THE REPRODUCTIVE PATTERN OF BARRED SAND BASS (*PARALABRAX NEBULIFER*) FROM SOUTHERN CALIFORNIA

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ABSTRACT

Sea basses of the genus *Paralabrax* are either gonochores or protogynous hermaphrodites. This study sought to determine which, if either, reproductive strategy prevails in barred sand bass (*Paralabrax nebulifer*). A total of 436 specimens were collected from June 1996 through April 1997 from seven different locations along the southern California coast. Of these, only two (< 0.1%) transitional individuals were found. All males examined exhibited testes with a membrane-lined central cavity and a sperm sinus in the gonadal wall; our findings suggest that these individuals may have passed through a female-like juvenile stage prior to maturation. In addition, 52% of the males examined had atretic bodies. However, males and females were equally distributed throughout the age classes collected. Based on all evidence, we conclude that barred sand bass in southern California are functional gonochores that possess a latent, perhaps ancestral, ability to change sex.

INTRODUCTION

The barred sand bass (*Paralabrax nebulifer*) is a common species in the nearshore marine environment and an important part of the marine recreational fishery of southern California. It ranked second in the number of fish taken by sport-fishers in the state in 1989 and remains one of the most frequently caught species (Oliphant 1990). The barred sand bass together with the kelp bass (*Paralabrax clathratus*) comprised more than 90% of the general "rock bass" recreational catch in the first half of the twentieth century (Frey 1971). Barred sand bass range from Santa Cruz, California, south to Magdalena Bay, Baja California Sur, including Guadalupe Island (Miller and Lea 1972), and occupy a variety of different habitats, including kelp beds and sand flats on the open coast to inland harbors and bays. They are benthic, relatively sedentary fish and are rarely found more than 3 m above the substrate, closely associated with bottom structure and sand (Turner et al. 1969; Feder et al. 1974; Larson and DeMartini 1984). Barred sand bass are oviparous broadcast batch spawners that breed from April to August, with a peak in July (Love et al. 1996). They tend to form large breeding aggregations over soft bottom areas at depths of 15–30 m during their spawning season. Their eggs and larvae are pelagic, drifting in open water, and juveniles appear in shallow water from late summer to early winter (Love 1991). In this species, 50% of males matured at 219 mm, and 50% of females matured at 239 mm. Males mature between the ages of 2 and 4 years and females between the ages of 2 and 5 years (Love et al. 1996).

The barred sand bass is a warm temperate member of the family Serranidae (sea basses) and one of the three species of *Paralabrax* that occur along the southern California coast. Many species of the family Serranidae have specialized reproductive strategies. Some species are protogynous hermaphrodites and hence spend the early part of their lives as females and later change sex to conclude their lives as males. Oda et al. (1993) suggested that barred sand bass might be protogynous hermaphrodites based on a relatively small number of specimens (*N* = 81). They found six specimens that had gonads containing both ovarian and testicular tissue. Four of these were believed to be active males with primary oocytes in the testes. Proliferating testicular tissue was found in the remaining two specimens, one of which was an immature individual. This finding led Oda et al. (1993) to conclude that barred sand bass may be capable of both pre- and post-maturational sex change. However, based on the criteria for hermaphroditism established by Sadovy and Shapiro (1987), Oda et al. concluded that there was insufficient evidence to identify barred sand bass as a protogynous hermaphrodite. Their histological evidence was certainly suggestive of that conclusion. The major weakness of this study was born out of the relatively small sample size, which made it impossible to examine sex ratios over size classes and, particularly, age classes. We sought to address these shortcomings by using a much larger sample and determining the age of each specimen.

Studies on the other two temperate *Paralabrax* species have shown varying results (DeMartini 1987). Kelp bass (*P. clathratus*) are found in nearshore coastal waters usually associated with hard substrate and kelp. They have been termed "secondary gonochores," believed to have evolved from protogynous ancestors and do not change sex (Smith and Young 1966). Spotted sand bass (*P. mac-*

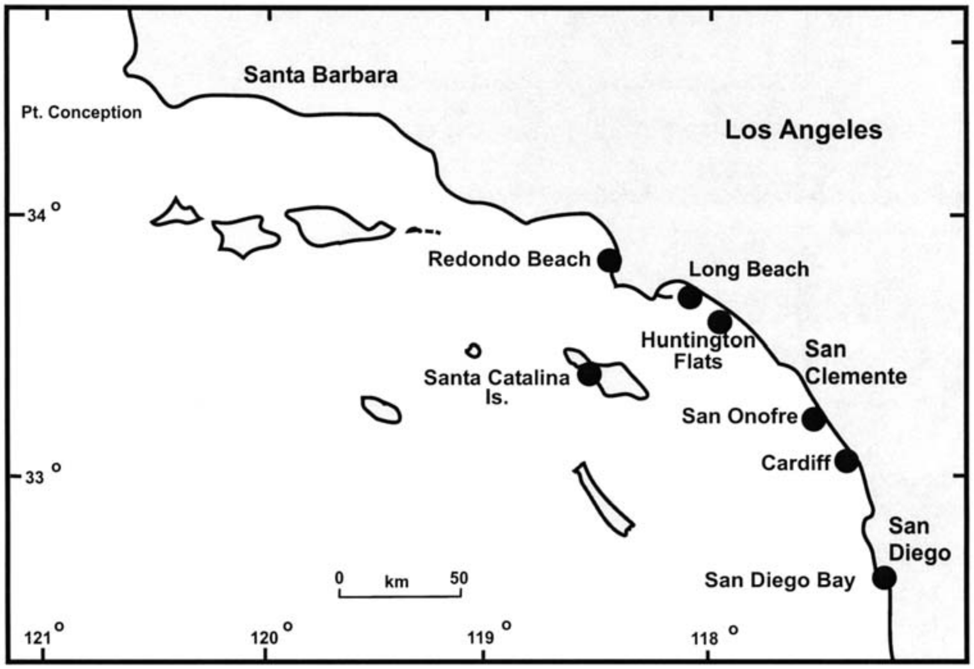

Figure 1. Sampling locations along the southern California coast for barred sand bass (*Paralabrax nebulifer*) reproductive study.

ulatofasciatus) found in harbors and bays (Allen et al. 1995) have specific populations that are protogynous hermaphrodites and others that appear to be gonochoric (Hastings 1989; Hovey and Allen 2000). The presence of protogynous hermaphrodites in *P. maculatofasciatus* was hypothesized to be related to population structure and mating system at various locations.

Our main objective in this study was to determine whether protogynous hermaphroditism occurs in barred sand bass and, if so, to what extent. To that end, we will present both histological evidence and age structure data from a large sample of barred sand bass collected primarily from breeding aggregations off the coast of southern California.

Knowledge of the reproductive strategy used by a species is important for proper management of the fishery. Fisheries are managed to protect the species and to provide sustainable yields. Management strategies assuming gonochorism may not be appropriate for fish populations with protogynous hermaphroditism. Species with the latter may have numerous small females and fewer larger males rather than equal ratios of males and females by length and age. Such species may require special protective measures to ensure sufficient numbers of both genders.

METHODS

A total of 436 specimens were collected by hook and line from June 1996 through April 1997 along the coast of southern California at Redondo Beach, Long Beach, Huntington Flats, Santa Catalina Island, San Onofre, Cardiff, and San Diego Bay (fig. 1). Each specimen was weighed to the nearest gram, measured to the nearest millimeter standard length, and preliminarily sexed. Gonads were removed, weighed, and preserved in 10% formalin for later histological examination.

Gonads were fixed by immersion in Davidson's solution (20% formaldehyde, 10% glycerin, 30% EtOH, 10% glacial acetic acid, and 30% deionized water) for two weeks then transferred to 70% ethyl alcohol. Cross-sectional tissue samples (4 mm thick) were removed from the mid area of each gonad and placed in Tissue Tek tissue cassettes. Using the Tissue Tek tissue-fixing unit, cassettes were emersed in a series of increasing concentrations (70%, 80%, 95%, and 100%) of ethyl alcohol with a final emmersion in xylene. The processed tissue was embedded with paraffin (at 56°C) and placed in a vacuum infiltrate for 1 h. The surface of the tissue block was then exposed and samples were soaked in a detergent bath for 10–14 days. Samples were sectioned at 6–12μm using an 820 Spencer microtome and mounted on slides. The slides were placed in an oven and allowed to dry for 7 days at 35°C. They were then stained using Mayer's alum hematoxylin and eosin, allowed to dry, and sealed with cytosol and a cover slip. Gonad morphology was examined using a compound microscope under 100–400× power. Initial sex determination was confirmed based on the finding of ovarian tissue (females), testicular tissue (males), and indeterminate tissue (immature individuals).

TABLE 1
Number, Size, and Sex of Barred Sand Bass (*Paralabrax nebulifer*) Collected in Reproductive Biology Study, June 1996–April 1997

Location	Total no. specimens	Size range (mm)	No. of males	Size range (mm)	No. of Females	Size range (mm)	No. of Immatures	Size range (mm)	No. of transitionals	Size range (mm)
Cardiff	80	170–310	46	200–290	33	170–310	1	183	0	
Catalina	18	158–395	11	158–395	6	171–300	1	178	0	
Huntington Flats	233	191–362	106	191–322	127	198–362	0		1	273
Long Beach	4	202–328	3	202–297	1	328	0		0	
Redondo	6	250–405	5	250–405	1	285	0		0	
San Diego	51	78–335	17	121–308	12	169–335	22	78–201	1	306
San Onofre	44	155–460	20	230–460	19	230–375	5	155–190	0	
Total	436	78–460	208	121–460	199	169–375	29	78–201	2	273–306

Potential hermaphroditism was diagnosed following the criteria outlined in Sadovy and Shapiro (1997). The following features were considered strongly indicative of protogynous hermaphroditism: (1) transitional individuals; (2) atretic bodies in stages 1, 2, or 3 of oocytic atresia within the testes; (3) a membrane line central cavity in the testes; and (4) sperm sinuses in the gonadal wall. A fifth feature, population structure, can also add support to a diagnosis of hermaphroditism, if indicated. We examined all samples to determine the presence of any of these features.

Sagittal otoliths were also removed from each specimen and mounted onto wood blocks using cyanoacrylate and sprayed with an accelerant for instant curing. Otoliths were then sectioned using a Buehler isomet low-speed saw. A dorso-ventral, 1.0 mm cross section was cut through the focus and perpendicular to the sulcus, using two diamond-edge blades separated by a stainless steel shim. Sections were polished with 3M lapping film and water. Sections were then placed in a black bottom, water-filled dish and read under a dissecting microscope at 50× magnification. Sections were aged by identifying the first translucent annulus and counting sequential growth zones from the center to the dorsal edge. Otoliths were then re-read by a second reader, with 85% agreement as outlined by Allen et al. (1995). These ages were then used to calculate sex ratios based on age class. Sex ratios were also calculated based on standard length. Sex ratios were examined for each individual location as well as for the population as a whole.

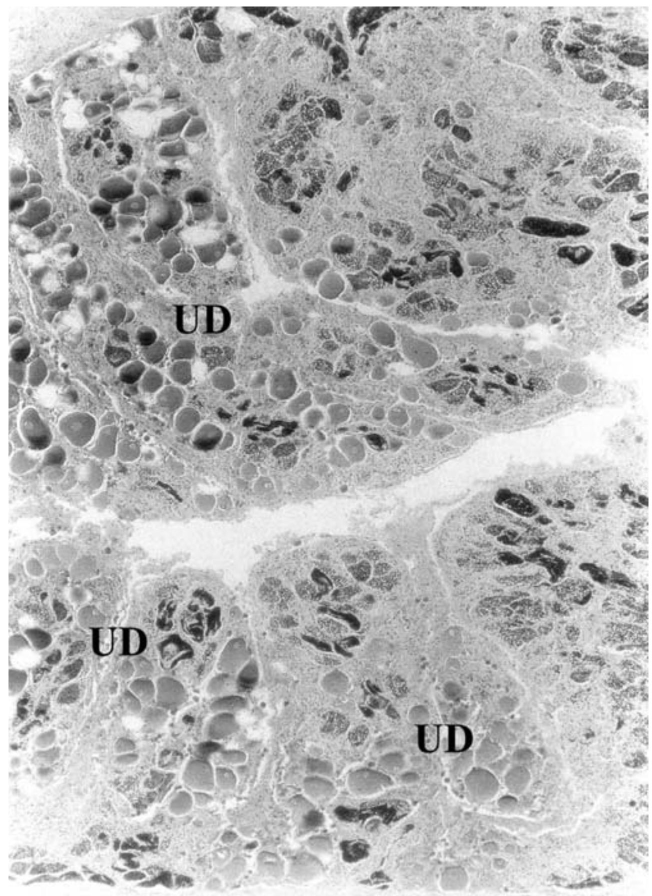

Figure 2. Immature gonad morphology in barred sand bass (*P. nebulifer*) showing undeveloped tissue (UD) resembling immature female features.

RESULTS

A total of 208 males, 199 females, 29 immatures, and 2 transitional individuals were examined (tab. 1). The majority of the fish (males and females) were collected from breeding aggregations at Huntington Flats, just southeast of Los Angeles–Long Beach Harbor. Immature individuals were only taken at Cardiff, Catalina, San Diego and San Onofre, with the majority being collected in San Diego. Overall, fish ranged in size from 78–460 mm SL. A 5-year-old, 273 mm SL transitional individual was taken from Huntington Flats and a 4-year-old, 250 mm SL transitional individual was taken from San Diego. Both transitional individuals were larger than the size at first maturity (Love et al. 1996).

Histological sections of ovaries in females showed typical primary, secondary, and vitellogenic oocytes. Testes of males exhibited typical spermatocytes, seminiferous

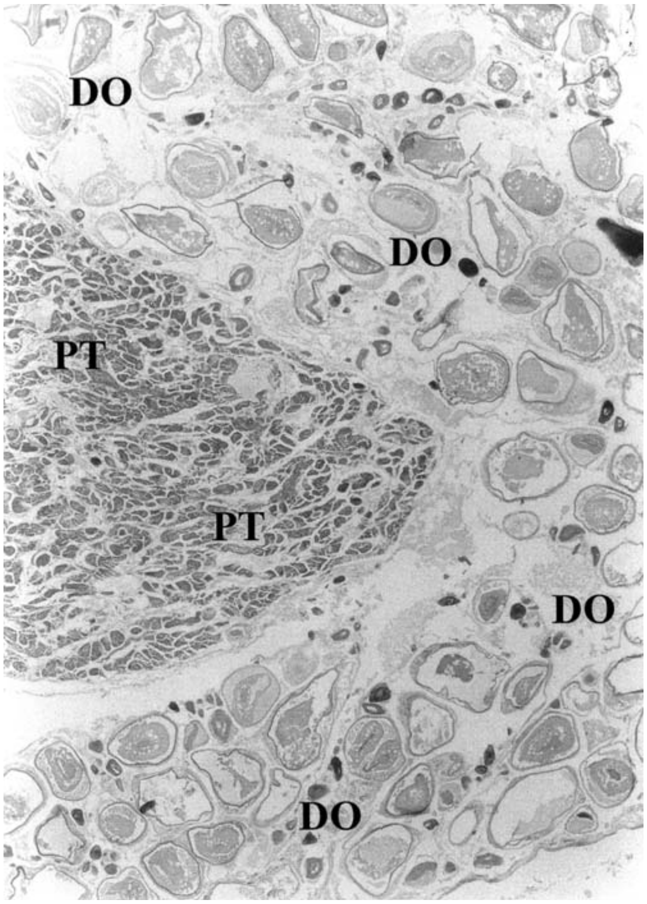

Figure 3. Transitional individual in barred sand bass (*P. nebulifer*) showing degenerating ovarian tissue (DO) surrounding proliferating testicular tissue (PT).

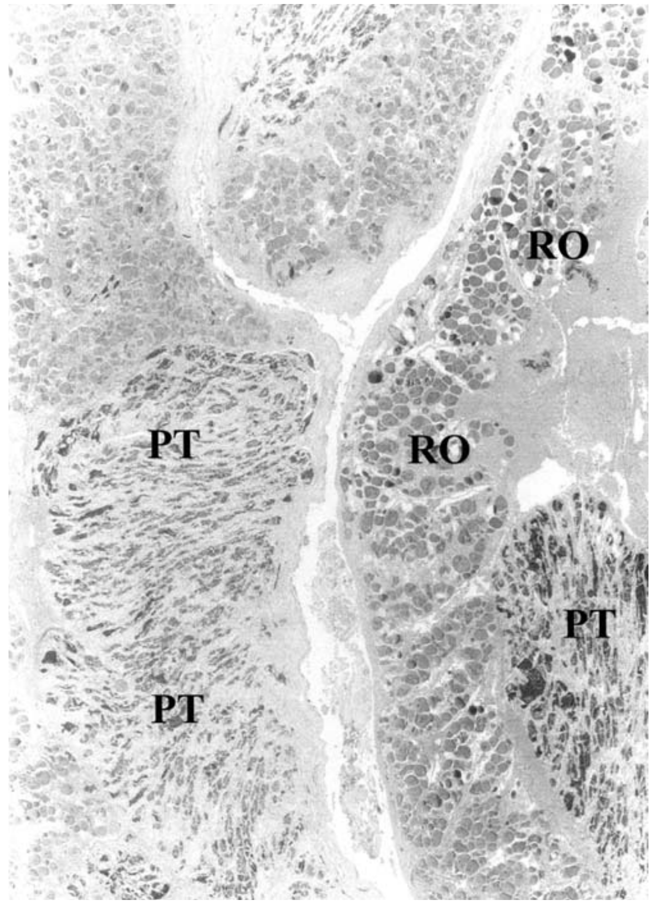

Figure 4. Transitional individual in barred sand bass (*P. nebulifer*) showing resting ovarian tissue (RO) and proliferating testicular tissue (PT).

tubules, sperm sinuses, and the crypts containing the developing sperm. All immature individuals were found to pass through a female-like stage of developing tissue with female features (fig. 2).

The gonad of the first transitional individual contained proliferating testicular tissue (developing sperm, sperm sinuses, and spermatocytes) and vitellogenic oocytes representing the degenerating ovarian tissue (fig. 3). This specimen was taken from Huntington Flats in August 1996 (during the later part of the breeding season). The second transitional individual exhibited resting ovarian tissue (rather than vitellogenic oocytes) and proliferating testicular tissue (fig. 4). This individual was taken off the coast of San Diego in April 1997 outside the breeding season.

A membrane-lined central cavity (gonadal lumen) with squamous epithelium cells was found in all males examined (fig. 5). The gonadal lumen was found to be used for the transportation of sperm in some of the males examined (fig. 6). The presence of a sperm sinus in the gonadal wall containing developing sperm surrounded by a muscle layer was also found in all males examined (fig. 7). Atretic bodies, found in 52% of all males examined, showed the characteristic yellow-brown pigment, granulose cells, and basophilic nuclei (fig. 8).

Sex ratios were examined based on standard length and age class (fig. 9). Individual locations showed comparable proportions of males and females throughout the size (ANCOVA, $F = 0.90$, $p = 0.35$) and age ($F = 0.88$, $p = 0.36$) classes allowing locations to be combined. In general, males and females were found to be distributed throughout the size and age classes for the entire sample (fig. 10). Most importantly, sex ratios did not differ significantly with age ($\chi^2 = 12.48$, df = 9, $p = 0.19$). Interestingly, females were actually better represented than males in the larger size classes ($\chi^2 = 25.59$, df = 8, $p < 0.001$). This distribution pattern is the opposite of what would be expected in a protogynous strategy. The presence of large females actually argues against protogyny and is likely due to sampling bias in this case. Samples were obtained primarily by hook and line in breeding aggregations where smaller, aggressive males tend to be captured at higher frequencies. Furthermore, these aggregations, once found, are targeted by commercial

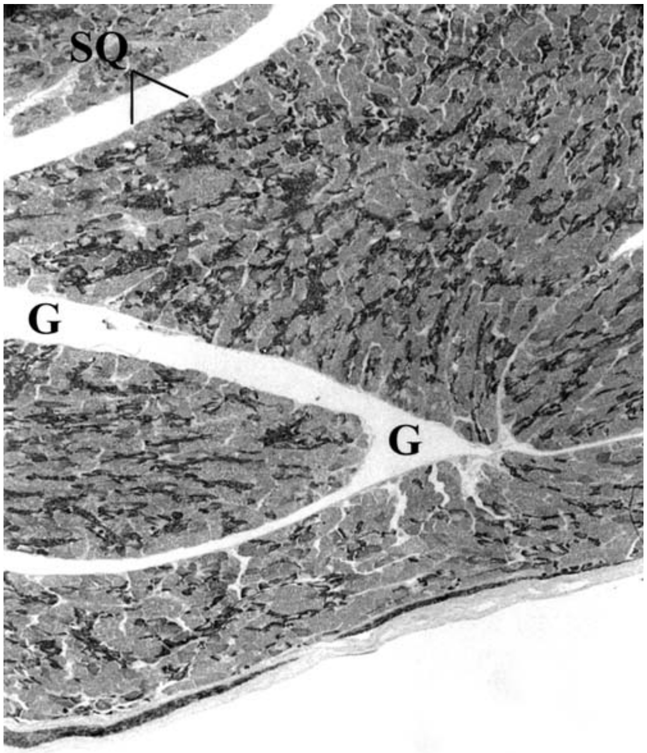

Figure 5. Gonadal lumen in male tissue of barred sand bass (*P. nebulifer*) showing gonadal lumen (G) and squamous epithelium cells (SQ).

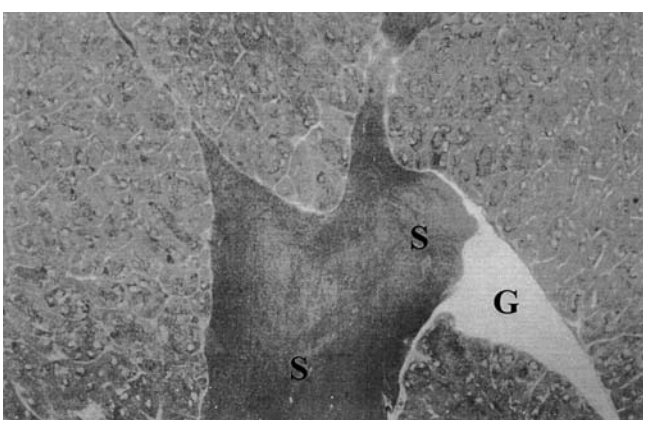

Figure 6. Gonadal lumen (G) functioning for the transport of sperm (S) in male tissue of barred sand bass (*P. nebulifer*).

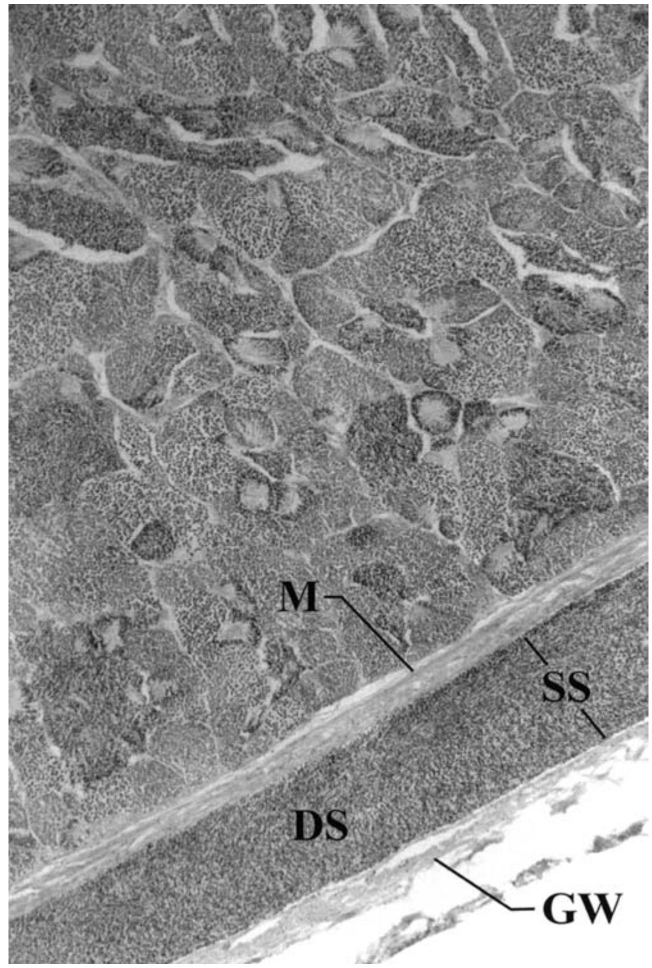

Figure 7. Sperm sinus (SS) in the gonadal wall of a male barred sand bass (*P. nebulifer*) containing developing sperm (DS) surrounded by the muscle layer (M) and gonadal wall (GW).

passenger fishing vessels (CPFVs) over successive days, which probably removes males preferentially over time on the spawning grounds.

DISCUSSION

The first and strongest criterion used to indicate sex change is the presence of transitional individuals (i.e., individuals in the process of changing sex) (Sadovy and Shapiro 1987). The gonadal tissue of protogynous hermaphrodites will show a degeneration of the initial ovarian tissue and a proliferation of testicular tissue. Our finding two transitional individuals during this study was predictable because transitional individuals are relatively uncommon in field studies. They are more typically seen in laboratory studies where sex change is experimentally induced (Sadovy and Shapiro 1987). The fact that we found transitional individuals at all supports the hypothesis that at least a portion of the population of barred sand bass can change sex.

The second criterion examined was the presence of a membrane-lined central cavity (gonadal lumen) in the testes. The gonadal lumen is thought to be a remnant of the ovarian lumen, which is used for egg transportation in females. All males examined were found to have gonadal lumens. The presence of a gonadal lumen is only a valid criterion if it is nonfunctional in males. Sperm was found in this cavity in some of the males ex-

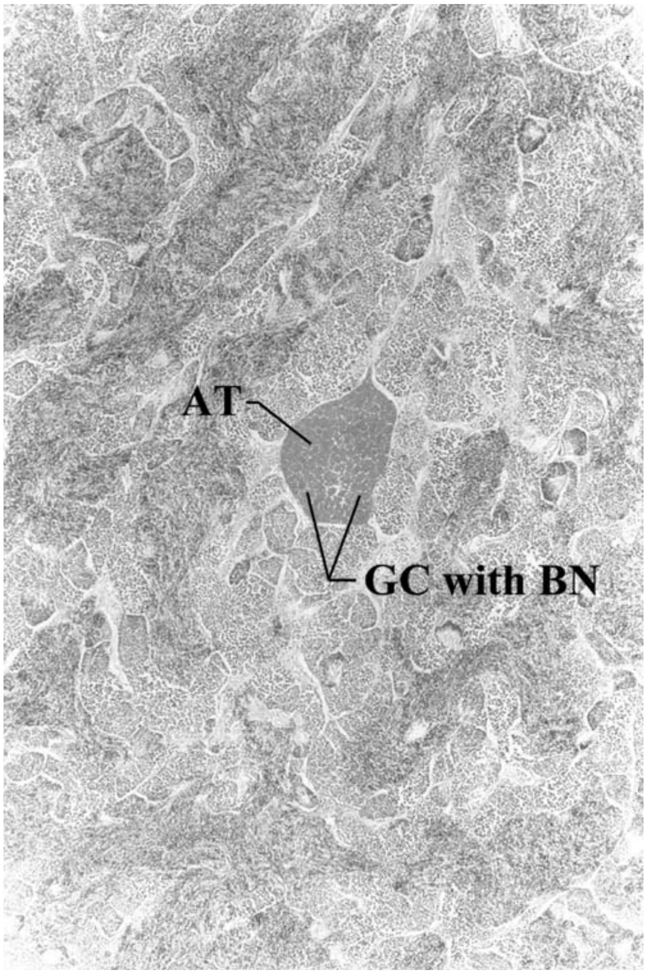

Figure 8. Atretic bodies (AT) in male barred sand bass (*P. nebulifer*) tissue showing the characteristic yellow-brown pigment, granulose cells (GC), and basophilic nuclei (BN) surrounded by advancing follicle degeneration.

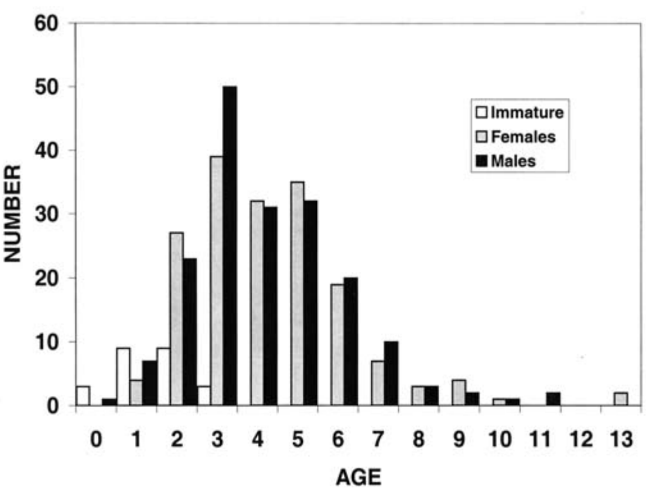

Figure 9. Number of immature, female, and male barred sand bass (*P. nebulifer*) specimens from southern California used in this study by age class ($N = 436$).

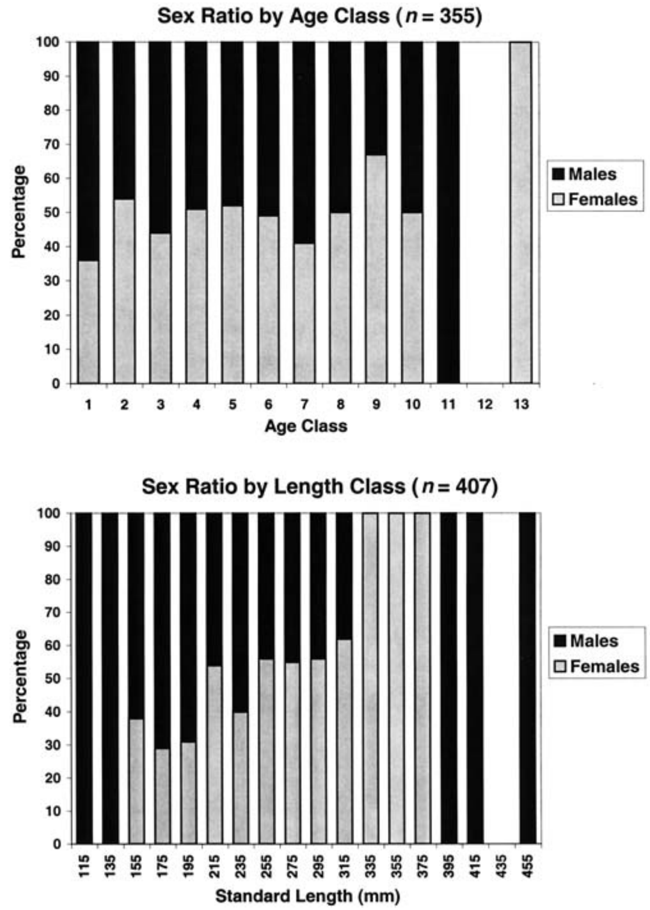

Figure 10. Sex ratios for the total sample of barred sand bass (*P. nebulifer*) showing the percentages of females and males by age class (*above*) and by length class (*below*).

amined, indicating that the cavity was used for the transportation of sperm in some individuals. Thus this criterion was not met.

The third criterion examined was the presence of the sperm sinus in the gonadal wall of the males. This sperm sinus is thought to develop from the splitting of the muscle layers of the ovarian capsule. All males examined were found to have a sperm sinus in the gonadal wall.

All males examined in this study contained gonadal lumens and sperm sinuses in the gonadal wall. There are two hypotheses other than hermaphroditism that can account for the presence of these two features in all males examined. First, these features may be evolutionary remnants from hermaphroditic ancestors. As mentioned earlier, species falling into this category have been termed "secondary gonochores" (Smith and Young 1966). Second, these features may result from all individuals passing through a female-like juvenile stage (Sadovy and Shapiro 1987). The gonads of all immature individuals examined in this study appeared to be passing through a female-like stage, supporting the second hypothesis.

The final criterion examined was the presence of atretic bodies. Atretic bodies were found in 52% of males examined. These structures are thought to be germ cell remnants of the initial sex that are retained after the sex change has occurred. Atretic bodies are not retained indefinitely, and as more time passes following change of sex, fewer germ cell remnants are present. These remnants can be caused by degenerating oocytes (going through atresia) but may also result from the degeneration of other types of cells (Smith and Young 1966). Alternative explanations for the presence of atretic bodies other than oocytic atresia include parasitic encystation (Atz 1964), sperm degeneration (Warner 1975b), and nonspecific tissue degeneration (Smith 1965).

Typical protogynous populations exhibit a bimodal distribution, with females making up a greater portion of the smaller, younger individuals and males making up a greater portion of the larger, older individuals (Sadovy and Shapiro 1987). We found an equal distribution of males and females throughout the age classes. While the sex distribution among length classes was significantly different, this is not the pattern of bimodality typically seen in protogynous populations. Factors that may obscure bimodality in protogynous populations are (1) lack of sexual dimorphism, (2) alternative pathways of male sexual development, (3) bisexual juveniles, (4) variation in size at sex change (Sadovy and Shapiro 1987), and (5) perhaps in this case, sampling bias. Thus, protogyny may not be completely ruled out on the basis of the sex-frequency distributions. However, the distributions found in this study favor a functionally gonochoric pattern of reproduction.

Typically in species that form breeding aggregations, individuals gather together during the breeding season in a central location to release their gametes. Protogyny is advantageous where larger males are able to dominate access to females (Warner 1975). In large breeding groups with an increased number of males present, it becomes difficult for larger males to monopolize access to females. Through mobbing and sneaking techniques, smaller males are able to gain access to females. All individuals may potentially release their gametes, and size does not appear to be an issue. Therefore, this arrangement tends to favor a gonochoric reproductive strategy. Kelp bass and barred sand bass are known to gather in large schools during spawning off California (Limbaugh 1955), and both species appear to be functionally gonochoric. Studies conducted on the spotted sand bass showed protogynous hermaphroditism to be most prevalent in large isolated populations where spawning may occur over large spatial and temporal scales (Hovey and Allen 2000).

In conclusion, the barred sand bass appears to possess many of the gonadal attributes of hermaphroditism, including the presence of transitional individuals and a large percentage of males possessing atretic bodies. However, transitional individuals are present very infrequently in their populations. Additionally, the presence of atretic bodies has been shown to be a weak indicator of sex change because atretic bodies may be formed by methods other than oocytic atresia, and a designation of a reproductive strategy cannot be determined using this characteristic alone. Barred sand bass were found to go through a female-like juvenile stage that may account for the presence of female tissue structures (gonadal lumen and sperm sinus) in all male individuals. The population-sex-by-age structure clearly follows a gonochoric pattern. Based on this population structure, the infrequent, hermaphroditic tissue conditions, and the data presented by Oda et al. (1993), we conclude that barred sand bass in southern California are functional gonochores that exhibit a latent ability to change sex.

The presence of hermaphroditic gonadal attributes, whether pre- or post-maturational, lends support to the "secondary gonochore" designation as proposed by Smith and Young (1966). However, additional information regarding the phylogeny of the genus *Paralabrax* is necessary before the validity of this term can be judged.

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