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REPRODUCTION OF YELLOWEDGE GROUPER, *EPINEPHELUS FLAVOLIMBATUS,* FROM THE EASTERN GULF OF MEXICO

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The yellowedge grouper, *Epinephelus flavolimbatus,* is a deep-water (100–300 m) western Atlantic serranid that occurs from North Carolina to Florida, throughout the Gulf of Mexico, in the West Indies, and off the coasts of Central and South America to Brazil (Carpenter and Nelson, 1971; Smith, 1971; Huntsman, 1976; Bullock and Smith, 1991). In the South Atlantic Bight, yellowedge grouper are exploited by commercial longliners and recreational anglers (Huntsman, 1976; Keener, 1984). During the late 1970s, interest in deep-water grouper stocks developed among Florida Gulf-coast commercial fishermen based on reports of large, unexploited concentrations of yellowedge grouper and other species such as snowy grouper, *E. niveatus*; warsaw grouper, *E. nigritus*; speckled hind, *E. drummondhayi*; tilefish, *Lopholatilus chamaeleonticeps*; and blueline tilefish, *Caulolatilus microps,* on the outer continental shelf. During 1980–1981, some commercial longline catches of yellowedge grouper and snowy grouper in the eastern Gulf exceeded 13 metric tons per 10-day trip (Bullock and Smith, 1991). Commercial grouper annual landings prior to 1986 were not recorded by species, but from 1986–1994, yellowedge grouper commercial landings on Florida's Gulf coast ranged from 212,008 to 421,007 kg per year (Florida Department of Environmental Protection annual fishery landings summaries). The recreational catch is negligible (U.S. Dept. Commerce, NMFS, Marine Recreational Fishery Statistics Survey), probably due to the great distance from ports (>120 km) to offshore depths (100–300 m), where yellowedge grouper typically occur. Yellowedge grou-

per is managed as a part of the deep-reef species complex and is included in the 0.7-million-kg annual quota allowed for this species complex in the Gulf of Mexico (Gulf of Mexico Fishery Management Council, 1981, 1994).

Little published information is available on the biology of yellowedge grouper. Bullock and Smith (1991) included preliminary data on yellowedge grouper in a description of eastern Gulf of Mexico serranids. The only comprehensive study is by Keener (1984), who described the spawning season and used otoliths to age yellowedge grouper commercially landed from South Carolina waters. Our study describes length-weight relations, size composition of the commercial catch, mode of reproduction, size at sexual maturity, and temporal spawning patterns for yellowedge grouper from the eastern Gulf of Mexico. We also attempted to age yellowedge grouper by examining sectioned otoliths.

Materials and Methods

From November 1977 to November 1980, we examined 1,140 yellowedge grouper from the eastern Gulf of Mexico. We sampled as many grouper as possible during the study period without regard to size. Grouper were caught by commercial fishermen using either hook-and-line gear or bottom longlines. For each specimen, we measured standard length (SL), fork length (FL), and total length (TL) in millimeters, and individual weights were taken in pounds and later converted to grams. All lengths reported in the text are TL. Otoliths were removed and stored in glycerin, and whole gonads were fixed in Davidson's solution. An additional 2,437 specimens were measured to determine the size structure of the commercial catch. We also obtained lengths of 621 yellowedge grouper that were measured as part of the Florida Department of Environmental Protection's Trip Interview Program during 1993–1994.

Fixed gonads were weighed to the nearest gram, transferred to 70% ethanol, and then dehydrated in a graded ethanol series. Histological sections of gonads from 1,107 grouper were prepared with the following procedure: tissue was embedded in paraffin, sectioned, and stained with "Harris" hematoxylin and eosin. Each tissue sample was examined microscopically to determine reproductive state. Gonads were classified based on the maturity criteria that Moe (1969) developed for *E. morio* (red grouper). The class "ripening" included both immature females ripening to spawn for the first time and mature-resting females that had spawned previously and were ripening to spawn again. The diameters of 10 of the largest oocytes in each histological section were measured with an ocular micrometer to estimate the mean maximum oocyte diameter. Oocytes were measured only if the nucleus was visible, indicating that the oocyte was sectioned approximately through the center of the oocyte. Monthly mean oocyte diameters were plotted to depict seasonal reproductive patterns. Gonadosomatic indices (GSI) were calculated for 788 grouper as

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

where GW = total gonad weight (g) and TW = total fish weight (g). Median gonadosomatic indices were plotted to show seasonal reproductive patterns.

We estimated the length at which 50% of the females in the population reached sexual maturity by determining the inflection point of a logistic function fit to the female maturity and length data. A similar function was fit to the sex and length data to estimate the length at which 50% of the females in the population had transformed into males. The parameter b from the logistic equation in Table 1 is the inflection point.

Transverse sections approximately 0.5 mm in width were cut from 1,033 otoliths (sagittae) with a Buehler Isomet low-speed saw. Three sections were cut from each otolith to ensure that one section contained the core. Sections were mounted on microscope slides and then examined with a dissecting microscope equipped with polarized light. Otoliths were independently examined by three readers.

Results and Discussion

During 1977–1980, we measured 3,577 yellowedge grouper that ranged from 360 to 1,083 mm (mean = 758 mm, SD = 121.6; Fig. 1A). Among fish that we sexed (N = 1,090), males ranged from 580 to 1,083 mm (mean = 880 mm, SD = 69.5, N = 396) and were significantly larger than females, which ranged from 360 to 1,065 mm (mean = 676 mm, standard deviation = 123.0, N = 694; t-test, $t = 30.50$, $P < 0.001$; Fig. 1B). The relations between standard length, fork length,

Table 1. Linear length-length and length-weight equations, and logistic equations relating the percentage of females that were sexually mature with total length and the percentage of the population made up by females with total length for yellowedge grouper from the eastern Gulf of Mexico. SL = standard length (mm), FL = fork length (mm), TL = total length (mm), WT = whole weight (kg), and GWT = gutted weight (kg). Values in parentheses are standard errors.

Y	X	N	a (1 SE)	b (1 SE)	r^2	TL range for regressions
			$Y = a + bX$			
SL	FL	1,408	−12.863 (0.9190)	0.849 (0.0012)	0.997	360–1,083
SL	TL	1,507	2.465 (1.1788)	0.789 (0.0015)	0.994	360–1,083
FL	SL	1,408	17.289 (1.0573)	1.174 (0.0017)	0.997	360–1,083
FL	TL	1,393	18.805 (1.1257)	0.928 (0.0015)	0.997	360–1,083
TL	SL	1,507	1.136 (1.4918)	1.260 (0.0024)	0.994	360–1,083
TL	FL	1,393	−17.612 (1.2401)	1.074 (0.0017)	0.997	360–1,083
$\log_{10}$WT	$\log_{10}$TL	465	−7.528 (0.0446)	2.861 (0.0156)	0.986	370–1,065
$\log_{10}$GWT	$\log_{10}$TL	713	−7.572 (0.0439)	2.874 (0.0153)	0.980	368–1,083
			$Y = (1/(1 + e^{(a(X + b))})) \cdot 100$			
% Mature (females)	TL	692	−0.26 (0.0022)	−568.6 (3.55)	0.586	360–1,065
% Females	TL	1,090	0.025 (0.0018)	−816.8 (2.923)	0.574	360–1,083

total length, and weight are presented in Table 1. Yellowedge grouper measured during 1993–1994 ranged from 273 to 1,107 mm and had a mean length of 650 mm (standard deviation = 160.1, N = 621; Fig 1C). The length-frequency distributions from 1977–1980 and 1993–1994 were significantly different (Kolmogorov-Smirnov two-sample test, $D = 0.340$, $P < 0.001$); the mean length of grouper caught during 1977–1980 was significantly larger than that of grouper caught during 1993–1994 (t-test, $t = 19.32$, $P < 0.001$).

Monandric protogynous hermaphroditism was suggested for *E. flavolimbatus* based on histological analyses of gonads following the criteria of Sadovy and Shapiro (1987). Protogyny was suggested by the presence of peripheral sperm-collecting sinuses in lieu of the centrally located ducts typical of gonochorists. Conclusive evidence was the presence of developing sperm cysts in a gonad containing degenerating oocytes (Fig. 2A). In addition, a vestigial ovarian lumen remained in the testis but appeared to be nonfunctional.

Recently, Sadovy and Colin (1995) suggested that particular care is needed in the diagnosis of functional sex change in serranids. Based on a histological study of Nassau grouper, *Epinephelus striatus,* they concluded that the presence of an ovarian-like lumen in males cannot confirm protogyny in the absence of other evidence. Their conclusion that Nassau grouper are "essentially gonochoristic" was supported by data on sex ratios and the mean sizes of males and females. The sex ratio of unexploited populations of Nassau grouper was not significantly different from 1:1 although the sex ratio of exploited populations was 2.2:1 (females:males). A sex ratio biased towards females is a characteristic of monandric protogynous species (Sadovy and Shapiro, 1987). Furthermore, Sadovy and Colin

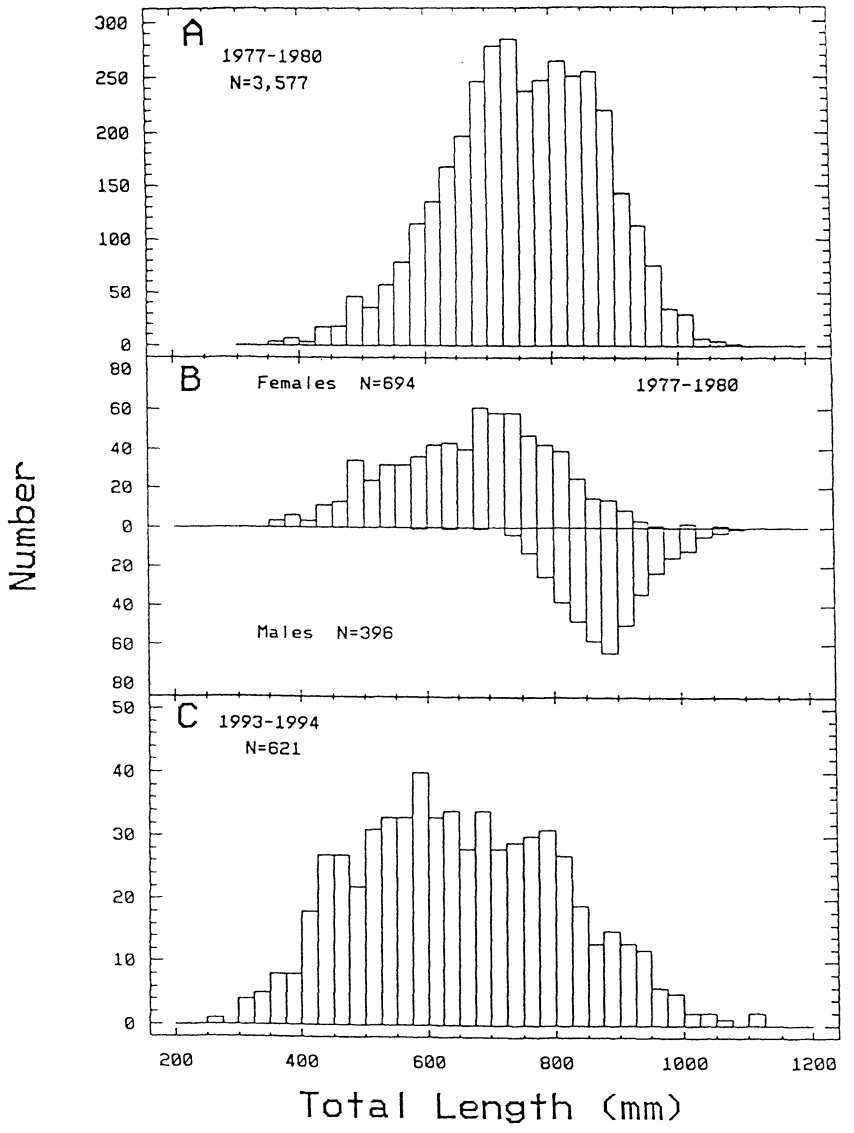

Figure 1. A) Length-frequency distribution of the commercial yellowedge grouper catch 1977–1980. B) Length-frequency distributions of female and male yellowedge grouper sampled from the commercial catch during 1977–1980. C) Length-frequency distribution of the commercial yellowedge grouper catch 1993–1994.

(1995) found little difference in the mean sizes of male and female Nassau grouper, inconsistent with protogyny.

We have no data on the sex ratio of unexploited yellowedge grouper populations, but the sex ratio of our sample was 1.8:1 (females : males), significantly different from 1:1 ($\chi^2 = 81.47$, $P < 0.001$) but similar to that of exploited Nassau grouper populations. An important difference between our yellowedge grouper data and those for Nassau grouper is that the size distributions of male and female yellowedge grouper were significantly different. Although there was considerable

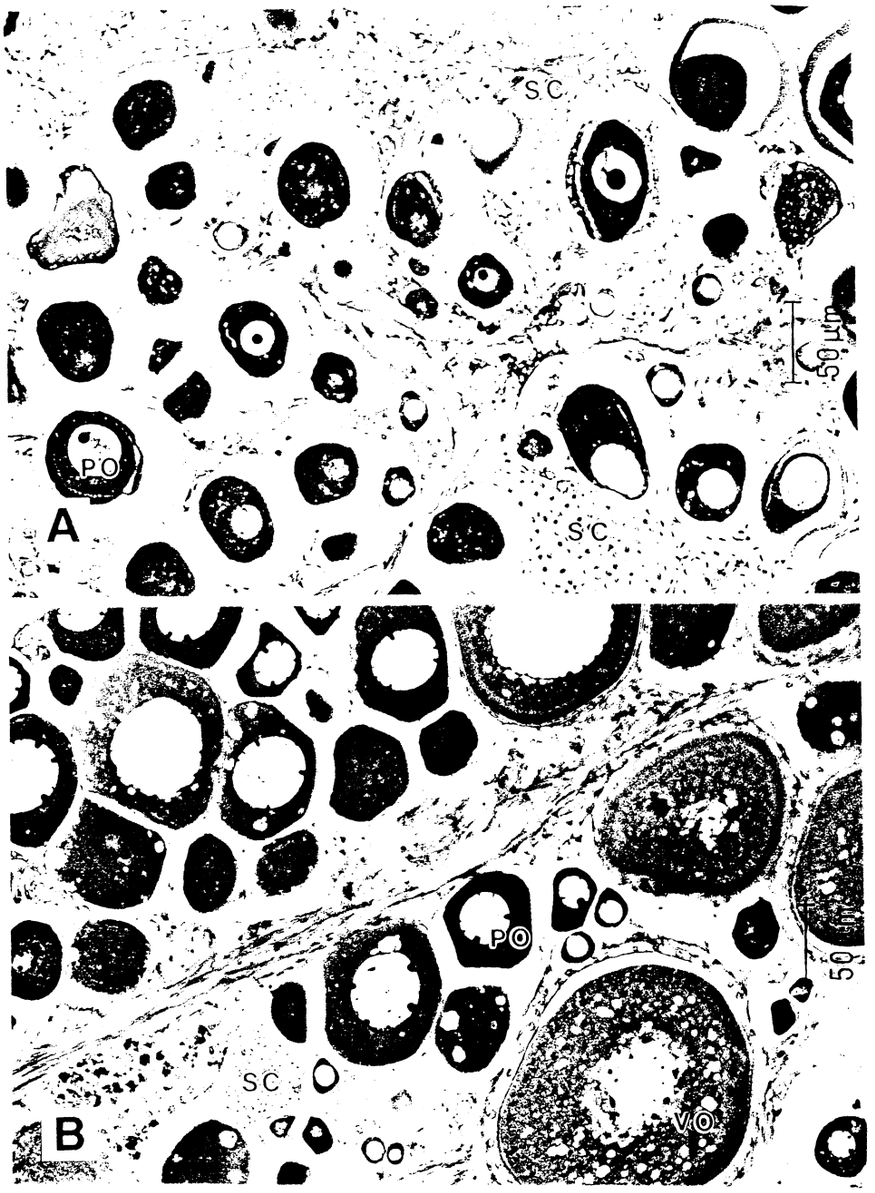

Figure 2. A) Photomicrograph of a transitional gonad from a yellowedge grouper (664 mm) showing proliferation of sperm cysts in degenerating ovarian tissue. B) Precocious sperm cyst in a ripening ovary from a yellowedge grouper (733 mm). PO = previtellogenic oocyte; SC = sperm cyst; VO = vitellogenic oocyte.

overlap between males and females in size distribution, males occupied the largest size-classes and were unrepresented in the smallest size-classes (Fig. 1B). This finding supports our hypothesis of monandric protogyny and is consistent with patterns observed for other grouper species that have been diagnosed as protogynous hermaphrodites (Moe, 1969; Collins et al., 1987; Hood and Schlieder, 1992; Sadovy et al., 1992; Bullock and Murphy, 1994).

The presence of scattered sperm cysts in functional ovaries was quite common in yellowedge grouper females (Fig. 2B). Other researchers have observed sperm cysts in functional ovaries in groupers. Smith (1965) reported what he termed "precocious crypts" (=cysts) in *Cephalopholis fulva* and *Petrometopon cruentatus*. Ferreira (1995) noted the presence of precocious sperm cysts in immature and mature ovaries of the common coral trout, *Plectropomus leopardus*. Neither Smith (1965) nor Ferreira (1995) believed that the presence of these cysts interfered with spawning as a female.

We estimated the length at which 50% of the females in the population had reached sexual maturity to be 569 mm (Table 1). Keener (1984) observed that 97% of females larger than 409 mm caught off the South Carolina coast were mature, suggesting a smaller size at sexual maturity for South Carolina fish than for those from the eastern Gulf of Mexico. Transition from female to male was also a function of size. At a length of 817 mm, 50% of the females in our Gulf sample had transformed into males (Table 1). We found only four transitional fish ranging from 525 to 871 mm and three immature males ranging from 732 to 898 mm. The scarcity of transitional fish and immature males in our samples suggests that transition occurs quickly and that males mature soon after transition. These results are similar to those of Keener (1984) who found transitional fish (700–809 mm) off South Carolina and reported that 71% of the grouper from 830 to 1,040 mm were males. The largest female we observed was 1,065 mm in length; the largest female reported by Keener (1984) was 990 mm. The presence of large females in the population suggests that not all females transform into males.

The shift in the length-frequency distributions of the yellowedge grouper commercial catch towards smaller sizes in 1993–1994 than in 1977–1980 suggests that the cumulative lifetime mortality rate experienced by the fish sampled in 1993–1994 was greater than that experienced by the fish sampled in 1977–1980. The percentage of the population made up by fish larger than 817 mm, the size at which we estimated that 50% of the females had transformed into males, was less in 1993–1994 (16.3%) than in 1977–1980 (34.5%). A consequence of this decrease in the numbers of large fish could be a decrease in the percentage of males present in the population, unless the sex ratio was conserved by a decrease in the size at transition. Because sex was not recorded for the fish measured during 1993–1994, we do not know if the population's sex ratio has changed or if the size at which transition occurs has changed. Furthermore, there are no estimates of fishing mortality rates available for yellowedge grouper that might suggest overfishing. Among other deep-water grouper, snowy grouper, *Epinephelus niveatus*, are thought to be overfished in many areas, and some populations show reductions in mean fish size similar to those that we observed for yellowedge grouper (Epperly and Dodrill, 1995).

Yellowedge grouper gonadal activity peaked during May–September. Immature (N = 160) and resting females (N = 96) were found during each month that we sampled. Ripe females (N = 311) were sampled January–October but were most abundant during May–September. Spent females (N = 137) were most abundant during October, the end of spawning season, but were present during July–March (Fig. 3). Resting females were most abundant during December–March, following

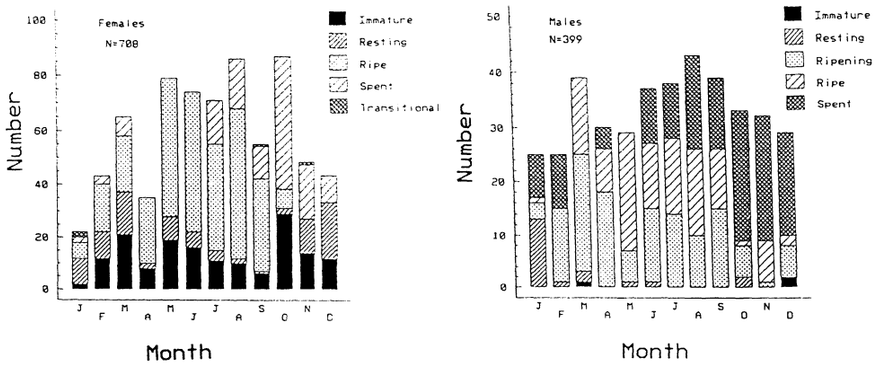

Figure 3. Monthly distributions of gonad classes for female and male yellowedge grouper caught during 1977–1980 from the eastern Gulf of Mexico.

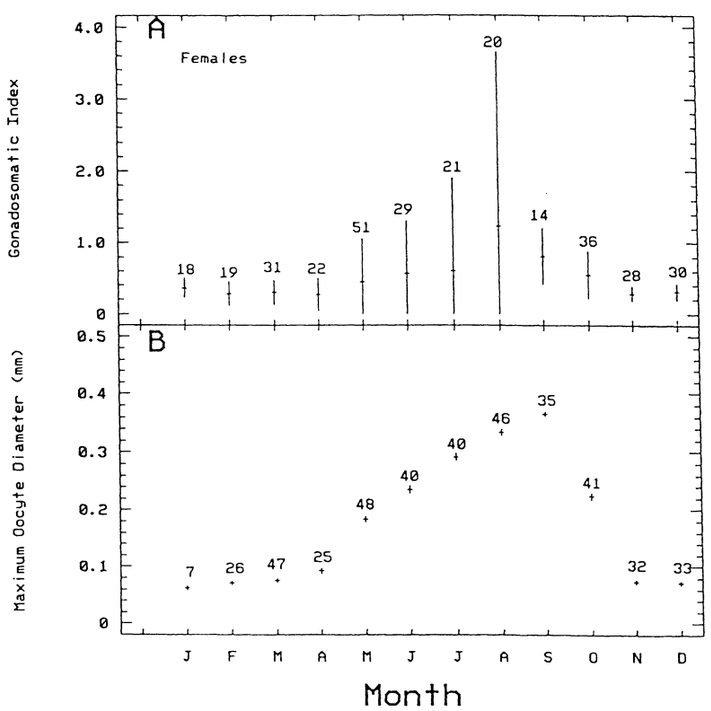

Figure 4. A) Monthly median GSI values with interquartile ranges and sample sizes for mature females, and B) monthly mean diameters (with standard errors) of the largest oocytes present in ovaries and number of fish examined for yellowedge grouper caught during 1977–1980 from the eastern Gulf of Mexico.

the completion of spawning in October. Ripe males (N = 109) were most abundant during March–September, but spent males (N = 138) were most abundant during October–December (Fig. 3). Female GSIs increased during May–August and then declined during September–October (Fig. 4A). Male GSIs showed no obvious pattern. Mean diameters of the largest oocytes present in individual ovaries peaked during September (Fig. 4B). Keener (1984) reported a similar seasonal gonadal activity pattern for yellowedge grouper caught off the South Carolina coast; ripe and ripening gonads occurred during April–September.

Our attempts to age yellowedge grouper using sectioned otoliths (sagittae) were unsuccessful. Annuli were more difficult to distinguish than those on otoliths of other grouper species we have examined at our laboratory (Bullock et al., 1992; Hood and Schlieder, 1992; Bullock and Murphy, 1994). We considered most yellowedge grouper otoliths to be unreadable. Typical otoliths had irregular and poorly defined features that precluded our making precise annulus counts. Keener (1984) noted similar problems and could estimate ages for only 27% of the 590 yellowedge grouper otoliths she examined. Keener reported ages as great as 15 years for grouper caught off the South Carolina coast and suggested that because of the uncertainties associated with ageing older fish, ages of over 20 years might be attained.

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MORE GASTROPODS FEEDING AT NIGHT ON PARROTFISHES

Philippe Bouchet and Doug Perrine

The marginellid gastropod *Hydroginella caledonica* (Jousseaume) has been reported to parasitize fishes of the families Scaridae, Serranidae and Pomacentridae (Bouchet, 1989). This occurs when these fishes are sleeping at night in small cavities of the coral reefs in New Caledonia. The marginellid *Tateshia yadai* Kosuge is associated with a scorpaenid in Japan (Kosuge, 1986). Furthermore, a cancellarid gastropod was reported to be associated with an electric ray in California (O'Sullivan et al., 1987). We here report additional such fish/gastropod associations in the Andaman Sea, involving two different gastropod families, a marginellid and a colubrariid.

The first observation was made by the junior author at about 2045 at the southeast corner of Narcondam Island [approximately 13°N, 93°E], in the northern part of the Andaman Islands, India, in February 1994, at a depth of 9 m. A sleeping bridled parrotfish, *Scarus frenatus,* without mucus cocoon, had about 20–30 marginellids crawling on it, about half on the left side, both on, and close to, the left pectoral fin, and the rest on the lower left side of the head. The fish was sleeping in a position that did not permit examination of its right side, so more specimens may have been present. The marginellids stayed on the fish while they were exposed to the diver's torch, and at least some had their proboscis extended to the parrotfish. The gastropods were photographed in situ (Fig. 3), but they were not collected. Additionally, there was a larger snail, presumed to be *Colubraria* sp. (see below), about 30–40 mm long, resting on the substrate nearby, which had its proboscis extended to the parrotfish, near the base of the left pectoral fin, in the same area where one group of marginellids was clustered. After a few seconds under the diver's light, the snail retracted its proboscis and crawled away.

The second observation (also by the junior author) was made at about 2100 in