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Reproductive biology of blacktip grouper, *Epinephelus fasciatus*, in Sulu Sea, Philippines

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Abstract : The maturation size, spawning season, sex ratio and hermaphroditism were studied in blacktip grouper, *Epinephelus fasciatus*, caught from Sulu Sea and landed at the Puerto Princesa City Market, Palawan, Philippines. Histological observations on gonads extracted from 1,119 individuals (105–330 mm in total length, TL) showed 542 individuals (125–280 mm TL) to be females, with 347 (153–330 mm TL) males, 139 (148–310 mm TL) hermaphrodites and 91 indeterminable individuals. Spawning occurred year round, although the gonadosomatic indices of females declined at the ends of both the dry (May) and rainy (October) seasons. The minimum size at maturity was determined to be 138 and 175 mm TL for females and males, respectively. Hermaphrodites were judged to function as males, because 127 out of 139 hermaphroditic individuals possessed mature testes. The sex ratio was 0.640 or 0.897 (hermaphrodites included as functioning males).

Keywords : Epinephelus fasciatus, *Spawning season, Sex ratio, Hermaphrodite, Gonad development*

1. Introduction

Groupers belonging to the subfamily Epinephelinae are commercially important tropical/subtropical fishes in the Indo-Pacific Ocean, especially in southeast Asian countries. The reproductive biology of the group has been frequently investigated, including studies by SHAPIRO (1987: review of grouper reproduction), ABU-HAKIMA (1987: reproductive biology of *Epinephelus tauvina*), KUO *et al.* (1988: artificial sex reversal in *E. fario*), MISHINA and GONZARES (1994: reproductive biology of *Cromileptes altivelis*), TAN-FERMIN *et al.* [1994: artificial sex reversal in *E. suillus* (= *coioides*)], ADAMS *et al.* (2000: sex ratio of *Plectropomus leopardus*) and LEE *et al.* (2002: reproductive biology of *E. merra*). The blacktip grouper, *E. fasciatus*, is one of the most common and the most widespread *Epinephelus* species, ranging from the coast of East Africa to the southeastern Pacific Ocean (HEEMSTRA and RANDALL, 1993). The species' reproductive biology of the species, including the spawning season, spawning behavior, sex ratio and seasonal changes of gonadosomatic index has been well studied, especially in Japan (MURAI *et al.*, 1984; OKAMURA, 1991, 1992; HAZAMA, 1993; KAWABE *et al.*, 2000). However, the study area has been limited to temperate/subtropical waters on the distributional margin of the fish. Moreover, no histological studies of the gonads have been carried out. Although the tropical waters around the Philippines are central to the species' distribution, blacktip grouper being locally abundant and commercially important (INGLES and PAULY, 1984; KOHNO, 1987), little information is available on their

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reproductive biology in that area. Therefore, this study investigated the maturation size, spawning season, sex ratio and hermaphroditism of *E. fasciatus* from the Philippines, based mainly on histological observations of the gonads.

2. Materials and Methods

Specimens used in the present study totaled 1,119 individuals (105–330 mm in total length, TL), having been caught by fishermen, using hook and lines, in Puerto Princesa Bay, depth of ca. 30m, and landed at Puerto Princesa City Market, Palawan Island, Philippines, in the period from 1995 to 2000. Sampling was conducted irregularly from 1995 to 1998 and essentially monthly from 1999 to 2000. The TL and the body and gonad weights (*BW*, *GW*), measured nearest to 1 mm and 0.01 g, respectively, of each specimen were measured while fresh. Gonadosomatic index (GSI) was calculated as $GSI = [GW / (BW-GW)] \times 1{,}000$.

For sex determination and histological observation, extracted gonads were fixed in 10% buffered formaldehyde seawater solution formalin immediately after the samplings, and the middle portions were dehydrated by ethanol and embedded in meta-acrylic resin (Technovit 7100), sectioned at 6μm, and stained with basic fuchsin and methylene blue. Gonad phases were defined by the most developed germ cells. Nomenclature for description of the stages of oogenesis and spermatogenesis was taken from YAMAMOTO *et al.* (1965) and OOTA *et al.* (1965), respectively. The criteria outlined by SADOVY and SHAPIRO (1987) were used in the diagnosis of protogynous hermaphroditism.

3. Results

3.1. Fish size and gonad development

Out of 1,119 individuals examined, 91 (8.1%) were not sexually determined even based on histological observations. The residual 1,028 were classified into three sexual categories: females, males and transitions.

The mean TL of females was 190 mm (n = 542, 52.7% of sex-determined specimens), ranging from 125–280 mm (Fig. 1) and the mean BW 115 g ranging from 30.4–366 g. Their ovaries were classified into the following five phases (Fig. 2), based on the most developed egg-cells in each ovary: phase 1–until perinucleolus stage (Fig. 2A); phase 2–until yolk vesicle stage (Fig. 2B); phase 3–until yolk globule stage (Fig. 2C); phase 4–until ripe-cell stage (Fig. 2D); phase 5–until atretic oocyte stage (Fig. 2E).

The mean of males were 230 mm (n = 347, 33.8%, range 153–330 mm TL) (Fig. 1) and 202 g (62.0–580 g BW), respectively. Their testes were divided into the following five phases, based on the most developed germ-cells in each testis (Fig. 3) : phase 1–until spermatogonium stage; phase 2–until spermatocyte stage; phase 3–until spermatid stage; phase 4–until mature spermatozoa stage; phase 5–empty crypts with residual spermatozoa. In many males (344 out of 347 specimens), ovigerous lamella and an ovarian cavity were clearly apparent. Atretic oocytes were observed in the gonads of 109 males, and gonads of large males were covered with thick tunica.

Transitions numbered 139 (13.5%) with a mean TL and BW of 208 mm (range 148–310 mm) (Fig. 1) and 154 g (53.0–535 g), respectively. Transitional individuals possessed both the spermatozoa and oocytes as follows (Fig. 4) : in a 180 mm TL specimen, peri-nucleolus stage oocytes and crypts filled with mature spermatozoa were observed, and ovarian lumen and sperm sinuses were also present (Fig. 4A) ;

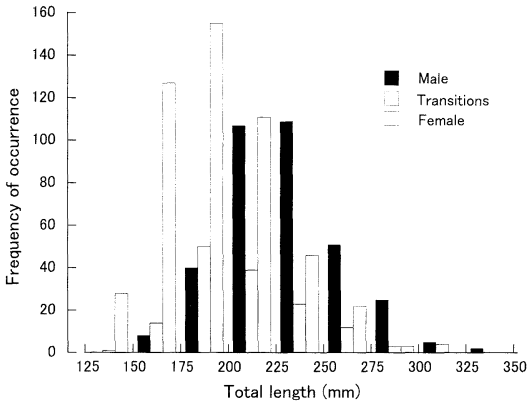

Fig. 1. Size-frequency distributions for female, transitional individuals, and male blacktip grouper *Epinephelus fasciatus* caught at Palawan Island, Philippines, between 1995 and 2000.

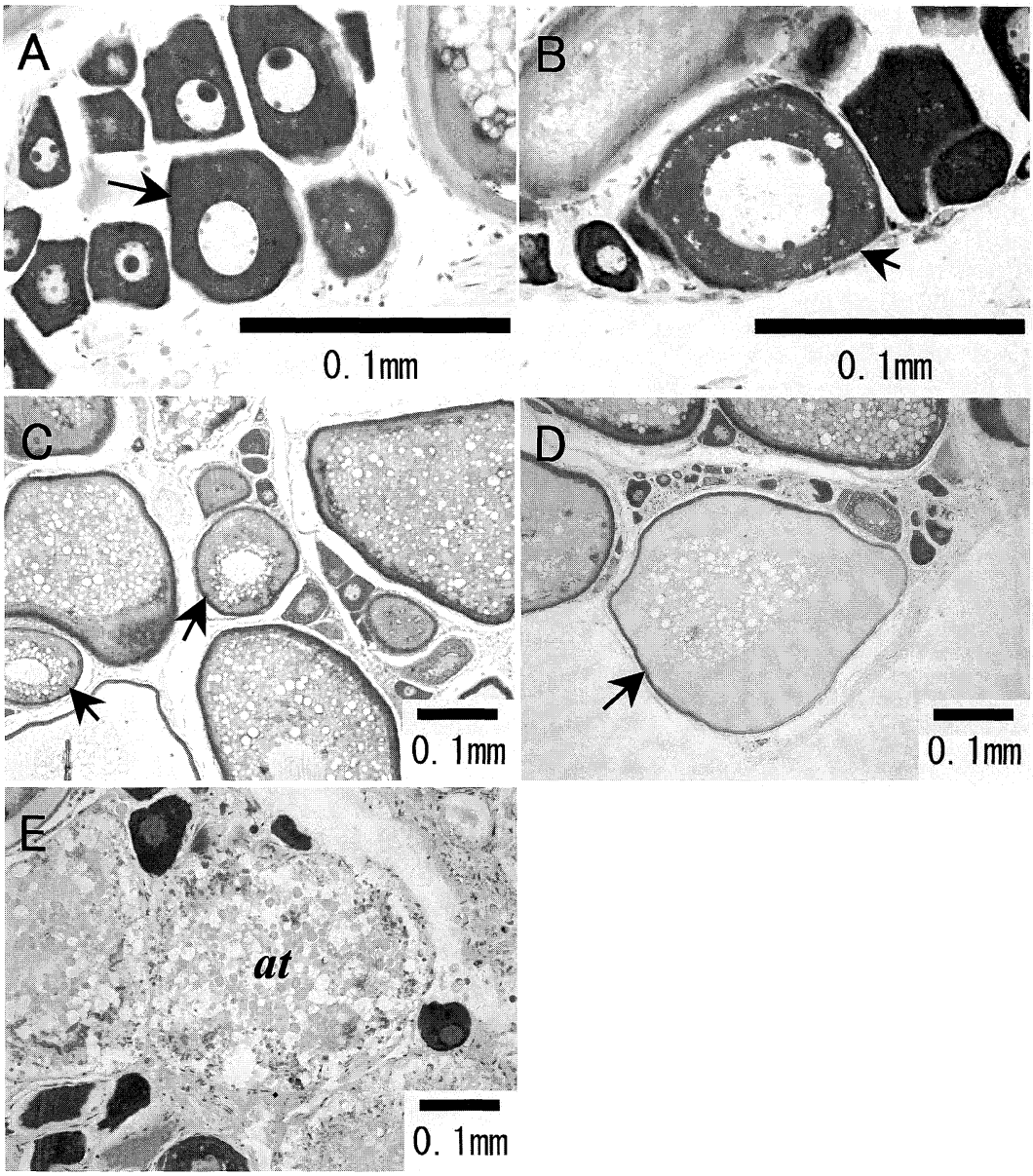

Fig. 2. Histological sections of female blacktip grouper, *Epinephelus fasciatus*, gonads at different developmental stages. A, peri-nucleolus stage oocyte (arrow) ; B, yolk vesicle stage oocyte (arrow) ; C, yolk globule stage oocytes (arrows) ; D, ripe cell stage oocyte (arrow); E, atretic oocyte (*at*).

and the yolk globule stage oocytes with crypts filled with spermatids were recognized in a 235 mm SL specimen (Fig. 4B). In most of the transitions (138 of 139), ovigerous lamella and an ovarian cavity were clearly apparent. Atretic oocytes were observed in the gonads of 39 transitions.

3.2. Sex ratio

Females comprised more than 80% of specimens of 100–150 mm TL, their ratio decreasing with growth to 15.7% of 250–300 mm TL specimens (Fig. 5). On the other hand, males were not identified among specimens <150 mm TL, but comprised 12.9% of 150–200 mm TL

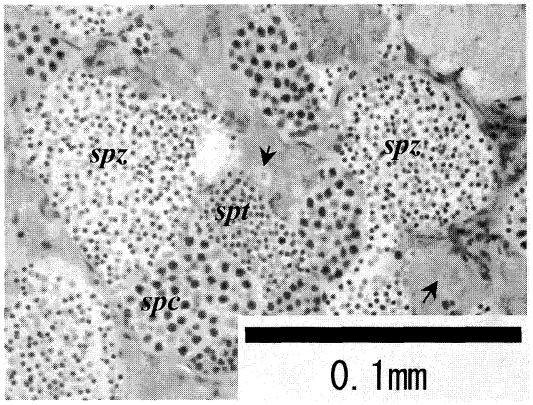

Fig. 3. Histological sections of male blacktip grouper, *Epinephelus fasciatus*, gonads. *spc*, spermatocytes; *spt*, spermatids; *spz*, spermatozoa. Arrows indicate spermatogonia.

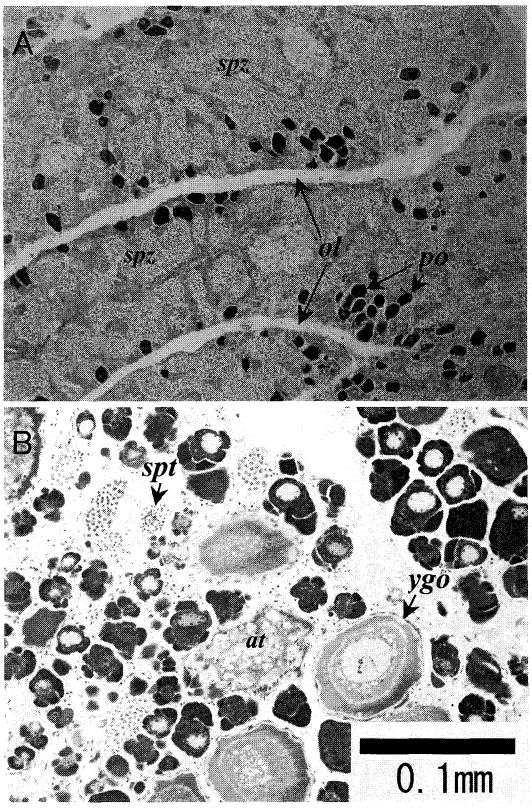

Fig. 4. Histological sections of transitional gonads of blacktip grouper, *Epinephelus fasciatus* (A, 180 mm TL ; B, 235 mm TL). *at*, atretic oocyte; *ol*, ovarian lumen; *po*, peri-nucleolus oocytes; *spt*, spermatids; *spz*, spermatozoa; *ygo*, yolk globule oocytes.

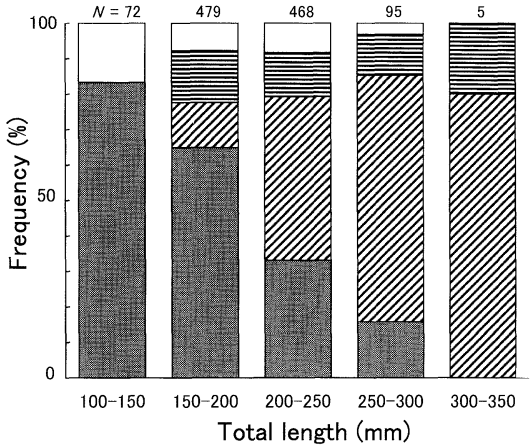

Fig. 5. Sex frequencies in blacktip grouper, *Epinephelus fasciatus*, caught at Palawan Island, Philippines, shown by 50-mm-TL-interval size classes. Open area, sex unidentified; shaded area, females; oblique-lines, males; horizontal-lines, hermaphrodites.

specimens, the ratio increasing with growth and reaching 80% of specimens of 300–350 mm TL. Transitions occupied about 10% of each size class greater than 150 mm TL.

3.3. GSI development with growth

The GSI values of females increased in specimens from ca. 140 mm TL, reaching the highest value (ca. 100) in specimens of ca. 200 mm TL and decreasing suddenly in specimens > 270 mm TL (Fig. 6). Ovarian developmental phase 1 appeared in specimens from 125–270mm TL, phase2 from 128–240 mm TL, phase 3 from 138–280 mm TL, phase 4 from 142–275 mm TL and phase 5 from 145–255 mm TL. Specimens determined as mature (developmental phases ≥ 3) showed GSI values from 2.0 to 100.0.

On the other hand, male GSI values were low, ranging from 0.1 to 16.0 (Fig. 7). Testicular developmental phase 1 appeared in specimens from 160–290 mm, phase 2 from 160–240 mm, phase 3 from 175–280 mm, phase 4 from 160–330 mm and phase 5 from 153–305 mm.

GSI values of transitions ranged from 0.1 to 14.7 (Fig. 8). Ovarian development in 127 specimens was recognized as phase 1, in 7 specimens as phase 2, in 5 specimens as phase 3 and 1 specimen as phase 5. Testicular development in

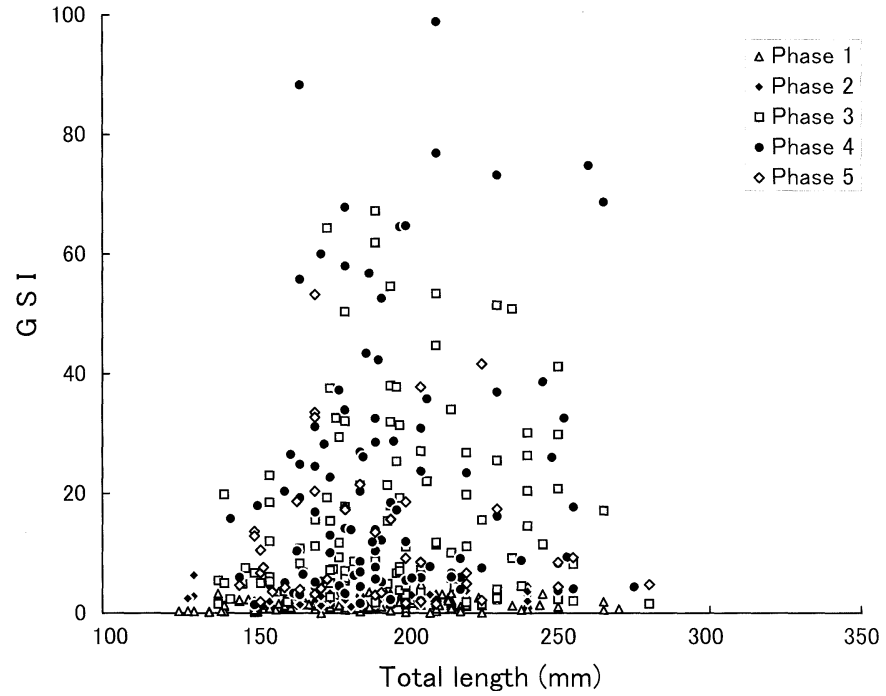

Fig. 6. Relationship between gonadosomatic index (GSI) and total length in female blacktip grouper, *Epinephelus fasciatus*, caught at Palawan Island, Philippines, with gonad developmental phases (see text).

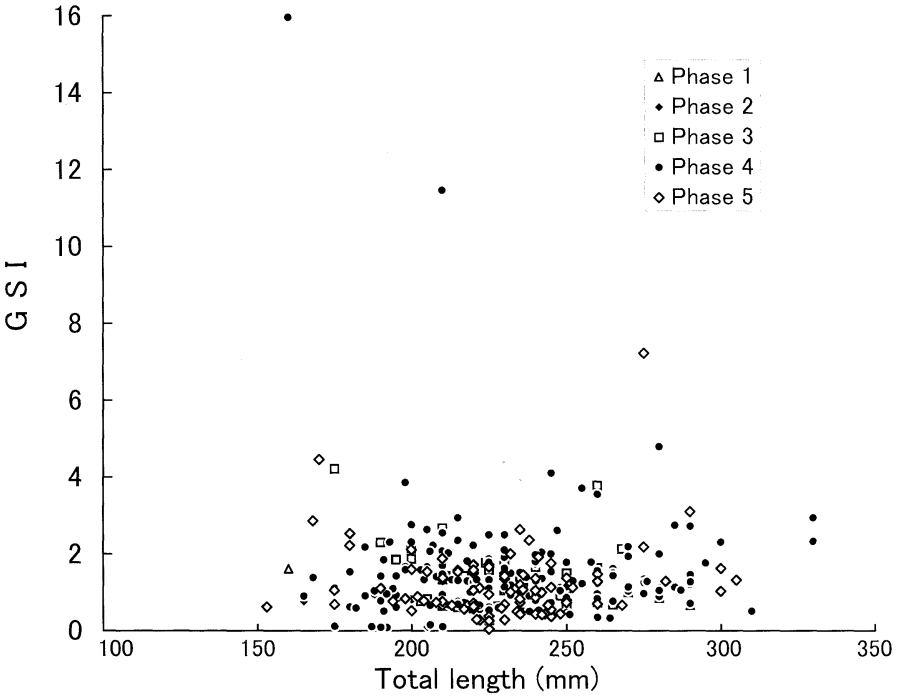

Fig. 7. Relationship between gonadosomatic index (GSI) and total length in male blacktip grouper, *Epinephelus fasciatus*, caught at Palawan Island, Philippines, with gonad developmental phases (see text).

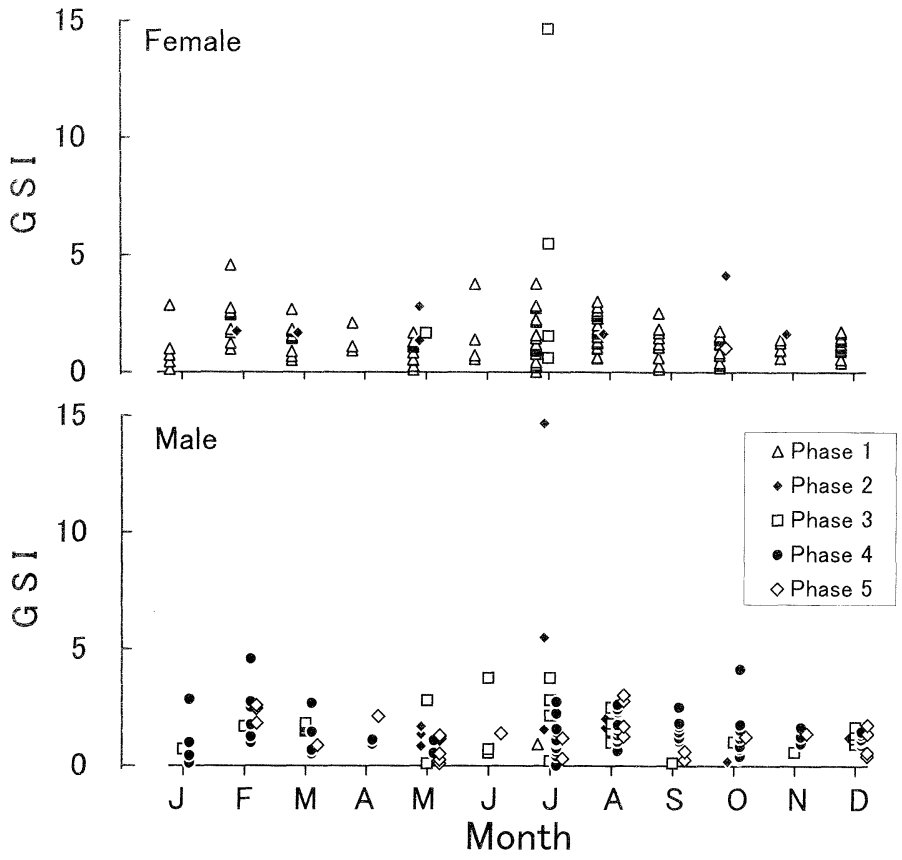

Fig. 8. Monthly changes of gonadosomatic index (GSI) in "female" (upper) and "male" (lower) hermaphroditic blacktip grouper, *Epinephelus fasciatus*, with gonad developmental phases (see text).

28 specimens was phase 3, being phases 4 and 5 in 72 and 27 specimens, respectively.

3.4. Seasonal changes of GSI and gonad developmental phase

The mean GSI values fluctuated from month to month, those in females varying from 2.3 in May to 19.9 in December and from 0.7 in March to 2.0 in August in males (Fig. 9). The mean GSI values were relatively low at the ends of both the dry (May) and rainy (October) seasons in both sexes.

Both female and male specimens with gonad developmental phases ≥ 3 were found all year round (Fig. 10). In females, individuals possessing the gonad developmental phases ≥ 3 occupied more than 70% in February, July and December, while in males those with the gonad developmental phases ≤ 2 appeared only in January, May, June, August and October.

4. Discussion

The present study confirmed that the blacktip grouper, *Epinephelus* fasciatus, is a protogynous hermaphrodite. This is provided by the fact that transitional atretic oocytes were present within the testes and that sperm sinuses were found in the gonadal wall, all of which being features strongly indicative of protogeny (SADOVY and SHAPIRO, 1987). Furthermore, the gonadal and population structures of the species were characteristic of a monandric species (SADOVY and SHAPIRO, 1987; NAKAI and SANO, 2002) ; many males had gonads retaining the ovigerous lamella and an ovarian cavity, and bi-modal size frequency distributions were observed, in which modal size of females being less than that of males.

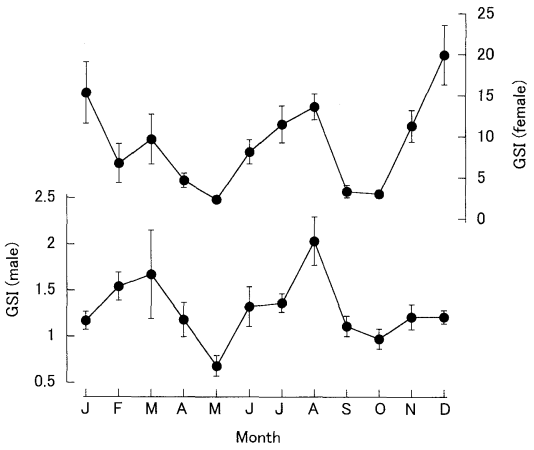

Fig. 9. Monthly changes of the mean gonadosomatic index (GSI) with standard error (bars) in female (upper) and male (lower) blacktip grouper, *Epinephelus fasciatus*, caught at Palawan Island, Philippines, between 1995 and 2000.

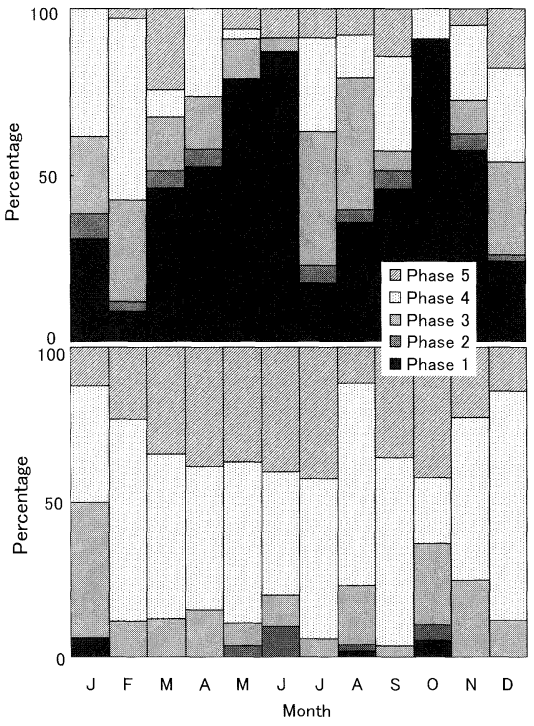

Fig. 10. Monthly changes of percentage frequency distribution of gonad developmental phases in female (upper) and male (lower) blacktip grouper, *Epinephelus fasciatus*, caught at Palawan Island, Philippines, between 1995 and 2000.

The spawning of blacktip grouper occurs year round in Palawan waters, Philippines, specimens of both sexes with gonad developmental phases ≥ 3 appearing throughout the year. High GSI were maintained in both sexes year round with much higher values in females than in males. Spawning activities would decrease, however, at the ends of both the dry and rainy seasons (May and October) when female GSI values were lower.

In captivity in Japan, the spawning season of this species was reported as June-November in Kochi Prefecture (OKAMURA, 1992), June and July in Wakayama Prefecture (HAZAMA, 1993) and throughout the year at the Ogasawara (Bonin) Islands (KAWABE *et al.*, 2000), the former two sites being temperate and the latter, subtropical. However, wild specimens caught by hook and line in Ogasawara indicated that the spawning season would be limited to April to June (MURAI *et al.*, 1984). According to SHAPIRO (1987), spawning occurs from September to February in New Caledonia, tropical southern hemisphere waters. These findings indicate that the spawning season of the species depends mainly on water temperature, higher temperatures having a prolonging effect; thus the spawning season is longer in lower latitude (tropical) waters. However, especially in captivity, the nutritional condition of the fish, as well as water temperature, would affect the spawning period, as pointed out by KAWABE *et al.* (2000).

Although sex was determined in specimens as small as 125 and 153 mm TL (females and males, respectively) in the present study, the smallest specimens with the mature gonads (developmental phases ≥ 3) were 138 and 175 mm TL for the respective sexes. Therefore, the former sizes are recognized as the biological minimum size for each sex in blacktip grouper. Hermaphrodites, the smallest recorded being 148 mm TL, are believed to function mainly as males, because only a few (6 out of 139 specimens) possessed ovaries at developmental phases ≥ 3, in contrast to those having testes of phase 3 or greater (127 out of 139 specimens).

Grouper sex ratios are known to be less than 1.0, males being fewer than females, because of protogynous hermaphroditism (TAN and TAN,

1974; SADOVY and SHAPIRO, 1987; SHAPIRO, 1987; TANAKA *et al.*, 1990). In the present study, out of 1,028 sex-determined specimens, female, male and hermaphrodite numbers were 542, 347 and 139, occupying 52.7%, 33.8% and 13.5%, respectively, resulting in a sex ratio of 0.640. Including the hermaphrodites as functioning male results in the sex ratio increasing to 0.897. The reported sex ratio of wild blacktip grouper at the Ogasawara Islands is 0.743 (MURAI *et al.*, 1984), compared with 1.238 in captivity in Kochi (OKAMURA, 1991, 1992).

In other grouper species, reported sex ratios are: 0.075 for *Cromileptes altivelis* (MISHINA and GONZARES, 1994), 0.075 for *Epinephelus* tauvina (ABU-HAKIMA, 1987), 0.18–0.35 for *E. guttatus* and 0.53–1.0 for *E. striatus* (COLIN *et al.*, 1987), and 0.193 for *E. morio* (BRULE *et al.*, 1999). According to HEEMSTRA and RANDALL (1993), the maximum sizes of the aforementioned species range between 700 and 1,000 mm TL. Although the sex ratio of these larger grouper species (maximum TL >700 mm) is usually much lower than 1.0, it is generally higher (0.640–0.897) in blacktip grouper [maximum TL 400 mm, according to HEEMSTRA and RANDALL (1993)]. Such differences in sex ratios between large and small grouper species may reflect different reproductive strategies. Further studies are necessary for confirmation of this.

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