Reproductive and sexual characteristics in five *Lethrinus* species in waters off the Ryukyu Islands

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Abstract Gonads of five lethrinids, viz., *Lethrinus harak*, *L. miniatus*, *L. obsoletus*, *L. ornatus*, and *L.* sp. 2, were collected monthly in waters off the Ryukyu Islands and observed histologically to reveal their spawning periods and size at sexual maturation and sexual transition. The spawning period was from April to November for *L. harak*, from April to July for *L. miniatus*, from April to October for *L. obsoletus*, from May to November for *L. ornatus*, and from April to October for *L.* sp. 2. Sexual patterns in the five species were determined by fork length at both sexual maturation and sexual transition. The body size (fork length, FL) and sex ratio (% of female) at 90% maturity in *L. harak* were 21.1 cm FL and 90%, respectively; those of *L. miniatus* were 42.2 cm FL and 80%; 25.7 cm FL and 60% for *L. obsoletus*; about 20 cm FL and 90% for *L. ornatus*; and about 26 cm FL and 90% for *L.* sp. 2. Because the sex ratios decreased to 0% at the maximum size classes in *L. miniatus*, *L. ornatus*, and *L.* sp. 2, the sexual patterns in these species were considered to be protogynous hermaphrodite. Although the sex ratio (% of female) once decreased to about 30% at 28 cm FL, rapid increase occurred in the larger size class in *L. harak*. However, the increase did not result from sexual transition. Hence, the sexual pattern of *L. harak* was considered to be protogynous hermaphrodite. Because the sex ratio (% of female) at body sizes larger than 23 cm FL was stable at about 60% in *L. obsoletus*, the sexual pattern was determined to be one of juvenile hermaphrodites.

Key words *Lethrinus* · Spawning season · Sexual maturation · Sex change · Protogynous hermaphroditism

Lethrinids of genus *Lethrinus* are commercially very important food fishes in countries of the Western Pacific Ocean to the Indian Ocean (Carpenter, 2001). Research carried out in recent decades on their reproduction (Toor, 1964a; Loubens, 1980a; Ebisawa, 1990; Church, 1995; Sumpton and Brown, 2004), growth (Loubens, 1980b; Church, 1995; Brown and Sumpton, 1998; Williams et al., 2003), feeding behavior (Toor, 1964b; Walker, 1978; Church, 1995), and sexuality (Young and Martin, 1982; Church, 1995; Bean et al., 2003), as well as stock assessment (Lebeau and Cueff, 1975; Baddar, 1987; Salem, 1990a; Church, 1995), has gradually revealed an ecological profile of the genus. Most studies have suggested protogynous hermaphroditism as the typical mode of sexuality in lethrinids based on criteria such as bimodal size distributions, with males dominating larger size classes and females dominating smaller size classes (Loubens, 1980a; Young and Martin, 1982), and histological features such as the presence of atretic oocytes and concurrent oocyte degeneration and spermatocyst proliferation (Young and Martin, 1982; Church, 1995; Bean et al., 2003).

Spawning periods, size at maturity, and sexual transition based on histological observations have been reported in *Lethrinus nebulosus* (see Ebisawa, 1990), *Lethrinus rubrioperculatus* (see Ebisawa, 1997), and *Lethrinus atkinsoni* (see Ebisawa, 1999) in Okinawa, southern Japan. According to those studies, the sexuality of *Lethrinus* species is complicated. Sexual transition from ovary to testis in *L. rubrioperculatus* and *L. atkinsoni* in the Okinawa population occurs after ovarian maturation, hence protogynous hermaphroditism, whereas that in *L. nebulosus* and *L. atkinsoni* in the Yaeyama population occurs before ovarian maturation, hence juvenile hermaphroditism as defined by Yogo (1987). Testes in *L. nebulosus* and *L. atkinsoni* in the Yaeyama population develop to gonadosomatic index (GSI) values of 5%–10% that are nearly equal to those of ovaries, whereas that of *L. rubrioperculatus* develops to a maximum of 1%. Two different spawning styles have been predicted in the *L. atkinsoni* Okinawa population because the testis in the earlier half of the spawning period develops GSI value of about 3%, whereas that in the latter half develops to about 1% but is nevertheless mature. Intensive catches carried out in the high testicular GSI periods in lethrinids indicate a strong connection between spawning style and fishing; thus, this is a topic that deserves further attention and research.

To differentiate between protogynous and juvenile hermaphroditism, sizes at maturity during the spawning period and at sexual transition must be clarified. In addition, providing information (spawning period, body size at sexual maturation, and sexuality) is very important for the rational use of the resource. This study, for the purpose of the rational use of these resources, investigates spawning periods, body sizes of both sexual maturation and sexual transition, and sexuality based on histological observations of gonads in five commercially important species: *Lethrinus harak*, *Lethrinus miniatus*, *Lethrinus obsoletus*, *Lethrinus ornatus*, and *Lethrinus* sp. 2 (sensu Carpenter, 2001; drab emperor is the English name). Factors assuming to affect sexuality in *Lethrinus* species are discussed.

Materials and Methods

Scientific names follow Carpenter (2001). Most specimens were obtained from commercial fishermen. The number of specimens (periods, locations) were as follows: 452 *L. harak* (August 1988–March 1992, off Yaeyama); 454 *Lethrinus miniatus* (February 1987–March 1988, off Okinawa Island); 523 *L. obsoletus* (July 1988–March 1992, off Yaeyama); 80 *L. ornatus* (April 1989–May 1992, off Yaeyama); 241 *L.* sp. 2 (April 1985–July 1987, off Okinawa Island). Fork length (FL) and body weight (BW) were measured. Gonads were extracted, weighed (gonad weight, GW) to the nearest 0.01 g, and then fixed with Bouin's solution (15 vol saturated picric acid solution, 5 vol formalin, and 1 vol acetic acid). A sample dissected from the middle portion of the gonad was embedded in paraffin, transversely sectioned at 6 μm, and stained with Mayer's hematoxylin and eosin for histological observations. Gonadosomatic index (GSI) was calculated for each sample using $GSI = GW/BW \times 100$. The stages of oocyte development were determined following Yamamoto and Yamazaki (1956), using histological preparations; viz., chromatin nucleolus (CN), early perinucleolus (EPN), late perinucleolus (LPN), yolk vesicle (YV), primary yolk globule, secondary yolk globule, tertiary yolk globule (TYG), migratory nucleus, prematuration, maturation, and ripe egg. However, some of these classes were combined and renamed as follows for the analysis, viz., early yolk globule stage (EYG) for both primary and secondary yolk globule stages and hydrated stage (Hy) for the migratory nucleus to ripe stages. The stage of maturity of ovaries was determined based on the most advanced oocyte stage appearing in the gonad. An atretic ovary (At) was defined as one in which atresia was observed in more than 70% of oocytes at the most advanced stage.

Thus far, it has been revealed that GSI values for ovaries greater than 1.0% indicate accumulated yolk globule in some lethrinids (Ebisawa, 1990, 1997, 1999). Therefore, body size of specimens used for estimating spawning period was determined by the following process: count the frequency of each maturity stage in each 1-cm FL class using the specimens obtained in periods when GSI values greater than 1.0% appeared; determine the smallest length interval at which the percentage of ovaries belonging to the early yolk globule to hydrated stages exceed 50%; and use all female specimens larger than or belonging to the length class in estimates of spawning season. The body size of each species was as follows: *L. harak*, ≧20.0 cm FL; *L. miniatus*, ≧38.0 cm FL; *L. obsoletus*, ≧24.0 cm FL; *L. ornatus*, ≧19.0 cm FL; and *L.* sp. 2, ≧23.0 cm FL. The existence of postovulatory follicles (POF), which provide evidence of spawning before sampling, was judged by examining ovaries, the maturity stage of which in the body size groups listed above is either TYG or Hy stage (excluding ripe stage). Some mature ovaries of *L.* sp. 2 collected in April and May and frozen before fixation were excluded from POF confirmation. The spawning period is defined as the period between the first and last months of either hydrated or POF-bearing ovaries.

Hermaphroditic gonads (He), defined as those in which both oocytes and testicular cells exist simultaneously, were treated as one of the ovarian maturity stages to examine when sexual transition occurs relative to the spawning cycle. Gonads with sperm in vas deferens and with numerous testicular cell stages scattered throughout the gonad were defined as testes even if oocytes were also present. The fork length, which was divided into 2-cm intervals in *L. miniatus* and 1-cm intervals in the other four species, is expressed by the minimum integer in the class (Lf). The ovarian maturation rate (OMR) was determined by the percentage of the total number of ovaries belonging to the EYG, TYG, and Hy stages relative to the number of whole ovaries in the length class. The sex ratio (SR) was determined by the percentage of the number of ovaries to the number of whole specimens in the length class. Relationships between Lf-OMR and Lf -SR are fitted using the logistic equation by $OMR = 1 \times (1 + e^{(a-b\times Lf)})^{-1}$ and $SR = 1 - C \times (1 + e^{(d-g\times Lf)})^{-1}$, where a, b, c, d, and g are regression coefficients, but $c = 1$, except in the case of *L. obsoletus*. The sizes at which 50% and 90% of females were sexually mature ($Lf{:}m_{50\%}$ and $Lf{:}m_{90\%}$), the size at which 50% were female ($Lf{:}s_{50\%}$), and the sex ratio at $Lf{:}m_{90\%}$ are calculated based on these logistic equations. The sexual pattern, either protogyny or juvenile hermaphroditism, was determined whether the sex ratios at sizes larger than $Lf{:}m_{90\%}$ decreased drastically. Because specimens have been collected throughout multiple years for all species except *L. miniatus*, monthly data were pooled across years for the other four species.

Results

Seasonal changes in ovarian development. *Lethrinus harak.*—Testicular GSI values were low in January and February, started to increase in March, maintained high values until November (except for September, when slight decreases were observed), then decreased to a minimum in December (Fig. 1A). The maximum testicular GSI value obtained was about 1.2. Ovarian GSI values were low from January to March, rapidly increased in April and May, subsequently decreased toward September, then increased again from October to November before the sudden decreases in December (Fig. 1B). GSIs greater than 1.0 were obtained from February to December. In the transitions of the ovarian maturity stages, almost all ovaries were either at the stage of TYG or Hy from April to October (Fig. 1C). In contrast, various stages of ovaries were obtained from November to March. Gonads of He were obtained continu-

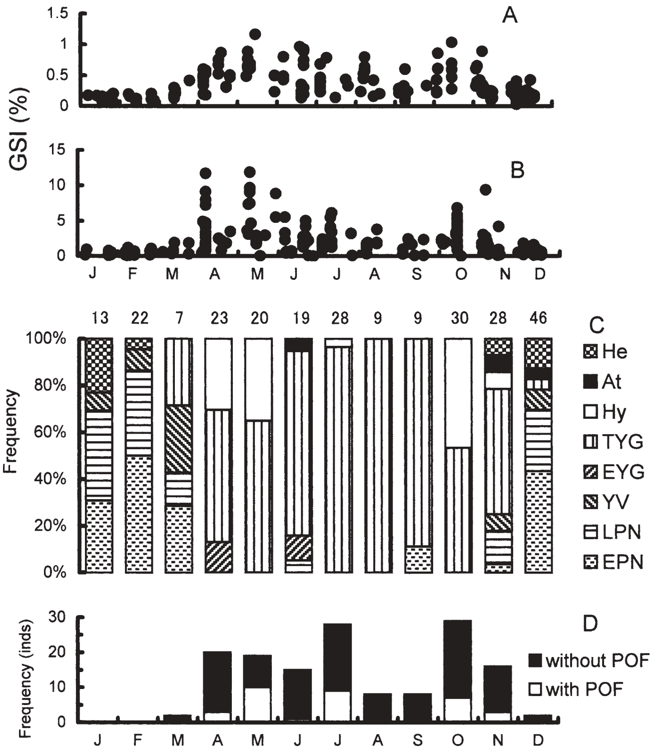

Fig. 1. Monthly changes of gonadal condition in *Lethrinus harak*. **A** Testicular gonadosomatic index (*GSI*); **B** ovarian GSI; **C** relative frequency of each ovarian maturity stage with the sample size *above bar*; **D** frequency of ovaries bearing postovulatory follicles (POF) (*open bar*) in mature ovaries. *At*, atretic ovary; *EPN*, early perinucleolus stage; *EYG*, early yolk globule stage; *He*, hermaphroditic gonad; *Hy*, hydrated stage; *LPN*, late perinucleolus stage; *TYG*, tertiary yolk globule stage; *YV*, yolk vesicle stage

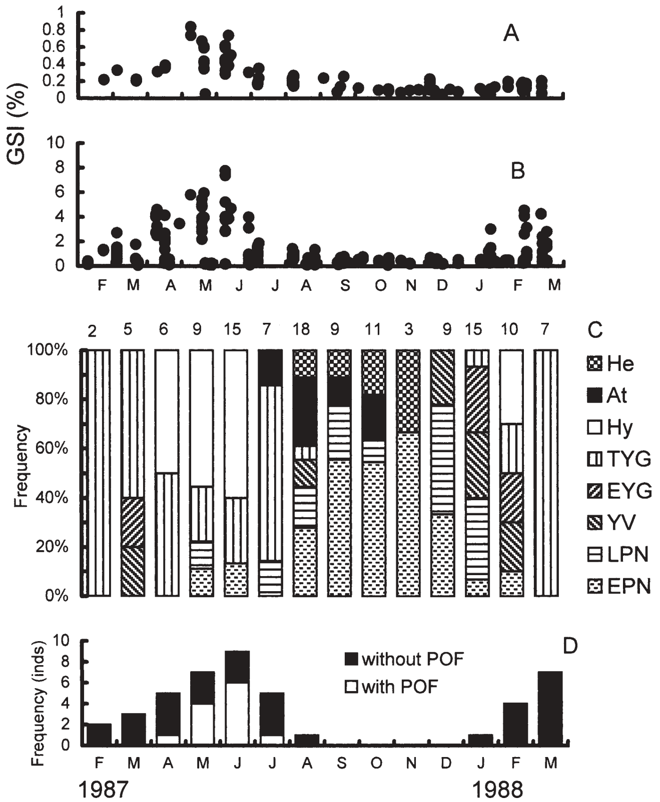

Fig. 2. Monthly changes of gonadal condition in *Lethrinus miniatus*. **A** to **D** and abbreviations are as same as those of *Fig. 1*

ously from November to February. Ovaries bearing POF were observed from April to November, except August and September (Fig. 1D). Spawning seems to be continuous from April to November; in August and September, GSI decreased and neither Hy stage nor POF-bearing ovaries were observed because almost all ovaries are in the TYG stage during these 2 months. Accordingly, the spawning period of the species is determined to be from April to November by the first and the last confirmation of both Hy stage and POF-bearing ovaries.

Lethrinus miniatus.—Testicular GSI peaked in May and June, then decreased in July, and stayed at low levels until March (Fig. 2A). The maximum testicular GSI value obtained was about 0.8. Ovarian GSI values peaked in June and subsequently decreased, reaching a low from August to December (Fig. 2B). Successively, GSI values greater than 3.0 appeared in January, and the number of individuals with GSI values greater than 1.0 increased again through February and March. The periods of months when GSI values were greater than 1.0 lasted from February to August in 1987 and from January to March in 1988. In the monthly changes of the ovarian maturity stage, ovaries at the stage of either TYG or Hy dominated from February to July 1987 and in March 1988 (Fig. 2C). In particular, ovaries at the Hy stage made up more than 50% of the total from April to June 1987. In contrast, various stages of ovaries such as EPN, LPN, and At were obtained from August to February. Gonads of He were obtained continuously from August to November. Ovaries bearing POF were obtained from April to July and comprised more than half of the mature ovaries in May and June (Fig. 2D). There were no POF-bearing ovaries in 1988; nevertheless, ovaries at the Hy stage were obtained in February. Thus, the spawning period of *L. miniatus* started in April in 1987 when both ovaries at the Hy stage and ovaries bearing POF were first confirmed and lasted until July of that year, when the last confirmation of ovaries bearing POF occurred. In 1988, the spawning period started in February with the first confirmation of ovaries at the Hy stage.

Lethrinus obsoletus.—Testicular GSI values were low until early March, rapidly increased after late March, maintained high values until July, then decreased toward December (Fig. 3A). The maximum testicular GSI value obtained was about 3.5. Ovarian GSI values were low from January to early March, slightly increased beginning in late March, maintained high values from April to August, then slightly decreased in September, reaching a minimum in December (Fig. 3B). The period when GSIs larger than 1.0 were obtained was from March to November. In the monthly changes of ovarian maturity stage, ovaries at the TYG stage dominated from April to October; however, in contrast to

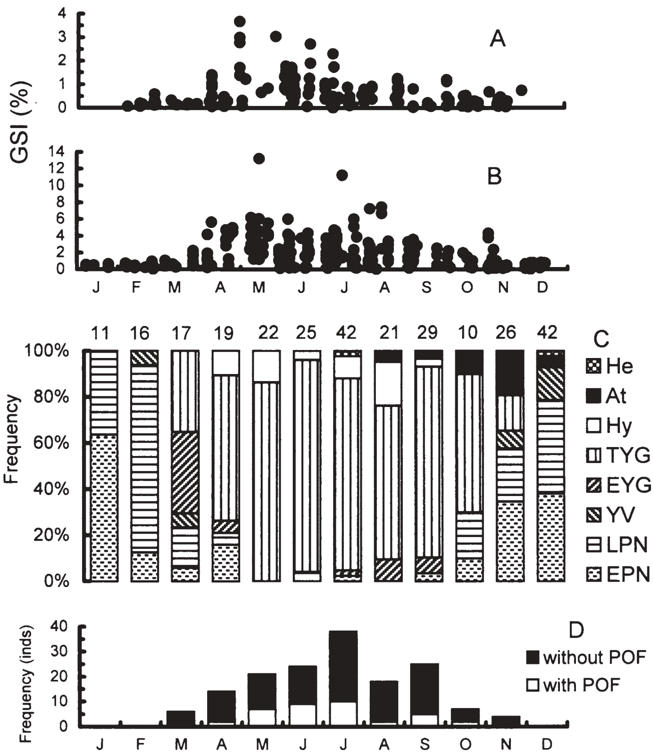

Fig. 3. Monthly changes of gonadal condition in *Lethrinus obsoletus*. **A** to **D** and abbreviations are as same as those of *Fig. 1*

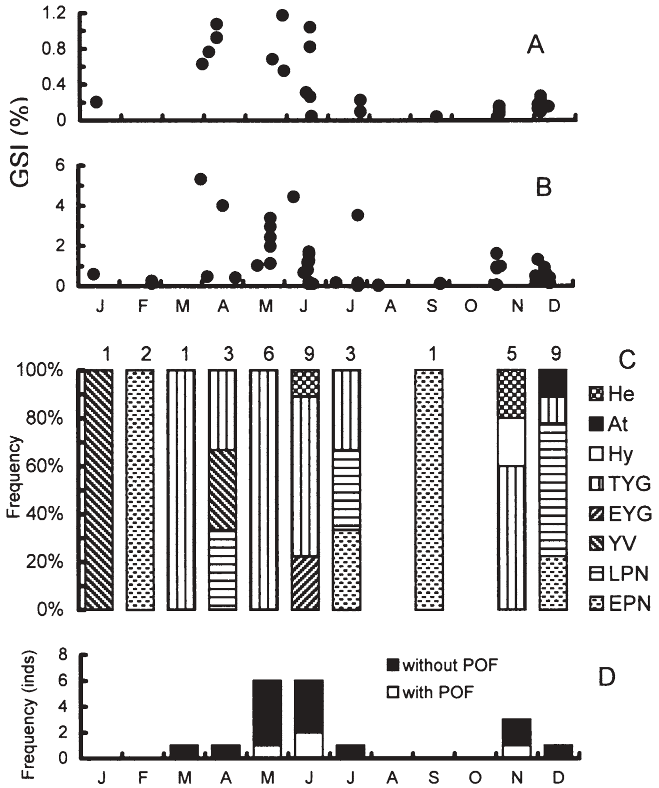

Fig. 4. Monthly changes of gonadal condition in *Lethrinus ornatus*. **A** to **D** and abbreviations are as same as those of *Fig. 1*

the preceding two species, few ovaries at the Hy stage were seen in this period (Fig. 3C). Ovaries of At were obtained continuously from August to December. Gonads of He were obtained in July and December in small numbers. Ovaries bearing POF were obtained continuously from April to October (Fig. 3D). The spawning period of the species was determined to be from April to October by the first and the last confirmation of both Hy stage and POF-bearing ovaries.

Lethrinus ornatus.—Although the number of specimens obtained was fewer than those of the other four species, both the spawning period and sexuality were judged to be adequately determined; therefore, this species is included in this report. Testicular GSI values were high between March and June and low from July to December and in January (Fig. 4A). The maximum testicular GSI value obtained was about 1.1. Ovarian GSI values were small in January and February, rapidly increased from late March, and maintained values greater than 1.0 until December in some portions (Fig. 4B). The period when GSIs greater than 1.0 were obtained was from March to December. Although the number of specimens was so small that obvious characteristics in the transition in ovarian maturity stages were not observed, ovaries at the TYG stage were obtained from March to December except in September, at the Hy stage in November, and at the At stage in December (Fig. 4C). Gonads of He were obtained in June and November. Ovaries bearing POF were obtained in May, June, and November (Fig. 4d). Accordingly, the spawning period of the species started from May at the latest and lasted until November.

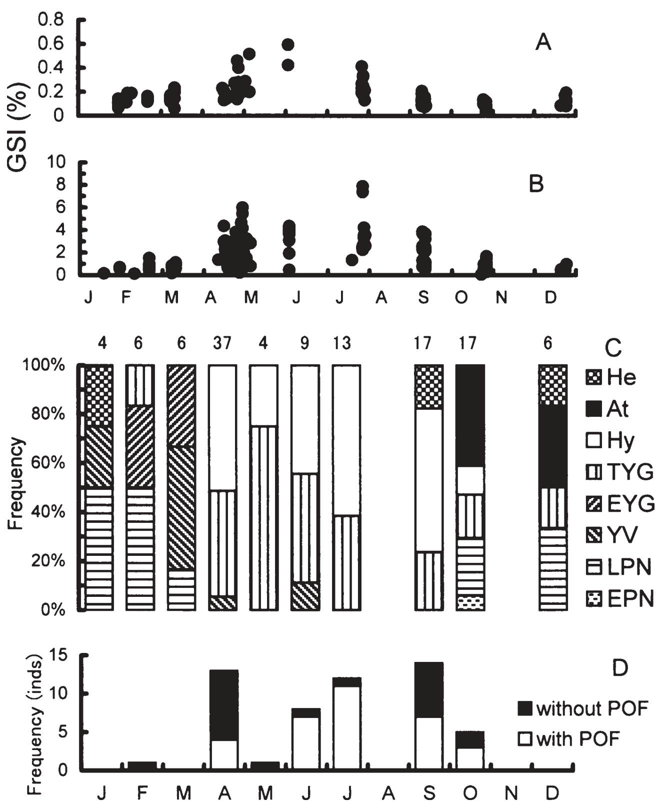

Fig. 5. Monthly changes of gonadal condition in *Lethrinus* sp. 2 (drab emperor). **A** to **D** and abbreviations are as same as those of *Fig. 1*

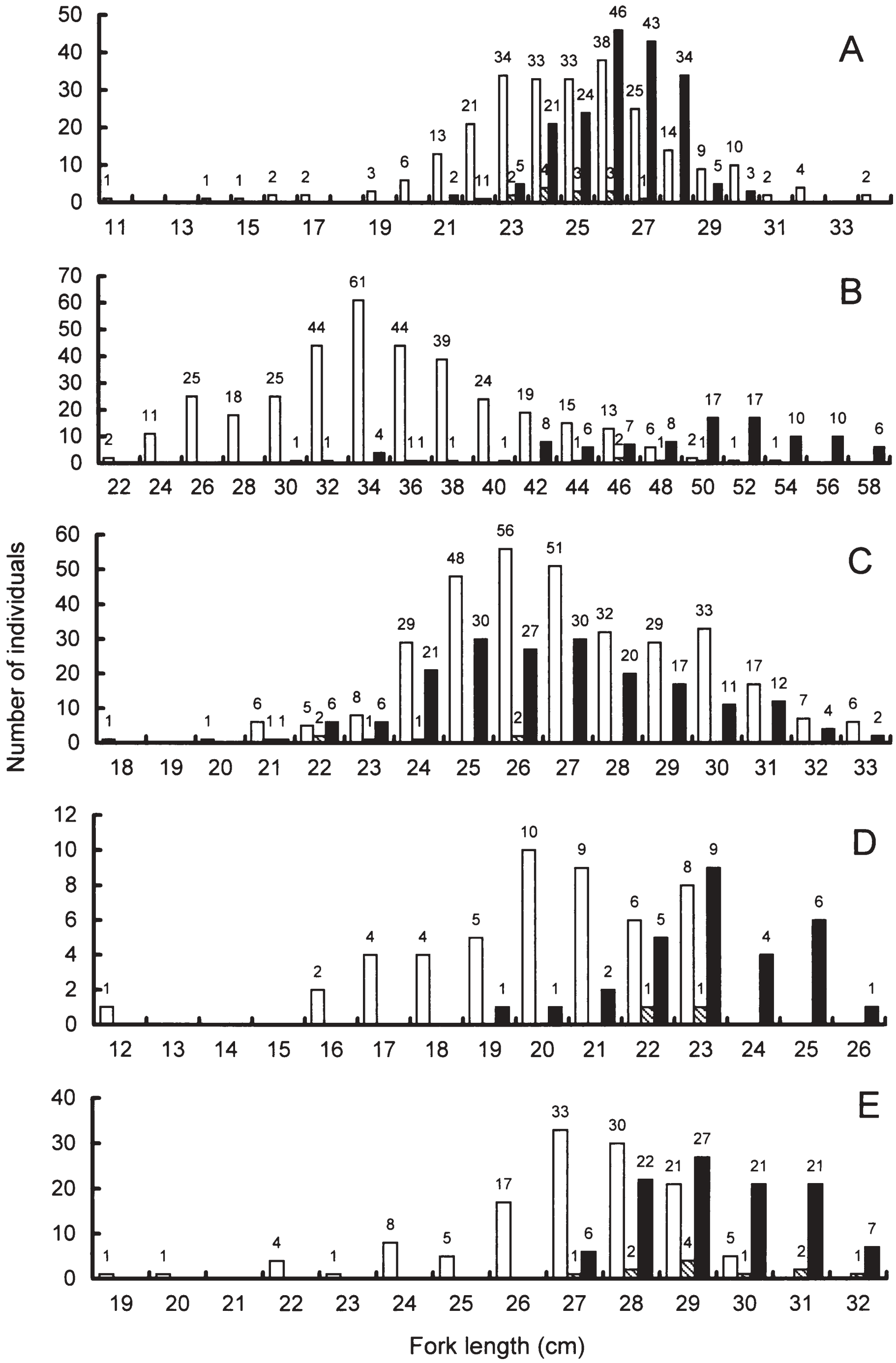

Fig. 6. Fork length frequency in specimens in *Lethrinus harak* (**A**), *L. miniatus* (**B**), *L. obsoletus* (**C**), *L. ornatus* (**D**), and *L.* sp. 2 (**E**). Number of specimens in each sex is indicated *above bars:* female (*open bar*), hermaphrodites (*lined*), and male (*solid*)

Lethrinus sp. 2.—Testicular GSI change was small with the peak value of about 0.6% in June (Fig. 5A). Ovarian GSI values were low from January through March, suddenly increased in April, remained high until September, and subsequently decreased (Fig. 5B). The period when GSI values greater than 1.0 were obtained lasted from February to October. In the monthly changes of ovarian maturity stage, almost all ovaries were at the stage of either TYG or Hy from April to September (Fig. 5C). Rates of both stages of ovaries decreased in October; conversely, ovaries at the At stage prevailed at that month and in December. Gonads of He were obtained in September, December, and January. Ovaries bearing POF were observed continuously from April to October, except for May, when very few POF-bearing ovaries were confirmed (Fig. 5D). Accordingly, the spawning period in *L.* sp. 2 was determined to be between April and October, during which time both Hy stage and POF-bearing ovaries were obtained.

Sex ratio, length of female at sexual maturation, and sexual pattern. *Lethrinus harak*.—A total of 254 ovaries were obtained in the range between 11.6 and 34.3 cm FL with a mode at 26 cm FL, 14 He gonads between 22.0 and

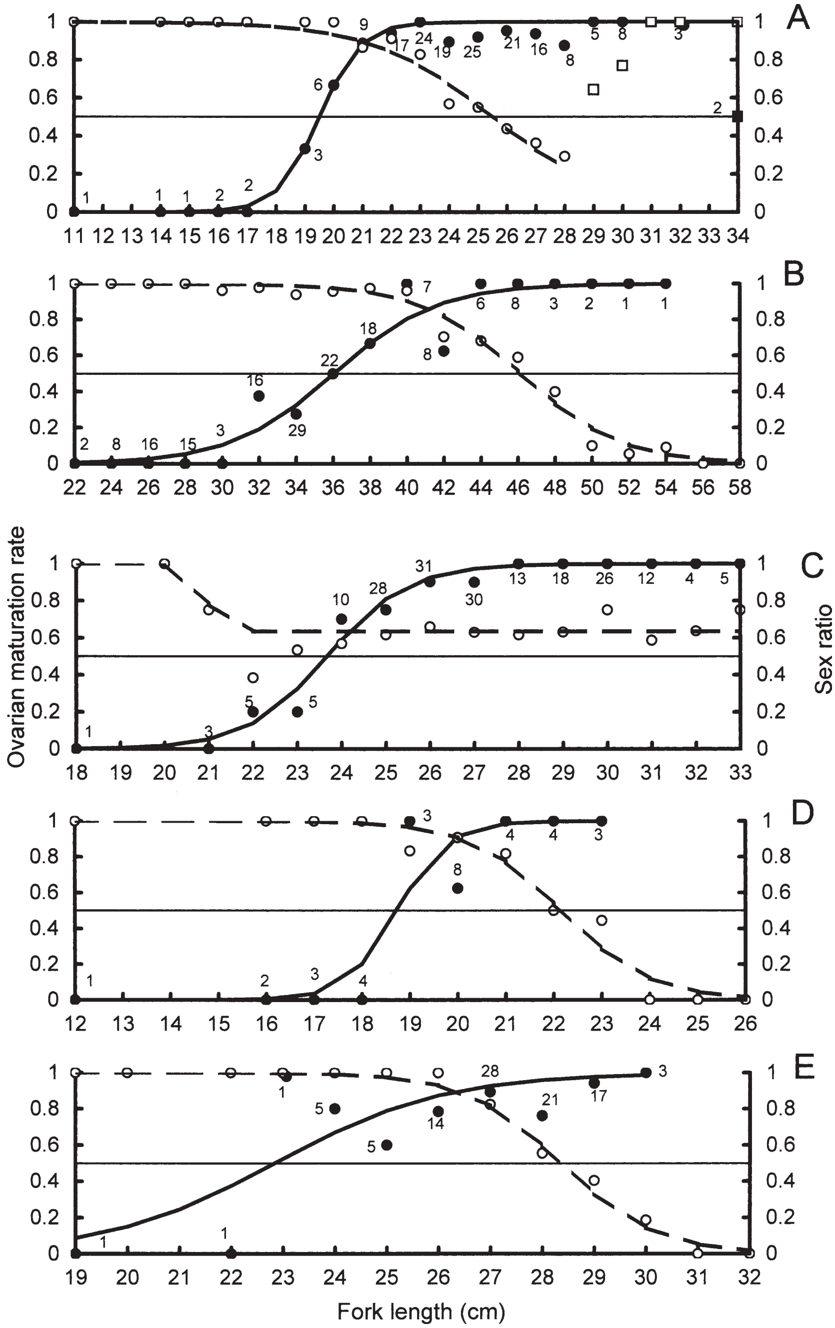

Fig. 7. Relationships of ovarian maturation rate (OMR) and sex ratio (SR) against fork length in *Lethrinus harak* (**A**), *L. miniatus* (**B**), *L. obsoletus* (**C**), *L. ornatus* (**D**), and *L.* sp. 2 (**E**). *Solid circles* and *solid lines* indicate OMR; *open circles* and *broken lines* are SR. *Open squares* and *solid square* in **A** were excluded from the logistic regression estimate. *Numbers beside solid circles* indicate sample sizes. Sample sizes for *open circles* (total number of female, male, and hermaphrodite specimens) are the same as in Fig. 6

27.7 cm FL, and 184 testes between 21.4 and 30.7 cm FL, with a mode at 26 cm FL (Fig. 6A). Previtellogenic atresia and speramatogenic cells coexisted in hermaphroditic gonads (Fig. 8A,B). SRs gradually decreased from 100% in classes smaller than 20 cm FL to about 30% at 28 cm FL (Fig. 7A). However, SRs rapidly increased toward 100% from 29 cm FL to 31 cm FL. The SR increases in classes larger than 29 cm FL were not caused by sexual transition from male to female but by the difference in the maximum size between the sexes. Lf:$s_{50\%}$ calculated using the logistic regression estimates excluding the sex ratio from 29 to 34 cm FL was 25.5 cm FL.

The OMR started to increase from 19 cm FL, reached about 90% at 21 cm FL, and varied between about 90% and 100% in the larger classes, except that of 50% in the maximum length class (Fig. 7A). The calculated Lf:$m_{50\%}$ and Lf:$m_{90\%}$ were 19.5 and 21.1 cm FL, respectively. SR at Lf:$m_{90\%}$ was about 90%. Accordingly, the sexuality of the species was considered to be protogynous hermaphroditism.

Lethrinus miniatus.—A total of 350 ovaries were obtained in the range between 22.5 and 54.0 cm FL (mode, 34 cm FL), 8 He gonads between 32.7 and 50.9 cm FL, and 96 testes between 31.0 and 59.2 cm FL (mode, 50–52 cm FL)

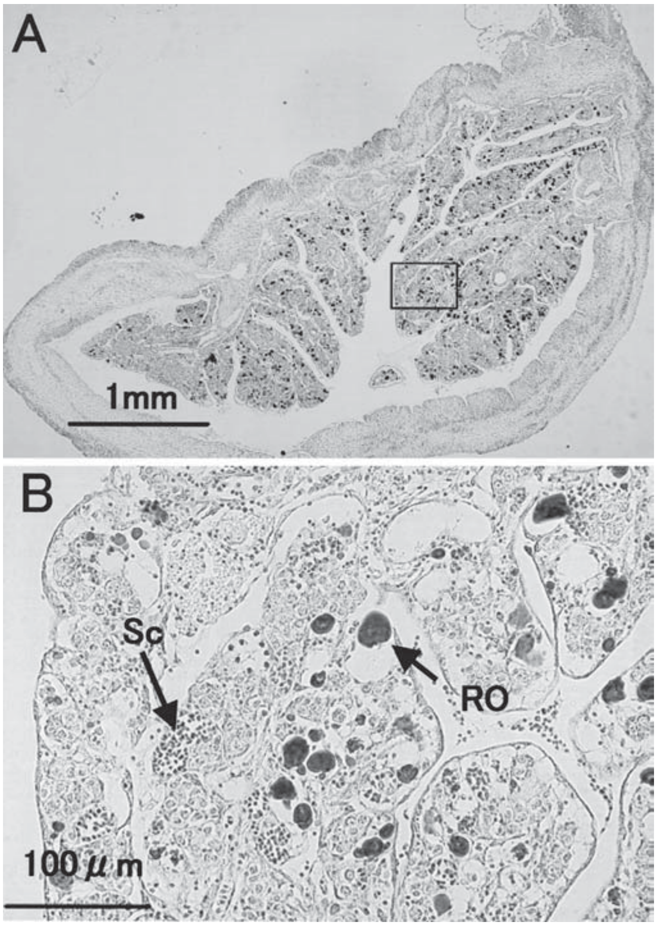

Fig. 8. Transverse sections of hermaphroditic gonad in *Lethrinus harak*. **A** Whole section in a 25.6cm FL specimen collected on 16 November 1990; **B** higher magnification of the area enclosed in **A**. *RO*, residual oocyte; *Sc*, spermatocytes

(Fig. 6B). A few testes were obtained in classes smaller than 40cm FL; i.e., SRs were larger than 90% in these classes (Fig. 7B). SRs rapidly decreased in classes larger than 42cm FL, attained 50% ($Lf{:}s_{50\%}$) at 46.1cm FL, and reached 0% at 56cm FL. In the histological specimens of hermaphroditic gonads, a gonad thought to be prematurational with a very thin gonadal wall (Fig. 9A,B) and other gonads thought to be postmaturational with very thick gonadal walls (Fig. 9C,D) were obtained. However, no hermaphroditic gonads with vitellogenic stage oocyte atresia, which indicates direct evidence of protogyny, were obtained.

Ovaries were immature in classes smaller than 30cm FL and were fully mature in classes larger than 44cm FL (Fig. 7B). $Lf{:}m_{50\%}$ and $Lf{:}m_{90\%}$ were 36.1cm FL and 42.2cm FL, respectively. SR at $Lf{:}m_{90\%}$ was about 80%. Accordingly, the remaining 80% of sex changes occur at sizes larger than 42.2cm FL; hence, the sexuality in *L. miniatus* was considered to be protogynous hermaphroditism.

Lethrinus obsoletus.—A total of 329 ovaries were obtained in the range between 18.6 and 33.9cm FL (mode, 26cm FL), 7 He gonads between 21.0cm and 26.8cm FL, and 187 testes between 21.1 and 33.2cm FL (modes, 25–27cm FL) (Fig. 6C). Because a single specimen was obtained in each 18cm FL class and 20cm FL class, SR was 100% in the two classes. SR rapidly decreased to 75% at 21cm FL, 40% at 22cm FL, then slightly increased to 50% at 23cm FL, and exhibited stable values around 60% in classes larger than 24cm FL. $Lf{:}s_{50\%}$ was not obtained from the logistic curve between Lf and SR. The total sex ratio was 63%.

The OMR started to increase from 22cm FL and attained a level of about 90% at 26cm FL (Fig. 7C). $Lf{:}m_{50\%}$ and $Lf{:}m_{90\%}$ were 23.7 and 25.7cm FL, respectively. The value of SR at $Lf{:}m_{90\%}$ was about 63%. An SR decrease was observed only in the range between 20 and 22cm FL. In

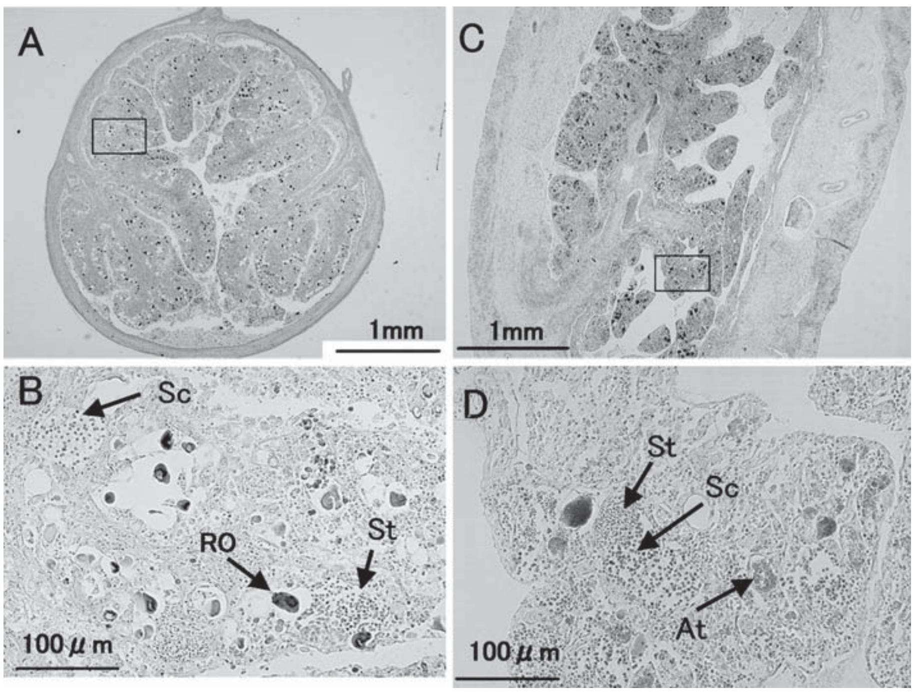

Fig. 9. Transverse sections of hermaphroditic gonads in *Lethrinus miniatus*. **A** Gonad showing premature sexual transition with a thin gonadal wall in a 32.7cm FL specimen collected on 16 December 1987; **B** higher magnification of the area enclosed in **A.** **C** Gonad showing postmature sexual transition with hypertrophied gonadal wall in a 47.8cm FL specimen collected on 26 November 1987; **D** higher magnification of the area enclosed in **C**. *At*, atresia; *St*, spermatid

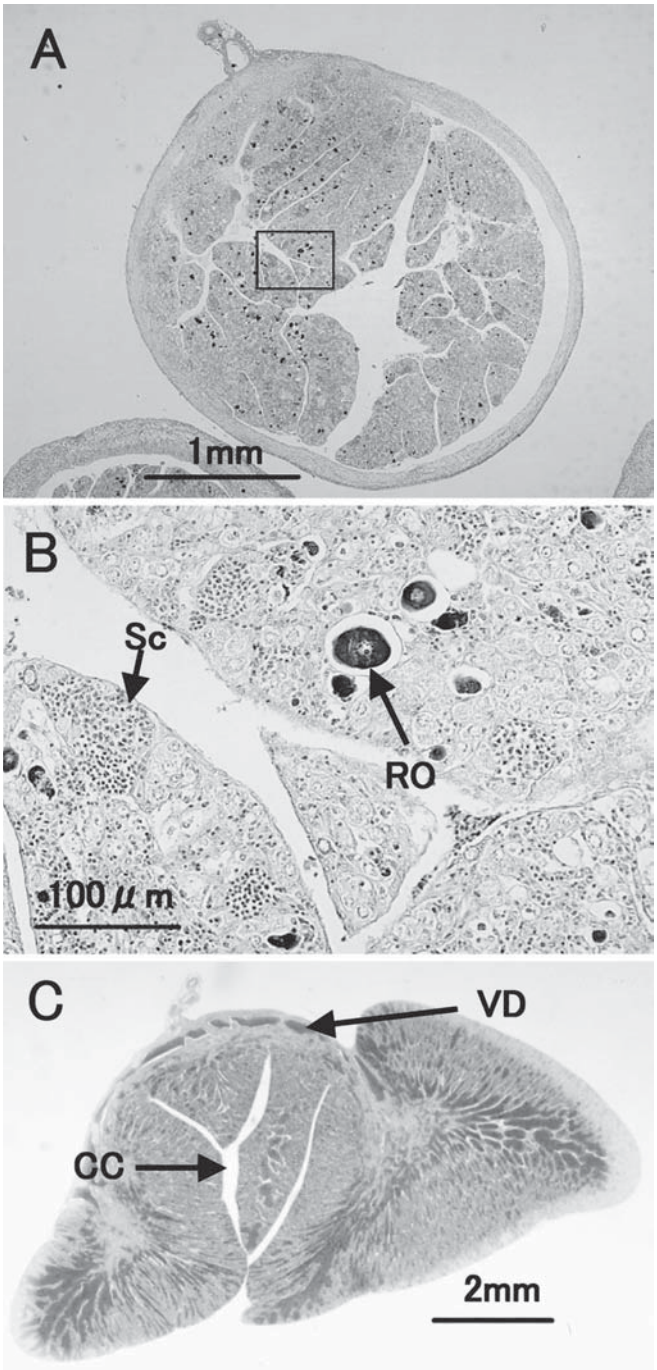

Fig. 10. Transverse sections of gonads in *Lethrinus obsoletus*. **A** Whole section of the differentiating gonad in a 22.6 cm FL specimen collected on 21 May 1991; **B** higher magnification of the area enclosed in **A. C** Testis in a 30.0 cm FL specimen collected on 14 August 1991. *CC*, central cavity, *VD*, vas deferens

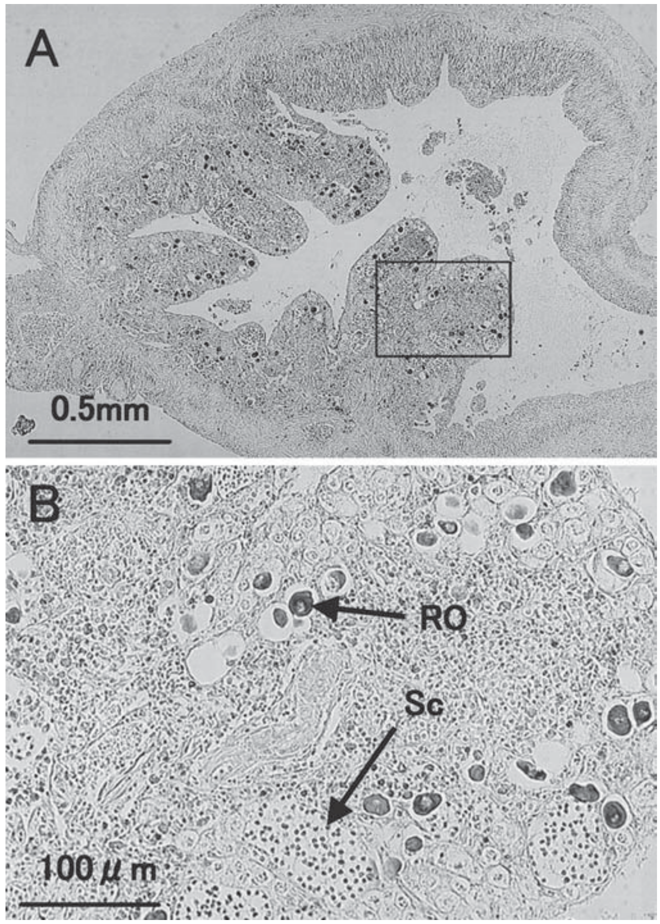

Fig. 11. Transverse sections of hermaphroditic gonad in *Lethrinus ornatus*. **A** Whole section in a 22.1 cm FL specimen collected on 19 June 1990; **B** higher magnification of the area enclosed in **A**

addition, all hermaphroditic gonads were very narrow with thin gonad walls (Fig. 10A,B), indicating that the sexual transition occurred before ovarian maturation. All testes were judged to be formed from ovaries because all testes had cavities derived from ovarian lumen and vasa deferentia in the gonadal wall (Fig. 10C). Accordingly, the sexuality of the species was considered to be juvenile hermaphroditism.

Lethrinus ornatus.—A total of 49 ovaries were obtained in the range between 12.0 and 23.9 cm FL (mode, 20 cm FL), and 29 testes between 19.8 and 26.8 cm FL (mode, 23 cm FL) (Fig. 6D). Two hermaphroditic gonads were obtained in specimens of 22.1 (Fig. 11A,B) and 23.1 cm FL. SRs maintained high values up to 21 cm FL, then suddenly dropped to about 50% at 22 cm FL and 23 cm FL, and to 0% in the classes larger than 24 cm FL. Lf : $s_{50\%}$ was 22.1 cm FL.

OMR was either 0% or 100% with the exception of 60% at 20 cm FL because of the small number of specimens. The logistic curve between OMR and Lf (see Fig. 7D) was drawn empirically because the curve obtained from the regression estimate on the dataset (Fig. 7D) was visibly inadequate. OMR seemed to attain a value of 90% at around 20 cm FL. SR at 20 cm FL was about 90%. Accordingly, the sexuality of the species was considered to be protogynous hermaphroditism.

Lethrinus sp. 2.—A total of 126 ovaries were obtained in the range between 19.1 and 30.9 cm FL (mode, 27 cm FL), 11 He gonads between 27.7 and 32.2 cm FL, and 104 testes between 27.0 and 32.4 cm FL (mode, 29 cm FL) (see Fig. 6E).

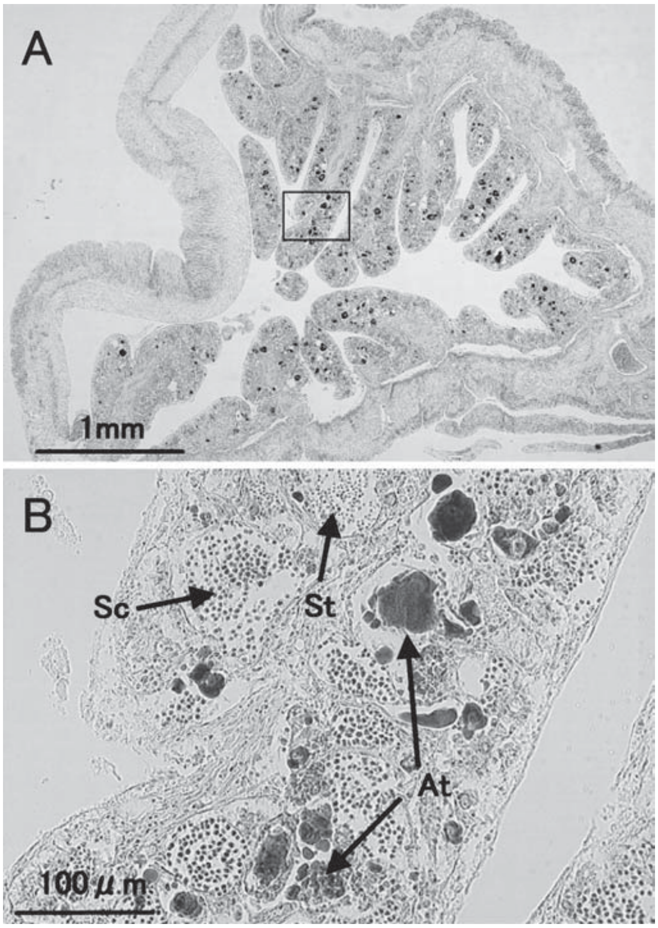

Fig. 12. Transverse sections of hermaphroditic gonad in *Lethrinus* sp. 2 (drab emperor). **A** Whole section in a 27.7 cm FL specimen collected on 9 September 1985; **B** higher magnification of the area enclosed in **A**

Numerous degenerating previtellogenic oocytes and spermatogenic cells at various stages coexisted in the hermaphroditic gonad (Fig. 12A,B). SRs started to decrease at 27 cm FL and attained 0% in classes larger than 31 cm FL (see Fig. 7E). The calculated Lf : $s_{50\%}$ was 28.4 cm FL. Because a single specimen was obtained in each 19 cm, 22 cm, and 23 cm FL class, the OMR was 0% in the former two classes and 100% in the latter class. The OMRs varied between 60% and 100% in classes larger than 24 cm FL. Therefore, logistic estimation between Lf and OMR seems to be inadequate because there are fewer specimens in smaller classes and because of the OMR variation in the larger classes; however, the OMR seems to reach about 90% at 26 cm FL. The value of SR at 26 cm FL was 93%. Accordingly, the sexuality in *L.* sp. 2 was considered to be protogynous hermaphroditism.

Discussion

Spawning periods. During the spawning period of *L. harak* (April to November), neither hydrated ovaries nor ovaries bearing POF were obtained in August and September, with relatively small GSI values. Therefore, the possibility of inactive spawning in these 2 months may exist. In the case of *L. ornatus*, although spawning in May, June, and November has been confirmed, that from August to October is yet unknown because of inadequate sampling in these months. Many *Lethrinus* species inhabit seagrass beds during their juvenile period (Kanashiro, 1998; Nakamura and Sato, 2004). Both from visual censuses and seine sampling carried out on seagrass beds in the Yaeyama area in 1995–1997, juveniles of *L. harak*, *L. obsoletus*, and *L. ornatus* were observed every month except for April and May, and juveniles of *L. harak* were captured every month, those of *L. ornatus* continuously from May to January, and those of *L. obsoletus* in May and from August to March (Kanashiro et al., 1997, 1998; Kanashiro and Nakamoto, 1999). Their spawning periods in the present study seem to fit well with periods of juvenile appearance. Accordingly, the spawning period in *L. ornatus* seems to last continuously until November, and that of *L. harak* would last continuously until November without the 2-month pause. A similar comparison was impossible in *L. miniatus* and *L.* sp. 2 because their juvenile habitats are yet unknown.

The start of spawning of *L. miniatus* was the earliest among *Lethrinus* species around the Ryukyu Islands as spawning started from February in 1988 (Ebisawa, 1990, 1997, 1999). The average water temperature in 1979–1989 at the 30-m layer of about 50 m depth off Okinawa Islands is lowest (= 20.9°C) in February (Okinawa Prefectural Fisheries and Ocean Research Center web page: http://www.pref.okinawa.jp/fish/jihou/jihouindex.html). Water temperatures of the same locations in February and March 1987, when there was no evidence of spawning, were 20.2°C and 20.3°C, respectively, whereas temperatures were 22.0°C and 22.6°C, respectively, in 1988, when spawning was confirmed (Okinawa Prefectural Fisheries and Ocean Research Center web page: http://www.pref.okinawa.jp/fish/jihous62/63_89.pdf). The earlier start of the spawning period in 1988 must have resulted from the water temperature being higher than usual in February.

The main spawning period in *L. miniatus* so far reported varies widely depending on survey locations: July–August at about 19°S in the central Great Barrier Reef (GBR) (Walker, 1975), July–November at about 23.5°S in the southern GBR (Sumpton and Brown, 2004), and October–April at about 29°S in Norfolk Island (NI) (Church, 1995). Average GSI values in the species in New Caledonia at about 22.5°S (NC) are 4.1 in October, and 1.7 and 1.9 in November and in December, respectively, suggesting that the spawning there lasts until December (Loubens, 1980a). Water temperature in GBR reached a low in July–August and a high in January–February (Australian Institute of Marine Science web page: http://www.aims.gov.au/pages/facilities/weather-stations/ytd-2002/ytd-cleveland.html). The spawning periods of *L. miniatus* are strongly affected by the latitude of their habitats; at low latitudes (central GBR), the spawning period occurs around the period with the lowest water temperature, at high latitudes (NI), it occurs around the period with the highest water temperature, and in the intermediate area (southern GBR and NC), it occurs in the period of increasing water temperature following the nadir. This observation indicates that the water temperature zone suitable for spawning is relatively narrower and lower in this species than in other lethrinids. Given the many other *Lethrinus* species that spawn in the period of highest water temperature and are distributed from the Ryukyu Islands to Australia continuously, the restricted distribution only from Australia to NC, and off the Ryukyu Islands, shown by *L. miniatus* (see Carpenter, 2001) is a very interesting issue.

Sexual pattern. Sadovy and Shapiro (1987) described features of gonads strongly indicative of protogyny as follows: (1) presence of membrane-lined central cavities in testes; (2) individuals with transitional gonads; (3) atretic bodies of yolked oocytes within testes; and (4) sperm sinuses in the gonadal wall. Gonads fulfilling these criteria except "atretic bodies of yolked oocytes within testes" found in this study indicate occurrences of a sex change from female to male in these five species. Protogyny was also supported by results in body size at sexual maturity and at sexual transition in the four species, except in *L. obsoletus*, where sexual transition occurred before ovarian maturation. *Lethrinus miniatus* was found to exhibit protogyny in GBR (Bean et al., 2003; Sumpton and Brown, 2004), NC (Loubens, 1980a), and NI (Church, 1995) populations, with a small portion of premature sexual transition occurring in GBR (Sumpton and Brown, 2004) and NI (Church, 1995). Thus, the sexuality is fundamentally the same between these populations even if the distribution of species between Northern and Southern Hemispheres is discontinuous.

Considerable oocyte degeneration before spermatogenic tissue proliferation has been reported in the GBR population (Bean et al., 2003). In the present study, vitellogenic stage oocyte atrasia was never observed in hermaphroditic gonads. The considerable degeneration of vitellogenic and previtellogenic oocytes before the proliferation of spermatogenic tissue seems to be characteristic of lethrinid sexual transition. Because no published report exists for the sexual pattern in the other four species, comparisons of the present results to other populations were not possible.

The sexuality of *Lethrinus* species so far revealed are summarized in Table 1. Each sexual condition listed in the table was determined by whether a different modal size

Table 1. Sexuality of *Lethrinus* species reported in the literature

Species	Location	Sexuality	Source
Lethrinus atkinsoni	Okinawa	P	Ebisawa (1999)
	Yaeyama	J	Ebisawa (1999)
	New Caledonia	J	Loubens (1980a), appeared as *L. mahsena*
L. borbonicus	Red Sea	J	Salem (1990b), appeared as *L. bungus*
L. choerorynchus	North West Shelf	P	Young and Martin (1982)
L. enigmatics	Indian Ocean	P	Lebeau and Cueff (1975)
L. genivitatus	Okinawa	J	Kanashiro et al. (1983), appeared as *L. nematacanthus*
	New Caledonia	P	Loubens (1980a), appeared as *L. nematacanthus*
	North West Shelf	P	Young and Martin (1982), appeared as *L. nematacanthus*
L. harak	Yaeyama	P	Present study
L. laticaudis	Gulf of Carpentaria	P	Young and Martin (1982), appeared as *L. fraenatus*
L. lentjan	Indian Ocean	J	Toor (1964a)
	New Caledonia	P	Loubens (1980a)
	North West Shelf	P	Young and Martin (1982)
	Gulf of Carpentaria	P	Young and Martin (1982)
L. miniatus	Okinawa	P	Present study
	New Caledonia	P	Loubens (1980a), appeared as *L. chrysostomus*
	Great Barrier Reef	P	Walker (1975), appeared as *L. chrysostomus*
	Great Barrier Reef	P	Bean et al. (2003)
	Great Barrier Reef	P	Sumpton and Brown (2004)
	Norfolk Island	P	Church (1995)
L. nebulosus	Okinawa	J	Ebisawa (1990)
	New Caledonia	J	Loubens (1980a)
	North West Shelf	J	Young and Martin (1982)
L. obsoletus	Yaeyama	J	Present study
L. ornatus	Yaeyama	P	Present study
	Okinawa	P	Ebisawa, unpublished data
L. rubrioperculatus	Okinawa	P	Ebisawa (1997)
	New Caledonia	P	Loubens (1980a), appeared as *L. variegatus*
L. variegatus	North West Shelf	P	Young and Martin (1982)
L. sp. 2 (drab emperor)	Okinawa	P	Present study

J, juvenile hermaphroditism; P, protogynous hermaphroditism distribution between sexes is obvious when there is no author's description of one of the two, because the modal difference is obvious in protogynous species (Loubens, 1980a; Young and Martin, 1982; Ebisawa, 1997), whereas it is not obvious in juvenile hermaphrodite species, as in *L. nebulosus* (see Ebisawa, 1990), *L. atkinsoni* (see Ebisawa, 1999), and in *L. obsoletus* (present study). Thus, juvenile hermaphroditism is not a rare case, and protogynous and juvenile hermaphroditism is not fixed to species.

Male juvenile hermaphrodites that function only as males throughout their lifetime are identical to primary males (sensu Reinboth, 1970). Emergences of primary males are related to the group density in other families. For example, the rates of emergence of the primary male in the bluehead wrasse *Thalassoma bifasciatum* (Labridae) vary from patch reef to patch reef, where higher density of the species in a patch reef leads to higher rates (Warner and Hoffman, 1980). The Nassau grouper *Epinephelus striatus* (Serranidae), which exhibits a gonocholistic sexual pattern and develops testes at the same volume as ovaries during its spawning period (Sadovy and Colin, 1995), is reported to form schools of 30–40 fish at the juvenile stage (250–350 mm in total length) (Tucker et al., 1993). Because schools of fish at the juvenile stage are as yet unknown in other protogynous serranids, a causal relationship between the school of fish at the juvenile stage and the gonocholist may exist. *Lethrinus nebulosus*, *L. atkinsoni* at Yaeyama, *L. obsoletus*, and *L. genivitattus*, which exhibit juvenile hermaphroditism, inhabit seagrass beds and adjacent areas in relatively high density during their juvenile stages (Kanashiro et al., 1997; Nakamura and Sato, 2004). *Lethrinus harak* also inhabits seagrass beds and adjacent areas with relatively high density (Nakamura and Sato, 2004) but exhibits protogynous hermaphroditism. *Lethrinus harak*, however, is found in solitude, or in groups comprising two to five individuals, and never in groups of several tens of fish on seagrass beds (Ebisawa, unpublished data). Therefore, population density in the juvenile stage seems to be a probable cue affecting the sexuality of hermaphroditic species including *Lethrinus*.

Intensive catches have been done from April to June in *L. obsoletus* and from February to June in *L. miniatus* at a number of sites known by local fishermen (Ebisawa, unpublished data). These two species probably aggregate to spawn at their own spawning site. The relationships between testicular size and spawning style revealed in many species (Robertson and Warner, 1978; Warner and Robertson, 1978) suggest that *L. obsoletus,* which has large testes, spawn in groups and that *L. miniatus,* which has small testes, spawn in pairs at their own spawning sites. Because the other three species have small body size and are mixed together with small amounts of commercial catch with other target species in fishing, information such as spawning aggregation and spawning sites is scarce.

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