tissue of a few individuals does occur in several species of *Serranus* (D'Ancona 1949; Reinboth 1962; Hastings and Bortone 1980).

Male germinal tissue originates outside testicular bands in a few species. Male tissue develops both within bands and along the edges of the germinal epithelium in female lamellae in *Pseudanthias squamipinnis* and *Hemanthias vivanus* (ventral band and germinal epithelium type, Table 2, Figure 1b; Suzuki et al. 1978; Hastings 1981). At least one species of serranine also shows this pattern, as Reinboth (1967a) noted that spermatogenic tissue develops and proliferates from both testicular islets and along the edges of germinal epithelium in *Chelidoperca hirudinacea*. In the anthiine genus *Hypoplectrodes*, male and female tissues are not spatially separated, as spermatogenic crypts develop throughout the germinal epithelium (germinal epithelium type, Table 2, Figure 1c; Jones 1980; Webb and Kingsford 1992).

Although testicular tissue in most serranids remains restricted to bands or islets in juveniles, females, and simultaneous hermaphrodites, it proliferates during development towards functional male tissue in gonochoric species and at sex change in protogynous species. During sexual transition in protogynous and protogynoid species, the proliferation of testicular tissue progresses anteriorly from the bands, where it grows outward in lobes that extend into the ovarian lumen (Atz 1964; Reinboth 1967a,b; Fishelson 1975; Hastings 1981; Wenner et al. 1986). Eventually, male tissue occupies the entire gonad as oocytes break down and are resorbed (Okada 1965a,b; Fishelson 1975; Hastings 1981; Hastings and Petersen 1986). In some anthiines, the gonadal lumen becomes completely occluded during masculinization, and the ripe testes of males show no evidence of a prior ovarian morphology (Jones 1980; Shapiro 1981; Moore et al. 2007). In males of gonochoric species, the same process occurs in juveniles, prior to sexual maturation (Sadovy and Domeier 2005).

Serranids show additional variations in the structure, location, and development of testicular tissue. In *Bullisichthys*, the anterior placement of the vent requires the rotation of the entire reproductive tract (Smith 1973). The testicular tissue in *Paralabrax* species is lined with dense columnar epithelium with basal nuclei. This tissue is not associated with female tissue and is unique to this genus (Smith and Young 1966; Hastings 1989). The testicular tissue in *Diplectrum* is confined to a wide band surrounding the gonad in the region just posterior to the junction of the ovarian lobes, and the shape of the band varies among species. The testicular band in *Diplectrum* is wider than most *Serranus* species (Smith 1965; Reinboth 1962; Bortone 1977b), although the size and shape of the band in *Serranus subligarius* is very similar to *Diplectrum* species (Hastings and Bortone 1980). In many species of *Serranus* (e.g., *S. cabrilla*, *S. phoebe*, *S. tabacarius*), *Hypoplectrus unicolor* and *Serraniculus pumilio* the testicular bands extend anterior of the junction of gonadal lobes, fuse anteriorly and form a loop along the margins of the alamellar region (Reinboth 1962; Smith 1965; Hastings 1973; Fischer 1981).

Epinephelidae. Unlike most serranid fishes but similar to the anthiine genus *Hypoplectrodes*, male and female germinal tissues in epinephelids are not distinctly separated or localized in different areas of the gonad, but rather they are intermixed throughout the gonads in bisexual juveniles, females, and males (germinal epithelium type, Table 2, Figure 1c). This type of configuration was termed the 'epinepheline' type by Smith (1965) and the 'undelimited type II' by Sadovy and Shapiro (1987). Spermatogenic crypts develop in scattered pockets with the ovarian lamellae during post-maturational sex change and in bisexual individuals of most species. Germinal changes associated with sex change usually occur simultaneously throughout the length of the gonads (Smith 1965; Johnson et al. 1998; Marino et al. 2001; Fennessy and Sadovy 2002; Adams 2003).

Although there is no distinct separation of spermatogenic and ovarian tissues, spermatogenic tissue does originate in specific portions of the gonads in some epinephelids. In a few species of *Epinephelus* and *Cephalopholis*, sperm tissue in juveniles and females exist as scattered groups of cells that occur along the edges of germinal epithelium of the lamellae (Smith 1965; Marino et al. 2001). In *E. merra* and *E. morio*, spermatogenic tissue first appears along the edges of lamellae near the gonadal lumen and proceeds towards the central region of lamellae during sex change (Johnson et al. 1998; Bhandari et al. 2003). In juvenile *E. striatus*, oocytes border lamellar margins and chords of seminiferous tubules and spermatogenic tissue arise centripetally (Sadovy and Colin 1995).

In other epinephelids, male germinal tissue arises parietally along the dorsal region of the gonads (dorsal germinal epithelium type, Table 2, Figure 1d). In *E. rivulatus*, all mature females contain male tissue, which is most common in aggregations near the dorsal blood vessel (Mackie 2003). Similarly, spermatogenic tissue in *E. andersoni* originates close to and on either side of the dorsal blood vessel from the posterior to mid-portion of the gonad and expands centripetally in lamellae and along the length of the gonad during masculinization (Fennessy and Sadovy 2002). In several *Plectropomus* species, sperm production begins in close proximity to the dorsal sperm sinuses and dorsal blood vessel in the proximal region of the gonad, although subsequent male development occurs throughout the length of the gonad (Ferreira 1995; Adams 2003). Also, precursory sperm sinuses develop in the dorso-medial region of the proximal, central, and distal portions of the gonadal lobes of juveniles and females, and these develop into sperm sinuses in developing males (Adams 2003). *Cephalopholis cyanostigma* also shows initial development of male tissue in the proximal portion of the female gonad during early sexual transition

(Mosse 2003). In *E. polyphekadion*, sperm crypts develop along the parietal areas of lamellae and proliferate outward in a centripetal pattern throughout the gonad during sex change (Brusle et al. 1989).

Species within two genera of epinephelids, *Pseudogramma* and *Rypticus*, exhibit patterns of gonad structure that are unique in the Epinephelidae. Smith (1965) performed a brief investigation into the reproductive biology of two species of *Rypticus* (*R. saponaceus* and *R. bistrispinus*). He found the gonad morphology of *Rypticus* to share characteristics with both serranine and epinepheline types. Similar to serranines, testicular tissue is initially confined to the base of the lamellae next to the alamellar junction at either side of the gonad (ventral germinal epithelium type, Table 2, Figure 1e). However, oocytes intermingle with spermatogenic crypts in these areas and thus are not clearly separated. The gonad structure of *Pseudogramma gregoryi* is by far the most unusual and bears little resemblance to that of other epinephelids. The testicular tissue is confined to a single median lobe within the dorsal wall of oviduct, posterior to the junction of the gonadal lobes (central lobe type, Table 2, Figure 1f)(Smith and Atz 1969; Sadovy and Liu 2008). Also, ovarian tissue within each gonadal lobe forms a single, medial ridge rather than multiple lamellae.

Accessory gonad structures

Eleven of 12 species of the serranine genus *Diplectrum* have a post-ovarian sinus at the posterior end of the gonad. This structure is well developed and forms an accessory reproductive structure (ARS) in at least two species, *D. pacificum* and *D.*

bivittatum (Bortone 1977a,b,c; Touart and Bortone 1980). The ARS consists of a post-ovarian sinus lined with cuboidal epithelial cells and numerous villae-like structures. The villae are made up of vascularized, elongated ovarian folds that project into a central lumen from the sinus wall. The ARS varies seasonally and is enlarged during the reproductive season. The exact function of this structure is not known, but it often contains mature ova and thus may be used to store ovulated oocytes prior to release during spawning (Bortone 1977a; Touart and Bortone 1980).

Several species of *Serranus* and one species of the anthiine genus *Hemanthias* have accessory reproductive structures, although they are less developed than in *Diplectrum*. The accessory structure in *S. atricauda* is simpler in structure but similar to that of *D. bivittatum* and *D. pacificum* in that it is present at the dorso-posterior region of the gonad, is surrounded by smooth-walled epithelium, is more developed in spawning individuals, and may contain a few mature oocytes (Bortone 1977c; Garcia-Diaz et al 2002). The floor of the post-ovarian sinus in *S. subligarius* is slightly convoluted, lined with epithelium, but rarely contains mature ova (Hastings and Bortone 1980). A similar but less developed structure occurs in the anthiine, *Hemanthias vivanus*, in which eggs pass through a post-ovarian sinus prior to entering the oviduct. The sinus is small, smooth-walled, and lined with epithelium (Hastings 1981). In male *S. psittacinus*, there are cul-de-sacs that may serve as sperm reservoirs or facilitate sperm release projecting from the vas deferens and lying within smooth muscle bands (Hastings and Petersen 1986).

Accessory reproductive structures have only been found in one epinephelid. *Pseudogramma gregoryi* contains an oviducal pouch that is similar in some ways to structures found in *Diplectrum* and some *Serranus* species. The structures found in all these fishes are lined with columnar epithelia, ventral-posterior in position, vascularized internally and more developed in spawning individuals (Smith and Atz 1969; Bortone 1977a). However, unlike the accessory structures found in some serranines and anthiines, the oviducal pouch of *P. gregoryi* is formed by the invagination of the common oviduct that connects directly to the body cavity through an opening in the antero-ventral portion of the oviduct. Also, the oviducal pouch of *P. gregoryi* lack the elongate villae present in *Diplectrum* (Smith and Atz 1969; Bortone 1977a; Touart and Bortone 1980).

II. Reproductive Development

Bisexual juveniles

The reproductive developmental pathways of serranids and epinephelids follow an "undifferentiated" sequence of gonadal development (Yamamoto 1969; Francis 1992). Gonadal differentiation begins as ovarian and development of male tissue occurs later during a bisexual juvenile stage (D'Ancona 1949; Reinboth 1970; Brusle 1983; Shapiro 1992; Siau 1994; Sadovy and Colin 1995; Erisman et al. 2007). Bisexual juvenile gonads are comprised mainly of previtellogenic oocytes with areas of undeveloped spermatogenic tissue (gonia and cysts of early spermatogenesis), and they show no evidence of prior spawning activity as either sex (Liu and Sadovy 2004b; Sadovy and Domeier 2005; Sadovy and Liu 2008). The bisexual juvenile stage leads to maturation of either or both sexes (for simultaneous hermaphrodites), and germinal tissue of the non-functional sex is retained from the juvenile stage but diminishes with size and age in gonochores and protogynous species (D'Ancona 1949; Siau 1994; Sadovy and Domeier 2005). There is no relationship between a bisexual juvenile stage and functional sexuality in teleost fishes, as this stage occurs in gonochores, simultaneous and sequential hermaphrodites, including both protandrous and protogynous species (Zohar 1978; Francis and Pankhurst 1988; Shapiro 1992; Sadovy and Colin 1995; Asoh and Shapiro 1997; Miura et al. 2003).

Within the Serranidae, bisexual juveniles have been identified in at least four genera of anthiines: *Hemanthias* (Hastings 1981), *Hypoplectrodes* (Webb and Kingsford 1992), *Pseudanthias* (Suzuki et al. 1978), and *Sacura* (Okada 1965b); and four genera of serranines: *Centropristis* (Wenner et al. 1986), *Diplectrum* (Bortone 1977c), *Paralabrax* (Hastings 1989; Borquez et al. 1998; Sadovy and Domeier 2005), and *Serranus* (Brusle 1983; Garcia-Diaz et al. 1997; Garcia-Diaz et al. 2003).

Bisexual juvenile gonads have been described in six genera of epinephelids: *Alphestes* (Erisman 2008), *Cephalopholis* (Smith 1965; Siau 1994; Chan and Sadovy 2002: Liu and Sadovy 2004b; Erisman 2008), *Epinephelus* (Moe 1969; Tanaka et al. 1990; Marino et al. 2001; Mackie 2003; Fennessy and Sadovy 2002; Rhodes and Sadovy 2002; Erisman 2008), *Mycteroperca* (Erisman et al. 2007), *Plectropomus* (Adams 2003), and *Pseudogramma* (Smith and Atz 1969). In some species, all fish pass through a juvenile bisexual phase prior to maturation as either a male, female, or a simultaneous hermaphrodite (Sadovy and Colin 1995, Chan and Sadovy 2002). In others, this stage is believed to be associated only with male development (Fennessy and Sadovy 2002; Rhodes and Sadovy 2002; Erisman et al. 2007). In *Epinephelus andersoni* and several species in the genus *Plectropomus*, precocious sperm sinuses develop in bisexual juveniles and females prior to sex change (Fennessy and Sadovy 2002; Adams 2003).

Bisexual adults

Bisexual adults are individuals with gonads that contain germinal tissue of both sexes, with the germinal tissue of one or both sexes being sexually mature. The term refers only to gonad morphology and is not meant to imply sexual function (Sadovy and Domeier 2005). Bisexual adult gonads have been reported in mature females in gonochoric and protogynous species in both subfamilies of serranids and in epinephelids. In anthiines, testicular tissue is present in mature females in several species of *Anthias*, *Hemanthias*, *Pseudanthias,* and in *Sacura margaritacea* but is absent in investigated species of *Epinephelides* and *Hypoplectrodes* (Reinboth 1963,1964; Atz 1964; Okada 1965a,b; Fishelson 1970; Suzuki et al. 1978; Jones 1980; Coleman 1981; Hastings 1981; Coleman 1983; Moore et al. 2007). Bisexual female gonads are also common among serranine fishes. Reinboth (1967a) noted that *Chelidoperca* have spermatogenic tissue in functional females. Similarly, spermatogenic tissue is present in females of all maturity states in species of

Paralabrax (Hovey and Allen 2000; Baca Hovey et al 2002; Sadovy and Domeier 2005). All species of simultaneously hermaphroditic serranines (e.g., *Hypoplectrus*, *Serranus*, *Diplectrum*) are characterized by bisexual adult gonads in which the germinal tissues of both sexes are active (Bortone 1977a; Fischer 1981; Brusle 1983; Fischer and Petersen 1987; Garcia-Diaz et al. 1997).

In the Epinephelidae, the presence of male germinal tissue in adult females has been reported in species of *Alphestes* (Erisman 2008), *Cephalopholis* (Smith 1965; Chan and Sadovy 2002; Erisman 2008), *Epinephelus* (Reinboth 1967; Brusle and Brusle 1975; Sadovy and Colin 1995; Bullock et al. 1996; Marino et al. 2001; Erisman 2008), *Mycteroperca* (Bullock et al. 1994; Brule et al. 2003), *Plectropomus* (Ferreira 1995; Adams 2002, 2003), and *Rypticus* (Smith 1965). Spermatogenic tissue is present in all female developmental stages in several species of *Cephalopholis* (Smith 1965; Siau 1994; Chan and Sadovy 2002) and *Epinephelus* (Marino et al. 2001; Fennessy and Sadovy 2002; Mackie 2003). In other epinephelids, male tissue is present in juvenile and inactive females but absent in active females (Moe 1969; Erisman et al. 2007). Testicular tissue was not found in females of two species of *Liopropoma* (Smith 1971b), although only five specimens from each species were examined.

Bisexual males, where the gonads consist of functional spermatogenic tissue with small amounts of scattered, inactive, ovarian tissue, occur in species of both families. Previtellogenic oocytes are common in males of many species and are thought to be remnants of the juvenile bisexual stage or of a prior functional female stage (van Oordt 1933; Smith 1959; Reinboth 1967b; Jones 1980; Coleman 1981;

Mackie 2003). In many cases, atretic vitellogenic oocytes also remain in males following post-maturational sex change (Hastings 1981; Adams 2003; Mackie 2006).

In the Serranidae, bisexual males have been reported in species of the anthiines *Epinephelides* (Moore et al. 2007), *Hemanthias* (Hastings 1981; Coleman 1983), *Hypoplectrodes* (Jones 1980; Webb and Kingsford 1992), *Odontanthias* (Coleman 1981), and *Pseudanthias* (Fishelson 1975), and the serranine genus *Paralabrax* (Sadovy and Domeier 2005). Bisexual males in the Epinephelidae have been described in several genera, including *Alphestes* (Erisman 2008), *Cephalopholis* (Smith 1965; Siau 1994; Chan and Sadovy 2002; Liu and Sadovy 2004b; Erisman 2008), *Epinephelus* (van Oordt 1933; Smith 1959; Reinboth 1967b; Brusle and Brusle 1975; Mackie 2003; Erisman 2008), *Liopropoma* (Smith 1971b), *Mycteroperca* (Smith 1959; Collins et al. 1987; Bullock and Murphy 1994; Brule et al. 2003a,b; Erisman et al. 2007), *Plectropomus* (Adams 2002, 2003) and *Pseudogramma* (Smith and Atz 1969). Female germinal cells are apparently absent in the ripe testes in several species of *Epinephelus* and at least one species of *Mycteroperca* (Marino et al. 2001; Rhodes and Sadovy 2002; Bhandari et al. 2003; Erisman et al. 2007; Erisman 2008).

Sexual Patterns

Four different sexual patterns have been reported in the Serranidae. All but one investigated species of anthiinea are believed to be protogynous hermaphrodites (Reinboth 1963, 1964; Okada 1965a,b; Fishelson 1970; Heemstra 1973; Fishelson 1975; Suzuki et al. 1978; Jones 1980; Hastings 1981, 1983). The exception is

Epinephelides armatus, which is reported to be gonochoric (Moore et al. 2007). The serranines exhibit gonochorism (*Paralabrax*), simultaneous hermaphroditism (*Bullisichthys*, *Diplectrum*, *Hypoplectrus*, *Serranus*, *Serraniculus*), protogynous hermaphroditism (*Chelidoperca*, *Centropristis*), and even protogynoid hermaphroditism (*Serranus baldwini* and *S. psittacinus*). Most species of *Paralabrax* are functional gonochores, although putative transitional individuals of *P. nebulifer* were reported by Baca-Hovey et al. (2002) and the sexual pattern of *P. maculatofasciatus* has been argued to be both protogynous and gonochoric by several authors (Hovey and Allen 2000; Sadovy and Domeier 2005; Hastings 1989). All investigated species of *Diplectrum* are reported as simultaneous hermaphrodites (Bortone 1977). All but two species of *Serranus* are simultaneous hermaphrodites. In *S. psittacinus* and *S. baldwini*, most individuals possess ovotestes and are functional simultaneous hermaphrodites. However the largest fish have only testicular tissue and function strictly as males (Hastings and Petersen 1986). This type of hermaphroditism was called "protogynoid hermaphroditism" by Harrington (1971), who coined the term in his descriptions of male *Kryptolebias marmoratus* (Cypridontiformes: Rivulidae) that developed from adult, self-fertilizing, simultaneous hermaphrodites.

Most epinephelids are protogynous hermaphrodites (Smith 1959, 1965; Moe 1969; Shapiro 1987; Sadovy and Liu 2008), but three species of groupers (*Cephalopholis furcifer*, *Epinephelus striatus,* and *Mycteroperca rosacea*) are now recognized as functional gonochores based on histological, demographic, and mating system data (Sadovy and Colin 1995; Erisman et al. 2007). Two species of

Liopropoma were investigated by Smith (1971) and both were classified as gonochores, although only a small number of specimens was examined. It is likely that several other species are gonochoric. For example, *E. itajara* and *E. polyphekadion* have 1:1 sex ratios, extensive overlap in the size and age range of sexes, and transitional individuals have not been identified from histological examinations of gonads (Smith 1959, 1971; Bullock and Smith 1991; Rhodes and Sadovy 2002). In this study (see below), these are called ‘unconfirmed gonochores’.

Male to female sex change (protandry) has been socially or hormonally induced in captive studies in *Epinephelus akaara* and *Cephalopholis boenak* (Tanaka et al. 1990; Okumura 2001; Liu and Sadovy 2004), but this form of hermaphroditism has not been found in the wild for any species. Smith (1965) presented the closest evidence of protandry for *Alphestes afer*: size distributions were bimodal, with the smallest size classes dominated by males and larger ones by females. Despite this, Smith concluded that the species was protogynous.

III. Evolutionary Patterns

Evolution of Gonad Morphology

The ventral band gonad type (VB, Figure 2) is the most common form in the Serranidae, as it occurs in most anthiines and all but one species of serranine. Phylogenetic reconstructions of gonad morphology evolution show that this character state is the ancestral form of gonad morphology in the family. The ventral band and germinal epithelium type (VB&GE) has evolved at least twice in two separate genera (*Pseudanthias* and *Hemanthias*) within the anthiine lineage and in the serranine genus *Chelidoperca*. The germinal epithelium gonad type (GE) evolved only once within the Serranidae in the genus *Hypoplectrodes*.

Four separate gonad morphology types have evolved in the Epinephelidae, and the ancestral character state was equivocal and could not be resolved by phylogenetic analyses (Figure 3). The GE gonad type is the most widespread character state of gonad morphology, where it is reported in 36 of 46 species from five separate genera (*Cephalopholis*, *Epinephelus*, *Alphestes*, *Hyporthodus*, and *Mycteroperca*). The dorsal germinal epithelium type (DGE) evolved independently five separate times within the family in three different genera (*Plectropomus*, *Epinephelus*, and *Mycteroperca*). Two character states evolved within the soapfish lineage. The ventral germinal epithelium type (VGE) evolved once in the genus *Rypticus*, and the central lobe (CL) gonad type evolved uniquely in the genus *Pseudogramma*.

Sexual Pattern Evolution

Protogyny is reported in all but one species of anthiine and in two genera in the serranines, *Chelidoperca* and *Centropristis* (Figure 4). A phylogenetic reconstruction of sexual pattern evolution predicts that protogyny is the plesiomorphic character state of sexual pattern of the Serranidae from which other states evolved. Gonochorism evolved only once from protogyny, within the genus *Paralabrax*. Simultaneous hermaphroditism evolved once in the lineage that includes species of *Hypoplectus*, *Serranus*, and *Diplectrum*, and it evolved from protogyny. Protogynoid hermaphroditism evolved once in two species of *Serranus* and is derived from the simultaneous hermaphroditic character state.

Protogynous hermaphroditism is the most pervasive sexual pattern in the Epinephelidae, where it is reported for 47 species from seven genera (*Alphestes*, *Cephalopholis*, *Epinephelus*, *Hyporthodus*, *Mycteroperca*, *Plectropomus*, *Rypticus*), and it is hypothesized as the ancestral character state of sexual pattern in all phylogenetic reconstructions (Figures 5-6). There is evidence of gonochorism in seven species, and it has evolved independently at least four and as many five times in three genera: *Cephalopholis*, *Epinephelus*, and *Mycteroperca*. The ACCTRAN reconstruction predicted four independent evolutions of gonochorism (Figure 5), whereas DELTRAN predicted five independent evolutions (Figure 6). No unequivocal instances of the transition from gonochorism to protogyny are evident in groupers; a reversal was reported for *Epinephelus fuscoguttatus* in only one ACCTRAN reconstruction. Simultaneous hermaphroditism arose once in the outgroup lineage, where it is reported only in *Pseudogramma gregoryi*.

Discussion

Reproductive anatomy

Serranid and epinephelid fishes exhibit a remarkable diversity with respect to reproductive anatomy. The internal arrangement of male and female tissues, and in particular the proliferation of spermatogenic tissue during masculinization, shows considerable variation in both families. Some gonad arrangements, such as the ventral band type in serranines and the germinal epithelium type in epinephelines are widespread and conserved throughout certain lineages. However, there are multiple tissue arrangement types in both families, and in some cases, multiple gonad morphology types are present within a single genus. In addition, evolutionary transformations in gonad types do not strictly follow taxonomic lines and remarkable changes in gonad morphology have evolved in closely related species and genera.

The testes of most species of epinephelids and serranids retain a gonadal lumen from an earlier ovarian phase, either as a juvenile bisexual stage, or as a functional female in those that change sex. However, in a few species the testes are completely solid with no evidence of a lumen (Smith 1965; Webb and Kingsford 1992; Sadovy and Liu 2008). The developmental history of these solid testes has not been examined. Likewise, males usually develop peripheral sperm sinuses in their testes that facilitate the transport of mature spermatozoa towards the main sperm duct for spawning. However, these structures are absent in at least one genus of each family. Both *Hypoplectrodes* (Serranidae) and *Pseudogramma* (Epinephelidae) utilize a central sperm sinus for the transport of sperm (Smith and Atz 1969; Webb and Kingsford 1992). A few representatives in each family have developed accessory reproductive structures for gamete storage (Smith and Atz 1969; Bortone 1977), and the entire reproductive tract is rotated with the vent anterior to the anus in the serranid genus *Bullisichthys* (Smith 1973).

Wide variation in the reproductive anatomy of serranid and epinephelid fishes appears to be typical of many teleost fishes, as other families show equivalent degrees of morphological diversity and many possess similar gonadal development and morphological traits. In families such as the Nemipteridae, Lethrinidae, Centracanthidae, Labridae, Pinguipedidae, Cirrhitidae, and Scaridae both primary males and secondary males possess a gonadal lumen and most develop peripheral sperm sinuses (Nakazono et al. 1985; Ebisawa 1990; Hoffman 1980; Cole 2003; Sadovy and Donaldson 1995; Sadovy and Liu 2008). However, some show considerable variation in this regard, as a few species of the Nemipteridae and Pomacentridae have solid testes containing no evidence of an ovarian lumen (Young and Martin 1988; Sadovy and Liu 2008). A bisexual juvenile phase is also common in many families that follow an undifferentiated sequence of gonadal development and the appearance of this phase bears no apparent relationship to sexual pattern (Yamamoto 1969; Francis 1992). Similarly, the presence of precocious sperm sinuses in bisexual juveniles is not unique to the Epinephelidae, as it has been described in gobies, damselfishes, and sparids (Brusle-Sicard and Reinboth 1990; Cole 1990; Brusle-Sicard and Fourcalt 1997). The arrangement of proliferation of male and female germinal tissues also differs among species within a number of families. For example, the Cirrhitidae and Nemipteridae show patterns similar to those described for serranid fishes, where spermatogenic tissue in most species arises at the junction of the alamellar ovarian wall and the lamellae but develops sporadically throughout the germinal epithelium in others (Sadovy and Donaldson 1995; Sadovy and Liu 2008).

The combination of patterns described in the Serranidae and Epinephelidae and those described for many other teleost families suggests that gonad morphology in teleost fishes is relatively complex and somewhat labile compared to other vertebrates. Therefore, some caution is warranted when using details of gonad morphology alone in discerning species relationships of closely related taxa.

Sexual Patterns

Differences in the sexual patterns among species within the Serranidae and Epinephelidae are striking. Four sexual patterns (gonochorism, simultaneous hermaphroditism, protogyny, and protogynoid hermaphroditism) are reported for the Serranidae and three (gonochorism, simultaneous hermaphroditism, protogyny) occur in the Epinephelidae. The evolution of sexual patterns in serranid fishes has intrigued researchers for several decades. In all cases, earlier workers included the Epinephelidae within an expanded Serranidae. I treat them separately based on results from recent phylogenetic analyses (Smith and Craig 2007), evaluating character evolution separately in the Epinephelidae and in the Serranidae *sensu stricto* (including the Serraninae and Anthiinae). However, it is important to point out that considering the Epinephelidae and Serranidae *sensu stricto* as sister groups (as done by earlier workers) would not alter the most parsimonious reconstruction of the evolution of sexual pattern in these fishes (see below).

Smith (1965) and Reinboth (1967) both reasoned that simultaneous hermaphroditism was the plesiomorphic character state of sexuality in the Serrranidae (*sensu lato*) from which protogynous hermaphroditism evolved. This was based in part on their interpretation of *Serranus* and related genera as morphologically generalized relative to other serranids (Smith 1971), as well as details of the reproductive anatomy of these fishes. Smith (1965) contended that the pattern of regionally separated but synchronously functioning male and female tissues in simultaneous hermaphrodites was the 'generalized condition.' From this, protogyny was said to have evolved in more 'advanced' species in which there is complete mixing of the male and female elements. Thus he constructed an evolutionary scenario in which members of the genus *Serranus* and other genera with simultaneous hermaphroditism were ancestral, with protogynous species (e.g., *Epinephelus*) as derived, and members of the genus *Rypticus* as intermediate between these groups. Based on Smith's criteria (1965), Reinboth (1967) concluded that *Chelidoperca* was also an intermediate between the 'primitive' *Serranus* form (territorial and simultaneous hermaphroditism) and the more derived *Epinephelus* form (non-territorial and protogynous).

My results do not support this scenario. Instead, the most parsimonious phylogenetic reconstructions of the evolutionary history of sexual patterns in the Serranidae (*sensu stricto*) and Epinephelidae indicate that protogynous hermaphroditism is the ancestral character state in both groups, and other forms of hermaphroditism evolved secondarily from this state. In the Epinephelidae, protogyny is the predominant and plesiomorphic condition, while simultaneous hermphroditism evolved uniquely in *Pseudogramma gregoryi* and gonochorism evolved independently multiple times. In the Serranidae, protogyny is the plesiomorphic condition being present in all members of the subfamily Anthiinae, as well as the early branching lineages of the Serraninae including the genera *Cheiloperca* and *Centropristis*. Gonochorism evolved uniquely in *Paralabrax*, while simultaneous hermaphroditism evolved once in the lineage leading to *Hypoplectrus*, *Diplectrum*, *Serranus* and related genera. Protogynoid hermaphroditism evolved once in the common ancester of a transisthmian species pair that is nested well within the otherwise simultaneous genus *Serranus*.

My results agree with those of Smith (1965), however, in the assessment of gonochorism and its evolutionary status within the Serranidae *sensu stricto*. Smith (1965) coined the term "secondary gonochorism" in his description of the genus *Paralabrax*. He recognized that functional sex change did not occur in some species but that juveniles contained bisexual gonads. As a result, he concluded that members of the genus *Paralabrax* were derived from hermaphroditic ancestors and that gonochorism was a derived sexual pattern in the lineage. In this study, I found phylogenetic evidence that gonochorism evolved uniquely in the Serranidae in the genus *Paralabrax* as hypothesized by Smith (1965) and repeatedly in the Epinephilidae.

More recently, Sadovy and Domeier (2005) offered an alternative hypothesis for the evolution of sexual patterns in the Serranidae. Based on the phylogenetic hypothesis proposed by Pondella et al. (2002) they concluded that the sexual pattern exhibited by *Paralabrax* reflected the ancestral sexual pattern for the family. More specifically, they hypothesized that the non-functional hermaphroditism (i.e., bisexual morphology without functional sex change) of several species in that genus indicated a proto-hermaphroditic ancestry in the Serranidae. A broader consideration of the phylogenetic relationships of these fishes, together with a strictly parsimony-based interpretation of character evolution (Maddison and Maddison 2000) does not support their conclusions. In addition to treating the Epinephelidae separately, I incorporated additional phylogenetic hypotheses in this study that place the subfamily Anthiinae as sister to the subfamily Serraninae (Meisler 1978; Baldwin and Johnson 1993) and include the genera *Paralabrax*, *Centropristis* and *Cheilidoperca* as sequential outgroups of the remainder of the Serraninae (Meisler 1978; Pondella et al. 2002). These relationships, with the first three lineages of the Serranidae as exclusively protogynous, supports my conclusion that protogyny is the ancestral sexual pattern in the family. Similarly, the prevalence of protogyny as the ancestral sexual pattern of all major lineages of the Epinephelidae, is consistent with a protogynous ancestor in that lineage as well.

Similar to morphological changes in gonad morphology, this diversity of sexual patterns reflects a larger pattern within the teleost fishes, as several families are known to exhibit multiple sexual patterns. Three different sexual patterns have been reported in the Platycephalidae, Muraenidae, Pseuodochromidae, Sparidae, Pomacentridae, Labridae, and the Gobidae, and multiple types of hermaphroditism occur within a single genus in the Sparidae (Fujii 1974; Cole 1990; Sadovy and Liu 2008).

Relationship of reproductive anatomy and sexual pattern

Two aspects of the gonad morphology of serranids and epinephelids appear to facilitate the evolution of their diverse sexual patterns. First, the presence of a juvenile bisexual stage in which presumptive male and female gonia are present, sets the stage of deflection of sexual expression in a variety of ways. It has been argued that this feature has led to the prevalence of hermaphroditsm in fishes in general, and it certainly applies to serranids and epinephelids (Sadovy and Liu 2008).

A second aspect of the gonad morphology of the Serranidae *sensu stricto* that appears to facilitate the evolution of their varied sexual patterns is the presence of presumptive male tissue in the ventral bands along the margin between the ovarian lamellae and the posterior alamellar region. This tissue band is inactive in functional females of protogynous species, but it develops and expands during sex change while ovarian tissue degenerates and is resorbed (Hastings 1981; Wenner et al. 1986; Bortone 1977a; Sadovy and Domeier 2005). It has been reported in a few protogynous serranids that testicular tissue also develops from areas scattered throughout the ovarian lamellae, but even in these the ventral bands contribute substantially to the testis of sex-changed males. In simultaneous serranids, these ventral bands are the sole location of active testicular tissue. They range in size from relative thin bands (e.g., *Serranus tigrinus*, Smith 1965) to broad masses of testicular tissue (e.g., *Diplectrum* species; Bortone 1977a,b). Regardless of its relative size, the testicular tissue of simultaneously hermaphroditic serranids remains restricted to the margin of the alamellar section and spatially segregated from the ovarian tissue. The protogynoid hermaphrodite *Serranus psittiscinus* reflects the patterns seen in both simultaneous and protogynous hermaphrodites: the testicular tissue is restricted the margin of the alamellar section of the ovotestis in hermaphrodites and it is from this region that testicular tissue proliferates in males (Hastings and Petersen 1986).

The predominant arrangement of presumptive male tissue in most epinephelids differs and may constrain the evolution of simultaneous hermaphroditism in these fishes. Unlike the condition in serranids, ventral testicular bands are absent in epinephelids and proliferation of testicular tissue occurs exclusively from cells scattered throughout the ovarian lamellae. This lack of spatial segregation of presumptive male and female tissues would seem to preclude the development of separate structures for collection and independent release of male and female gametes. The only known simultaneous species of epinephelid, *P. gregoryi*, has a different gonad structure with testicular tissue restricted to a central lobe and a central sperm duct (Smith and Atz 1969). It would be informative to know the sequence of character evolution leading to this species. This constraint hypothesis predicts that gonad morphology changed prior to or in concert with the evolution of simultaneous hermaphroditism in this lineage.

This and other issues in the evolution of serranid and epinephelid reproductive patterns await additional information on the anatomy and sexual pattern of these fishes. This is especially true for the soapfishes (*Rypticus* and relatives) and basslets (*Liopropoma*) in the Epinephelidae and *Hypoplectrodes* and *Chelidoperca* in the Serranidae. These poorly studied groups show considerable variation in reproductive anatomy and sexual pattern that may hold further clues to the evolution of reproductive strategies in these fishes.

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Table 5.1. Gonad types and sexual patterns reported for serranid and epinephelid fishes. CL = central lobe, DGE = dorsal germinal epithelium, G = gonochorism, GE = germinal epithelium, GR = group spawning, GU = gonochorism unconfirmed, MI = mixed spawning, NA = no data available, PA = paired spawning, P = protogynous hermaphroditism, PG = protogynoid hermaphroditism, S = simultaneous hermaphroditism, VB = ventral bands, VB & GE = ventral bands and germinal epithelium, VGE = ventral germinal epithelium. * Indicates that the species was excluded from phylogenetic analyses.

Family	**Subfamily**	**Species**	**Gonad Type**	**Sexual Pattern**	**References**
Serranidae	Anthiinae	*Anthias anthias*	VB	P	Reinboth 1963, 1964
		Anthias noeli	VB	P	Anderson and Baldwin 2000
		Anthias nicholsi	VB	P	Anderson and Baldwin 2000
		*Epinephelides armatus**	NA	G	Moore et al. 2007
		Hemanthias leptus	NA	P	Anderson and Baldwin 2000
		Hemanthias peruanus	VB	P	Coleman 1983
		Hemanthias vivanus	VB & GE	P	Hastings 1981
		Hypoplectrodes huntii	GE	P	Jones 1980
		Hypoplectrodes macullochi	GE	P	Webb and Kingsford 1992
		Odontanthias martinicensis	VB	P	Coleman 1981
		Pseudanthias bicolor	NA	P	Howe 1996
		Pseudanthias conspicuus	NA	P	Heemstra 1973
		Pseudanthias elongatus	VB	P	Reinboth 1963, 1964
		Pseudanthias squamipinnis	VB & GE	P	Fishelson 1975; Suzuki et al. 1978; Shapiro 1981
		Pseudanthias taeniatus		P	Katayama 1978
		Sacura margaritacea	VB	P	Reinboth 1963; Okada 1965a,b; Suzuki et al. 1974
Serranidae	Serraninae	*Bullisichthys caribbeus**	VB	S	Smith and Erdman 1973
		Centropristis ocyurus	VB	P	Link 1980; Wenner et al. 1986
		Centropristis philadelphica	VB	P	Link 1980; Wenner et al. 1986
		Centropristis striata	VB	P	Lavenda 1949; Link 1980; Wenner et al. 1986
		Chelidoperca hirudinacea	VB & GE	P	Reinboth 1967a
		Diplectrum bivittatum	VB	S	Bortone 1977b; Touart and Bortone 1980

Table 5.1. Continued.

Family	**Subfamily**	**Species**	**Gonad Type**	**Sexual Pattern**	**References**
		Diplectrum formosum	VB	S	Bortone 1971; Bortone 1977b
		Diplectrum macropoma	VB	S	Bortone 1977b,c
		Diplectrum pacificum	VB	S	Bortone 1977a,b,c
		Diplectrum rostrum	VB	S	Bortone 1974, 1977b
		Hypoplectrus chlorurus	VB	S	Barlow 1975; Ramirez 1984
		Hypoplectrus nigricans	VB	S	Fischer 1981; Petersen 1991
		Hypoplectrus puella	VB	S	Ramirez 1984
		Hypoplectrus unicolor	VB	S	Smith 1959; Barlow 1975; Fischer 1981; Ramirez 1984
		Paralabrax auroguttatus	VB	G	Sadovy and Domeier 2005
		Paralabrax clathratus	VB	G	Smith and Young 1966, Oda et al. 1993; Sadovy and Domeier 2005
		Paralabrax humeralis	NA	G	Borquez et al. 1998
		Paralabrax maculatofasciatus	VB	G	Hastings 1989; Oda et al. 1993; Hovey and Allen 2000; Sadovy and Domeier 2005
		Paralabrax nebulifer	VB	G	Oda et al. 1993; Baca-Hovey and Allen 2002; Sadovy and Domeier 2005
		Schultzea beta	VB	NA	Robins and Starck 1961
		Serraniculus pumilio	VB	S	Hastings 1973
		Serranus annularis	VB	S	Dantchakoff 1936; Longley and Hildebrand 1941
		Serranus atricauda	VB	S	Garcia-Diaz et al. 2002, 2006
		Serranus baldwini	VB	PG	Petersen and Fischer 1986; Petersen 1991; Leonard 1993
		Serranus cabrilla	VB	S	van Oordt 1929; Reinboth 1962, 1967b; Siau and Bouain 1994a; Garcia-Diaz et al. 1997
		Serranus hepatus	VB	S	van Oordt 1929; Dantchakoff 1936, D'Ancona 1949; Reinboth 1962, 1970; Brusle 1983

Table 5.1. Continued.

Family	**Subfamily**	**Species**	**Gonad Type**	**Sexual Pattern**	**References**
		Serranus notospilus	VB	S	Longley and Hildebrand 1941
		Serranus phoebe	VB	S	Longley and Hildebrand 1941; Smith 1959
		Serranus psittacinus	VB	PG	Hastings and Petersen 1986; Petersen 1991
		Serranus scriba	VB	S	van Oordt 1929; D'Ancona 1949; Reinboth 1962; Abd-el-Aziz and Ramadan 1990; Siau and Bouain 1994a; Zorica et al. 2005
		Serranus subligarius	VB	S	Clark 1959; Hastings and Bortone 1980; Oliver 1997
		Serranus tabacarius	VB	S	Smith 1959; Petersen 1991
		Serranus tigrinus	VB	S	Smith 1959, 1965; Pressley 1981; Petersen 1991
		Serranus tortugarum	VB	S	Longley and Hildebrand 1940; Fischer and Petersen 1987; Petersen and Fischer 1986; Petersen 1991
Epinephelidae	Epinephelinae	*Alphestes afer*	GE	P	Smith 1959
		Alphestes immaculatus	GE	NA	Erisman 2008
		Cephalopholis argus	NA	P	Shpigel 1985
		Cephalopholis boenak	GE	P	Chan and Sadovy 2002; Liu and Sadovy 2004a,b
		Cephalopholis cruentata	GE	P	Smith 1959, 1964, 1965
		Cephalopholis cyanostigma	GE	P	Mosse 2003
		Cephalopholis fulva	GE	P	Smith 1959, 1964, 1965
		Cephalopholis furcifer	GE	G	Smith 1959, 1965
		Cephalopholis hemistiktos	NA	P	Shpigel 1985; El-Etreby et al. 1993
		Cephalopholis miniata	NA	P	Shpigel 1985
		Cephalopholis panamensis	GE	P	Erisman 2008

Table 5.1. Continued.

Family	Subfamily	Species	Gonad Type	Sexual Pattern	References
		Cephalopholis taeniops	GE	P	Siau 1994
		Cephalopholis urodeta	GE	P	Nakai and Sano 2002
		Epinephelus adscensionis	GE	P	Smith 1965
		Epinephelus aeneus	GE	P	Brusle and Brusle 1975; Vadiya 1984; Hassin et al. 1997
		Epinephelus akaara	GE	P	Tanaka et al. 1990; Okumura 2001
		Epinephelus andersoni	DGE	P	Fennessy and Sadovy 2002
		Epinephelus bruneus	NA	P	Lee 1995
		Epinephelus chlorostigma	GE	P	Reinboth 1967; Moussac 1986
		Epinephelus coioides	GE	P	Quinitio et al. 1997; Tan-Fermin et al. 1994
		Epinephelus diacanthus	GE	P	Chen et al. 1980
		Epinephelus drummondhayi	GE	P	Brule et al. 2000
		Epinephelus fasciatus	GE	P	Vadiya 1984
		Epinephelus fuscoguttatus	NA	P	Pears et al. 2006
		Epinephelus guttatus	GE	P	Smith 1959, 1965; Shapiro et al. 1993; Sadovy et al. 1994
		Epinephelus itajara	GE	GU	Smith 1959; Bullock et al. 1992; Sadovy and Eklund 1999
		Epinephelus labriformis	GE	P	Erisman 2008
		Epinephelus malabaricus	NA	P	Sheaves 1995
		Epinephelus merra	GE	P	van Oordt 1933; Bhandari et al. 2003, 2004

Table 5.1. Continued.

Family	Subfamily	Species	Gonad Type	Sexual Pattern	References
		Epinephelus morio	GE	P	Moe 1969; Johnson et al. 1998; Brule et al. 1999
		Epinephelus polyphekadion	DGE	GU	Brusle et al. 1989; Rhodes and Sadovy 2002a,b
		Epinephelus rivulatus	DGE	P	Mackie 2000, 2003, 2006
		*Epinephelus sexfasciatus**	GE	P	van Oordt 1933
		Epinephelus striatus	GE	G	Smith 1959, 1965; Sadovy and Colin 1995; Sadovy and Eklund 1999
		Epinephelus summana	GE	P	van Oordt 1933
		Epinephelus tauvina	NA	P	Tan and Tan 1974; Lee et al. 1995
		Epinephelus tukula	GE	P	Yeh et al. 2003
		Hyporthodus flavolimbatus	GE	P	Keener 1984; Bullock et al. 1996
		Hyporthodus niveatus	GE	P	Wyanski et al. 2000; Moore and Labisky 1984
		Hyporthodus septemfasciatus	NA	P	Lee 1995
		Liopropoma mowbrayi	NA	GU	Smith 1971
		Liopropoma rubre	NA	GU	Smith 1971
		Mycteroperca bonaci	GE	P	Smith 1959; Garcia-Cagide and Garcia 1996; Crabtree and Bullock 1998; Brule et al. 2003
		Mycteroperca interstitialis	GE	P	Smith 1959; Bullock and Murphy 1994
		Mycteroperca marginatus	DGE	P	Brusle and Brusle 1975; Marino et al. 2001
		Mycteroperca microlepis	GE	P	McErlean and Smith 1964; Roberts and Schleider 1983; Collins et al. 1987; Collins et al. 1997; McGovern et al. 1998; Brule et al. 2003
		Mycteroperca phenax	GE	P	Coleman et al. 1996; Harris et al. 2002

Table 5.1. Continued.

Family	**Subfamily**	**Species**	**Gonad Type**	**Sexual Pattern**	**References**
		Mycteroperca rosacea	GE	G	Erisman et al. 2007
		Mycteroperca rubra	GE	P	Siau and Boauain 1994
		Mycteroperca tigris	GE	P	Smith 1959; Garcia-Cagide and Garcia 1999; White et al. 2002
		Mycteroperca venenosa	GE	P	Smith 1959; Garcia-Cagide and Garcia 1996
		Plectropomus laevis	DGE	P	Adams 2003
		Plectropomus leopardus	DGE	P	Goeden 1978; Ferreira 1995; Adams 2003
		Plectropomus maculatus	DGE	P	Ferreira 1993; Adams 2003
		Pseudogramma gregoryi	CL	S	Smith and Atz 1969
		Rypticus bistrispinus	VGE	P	Smith 1965
		Rypticus saponaceus	VGE	P	Smith 1959, 1965

Table 5.2. Gonad morphology types in serranid and epinephelid fishes, classified by the origination and proliferation of male germinal tissues.

Gonad Type	**Description**
Ventral Bands (VB)	Testicular tissue develops within discrete bands or islets that course that form along the junction of the alamellar region and the gonadal wall. Male tissues are topographically separated from female tissues.
Ventral Bands and Germinal Epithelium (VBGE)	Testicular tissue develops both within bands and along germinal epithelia of ovarian lamellae.
Germinal Epithelium (GE)	Testicular tissue develops in scattered crypts throughout the germinal epithelia of the ovarian lamellae.
Ventral Germinal Epithelium (VGE)	Testicular tissue develops in crypts in the ventral area of the gonad near the junction of the alamellar region and the gonadal wall. Male and female tissue are intermixed and not separated.
Dorsal Germinal Epithelim (DGE)	Testicular tissue develops first in the dorsal region of the gonad in close proximity to the dorsal blood vessel and proliferates outward during masculinization.
Median Lobe (ML)	Testicular tissue develops within a single lobe that is nested between ovarian lobes.

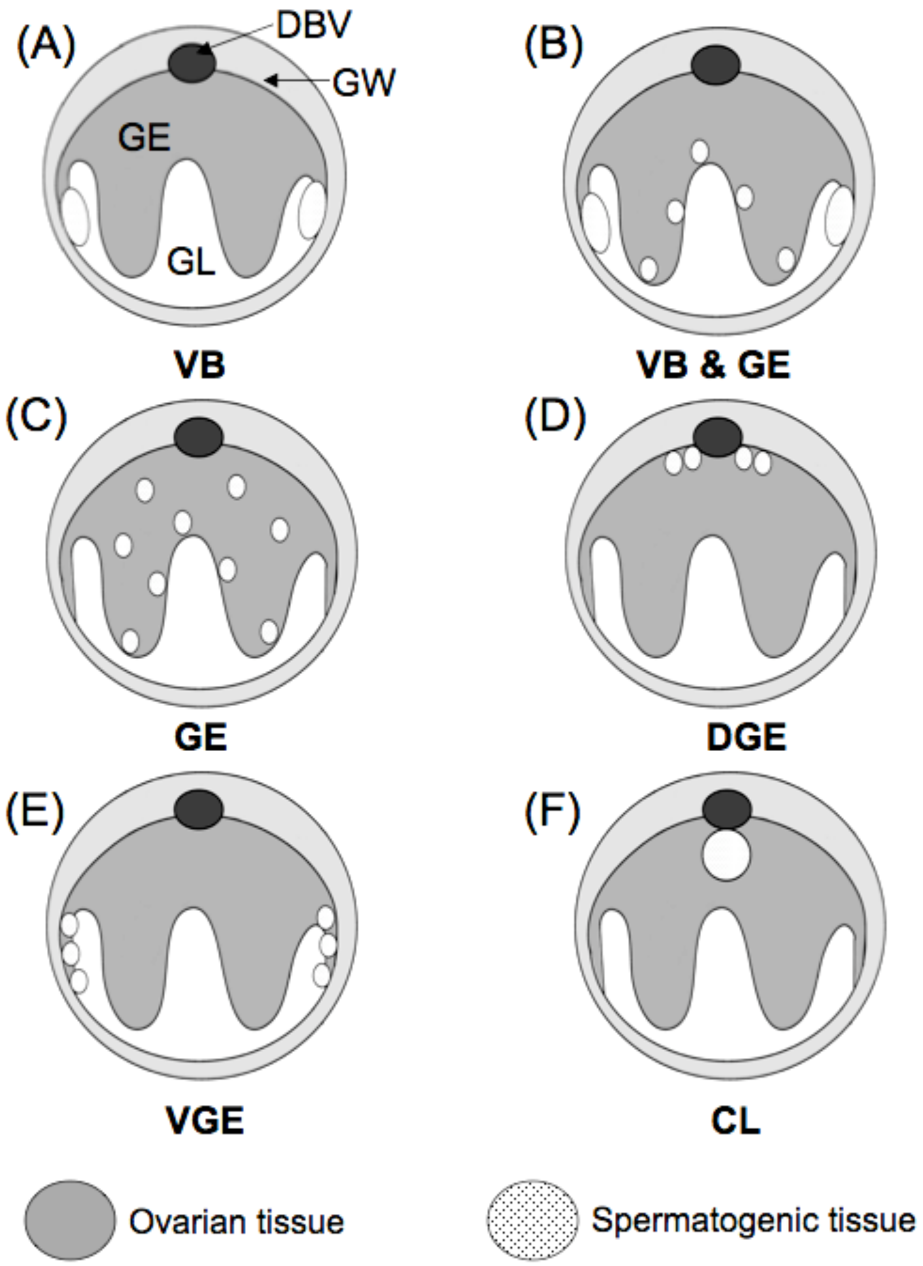

Fig. 5.1. Gonad morphology types in serranid and epinephelid fishes, shown in transverse sections. CL = central lobe, DBV = dorsal blood vessel, DGE = dorsal germinal epithelium, GE = germinal epithelium, GW = gonadal wall, GL = gonadal lumen, VB = ventral bands, VB & GE = ventral bands and germinal epithelium, VGE = ventral germinal epithelium.

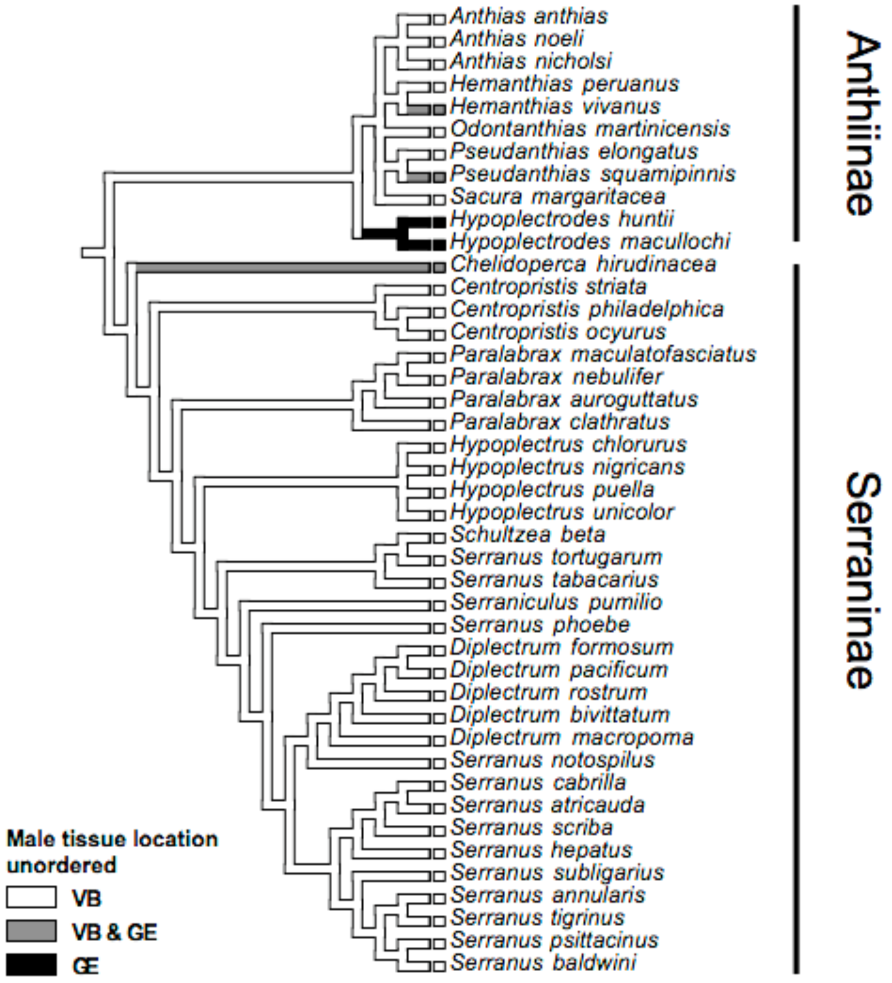

Fig. 5.2. Most parsimonious reconstructions of the transformations in character states for gonad morphology type in serranid fishes (Teleostei: Serranidae).

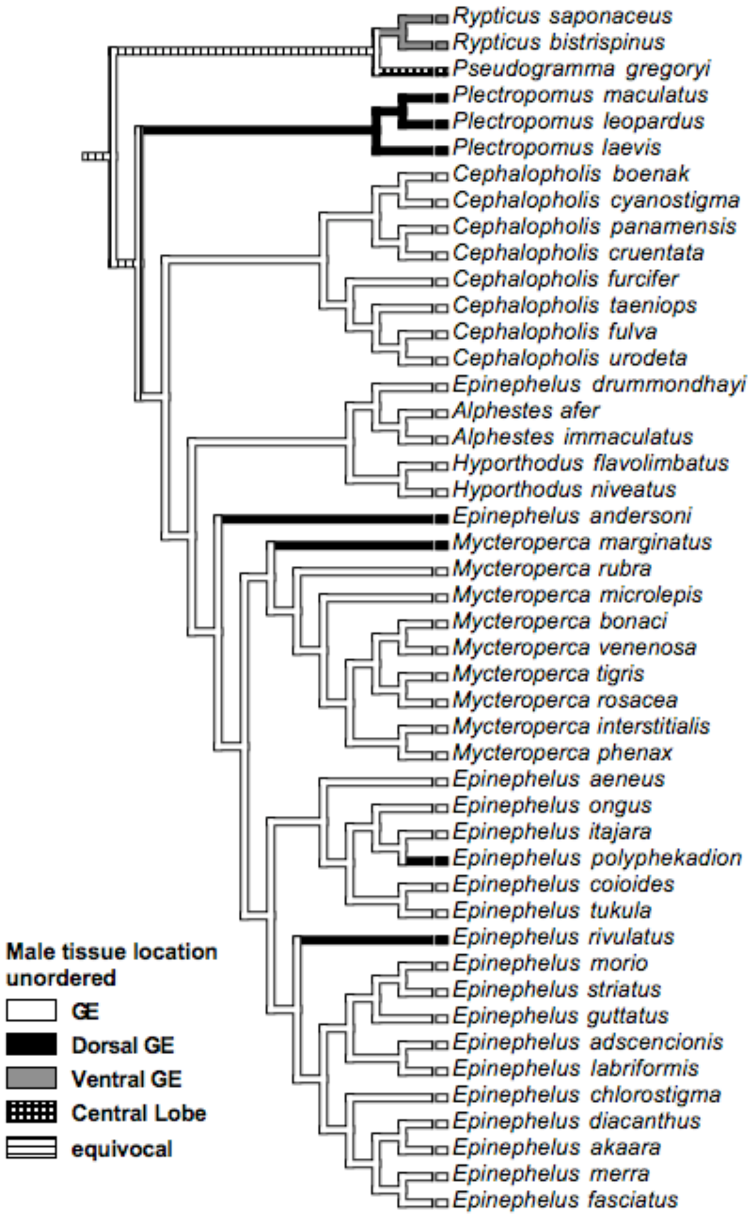

Fig. 5.3. Most parsimonious reconstructions of the transformations in character states for gonad morphology type in epinephelid fishes (Teleostei: Epinephelidae).

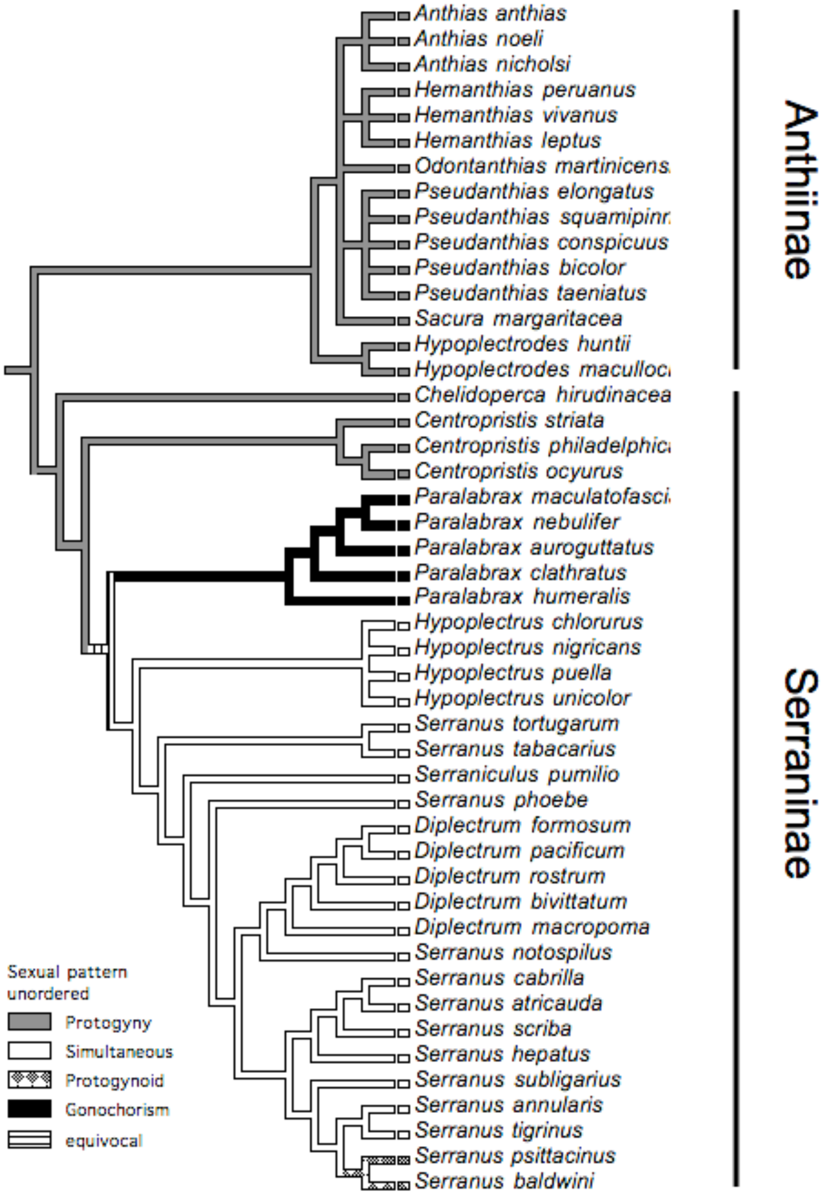

Fig. 5.4. Most parsimonious reconstructions of the transformations in character states for sexual pattern in serranid fishes (Teleostei: Serranidae)

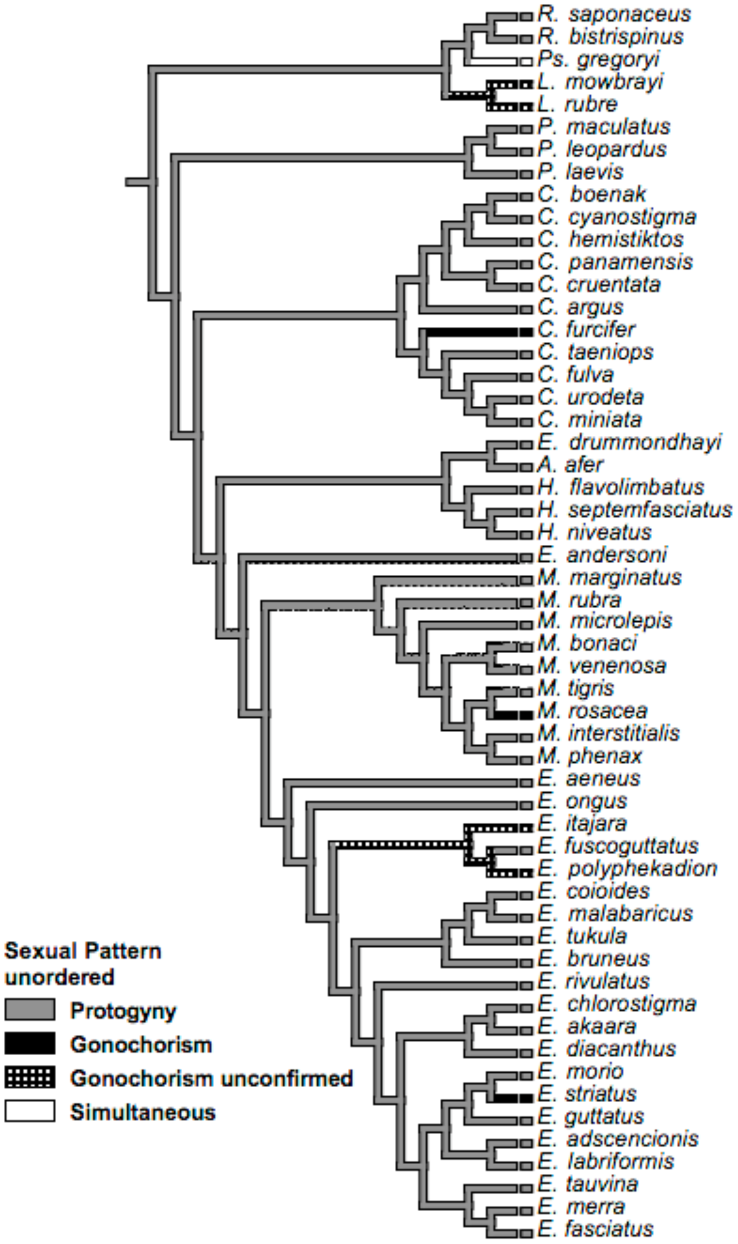

Fig. 5.5. Most parsimonious reconstructions of the transformations (ACCTRAN) in character states for sexual pattern in epinephelid fishes (Teleostei: Epinephelidae).

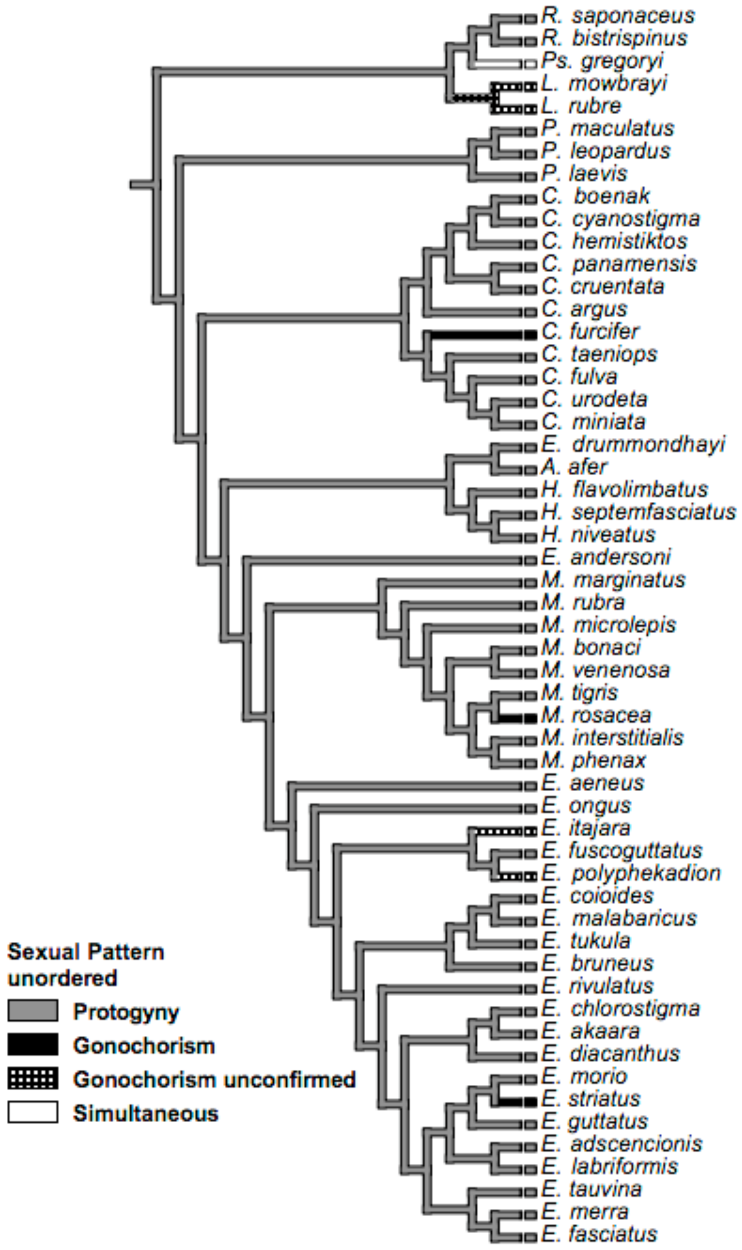

Fig. 5.6. Most parsimonious reconstructions of the transformations (DELTRAN) in character states for sexual pattern in epinephelid fishes (Teleostei: Epinephelidae).

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CHAPTER 6

Breakdown of the size-advantage: evolutionary changes in mating behavior influence the loss of sex change in groupers (Teleostei: Epinephelidae)

Abstract

The size-advantage model asserts that mating system characteristics influence the incidence and direction of sex change in animals. Although it is supported by numerous experimental and theoretical studies, none have tested predictions of the model within the context of a robust, species-level phylogeny. Using this approach, we tested whether changes in sexual pattern in groupers (Teleostei: Epinephelidae: Epinephelini), and in particular the loss of sex change, were related to changes in two traits related to the mating system: mating group structure and sperm competition intensity. All phylogenetic reconstructions indicated that protogyny and pair spawning are the plesiomorphic conditions for the lineage; both gonochorism and group spawning evolved independently at least four times in three different genera. Tests of correlated evolution showed that evolutionary transformations in sexual pattern from protogyny to gonochorism are significantly correlated with transformations in mating group structure from paired to group spawning, and transformations in mating group structure occurred prior to or simultaneously with transformations in sexual pattern. Sperm competition, as reflected by relative testes weights in males, is significantly higher in gonochoric species than protogynous species. This phylogenetic comparative study suggests that the loss of sex change in some groupers was influenced by changes in mating group structure from paired to group spawning and associated increases in sperm competition among males. Moreover, it provides phylogenetic support for predictions of the size-advantage model and empirical evidence for the influence of sperm competition on sex allocation in animals.

Introduction

Sex change, a form of hermaphroditism in which an individual functions first as one sex and then switches to function as the other sex, is widespread among plants, invertebrates, and fishes (Chan 1970; Policansky 1982; Sadovy and Liu 2008). Sex allocation theory provides explanations for the ways in which organisms allocate resources to male and female functions (Charnov 1982), and the size-advantage model is the most widely recognized theory on the adaptive significance and evolution of sex change in animals (Ghiselin 1969; Warner 1975; Muñoz and Warner 2003). The model proposes that selection for sex change exists when reproductive success increases with size or age more rapidly for one sex than for the other. It also contends that sexual pattern, including the timing and direction of sex change, is determined by aspects of the social and mating system of each species (Warner 1984; Warner 1988). Selection for protogyny, or female-first sex change, should occur in polygynous mating systems where large males are able to monopolize matings with females (Warner 1984). Under this scenario, large males realize a marked advantage in reproductive success in comparison with females and small males, and an individual can maximize its lifetime reproductive success by functioning as female when small and young and as a male when large and old.

Although the model focuses primarily on sex change, it also predicts conditions in which gonochorism (separate sexes) should be favored. For instance, the intensity of sexual selection for protogyny varies inversely with population size and the ability of small males to access females in matings (Warner and Hoffman 1980;

Warner 1982). Therefore, gonochorism rather than protogyny should be more common in populations or species where the density of males is high, because large males have less opportunity to monopolize matings in these situations (Warner 1984). Sperm competition, the conflict between two or more males to fertilize the eggs of a female (Parker 1970), is often intense at high population densities, with numerous males mating in groups with individual females (Birkhead and Moller 1998). When this occurs, the reproductive advantage of large males decreases due to mating interference and the dilution of gametes by other males, and selection for protogyny decreases as well (Muñoz and Warner 2003; Muñoz and Warner 2004).

Predictions of the size-advantage model are supported by numerous theoretical and empirical studies on the mating systems and sexual patterns in animals (e.g., Warner et al. 1996; Baeza et al. 2004; Collin et al. 2005), and especially reef fishes (Robertson 1972; Kuwamura and Nakashima 1998; Munday et al. 2006). However, few have examined the model within a phylogenetic context (Ross 1990; Allsop and West 2004; Molloy et al. 2007) and none have done so using a robust, species-level phylogeny. Early studies attempting to trace the evolutionary history of sex change in fishes suffered from a lack of resolution on the phylogenetic relationships of species and limited knowledge of their reproductive biology (Reinboth 1970; Smith 1975). Fortunately, information on the systematics and sexual development of fishes has increased dramatically over the last two decades, providing new opportunities to investigate the evolution of sexual patterns of this group. Recent, large-scale phylogenetic investigations on teleost fishes have demonstrated that sexuality is evolutionary labile and that shifts in sex-determining modes are frequent and have involved a variety of distinct ancestral pathways (Mank and Avise 2006; Mank et al. 2006). Comparative studies on fine-scale (i.e., species-level) evolutionary changes in sexual pattern are absent, yet they may hold important clues regarding the mechanisms that have shaped the evolution of sex allocation in animals.

The groupers (Teleostei: Epinephelidae: Epinephelini) provide an ideal opportunity to use the phylogenetic comparative method (Felsenstein 1985; Martins and Hansen 1996) to evaluate tenets of the size-advantage model. Scientific interest in the reproductive patterns of these fishes dates back to observations by Aristotle and is congruent with the earliest research on hermaphroditism in animals (Atz 1964). Due to their importance to fisheries and aquaculture, the sexual and mating patterns of many species are well known (Shapiro 1987; Sadovy 1996) and their phylogenetic relationships were recently hypothesized based on molecular data (Craig and Hastings 2007). Most grouper species are protogynous hermaphrodites; some (diandry) or all (monandry) individuals within populations reproduce first as females and change sex later in their lifetime to reproduce as males (Smith 1965; Sadovy and Liu 2008). However, a few species follow a gonochoric sexual pattern: individuals function either as male or female without post-maturational sex change (Sadovy and Colin 1995; Erisman et al. 2007). Mating group structure is equally diverse. Some species spawn in pairs, others spawn in groups, and a few species exhibit both behaviors (Sadovy et al. 1994; Whaylen et al. 2004; Erisman et al. 2007; Mackie 2007).

In this study, we used a comparative phylogenetic approach to reconstruct the evolutionary history of reproduction in groupers and evaluate the association between mating system characteristics (i.e., mating group structure and sperm competition) and sexual pattern in these fishes. More specifically, we tested whether evolutionary shifts in sexual pattern and, in particular the loss of sex change, were influenced by changes from paired to group spawning and associated increases in sperm competition among males. These analyses allowed us to determine whether predictions of the size-advantage model regarding the influence of mating behavior on sex allocation are congruent with fine-scale, evolutionary patterns of sex change within a resolved phylogeny.

Materials and Methods

Sources of Data. We searched published literature on groupers and other epinephelid fishes for information on their mating behaviors and sexual patterns. We used data from a total of 54 species of groupers and 7 species of soapfishes and related tribes, which are sister taxa to the grouper lineage (Craig and Hastings 2007; SI Table 1). Mating group structure was scored into three categories: paired, group, or mixed spawning. Paired spawning species were those in which adults are known to release their gametes in male-female pairs. Species that release gametes within groups containing several males and one or more females were classified as group spawners, and those reported to exhibit both behaviors were scored as mixed spawners.

Sexual pattern was divided into four categories: protogynous hermaphroditism, gonochorism, gonochorism unconfirmed, and simultaneous hermaphroditism. Those species for which there is histological evidence of post-maturational sex change from adult female to adult male and had been reported to change sex in this manner were classified as protogynous hermaphrodites (Sadovy and Shapiro 1987; Sadovy and Domeier 2005). For protogynous species, no distinction was made between monandric and diandric species, because this distinction is made infrequently in the literature. We categorized a species as a gonochore if histological or other analyses confirmed gonochorism. A species was categorized as an unconfirmed gonochore if either gonochorism was reported and histological evidence was incomplete, or if histological analyses found no evidence of post-maturational sex change. One outgroup species was classified as a simultaneous hermaphrodite, as reported by Smith and Atz (1969).

Tree Construction and Character Evolution. Phylogenetic trees of included taxa reflected results from the maximum-likelihood analyses of the species relationships presented by Craig and Hastings (2007). Species for which no data on either sexual or mating pattern could be located were pruned from the tree and excluded from this study. Likewise, species without information on their phylogenetic relationships (i.e., those not included in Craig and Hastings (2007)) were also removed from the analyses. The soapfishes and related tribes were used as the outgroup, in accordance with Craig and Hastings (2007). More distantly related fishes such as the anthiines (Serranidae: Anthiinae) and the sea basses (Serranidae: Serraninae) were excluded, because their close relationship to groupers has been called into question (Smith and Craig 2007).

We used the parsimony analysis from MacClade version 4 (Maddison and Maddison 2000) to reconstruct the evolution of sexual pattern (50 grouper and 5 outgroup species) and mating group structure (28 grouper and 5 outgroup species) within the epinephelid phylogeny. Evolutionary transformations of both characters were reconstructed using both ACCTRAN and DELTRAN resolving options in order to compare scenarios related to the origination and reversal of character states within the phylogeny. The ACCTRAN option chooses character state assignments that accelerate changes toward the root of the tree, maximizing early gains and subsequent reversals. Conversely, the DELTRAN option forces changes toward the tips of the tree and maximizes parallel changes or convergence (Maddison and Maddison 2000).

Tests of Correlated Evolution. We used MacClade (Maddison and Maddison 2000) to simultaneously reconstruct evolutionary changes in sexual pattern and mating group structure and determine whether the evolution of gonochorism was correlated with the evolution of group spawning. Character state for the sexual pattern of each species was scored according to the presence or absence of gonochorism, and character state for mating pattern was scored according to the presence or absence of group spawning. Two phylogenies were used to recreate the evolution of these characters. The first phylogeny was built using all species (54 grouper and 7 outgroup species) for which data exist for either sexual or mating pattern. A second phylogeny was constructed using only those species for which data exist for both characters (24 grouper and 3 outgroup species). For both, the evolutionary histories of sexual and mating pattern were rebuilt using ACCTRAN and DELTRAN options.

The concentrated-changes test (Maddison 1990; Lorch and Eadie 1999) was carried out in McClade (Maddison and Maddison 2000). The test is designed to calculate the expected distribution of changes to one character, given the state of a second causal character and the actual number of changes to the first character that occurred in the entire clade (Maddison and Maddison 2000; Peach and Rouse 2004). We used the exact probability formula to determine whether character transformations of sexual pattern were associated with changes in mating pattern. Group spawning was chosen as the derived state of the independent character, and gonochorism was chosen as the derived state of the dependent character. We had two a priori reasons to use character states associated with sexual pattern as the dependent variable. First, the appearance of gonochorism on all most parsimonious reconstructions of sexual pattern evolution within the tree occurred simultaneously or subsequent to changes in mating pattern, thus suggesting that the former character was influenced by the latter. Second, sex allocation theory contends that mating system characteristics influence sexual patterns in animals (Warner 1988; Munday et al. 2006).

The null hypothesis was that the gonochoric character state was uniformly distributed on the phylogenetic tree. The observed distribution of character transformations was then used to calculate the probability that these were associated with a group-spawning mating pattern (independent character). We ran the test multiple times using tree reconstructions built from all possible combinations of phylogeny types (all or reduced taxa), both types of resolving options (ACCTRAN and DELTRAN), and scenarios that included all gains in the independent variable (i.e., evolutions of group spawning) that occurred simultaneous to or subsequent to gains in the dependent variable (i.e., evolution of gonochorism) versus those that included only gains in the independent variable that occurred subsequent to changes in the dependent variable. For all runs, we chose a simulation sample size of 1,000,000 replications of actual changes.

Analysis of Sperm Competition and Sexual Pattern. The relative testes weights (RTW) of reproductively active males were used as a proxy of sperm competition intensity, as demonstrated in previous studies in fishes (Warner 1997; Taborsky 1998; Petersen and Warner 1998). In order to confirm this relationship for groupers, we first tested whether sperm competition was associated with RTW using a linear correlation. We found data on the RTW of males for 16 species of groupers (Table 2). RTW was calculated as: RTW = [gonad weight (g) / total body weight (g)] x 100. Each species was scored for the intensity of sperm competition (Petersen and Warner 1998), grouped as follows with increasing levels of sperm competition: (1) non-aggregating species that spawn in pairs with no sperm competition, (2) aggregating species that spawn in pairs, (3) aggregating species that spawn in both pairs and in groups, (4) aggregating species reported to spawn only in large groups.

Statistical analyses that treat species values as statistically independent points are questionable, because closely related species tend to share traits through common descent rather than through independent evolution (Harvey and Pagel, 1991). We used the Comparative Analysis by Independent Contrasts (CAIC) statistical package (Purvis and Rambaut 1995) to transform non-independent species data for RTW and sperm competition rank into values that were suitable for use with conventional statistical procedures. We constructed a phylogeny for the 16 species based on species relationships proposed by Craig and Hastings (2007) and set branch lengths as equal. Sperm competition rank was chosen as the independent variable and RTW was chosen as the dependent variable. The resulting contrasts for each variable were then analyzed by linear correlation (Purvis and Rambaut 1995).

Next, we compared the mean RTW of gonochoric species to gonochoric-unconfirmed and protogynous species in order to test the relationship between sperm competition intensity and sexual pattern. A Kruskal-Wallis test (non-parametric ANOVA) was used, because arc-sin transformed data did not conform to the assumptions of parametric analyses. Raw data for RTW and sexual pattern were used, because data transformations from CAIC produced too few independent contrasts to detect a statistical relationship. Mann Whitney U tests were used to make pair-wise comparisons in RTW between protogynous, gonochoric unconfirmed and gonochoric species.

Results

Evolution of sexual and mating patterns. Protogynous hermaphroditism is the most pervasive sexual pattern in groupers and is predicted as the ancestral character state of sexual pattern in all phylogenetic reconstructions (Figs. 1 and 2). Protogyny has been reported in 45 species of groupers (Table 1) and 2 outgroup species of soapfishes and related fishes (Family Epinephelidae; tribes Liopropomini, Grammistini, and Diploprionini). There is evidence of gonochorism in five species of groupers and two species of basslets in the genus *Liopropoma* (Table 1). For groupers, gonochorism evolved independently at least four and as many five times in three genera: *Cephalopholis, Epinephelus,* and *Mycteroperca*. The ACCTRAN reconstruction predicted four independent evolutions of gonochorism (Fig. 1), whereas DELTRAN predicted five independent evolutions (Fig. 2). No unequivocal instances of the transition from gonochorism to protogyny are evident in groupers; a possible reversal was predicted for *Epinephelus fuscoguttatus* in only one ACCTRAN reconstruction. Simultaneous hermaphroditism arose once in the outgroup lineage, where it is reported for *Pseudogramma gregoryi* (Table 1).

Pair spawning is the most common mating pattern in groupers and is predicted to be the ancestral character state in all phylogenetic reconstructions (Figs. 3 and 4). It is known in 23 species of groupers and five outgroup species (Table 1). Group spawning is reported for seven species of groupers. Two species, *Epinephelus itajara* and *Mycteroperca tigris*, are known to spawn in pairs and in groups (i.e., mixed spawners). Group spawning evolved four separate times within the phylogeny in both

ACCTRAN (Fig. 3) and DELTRAN (Fig. 4) reconstructions, occurring in species of *Cephalopholis*, *Epinephelus*, and *Mycteroperca*.

Tests of Correlated Evolution. Transformations in sexual pattern from protogyny to gonochorism were significantly correlated with transformations in mating pattern from paired to group spawning in six of eight simulations (Table 2). In all simulations, transformations from paired to group spawning occurred prior to or simultaneous with transformations from protogyny to gonochorism (Figs. 5-7). Changes in sexual and mating pattern were significantly correlated using all taxa with the ACCTRAN option when both simultaneous and subsequent gains were included (concentrated changes test; 4 gains and 1 loss; $p < 0.001$; Fig. 5). The relationship was also significant when simultaneous gains were removed (concentrated changes test; 2 gains and 1 loss; $p = 0.005$). DELTRAN simulations utilizing all taxa produced similar results when all gains were included (4 gains and 0 losses; $p < 0.001$; Fig. 6) and when simultaneous gains were removed (2 gains and 0 losses; $p = 0.022$). Simulations involving the shortened list of taxa were similar for ACCTRAN and DELTRAN options (Fig. 7). Both were highly significant when all gains were included (4 gains and 0 losses; $p < 0.001$) but were not significant when simultaneous gains were removed (1 gain and 0 losses; $p > 0.05$).

Sperm Competition and Sexual Pattern. Relative testes weights (RTW) of breeding males were positively correlated with sperm competition intensity (linear correlation of phylogenetically independent contrasts; $r^2 = 0.642$; $p < 0.001$; Fig. 8). Median RTW differed significantly among sexual pattern groups (Kruskal-Wallis; KW = 7.95; $p = 0.018$; Fig. 9). RTW of protogynous species (mean = 0.58%, SE = 0.14) was significantly lower than both gonochoric (mean = 7.00%, SE = 3.00)(Mann Whitney; U = 1.00; $p = 0.022$) and gonochoric unconfirmed species (n =2, mean = 3.00%, SE 1.5%) (Mann Whitney; U = 1.00; $p = 0.035$), and pooled RTW data for gonochoric and gonochoric unconfirmed species (Mann Whitney; U = 1.00; $p = 0.002$. Median RTW values were similar between gonochoric and gonochoric unconfirmed species, but data were too few to detect a significant statistical relationship.

Discussion

In this study, we reconstructed the evolutionary history of reproduction in groupers and compared it to predictions of the size-advantage model for sex change in animals. Placement of sexual pattern and mating system data within a phylogenetic context allowed us to determine the extent to which sexual pattern was shaped by ancestry in this group. Our results indicate that shifts in sexual pattern from protogyny to gonochorism were not phylogenetically constrained, but rather they were correlated with parallel changes in mating group structure from paired to group spawning. Comparisons of relative testes weights in males and sexual pattern indicated that sperm competition is more intense in gonochoric species than protogynous species. Taken together, these results provide phylogenetic support for the size advantage model and empirical support for the effects of sperm competition on sex change in animals.

The size-advantage model (Ghiselin 1969; Warner 1975; Warner and Muñoz 2003) contends that aspects of mating behavior influence sex allocation in animals, and it provides conditions under which different sexual patterns may evolve. Protogyny is adaptive when the reproductive success of large males is higher than both females and small males. This occurs in mating systems where large males monopolize matings with females and exclude smaller males from spawning (Warner 1988). Conversely, gonochorism is adaptive when the sexes show a similar increase in reproductive success with size (Warner 1984). Selection for gonochorism should occur in group spawning mating systems characterized by intense sperm competition, where multiple males compete to fertilize the eggs of females (Warner 1982; Muñoz and Warner 2003). Sperm competition should be lower in protogynous species, because large males reduce male-male competition during spawning through aggressive behavior (Munoz and Warner 2003; Molloy et al. 2007).

The mating systems and sexual patterns of groupers are remarkably consistent with predictions of the model. In protogynous species, large males establish mating territories and frequently engage in bouts of aggression with other males (Goeden 1978; Shpigel and Fishelson 1991; Zabala et al. 1997). Also, mating in protogynous species occurs in pairs, and large males often monopolize matings with several females (Colin et al. 1987; Donaldson 1995; Mackie 2007). Conversely, spawning in gonochoric species of groupers occurs in groups with several males and one or more females, and male territorial behavior is rare or absent (Sadovy and Colin 1995; Sala et al. 2003; Erisman et al. 2007). At least one protogynous species of grouper, *Epinephelus tigris*, has been reported to spawn in both pairs and in large groups (Sadovy et al. 1994; Whaylen et al. 2004). It is possible that this species is diandric, with functional sex change occurring in certain populations where males pair spawn with females and monopolize matings through aggressive behavior and functional sex change is absent or rare in populations where adults spawn in groups.

Evolutionary changes in sexual pattern and mating behavior in groupers were also congruent with predictions of the size-advantage model. In this study, we found that changes in sexual pattern from protogyny to gonochorism were correlated with equivalent changes in mating systems. In fact, all phylogenetic reconstructions of the evolution of sexual and mating patterns in groupers showed that transformations in mating group structure occurred prior to or simultaneous with transformations in sexual pattern, suggesting that the loss of sex change was associated with a switch from paired to group spawning behavior. Moreover, the evolution of gonochorism was not phylogenetically constrained, as it occurred independently in several genera and was not restricted to certain branches of the phylogenetic tree. Two outgroup species in the genus *Liopropoma* did not follow predictions of sex allocation theory, although this may be due to a lack of resolution on their reproductive patterns. Smith (1971) concluded that *L. mowbrayi* and *L. rubre* were gonochores, but for both species, only five specimens of either sex were examined histologically. Designation of sexual pattern based on such a low sample size is suspect (Sadovy and Domeier

2005; Sadovy and Liu 2008). Alternatively, it is possible that *Liopropoma* species are indeed gonochoric, and factors other than mating behavior (e.g., sex-related differences in growth rate, longevity and mortality) may drive sex allocation in these fishes (Warner 1988; Devlin and Nagahama 2002; Munday et al. 2006).

We suspect that the evolutionary link between mating systems and sex change are not unique to groupers and likely occur in other groups of fishes for which either data on reproductive patterns are sparse or the phylogenetic relationships of species are unavailable or incomplete. In several reviews on the mating systems and sexual patterns of labrid and scarid fishes, all protogynous species exhibited some form of mate monopolization by large males (Robertson and Choat 1974; Robertson and Warner 1978, Warner and Robertson 1978), and sex change is rare in species where large males have less opportunity to monopolize matings with females (Warner 1984; Hoffman 1985). In some species, sexual pattern varies considerably with local conditions and sex change is less frequent in habitats where population densities are high and group spawning is prevalent (Warner and Robertson 1978; Warner and Hoffman 1980; Warner 1982). A recent study on the reproductive evolution of sea basses (Teleostei: Serranidae), a family of fishes once thought to be closely related to the groupers (but see Smith and Craig 2007), produced results similar to those found in this study. Protogynous hermaphroditism is the ancestral condition of sexuality in the family (Erisman 2008) and the evolution of gonochorism from protogyny is known to occur only in the genus *Paralabrax* (Sadovy and Domeier 2005) and is associated with the evolution of group spawning in this lineage (Erisman and Allen 2006; Miller and Allen 2006**;** Erisman 2008).

Comparisons of sperm competition levels between protogynous and gonochoric species provide additional evidence of the influence of mating behavior on the sexual patterns of groupers. Sperm competition intensity, as indicated by mating group structure, was directly related to the relative testes weights of males and significantly higher in gonochoric species than in protogynous species. Similar results have been found in other studies. For example, Muñoz and Warner (2003) found that deferral of sex change was more common in parrotfishes where there was sperm competition. Serranid fishes show a similar pattern. In protogynous and simultaneously hermaphroditic species, sperm competition is rare or absent and the testes of reproductively active males are less than 1% of their body weight (Suzuki et al. 1978; Link 1980; Petersen and Warner 1998; Petersen 2006). However, male testes weights are relatively large (> 5% of body weight) in gonochoric species that spawn in large groups (Erisman and Allen 2006; Erisman 2008 -Appendix 1). Finally, a recent study by Molloy et al. (2007) that included 116 species of fishes found that testes sizes were significantly smaller in protogynous species than in gonochoric species.

To summarize, we contend that the loss of sex change in groupers was influenced by shifts in the mating systems of certain species, and we propose a scenario for its evolution (Fig. 10). As the shift from paired to group spawning occurred, the reproductive advantage of large males decreased because they were unsuccessful in excluding small males from mating with females. Small males increased their reproductive success relative to large males by accessing females via group spawnings. The increase in the number of male participants during matings equated to an increase in sperm competition, and in order to maximize their reproductive success, both large and small males responded by allocating more energy towards the production of gametes (via increased testes sizes). As a result of the changes in mating group structure and sperm competition levels, selection for sex change decreased and gonochorism evolved.

Factors that determine the mating systems of groupers and, in particular, the cause of the shift from paired to group spawning in certain species are not understood. Ecological variables such as the distribution, abundance, and predictability of resources directly affect the mating strategies of animals (Emlen and Oring 1977), including fishes (Fricke 1980; Jones 1981), and large social groups are more likely to form when resources are distributed uniformly or in especially dense aggregations (Crook 1972; Altmann 1974; Warner 1980). The social structure of some gonochoric groupers is congruent with these hypotheses. Adult *Cephalopholis colonus* form immense schools and feed in the water column on zooplankton blooms (Heemstra and Randall 1993). Similarly, adult *Mycteroperca rosacea* often occur in groups and aggregate to feed on schools of small fishes (Hobson 1968; Erisman et al. 2007). When food is abundant and supports large populations of aggregated adults, resource defense may not be a viable strategy for males, who instead invest their energy in sperm production to maximize their reproductive success. However, other gonochoric groupers only occur in groups during spawning periods (Domeier and Colin 1997), which suggests determinants of mating group structure vary among species.

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Table 6.1. Sexual and mating patterns reported for groupers and related fishes. GO = gonochorism, GR = group spawning, GU = unconfirmed gonochorism, MI = paired and group spawning, NA = no data available, PA = paired spawning, PR = protogynous hermaphroditism, SI = simultaneous hermaphroditism.

Tribe	Species	Sexual Pattern	Mating Pattern	References
Epinephelini	*Alphestes afer*	PR	NA	Smith 1959
(Groupers)	*Alphestes immaculatus*	NA	PA	Erisman 2008
	Cephalopholis argus	PR	PA	Shpigel 1985; Donaldson 1995
	Cephalopholis boenak	PR	PA	Donaldson 1989; Chan and Sadovy 2002; Liu and Sadovy 2004a,b; Liu and Sadovy 2005
	Cephalopholis colonus	NA	GR	Sala et al. 2003; Erisman 2008
	Cephalopholis cruentata	PR	PA	Smith 1959, 1964, 1965; Nagelkerken 1979
	Cephalopholis cyanostigma	PR	NA	Mosse 2003
	Cephalopholis fulva	PR	PA	Smith 1959, 1964, 1965; Thompson and Munro 1974, 1978
	Cephalopholis furcifer	GO	GR	Smith 1959, 1965
	Cephalopholis hemistiktos	PR	PA	Shpigel 1985; El-Etreby et al. 1993
	Cephalopholis miniata	PR	PA	Shpigel 1985
	Cephalopholis panamensis	PR	PA	Erisman 2008
	Cephalopholis taeniops	PR	NA	Siau 1994
	Cephalopholis urodeta	PR	PA	Donaldson 1995; Nakai and Sano 2002
	Dermatolepis dermatolepis	NA	GR	Erisman 2008
	Epinephelus adscencionis	PR	NA	Smith 1965
	Epinephelus aeneus	PR	NA	Brusle and Brusle 1975; Vadiya 1984; Hassin et al. 1997
	Epinephelus akaara	PR	PA	Tanaka et al. 1990; Lee 1995; Okumura 2001
	Epinephelus andersoni	PR	PA	Fennessy and Sadovy 2002
	Epinephelus bruneus	PR	NA	Lee 1995
	Epinephelus chlorostigma	PR	NA	Reinboth 1967; Moussac 1986
	Epinephelus coioides	PR	NA	Quinitio et al. 1997; Tan-Fermin et al. 1994; Sheaves 1995
	Epinephelus diacanthus	PR	NA	Chen et al. 1980; McIlwain et al. 2006
	Epinephelus drummondhayi	PR	NA	Brule et al. 2000
	Epinephelus fasciatus	PR	PA	Vadiya 1984
	Epinephelus fuscoguttatus	PR	NA	Pears et al. 2006
	Epinephelus guttatus	PR	PA	Smith 1959, 1965; Burnett-Herkes 1975; Shapiro et al. 1993a,b; Sadovy et al. 1994b; Shapiro et al. 1994
	Epinephelus itajara	GU	MI	Smith 1959; Bullock et al. 1992; Sadovy and Eklund 1999; Deloach and Humann 1999; F. Coleman and C. Koenig pers. comm.
	Epinephelus labriformis	PR	PA	Craig et al. 2006; Erisman 2008
	Epinephelus malabaricus	PR	NA	Sheaves 1995

Table 6.1. Continued.

Tribe	Species	Sexual Pattern	Mating Pattern	References
	Epinephelus merra	PR	PA	van Oordt 1933; Bhandari et al. 2003, 2004
	Epinephelus morio	PR	NA	Moe 1969; Johnson et al. 1998; Brule et al. 1999
	Epinephelus ongus	PR	NA	Craig 2007
	Epinephelus polyphekadion	GU	NA	Brusle et al. 1989; Rhodes and Sadovy 2002a,b
	Epinephelus rivulatus	PR	PA	Mackie 2000, 2003, 2006, 2007
	Epinephelus striatus	GO	GR	Smith 1959, 1965; Colin et al. 1987; Colin 1992; Sadovy and Colin 1995; Sadovy and Eklund 1999
	Epinephelus tauvina	PR	NA	Tan and Tan 1974; Lee et al. 1995
	Epinephelus tukula	PR	NA	Yeh et al. 2003
	Hyporthodus flavolimbatus	PR	NA	Keener 1984; Bullock et al. 1996
	Hyporthodus niveatus	PR	NA	Wyanski et al. 2000; Moore and Labisky 1984
	Hyporthodus septemfasciatus	PR	NA	Lee 1995
	Mycteroperca bonaci	PR	PA	Smith 1959; Garcia-Cagide and Garcia 1996; Crabtree and Bullock 1998; Brule et al. 2003; Paz and Sedberry 2008
	Mycteroperca interstitialis	PR	NA	Smith 1959; Bullock and Murphy 1994
	Mycteroperca marginatus	PR	PA	Brusle and Brusle 1975; Brusle 1985; Zabala et al. 1997a,b; Bertoncini et al. 2003; Marino et al. 2001; Hereu et al. 2006
	Mycteroperca microlepis	PR	PA	McErlean and Smith 1964; Roberts et al. 1983; Collins et al. 1987; Gilmore and Jones 1992; Coleman et al. 1996; Collins et al. 1997; Brule et al. 2003
	Mycteroperca phenax	PR	PA	Gilmore and Jones 1992; Harris et al. 2002
	Mycteroperca rosacea	GO	GR	Erisman et al. 2007a,b
	Mycteroperca rubra	PR	NA	Siau and Boauain 1994
	Mycteroperca tigris	PR	MI	Smith 1959; Sadovy et al. 1994; Garcia-Cagide and Garcia 1999; Matos-Caraballo et al. 2006; White et al. 2002; Whaylen et al. 2004
	Mycteroperca venenosa	PR	PR	Smith 1959; Garcia-Cagide and Garcia 1996
	Plectropomus areolatus	NA	PA	Pet et al. 2005
	Plectropomus laevis	PR	NA	Adams 2003
	Plectropomus leopardus	PR	PA	Goeden 1978; Samoilys and Squire 1994; Ferreira 1995; Adams 2003; Frisch et al. 2007

Table 6.1. Continued.

Tribe	Species	Sexual Pattern	Mating Pattern	References
Diploprionini (soapfishes)	*Diploprion bifasciatum*	NA	PA	Thresher 1984
Liopropomini (basslets)	*Liopropoma mowbrayi*	NA	PA	Smith 1971; Thresher 1984
	Liopropoma rubre	NA	PA	Smith 1971; Thresher 1984; Deloach and Humann 1999
Grammistini (soapfishes)	*Pseudogramma gregoryi*	SI	NA	Smith and Atz 1969
	Pseudogramma polyacanthus	NA	PA	Thresher 1984
	Rypticus bistrispinus	PR	NA	Smith 1965
	Rypticus saponaceus	PR	PA	Smith 1959, 1965; Deloach and Humann 1999

Table 6.2. Summary of results from tests of correlated evolution, organized by resolving option (ACCTRAN vs. DELTRAN), gains and losses used (all vs. subsequent), and numbers of taxa included (all vs. reduced).

Simulation	**Gains/Losses**	**All Taxa (61 species)**	**Reduced Taxa (27 species.)**
ACCTRAN	**4/1 (All)**	**$p < 0.001$***	**$p < 0.001$***
ACCTRAN	**2/1 (Subsequent)**	**$p = 0.043$***	**$p = 0.415$**
DELTRAN	**4/0 (All)**	**$p < 0.001$***	**$p < 0.001$***
DELTRAN	**2/0 (Subsequent)**	**$p = 0.038$***	**$p = 0.416$**

Table 6.3. Relative testes weights (RTW) of breeding males, sperm competition rank, and sexual pattern for 16 species of groupers. GO = gonochorism, GU = unconfirmed gonochorism, PR = protogynous hermaphroditism. Sperm competition rankings were as follows: (1) non-aggregating species that spawn in pairs and face no sperm competition, (2) aggregating species that spawn in pairs, (3) aggregating species that spawn in both pairs and in groups, (4) aggregating species reported to spawn only in large groups.

Species	**Mean RTW (%body wt.)**	**Sperm Competition Rank**	**Sex Pattern**	**References**
Cephalopholis boenack	0.5	1	PR	Donaldson 1989; Chan and Sadovy 2002
Cephalopholis panamensis	0.2	1	PR	Erisman 2008
Epinephelus diacanthus	0.5	1	PR	Chen et al. 1980; McIlwain et al. 2006
Epinephelus guttatus	1	2	PR	Colin et al. 1987; Shapiro et al. 1993; Sadovy et al. 1994a,b
Epinephelus itajara	1.5	3	GU	Sadovy and Eklund 1999; Deloach and Humann 1999; FC Coleman and K Koenig, personal communication
Epinephelus merra	0.4	1	PR	Lee et al. 2002; Bhandari et al. 2003; Soyano et al. 2003
Epinephelus morio	0.3	1	PR	Johnson et al. 1998; Brule et al. 1999
Epinephelus polyphekadion	4.5	3	GU	Rhodes and Sadovy 2002a,b
Epinephelus striatus	10	4	GO	Sadovy and Colin 1995; Sadovy and Eklund 1999
Mycteroperca bonaci	0.43	2	PR	Carter 1989; Brule et al. 2003; Whaylen et al. 2004; Paz and Sedberry 2008
Mycteroperca marginatus	0.6	2	PR	Zabala et al. 1997a,b; Marino et al. 2001
Mycteroperca microlepis	0.3	2	PR	Gilmore and Jones 1992; Coleman et al. 1996; Collins et al. 1997
Mycteroperca phenax	0.3	2	PR	Gilmore and Jones 1992; Coleman et al. 1996; Harris et al. 2002
Mycteroperca rosacea	4	4	GO	Erisman et al. 2007a,b
Mycteroperca tigris	2	3	PR	Sadovy et al. 1994; Whaylen et al. 2004
Plectropomus leopardus	0.4	2	PR	Samoilys and Squire 1994; Ferreira 1995; Frisch et al. 2007

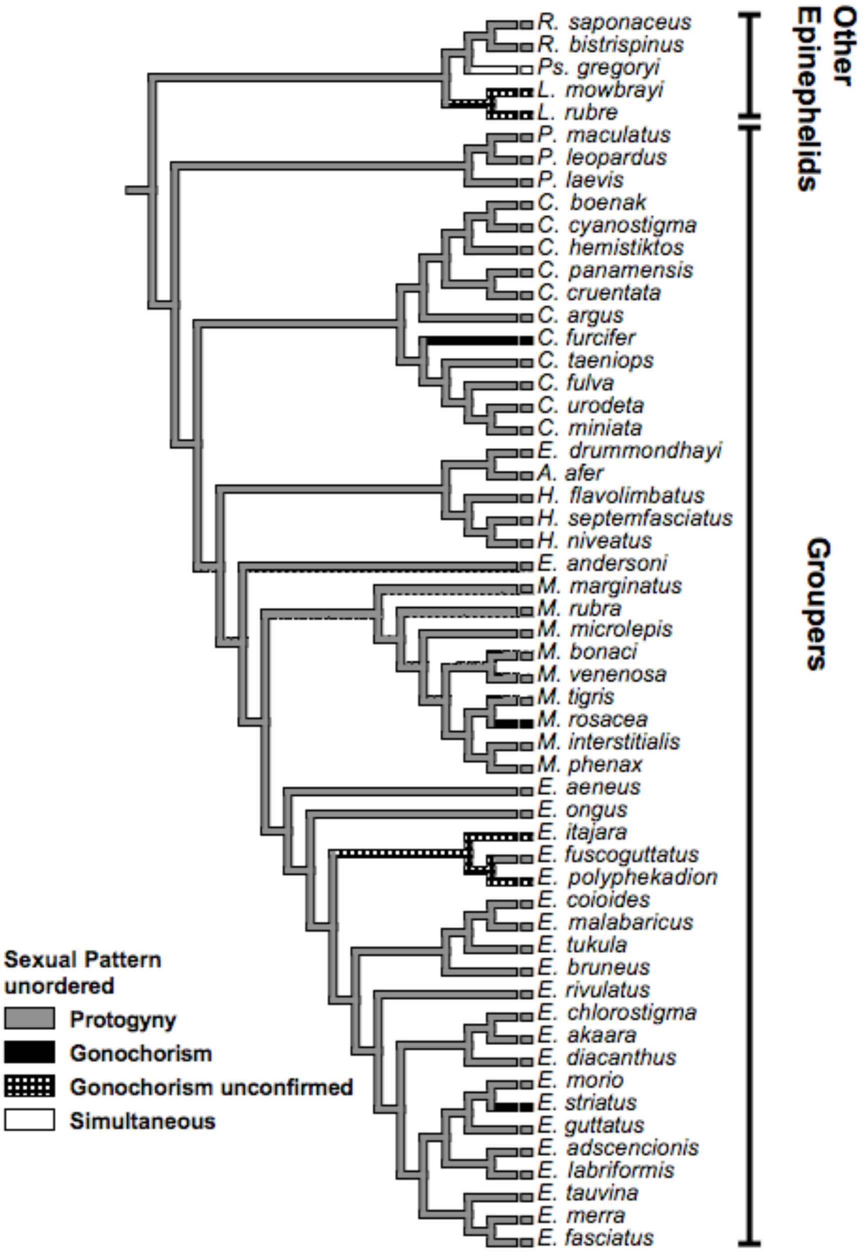

Fig. 6.1. Most parsimonious reconstructions of the transformations (ACCTRAN) in character states for sexual pattern in 55 species of epinephelid fishes.

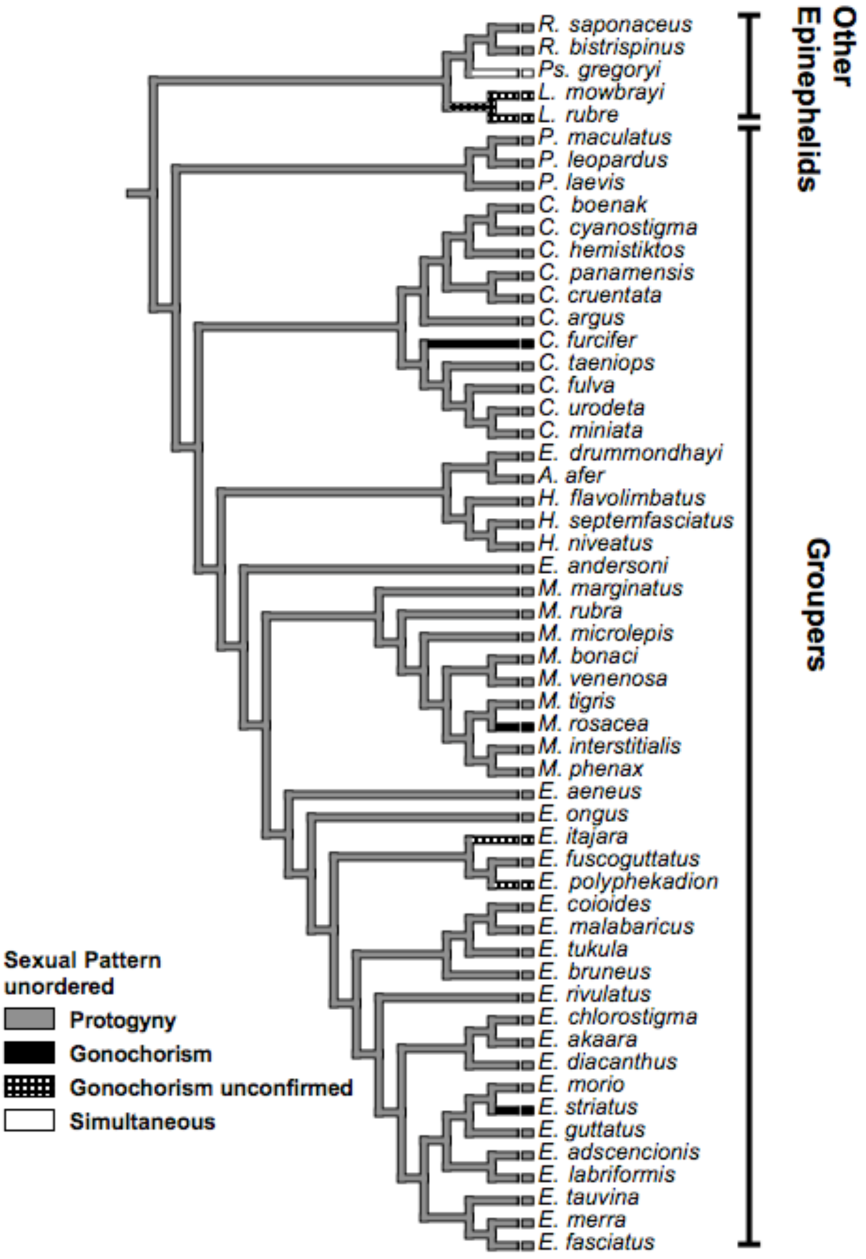

Fig. 6.2. Most parsimonious reconstructions of the transformations (DELTRAN) in character states for sexual pattern in 55 species of epinephelid fishes.

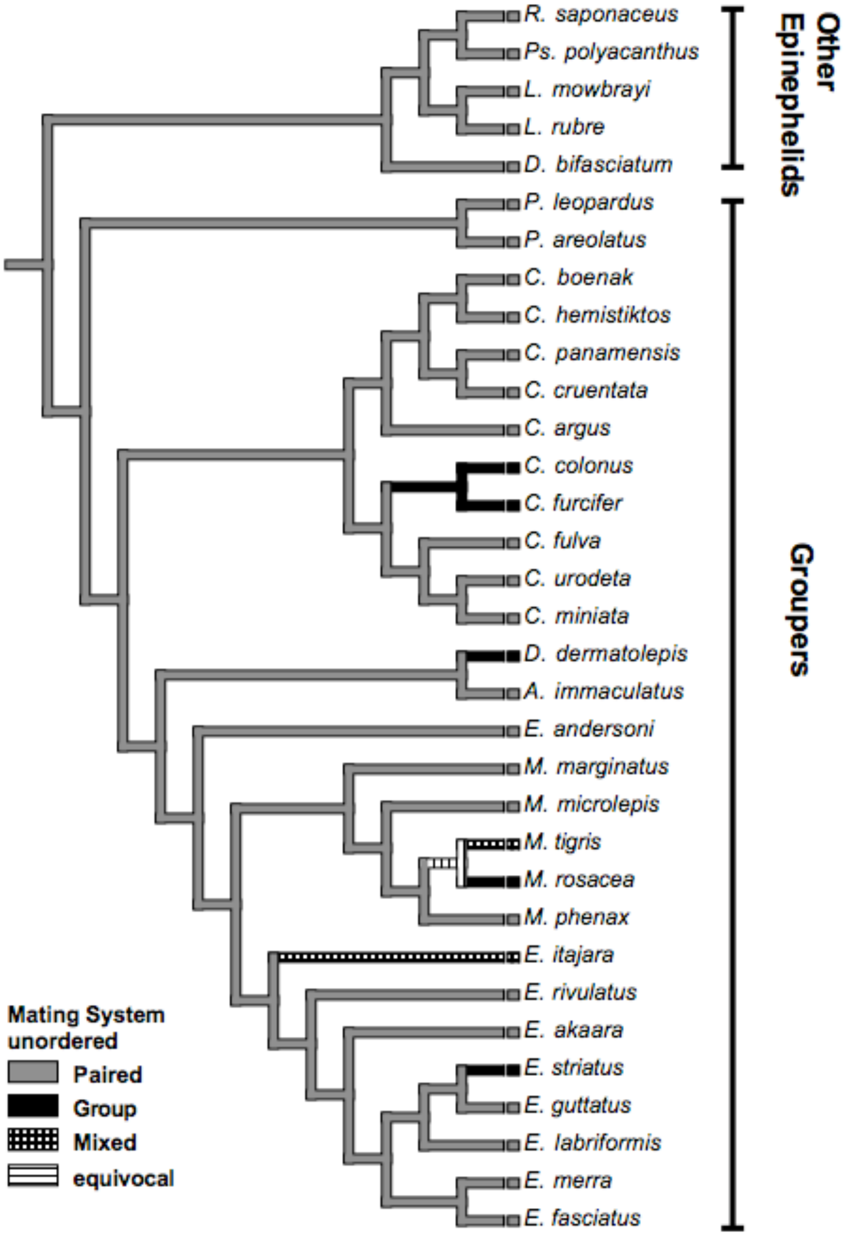

Fig. 6.3. Most parsimonious reconstructions of the transformations (ACCTRAN) in character states for mating pattern in 33 species of epinephelid fishes.

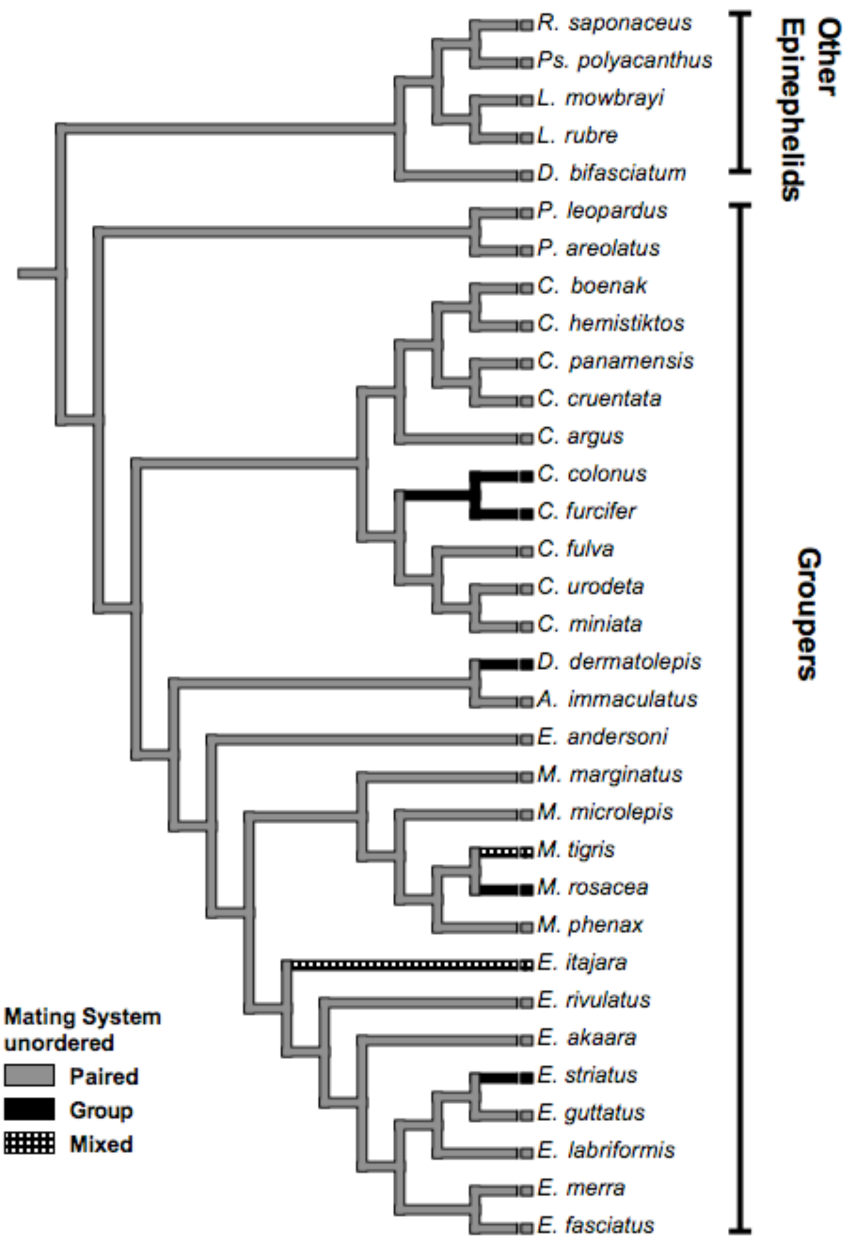

Fig. 6.4. Most parsimonious reconstructions of the transformations (DELTRAN) in character states for mating pattern in 33 species of epinephelid fishes.

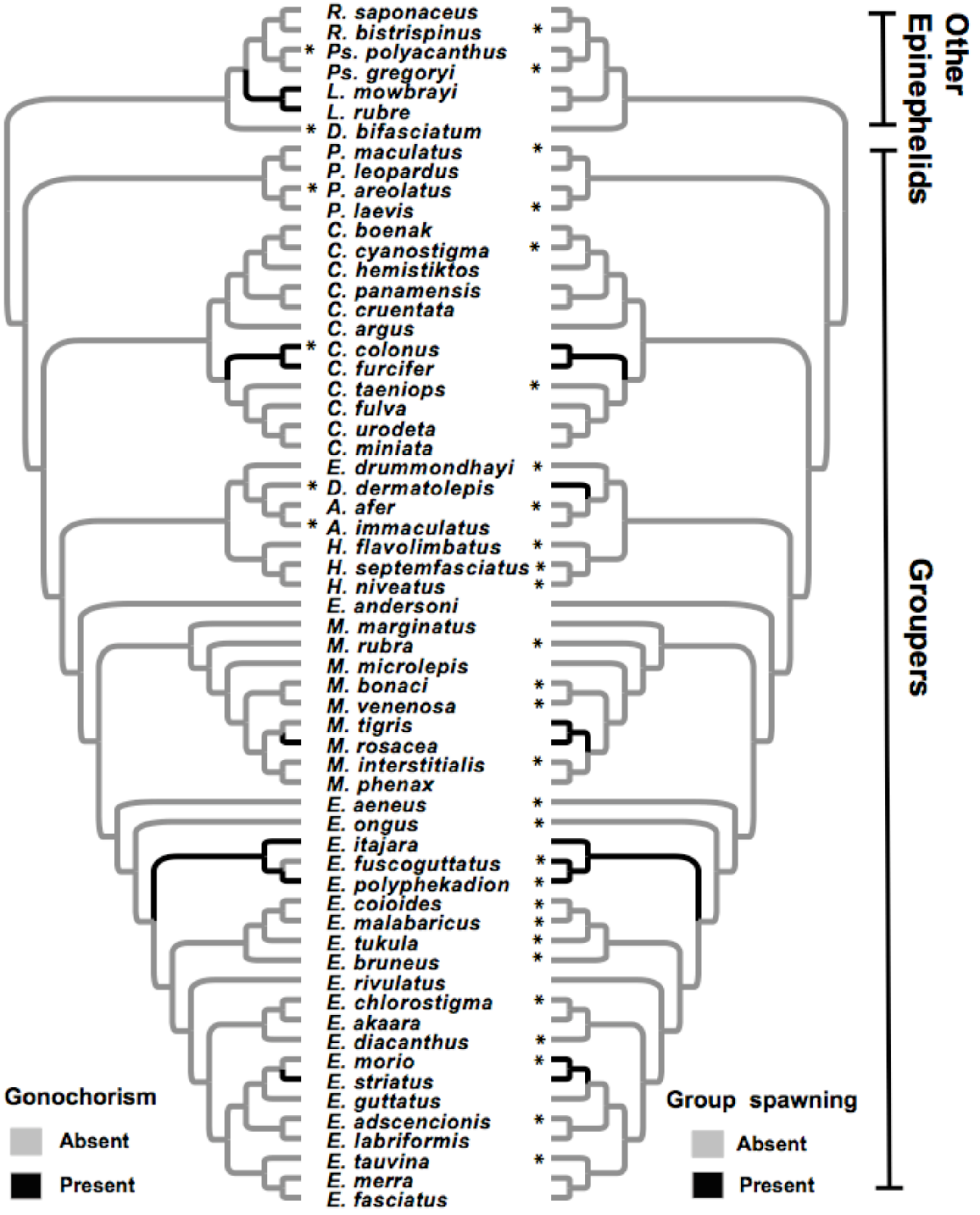

Fig. 6.5. Paired phylogenetic reconstructions (ACCTRAN) of the evolution of gonochorism and group spawning in 61 species of epinephelid fishes. * Indicates no data available.

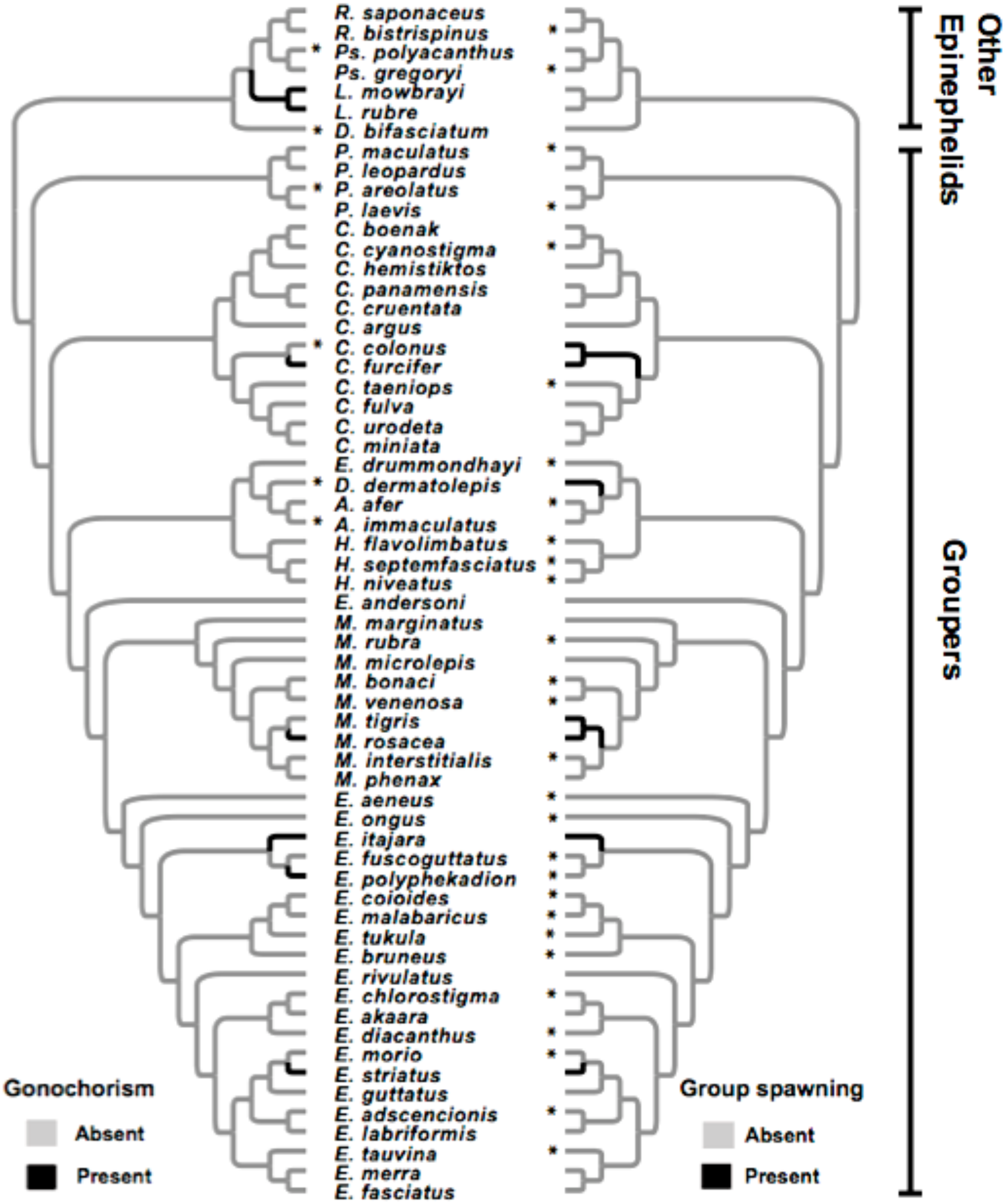

Fig. 6.6. Paired phylogenetic reconstructions (DELTRAN) of the evolution of gonochorism and group spawning in 61 species of epinephelid fishes. * Indicates no data available.

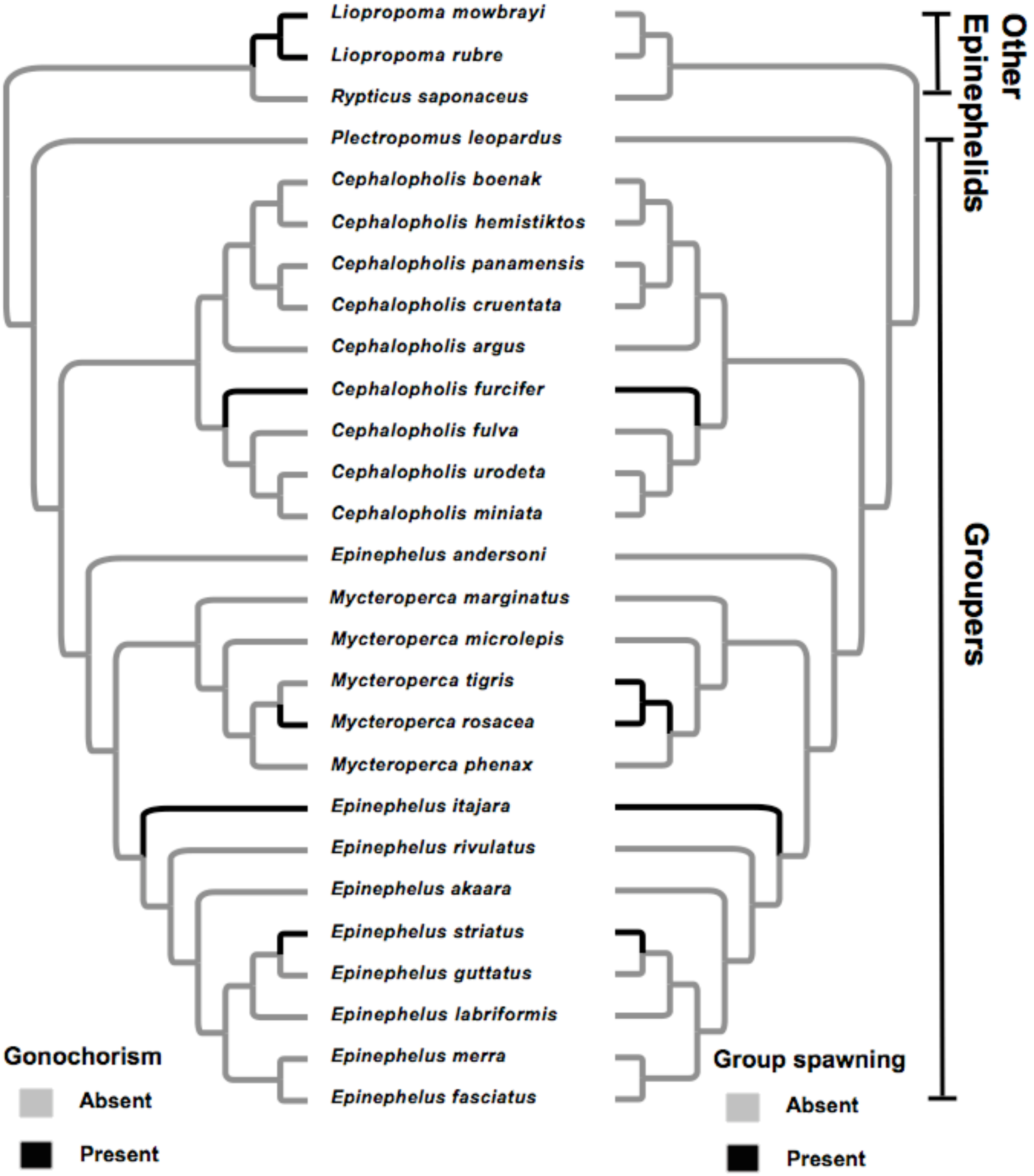

Fig. 6.7. Paired phylogenetic reconstructions (ACCTRAN and DELTRAN) of the evolution of gonochorism and group spawning in 27 species of epinephelid fishes.

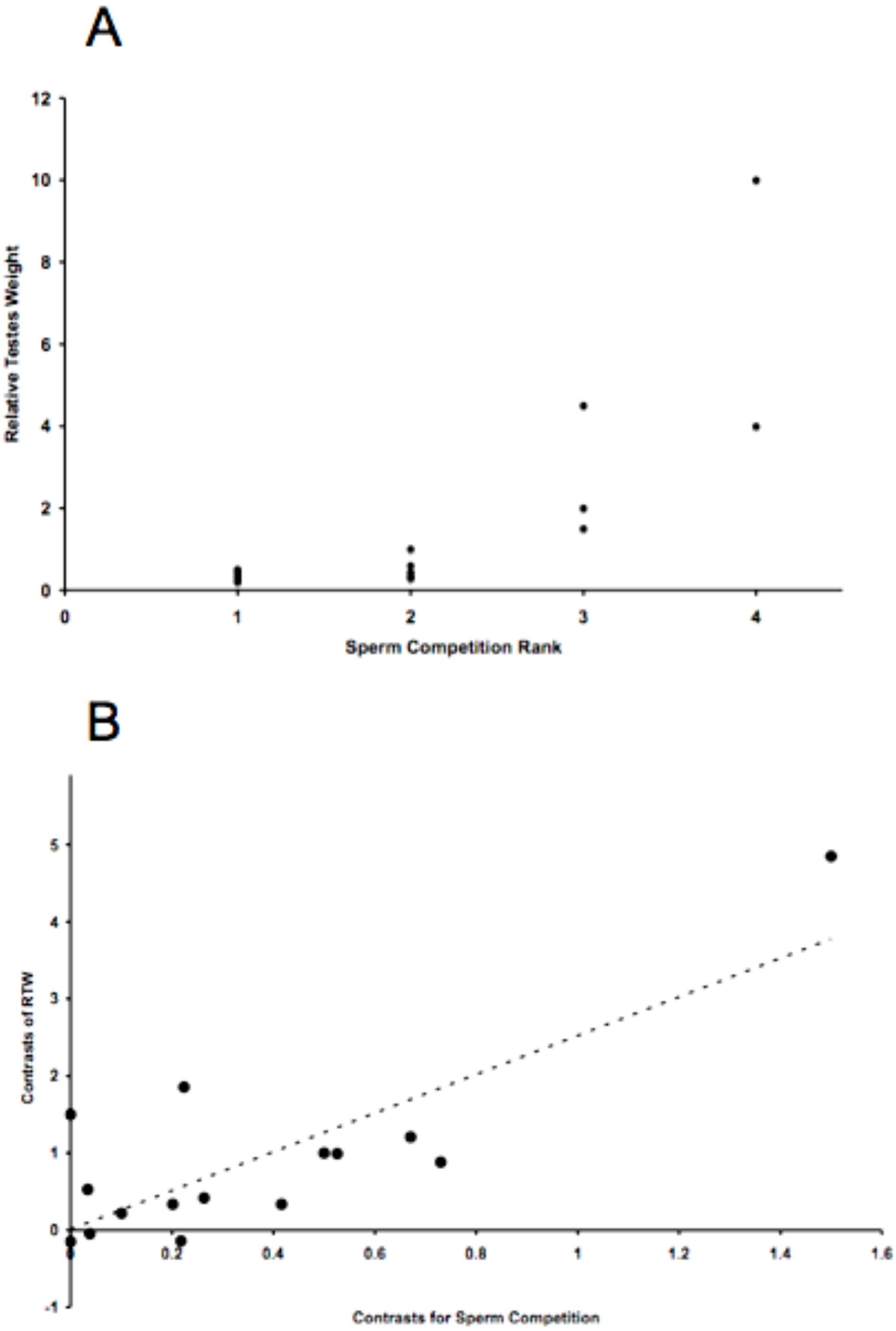

Fig. 6.8. Comparisons of relative testes weights (RTW) and sperm competition rank in 16 species of epinephelid fishes. a) Raw data, b) phylogenetically independent contrasts.

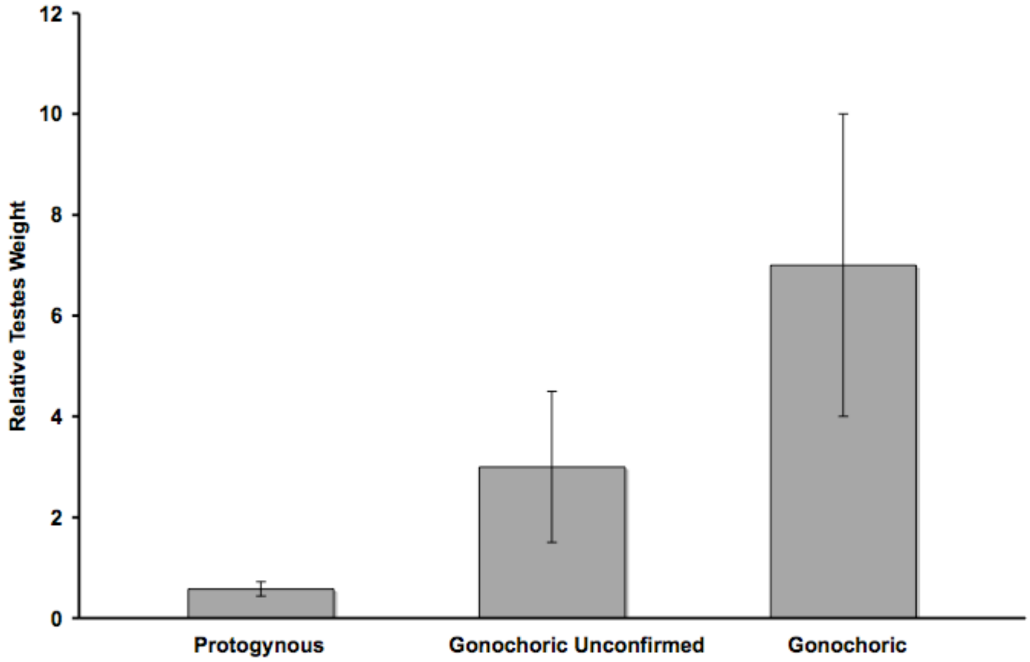

Fig. 6.9. Comparisons of median elative testes weights (± SE) in 16 species of epinephelid fishes by sexual pattern.

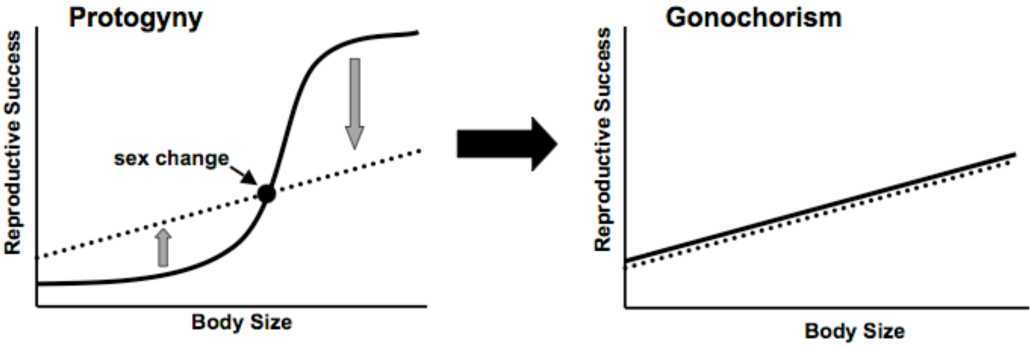

Fig. 6.10. Conceptual model showing how reproductive success of males (solid line) and females (dashed line) shifts as sperm competition increases and mating group structure changes from paired to group spawning, which in turn leads to the loss of selection for sex change and the evolution of gonochorism.

Appendix 1: Analysis of sperm competition in serranid fishes

Table 6.A1: Comparisons of relative testes weights (RTW), sperm competition intensity, and sexual patterns in 10 species of serranid fishes (Teleostei: Serranidae).

Species	**Mean RTW (%body wt.)**	**Sperm Competition Rank**	**Sex Pattern**	**References**
Centropristis striata	0.6	2	PR	Link 1980
Hypoplectrus sp.	0.05	1	SI	Petersen 1991; Petersen and Warner 1998
Paralabrax clathratus	5.8	4	GO	Erisman et al. 2006a,b
Paralabrax nebulifer	6	4	GO	Allen, unpublished data
Pseudanthias squamipinnis	0.5	2	PR	Suzuki et al. 1978
Serranus baldwini	0.09	1	SI	Petersen 1991; Petersen and Warner 1998; Petersen 2006
Serranus psittacinus	0.23	1	PD	Hastings and Petersen 1986; Petersen 1991; Petersen 2006
Serranus tabacarius	0.07	1	SI	Petersen and Warner 1998; Petersen 2006
Serranus tigrinus	0.08	1	SI	Petersen 1991; Petersen and Warner 1998; Petersen 2006
Serranus tortugarum	0.63	2	SI	Petersen and Warner 1998; Petersen 2006

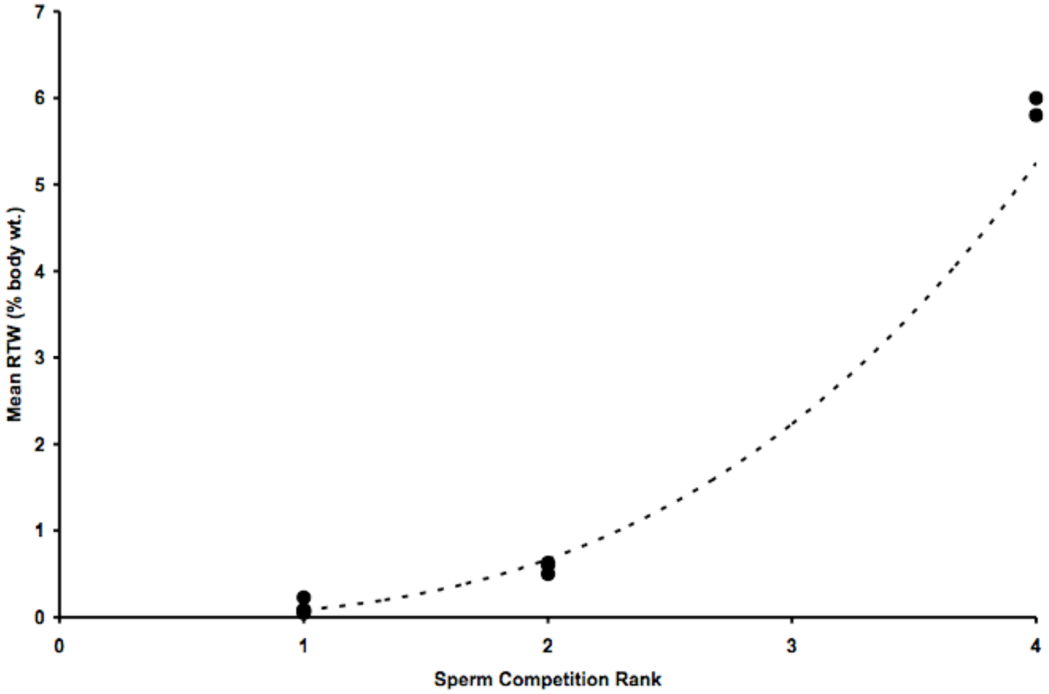

Fig. 6.A1. Comparisons of mean relative testes weights (RTW) by sperm competition rank in 10 species of serranid fishes (Teleostei: Serranidae). Mean RTW values were positively correlated with sperm competition intensity (Linear correlation; $r^2 = 0.912$; $p < 0.001$).

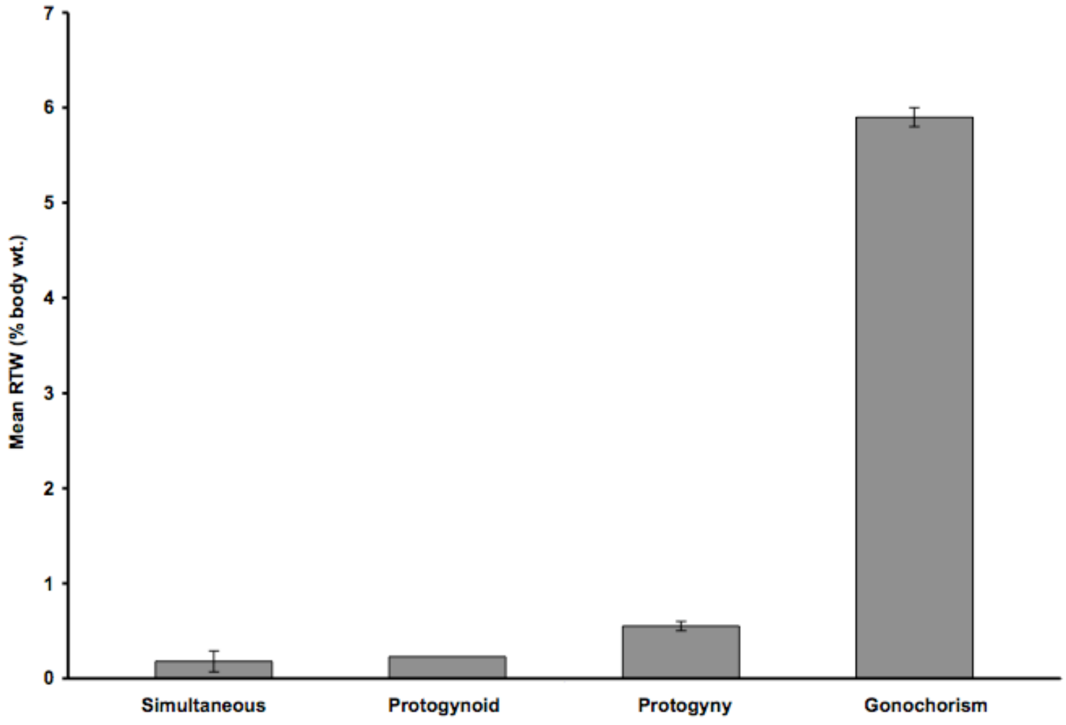

Fig. 6.A2. Comparisons of mean relative testes weights (RTW) by sexual pattern type in 10 species of serranid fishes (Teleostei: Serranidae). Differences in median RTW among sexual pattern types were significant (Kruskal-Wallis Test; KW = 5.705; p = 0.046). Median RTW of gonochoric species was significantly higher than hermaphroditic species (Mann Whitney U Test; hermaphroditic species pooled; U = 0.00, p = 0.041).

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