Studies on the age, growth and reproduction of Lophiomus setigerus and Lophius litulon in the East China Sea and the Yellow Sea

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PREFACE

Two species of anglerfish, *Lophiomus setigerus* and *Lophius litulon*, also known as goosefish or monkfish, are considered delicacies in Japan. The price of these anglerfish has been increasing over the last few decades, and now they are one of the most valuable food fishes during the winter season. In spite of their commercial importance, little is known about the biology of these two species. The purpose of this study is to increase our knowledge of the age, growth and reproduction of *L. setigerus* and *L. litulon* in the East China and Yellow Seas.

The current taxonomic classification of *L. setigerus* and *L. litulon* is:

Phylum: Chordata

Subphylum: Vertebrata

Grade: Pisces

Class: Osteichtyes

Subclass: Actinopterygii

Subdivision: Teleostei

Infradivision: Euteleostei

Superorder: Paracanthopterygii

Order: Lophiiformes

Suborder: Lophioidei

Family: Lophiidae

The family Lophiidae is divided into three distinct genera, *Lophiomus*, *Lophius* and *Lophiodes*. *Lophiomus* is monotypic, while *Lophius* and *Lophiodes* contain 8 and 14 species, respectively (Caruso, 1981, 1983; Saruwatari and Mochizuki, 1985). The

species of *Lophiomus* and *Lophius* are listed in Table 1.

L. setigerus and *L. litulon* are two morphologically similar but taxonomically distinct species that are distinguished on the basis of several external features and by the median number of vertebrae (Table 2, Caruso, 1983; Yamada 1986, 1993). *L. setigerus* is wide ranging throughout the western and Indo- Pacific, but is absent from the Atlantic and eastern Pacific (Table 1, Caruso, 1983). While, *L. litulon* is the only species of *Lophius* that inhabits the western Pacific Ocean. *L. setigerus* and *L. litulon* both occur in the East China and Yellow Seas. However, the former species lives predominantly in waters south of the 32th parallel, in the East China Sea, and the latter mainly north of 32° N, in the Yellow Sea (Yamada, 1986). Ueno (1966) reported that in Japanese waters *L. litulon* is caught mainly in the coastal waters of the Sea of Japan; while *L. setigerus* is taken mainly in the coastal waters of the Pacific Ocean. This distribution shows that *L. setigerus* prefers warmer waters than *L. litulon*.

Anglerfish have unique breeding habits. Female anglerfish, like other Lophiiformes fishes, extrude a gelatinous egg mass, within which individual eggs float in separate chambers, (Fulton, 1898; Gill, 1908; Connolly, 1920; Dahlgren, 1928; Berril, 1929; Breder, 1949; Bigelow and Schroeder, 1953; Mosher, 1954; Rasquin, 1958; Ray, 1961; Mito, 1963; Pietsch and Grobecker, 1980; Feinberg, 1984; Armstrong et al., 1992; Afonso-Dias and Hislop, 1996; Yoneda et al., 1997a). In *Lophius* spp., the egg masses are very large, up to 9-12 m in length and 0.6-1.5 m in width, and contain as many as1-2 million eggs (Connolly, 1920; Dahlgren, 1928; Berril, 1929; Feinberg, 1984; Armstrong et al., 1992; Afonso-Dias and Hislop, 1996). At the Oarai Aquarium, female *L. litulon* with total lengths between 700 and 850 mm were observed extruding egg masses reaching 7.5-8.5 m in

Table 1. Lists of *Lophiomus* and *Lophius* species

Genus	Species	Habitat
Lophiomus	*L. setigerus* (Vahl), [*Ankou* *]	Western and Indo- Pacific
Lophius	*L. litulon* (Jordan), [*Kiankou* *]	western Pacific
	L. americanus (Valenciennes)	western North Atlantic
	L. budegassa (Spinola)	Mediterranean Sea and eastern North Atlantic
	L. gastrophysus (Ribeiro)	western Atlantic
	L. piscatorius (Linnaeus)	Mediterranean Sea, Black Sea and eastern North Atlantic
	L. upsicephalus (Smith)	eastern South Atlantic and the southwestern Indian Ocean.
	L. vaillanti (Regan)	eastern Atlantic
	L. vomerinus (Valenciennes)	Bay of Bengal and eastern South Atlantic

Caruso (1983).

* Japanese name.

Table 2. Primary morphological characteristics of *Lophiomus setigerus* and *Lophius litulon*

	L. setigerus	*L. litulon*
Coloration		
dorsal surface	light to dark brown above with darker spots	light brown above with spots surrounded by darker pigment
ventral surface	white to light gray	light to dark gray
inside of mouth	dark brown or black with numerous light circular plates	gray
Humeral spine	2-5 branched tips	1-2 branched tips
Number of vertebrae	18-19	26-27
Maximum size	400 mm in total length	1000 mm in total length

length, that contained an estimated 2 million infertile eggs (Kofuji, K., Oarai Aquarium, pers. comm.). Fertile eggs develop and hatch within the egg mass, and the larvae break out of the egg mass (Connolly, 1920; Dahlgren, 1928; Berril, 1929; Mito, 1963). The larval anglerfish are initially planktonic and have elongated dorsal head spines and pelvic and pectoral fins before they settle down to a life on the bottom (Fig. 1, Connolly, 1920; Dahlgren, 1928; Berril, 1929; Mito, 1963; Matsuura and Yoneda, 1986; Minami, 1988; Watson, 1996).

Most studies on the age, growth and reproduction of anglerfish have been undertaken by European and American scientists, because most *Lophius* spp. are found in the Atlantic Ocean. These fish have been highly esteemed as a food fish in Europe for centuries (Bigelow and Schroeder, 1953; Caruso, 1983; Armstrong et al. 1992; Almedia et al., 1995; Afonso-Dias and Hislop, 1996). Fulton (1898, 1903) described the ovarian histology and growth rate of *Lophius piscatorius* in Scottish waters, for the first time. Connolly (1920) estimated the growth rate of *Lophius americanus* using field observations of the occurrence of young anglerfish and by counting rings on the surface of the vertebral centrum. In a review of the life history of *L. americanus*, Bigelow and Schroeder (1953) reported a seasonal progression of spawning from southern to northern waters, and discussed the location of the spawning grounds. Tsimenidis and Ondrias (1980) used the otoliths to describe the age and growth of *Lophius budegassa* and *L. piscatorius*. Subsequent studies used several bony structures to determine the age and growth of *L. budegassa*, *L. piscatorius* (Dupouy et al. 1986), *Lophius upsicephalus* (Griffiths and Hecht, 1986) and *L. americanus* (Armstrong et al., 1992). Recently, Armstrong et al. (1992) determined the spawning season, size and age at sexual maturity, and fecundity of *L. americanus* in the western North Atlantic using the first histological studies since

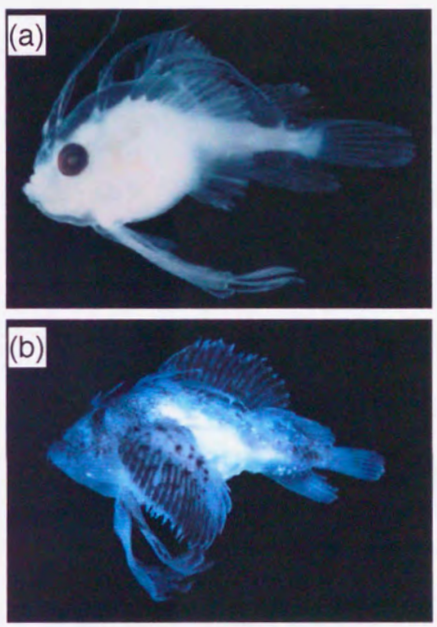

Fig. 1. Photographs of larvae of *L. litulon*. (a) Sampled in the East China Sea in May 1995, size = 21 mm in total length. (b) Sampled in Wakasa Bay in May 1997, size = 53 mm in total length.

those of Fulton (1898). Afonso-Dias and Hislop (1996) also studied the spawning season and size at sexual maturity of *L. piscatorius* from the waters off the northwest coast of Scotland.

In spite of their traditional importance as a food fish in Japan, only a few attempts have been made to study the age, growth and reproduction of *L. setigerus* and *L. litulon* to date. Mito (1963) gave a brief account of the structure of an ovary with mature oocytes in *L. litulon*. Kosaka (1966) described the age, growth, spawning season and size at sexual maturity of *L. litulon* in Sendai Bay. Yamada (1986) reported the size at sexual maturity and spawning season of *L. litulon*, as well as the spawning season of *L. setigerus* in the East China and Yellow Seas. This report is the first description of the reproduction of these two anglerfish in the East China and Yellow Seas. Minami (1988) reported the spawning season of *L. setigerus* and *L. litulon* in Japanese waters. In an unpublished report, Tokimura et al.[1] examined the age, growth and spawning season of *L. setigerus* and *L. litulon* in the East China and Yellow Seas. Suzuuchi[2] and Hori (1995) commented on the size of both sexes, the spawning season and the size at sexual maturity of *L. litulon* in the coastal waters of Hokkaido and Ibaraki. More recently, Yoneda et al. (1997a, b) reported the ovarian structure and batch fecundity in *L. setigerus*, and the age and growth

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[1] Tokimura, M., Fujita, H. and Kitajima, T. (1990). Abst. Metg. Japan. Soc. Fisheries Sci., April, p. 9 (in Japanese).

[2] Suzuuchi, T. (1993). Report on *Lophius litulon*. Hokusuishi Dayori, Hokkaido Central Fish Exp. Stn., 22, pp. 29-31 (in Japanese).

of *L. litulon*. Additional reports on the age and growth (Yoneda et al.[3]) and the reproductive cycle and sexual maturity (Yoneda et al.[4]) of *L. setigerus*, and the reproductive cycle, batch fecundity and seasonal distribution of *L. litulon* (Yoneda et al.[5]) have been made.

This study consists of four chapters. The first two chapters describe *L. setigerus* and the final two chapters *L. litulon*. In Chapter I, determinations of the age and growth of *L. setigerus* are made using the vertebral centrum. In Chapter II, the gonadal structure, reproductive cycle, sexual maturity and batch fecundity of *L. setigerus* are described. Spermatogenesis in male anglerfish is shown to be of the 'semi-cystic' type and, in females, spawning is demonstrated to have a lunar periodicity. In Chapter III, the vertebral centrum is used to study the age and growth of *L. litulon*. My age and growth estimates agree closely with field observations of the occurrence of young fish. In Chapter IV, the gonadal structure, reproductive cycle, sexual maturity, batch fecundity and seasonal

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[3] Yoneda, M., Tokimura, M., Fujita, H., Takeshita, N., Takeshita, K., Matsuyama, M. and Matsuura, S. (1997). Age and growth of the anglerfish *Lophiomus setigerus* in the East China Sea. *Fisheries Science,* under review.

[4] Yoneda, M., Tokimura, M., Fujita, H., Takeshita, N., Takeshita, K., Matsuyama, M. and Matsuura, S. (1997). Reproductive cycle and sexual maturity of the anglerfish *Lophiomus setigerus* in the East China Sea: with a note on specialized spermatogenesis. *Journal of Fish Biology,* under review.

[5] Yoneda, M., Tokimura, M., Fujita, H., Takeshita, N., Takeshita, K., Matsuyama, M. and Matsuura, S. 1997. Reproductive cycle, batch fecundity, and seasonal distribution of the anglerfish, *Lophius litulon*, in the East China Sea and the Yellow Sea. *Fishery Bulletin*, under review.

distribution of *L. litulon* are outlined. This chapter describes this species' spawning ground and the migratory pattern of sexually mature males and females.

CHAPTER I AGE AND GROWTH OF *LOPHIOMUS SETIGERUS*

Introduction

The anglerfish *Lophiomus setigerus* is widely distributed in the western Pacific, the Indian Ocean and the Red Sea (Caruso, 1983,1989). In the East China Sea, anglerfish are typically caught at depths of 75-85 m in water with a temperature ranging from 17-20 °C, during the autumn season (Yamada, 1986). Tokimura (1992) reported that the distribution of *L. setigerus* in the East China Sea does not change throughout the year. Yamada (1986) showed that spawning of *L. setigerus* occurs during the period from April through May in the East China Sea. Yoneda et al. (1997a), however, suggest that females undergo repeated spawnings between May and November.

Earlier reports on the size of *L. setigerus* include disparate information. One finding is that anglerfish larger than 30-35 cm in total length are rarely caught in the East China Sea (Yamada, 1986; Tokimura et al.[1]). Another is that anglerfish reach approximately 1 m in body length (Ochiai and Tanaka, 1986; Suzuuchi [2]). There has only been one study on age and growth of anglerfish in the East China Sea using analysis of length-frequency distributions (Tokimura et al.[1]). This study grouped the data from the two sexes together. To date, no study on age and growth of anglerfish using any hard structures has been performed. In this chapter, I examined the age and growth of anglerfish in the East China Sea using vertebral centra.

Materials and methods

A total of 1186 anglerfish were collected from a commercial trawl fishery and from three trawl surveys conducted by the Seikai National Fisheries Research Institute (SNFRI), National Fisheries University and Nagasaki University, during the period from March 1991 to February 1996 in the East China Sea (Fig. 2).

All specimens were measured to the nearest millimeter in total length (*TL*) and to the nearest gram in body weight (*BW*). The masses of the gonads and livers were determined to the nearest 0.1 g and the gonads were preserved in Bouin's solution or 10 % formalin for subsequent histological observations and fecundity estimates.

Vertebral centra were chosen as the optimal hard structure for use as a marker in age determination, based on a preliminary examination revealing that each centrum contained concentric bands that appeared to be ring marks (Fig. 3). Otoliths, basihyals, metapterygoids, pelvic girdles, actinosts and illiciums were also examined; however, these structures were either opaque or had extremely irregular outer margins, making it difficult or impossible to clearly discern ring marks.

Vertebra number 8 was used for age determination. Vertebra number 7 or 9 was used if number 8 was damaged in preparation. After the marker vertebrae were soaked in hot water and cleaned, they were sectioned mid-frontally and the dorsal parts used for examination. Ring marks on the anterior face of the centrum were counted and measured using a reflected light profile projector at 5-10 × magnification. A broad, opaque band and a narrow, translucent band appeared alternately on the centrum surface. Any translucent band that completely encircled the centrum surface was considered a 'true band' and was counted, and one that did not was considered a 'false band' and was not counted. A total of 963 specimens (81%) was used for age determination. The distance from the focus

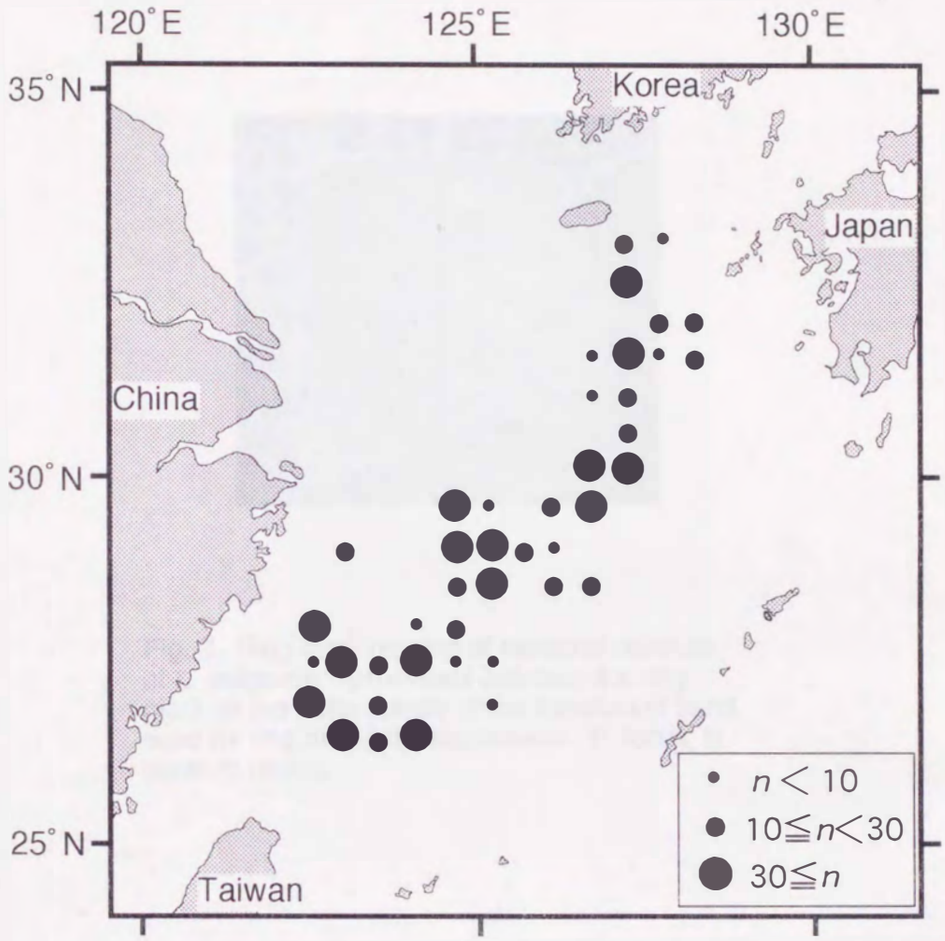


Fig. 2. Geographical distribution of specimens of *L. setigerus* collected in the East China Sea. *n*, number of fish examined.

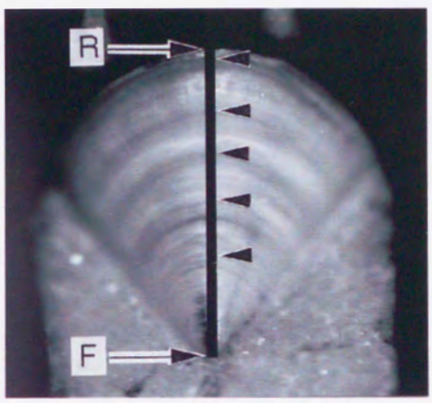


Fig. 3. Ring mark reading of vertebral centrum of *L. setigerus*. Arrowhead indicates the ring mark on the outer margin of the translucent band used for ring radius measurements. F, focus; R, centrum radius.

(F) to the outer margin of the translucent band of each ring mark (ring radius, r_n) and the centrum radius (R) were measured on a transverse plane along a straight line through the focus (Fig. 3).

The marginal increment of the centrum (MI) was examined using specimens possessing 1-6 ring marks based on the following equation:

$$MI = (R - r_{max}) / (r_{max} - r_{max-1})$$

where R is the centrum radius (mm) and r_{max} is the distance (mm) between the focus and the outer margin of the last translucent band.

To evaluate growth of males and females, the mean back-calculated *TL*s were fitted to the von Bertalanffy growth equation using the Marquardt method (Draper and Smith, 1966; Akamine, 1995).

Females (n = 765) were significantly larger in *TL* than males (n = 421; Mann-Whitney U-test, P<0.001; Fig. 4). Length frequency distributions clearly showed differences in the range of sizes within each of the sexes: most males sampled ranged from 200 to 300 mm *TL* and females from 300 to 400 mm *TL*. The maximum size of any of the males was 350 mm *TL* and the largest of the females had a body length of 428 mm *TL*.

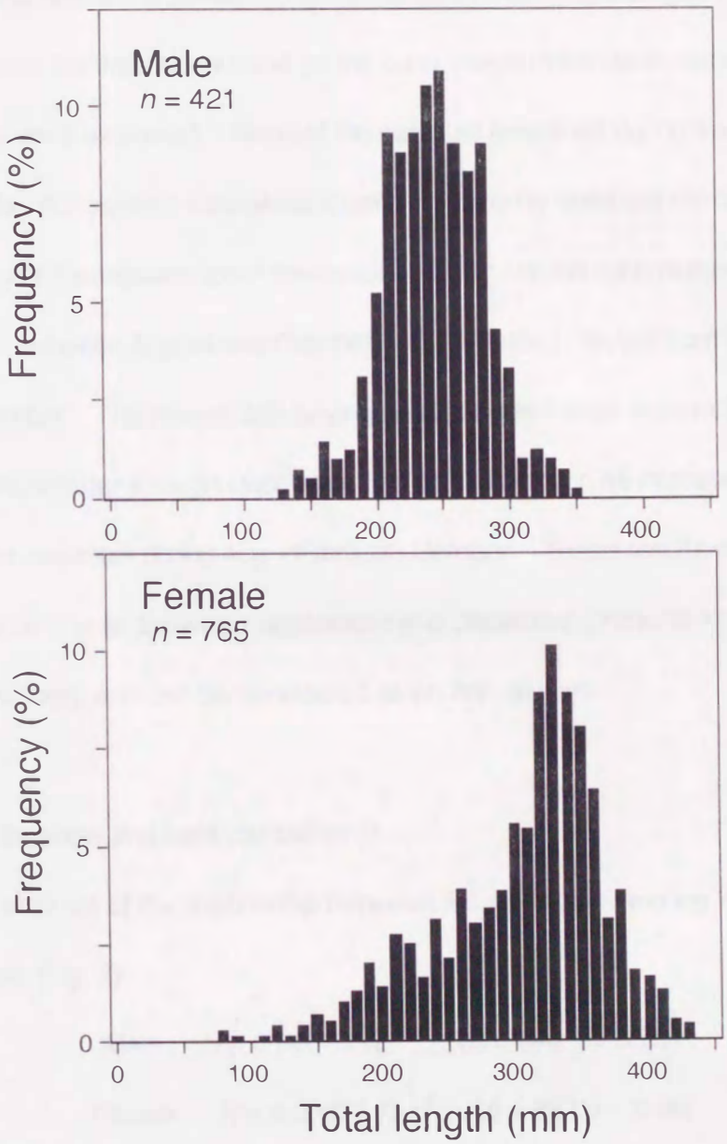


Fig. 4. Length-frequency distribution of *L. setigerus* sampled from the East China Sea. *n*, number of fish examined.

Results

Period of ring mark formation

In order to define clearly the period of ring mark formation, monthly changes in the frequency of appearance of the translucent band on the outer margin of the centrum as well as the *MI* of the centrum were examined. None of the samples examined during the period from January to May possessed a translucent band on the outer margin of the centrum (Fig. 5). The frequency of the appearance of the translucent band on the outer margin of the centrum remained high between August and October and decreased sharply from November through December. The fewest *MI*s having new opaque bands occurred during the period from September through December (Fig. 6). The mean *MI* increased in January and reached a maximum during August through October. These results suggest that ring marks form once a year, between September and December (primarily in November through December), and can be considered as an annual mark.

R-TL relationships and back-calculated TL

Regression analysis of the relationship between the log *R* (mm) and log *TL* (mm) for each sex was linear (Fig. 7) :

$$\text{Male}: \quad R = 0.00678\ TL^{1.16} \qquad (n = 340;\ r = 0.93)$$

$$\text{Female}: \quad R = 0.00630\ TL^{1.17} \qquad (n = 623;\ r = 0.96)$$

In the regression analysis of the relationship between the log *R* and log *TL*, significant differences occurred between sexes (ANCOVA, $P < 0.001$). The data were thus treated separately for each sex.

The mean ring radii of each successive centrum annulus at the time of annulus formation are shown in Tables 3 and 4. The specimens from males possessed 1-8 ring

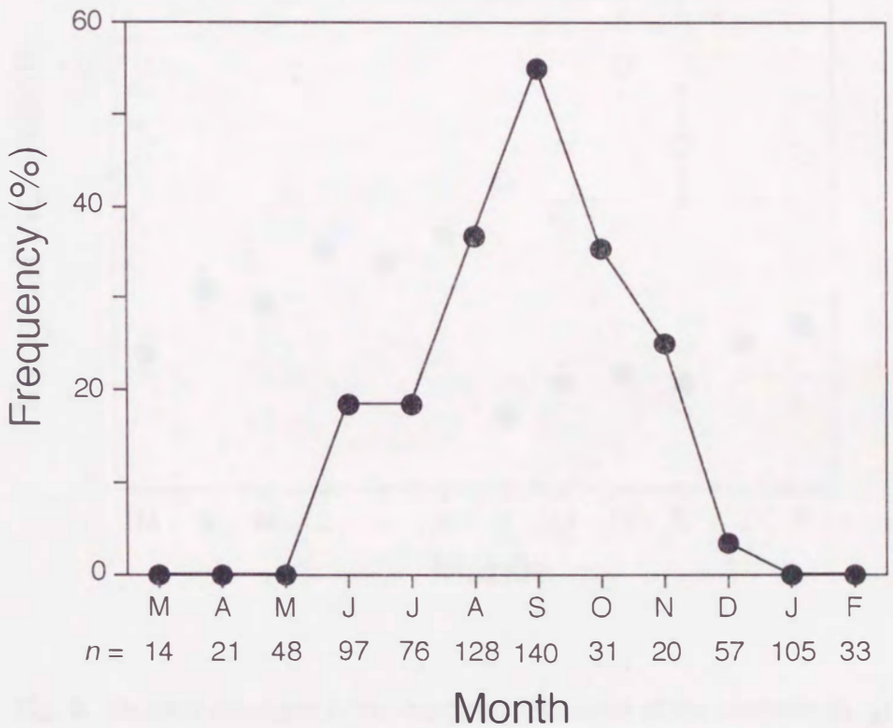


Fig. 5. Monthly changes in the frequency of appearance of a translucent band on the outer margin of the centrum of *L. setigerus*. *n*, number of fish examined.

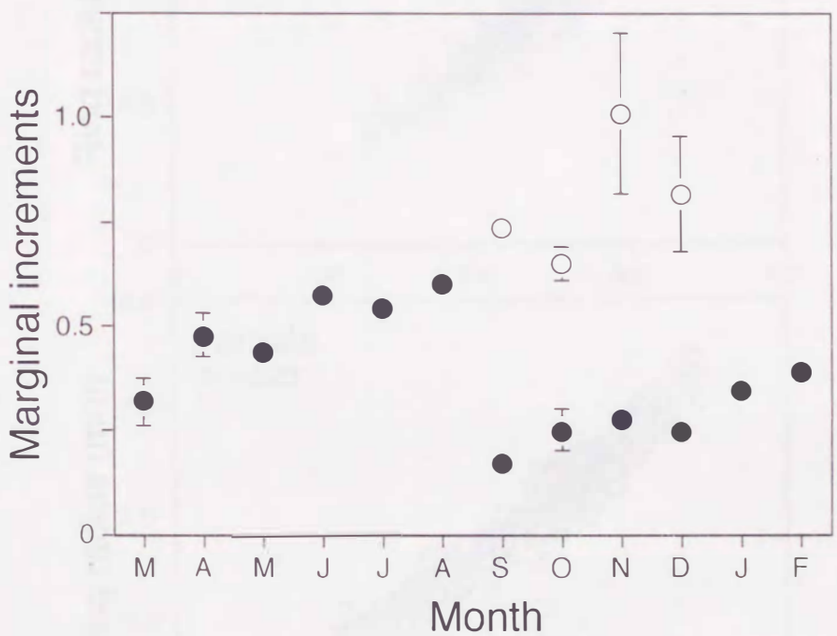


Fig. 6. Monthly changes in the marginal increment of the centrum (r_{1-6}) of *L. setigerus* (closed circles). Open circles indicate the mean marginal increments of centra without new opaque bands observed during the period between September and December. Vertical bars indicate standard error.

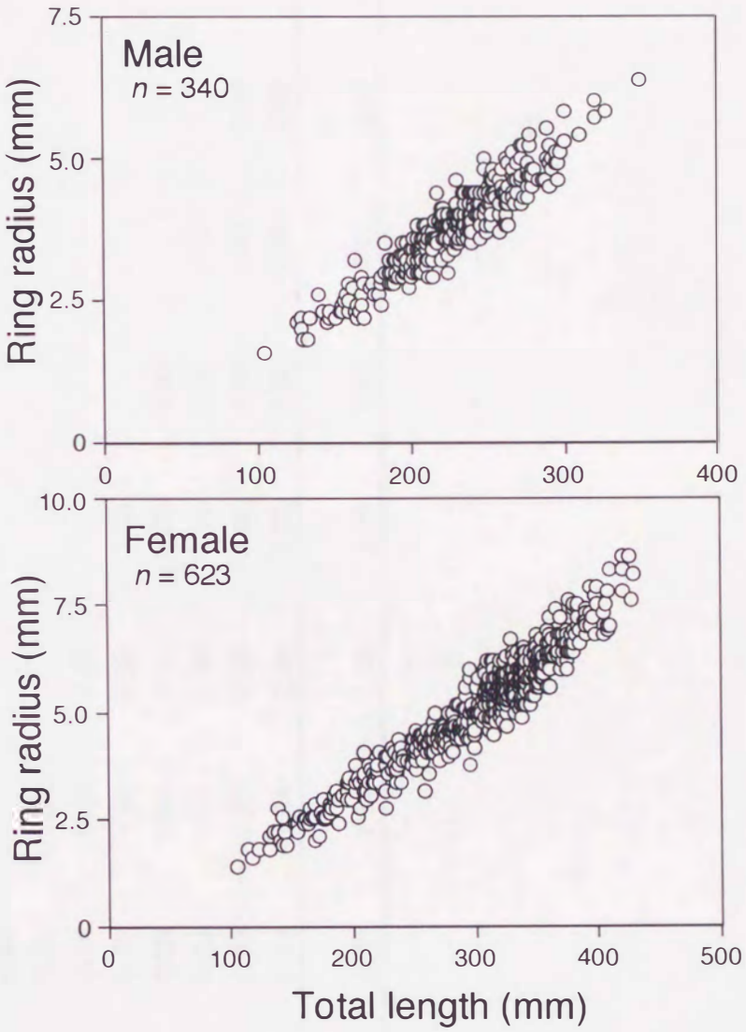


Fig. 7. Relationship between the centrum radius and total length for male and female *L. setigerus*. *n*, number of fish examined.

Table 3. Mean ring radii (mm) at each ring group for male *L. setigerus*

Ring group	*n*	r_1	r_2	r_3	r_4	r_5	r_6	r_7	r_8
1	2	0.89							
2	28	0.88	1.84						
3	76	0.74	1.82	2.73					
4	98	0.73	1.70	2.55	3.33				
5	82	0.69	1.65	2.51	3.26	3.93			
6	40	0.63	1.55	2.32	3.10	3.80	4.40		
7	9	0.78	1.62	2.38	3.08	3.80	4.44	4.94	
8	5	0.74	1.64	2.38	3.02	3.58	4.28	4.88	5.42
	340								
Mean (weighted)		0.72	1.71	2.55	3.25	3.87	4.40	4.92	5.42

n, number of fish examined.

Table 4. Mean ring radii (mm) at each ring group for female *L. setigerus*

Ring group	*n*	r_1	r_2	r_3	r_4	r_5	r_6	r_7	r_8	r_9	r_{10}	r_{11}
1	3	1.00										
2	36	0.92	1.95									
3	54	0.82	1.90	2.83								
4	75	0.81	1.93	2.90	3.81							
5	129	0.77	1.86	2.85	3.73	4.56						
6	159	0.80	1.85	2.80	3.69	4.49	5.23					
7	100	0.75	1.74	2.64	3.50	4.31	5.05	5.73				
8	42	0.71	1.67	2.56	3.41	4.24	4.95	5.68	6.30			
9	15	0.75	1.65	2.47	3.21	4.02	4.75	5.48	6.12	6.64		
10	7	0.81	1.84	2.74	3.56	4.33	5.03	5.82	6.61	7.24	7.40	
11	3	0.85	1.86	2.82	3.62	4.36	5.00	5.76	6.46	7.08	7.68	8.26
	623											
Mean (weighted)		0.79	1.84	2.77	3.64	4.43	5.11	5.70	6.30	6.86	7.67	8.26

n, number of fish examined.

marks, while females had 1-11 ring marks. Neither Lee's, nor reversed Lee's phenomenon was found for the mean ring radii of the centrum.

Growth

The spawning season of anglerfish in the East China Sea occurs from May to November (Yoneda et al., 1997a). However, the annual ring marks were formed between September and December (primarily in November through December), about three or four months after the middle of the spawning season. Thus, the mean back-calculated lengths for each sex and age ($t + 0.3$) were fitted to the von Bertalanffy growth equation (Fig. 8):

$$\text{Male}: \quad TL_t = 377.6\,(1 - e^{-0.193(t + 0.290)}) \quad (t \leq 8)$$

$$\text{Female}: \quad TL_t = 616.4\,(1 - e^{-0.109(t + 0.120)}) \quad (t \leq 11)$$

The growth curves indicated that the growth trends of both sexes were similar until age 2, but that females grew faster than males over age 3.

The relationship between BW (g) and TL (mm) for each sex was determined using the following equation (Fig. 9):

$$\text{Male}: \quad BW = (2.57 \times 10^{-5})\,TL^{2.95} \quad (n = 421;\ r = 0.95)$$

$$\text{Female}: \quad BW = (1.07 \times 10^{-5})\,TL^{3.12} \quad (n = 765;\ r = 0.97)$$

This analysis showed that significant differences occurred in both the intercept and the slope for both sexes (ANCOVA, $P < 0.01$).

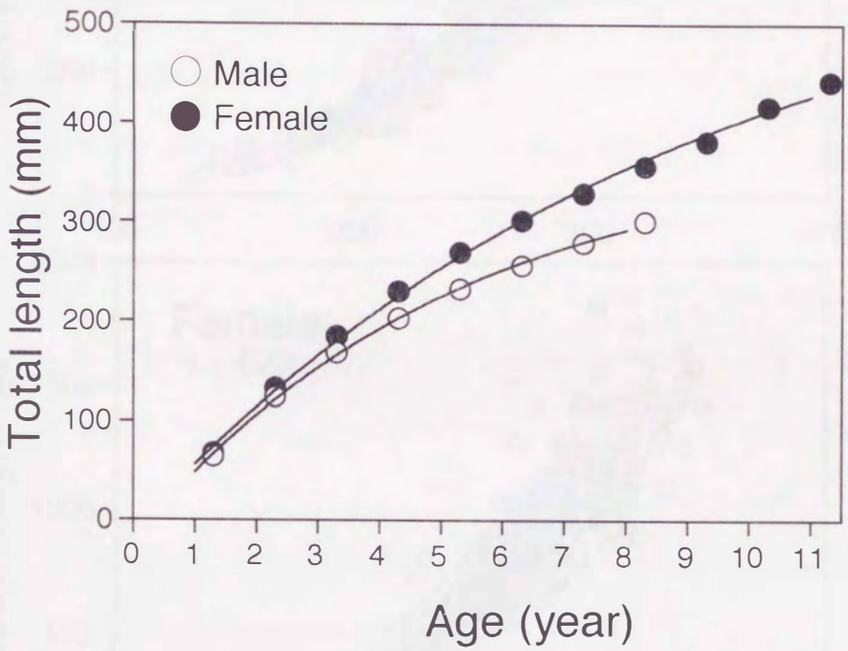


Fig. 8. von Bertalanffy growth curve for male and female *L. setigerus*. Circles are the back-calculated total length at ring formation.

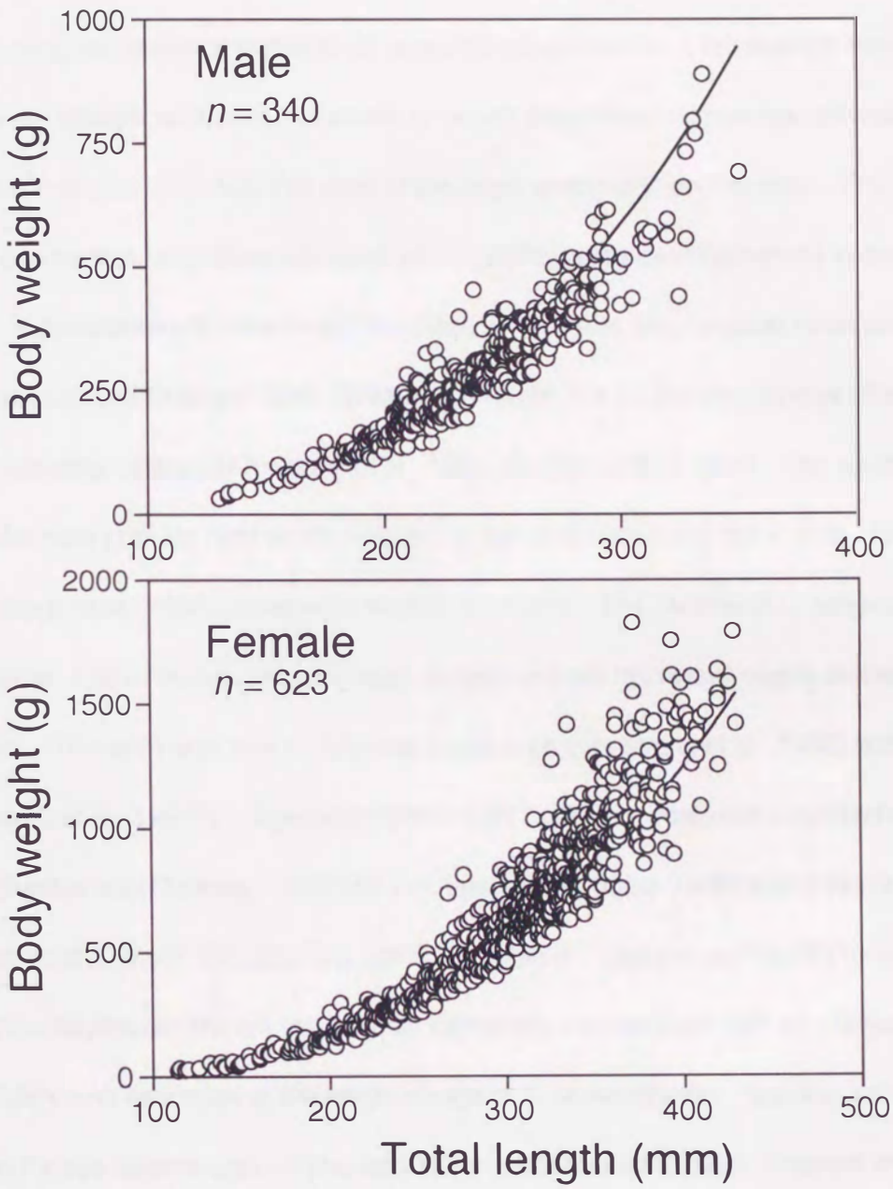


Fig. 9. Relationship between body weight and total length for male and female *L. setigerus*. *n*, number of fish examined.

Discussion

This is the first report on age and growth of male and female anglerfish in the East China Sea using the vertebral centra as an age determinant marker. My findings indicates that there are differences both in the growth increment and in the maximum age between the two sexes, reflected in the fact that most of the larger specimens are females. This was also found to be true for *L. litulon* (Yoneda et al., 1997b) in the East China and Yellow Seas.

Age determination markers of lophiidae fishes have been agreed upon as otolith (Tsimenidis and Ondrias, 1980; Griffiths and Hecht, 1986), illicium (Dupouy et al., 1986) and vertebral centrum (Armstrong et al., 1992; Yoneda et al., 1997b). The sagittal otoliths are the most popular hard structure used for age determination (Hattori et al., 1992; Tominaga et al., 1996; Lowerre-Barbieri et al., 1995). The otoliths of *L. setigerus*, however, had extremely irregular outer margins and did not reveal clearly discernible ring marks. This was also true for *Lophius americanus* (Armstrong et al., 1992) and *L. litulon* (Yoneda et al., 1997b). Age determinations for *Lophius piscatorius*, *Lophius budegassa* (Tsimenidis and Ondrias, 1980) and *Lophius upsicephalus* (Griffiths and Hecht, 1986) used otoliths, which are extremely variable in shape. Griffiths and Hecht (1986) conclude that the sagittal otoliths are the only structures that can be used with any degree of confidence for estimates of the length-at-age of *L. upsicephalus*. Spines are commonly used for age determination of sharks (Holden and Meadows, 1962; Beamish and McFarlane, 1985). Dupouy et al. (1986) developed the method of illicium sectioning for age determination in *L. budegassa* and *L. piscatorius*, but the illiciums of *L. setigerus* were not composed of concentric ring marks. The vertebral centra are the most commonly used age determinant for elasmobranches (Ishiyama, 1951; Daiber, 1960; Cailliet et al., 1990; Yudin and Cailliet, 1990; Yamaguchi et al., 1996). Even though false bands were often

observed on the surface of the centrum of *L. setigerus*, age determination in that species, as well as in *L. americanus* (Armstrong et al., 1992) and *L. litulon* (Yoneda et al., 1997b), was dependent on the occurrence of concentric bands (as true bands) on the surface of the centrum. The vertebral centrum may be a suitable structure for age determination of some lophiidae fishes.

The maximum size of any specimen from the 1186 sampled was 428 mm *TL* for a female. My length-frequency distributions showed that a large number of specimens of both males and females were less than 400 mm *TL*. This is in agreement with the report of Yamada (1986) and Tokimura et al.[1] on anglerfishes in the East China Sea. From the trawl survey conducted by SNFRI, all of the *L. setigerus* caught were less than 450 mm *TL* except for a single specimen of 500 mm *TL* over (Yamada, U. and Tokimura, M., Seikai Natl. Fish. Res. Inst., pers. comm.). These findings are quite different from previous information reporting anglerfish reaching approximately 1 m in body length (*BL*) (Ochiai and Tanaka, 1986; Suzuuchi [2]). In the herring *Clupea pallasii* (Kanno, 1989), the pacific cod *Gadus macrocephalus* (Hattori et al., 1992), the starspotted dogfish *Mustelus manazo* (Yamaguchi et al., 1996) and the pointhead flounder *Hippoglossoides pinetorum* (Tominaga et al., 1996), differences occur in growth rate and lifespan among the different waters and/or subpopulations. Furthermore, in the weakfish *Cynoscion regalis* (Lowerre-Barbieri et al., 1995) from Chesapeake Bay, historic changes in maximum size and age have been reported due to large fluctuations in year-class abundance. To my knowledge, no information about the age and growth of *L. setigerus* in waters other than those discussed here has been reported. Conversely, *L. litulon* (Yoneda et al., 1997b; Tokimura et al.[1]) do reach sizes greater than 1 m *TL*; and there is a close resemblance between *L. litulon* and *L. setigerus*. The previous finding that *L. setigerus* reach sizes of

approximately 1 m *BL* seems likely to be due to mistaking the two species of anglerfish.

The ring radius of each individual correlated very well with total length within each of the sexes, although regression analysis of the relationship between the log *TL* and log *R* demonstrated that significant differences occur between the sexes. Figure 7 shows that the growth rate of the ring radius in larger females (larger than about 300 mm *TL*) appears to be different from that of smaller females (smaller than about 300 mm *TL*). As a female anglerfish reaches 50 % sexual maturity at 303 mm *TL* in the East China Sea (see Chapter II), the relationship between *R* and *TL* for sexually immature (< 303 mm *TL*; $n = 252$) and mature (≥ 303 mm *TL*; $n = 371$) females was reexamined. The reanalysis demonstrated that statistically significant differences occurred between immature and mature individuals as reflected in the value of the slope (ANCOVA, $P < 0.001$). On the other hand, in male anglerfish no significant differences occurred in either the intercept or the slope between sexually immature (< 178 mm *TL*; $n = 37$; see Chapter II) and mature (≥ 178 mm *TL*; $n = 303$) individuals. The morphological changes and size variation of each vertebral centrum with respect to fish growth have been reported for many fishes (Clothier, 1946; Yamada, 1961a, b; Komada, 1980). In the jack mackerel *Trachurus japonicus* (Yamada, 1961b), transitional points in vertebral length growth rate are found twice during its life history; firstly at the fry stage and secondly at the end of 1 year, which is possibly related to sexual maturity. I found that the ovarian weight of anglerfish markedly increased to about half of the body mass just prior to spawning, and that the liver weight of mature females changed significantly with seasonal cycles and ovarian development. The vertebra used for age determination in the present study (No. 7, 8 or 9) is located at the midpoint of the vertebral column and above the posterior portion of the abdominal cavity. This suggests that the transition in the vertebral radius growth rate of females may be caused by the extraordinary

variance of the visceral condition between sexually immature and mature individuals.

My findings indicates that the estimated *TL* at each age for females is larger than that of males; and that the observed longevity for females is greater than for males, which is consistent with the sex-specific length-frequency distributions. This may contribute to the differences in size at sexual maturity (see Chapter II). This same finding applies to *L. litulon* (Yoneda et al., 1997b). Although the growth differences between the sexes of *L. americanus* (Armstrong et al., 1992) and *L. piscatorius* (Tsimenidis and Ondrias, 1980; Dupouy et al., 1986) are less than those observed for *L. setigerus*, in these *Lophius* spp. (Armstrong et al., 1992; Afonso-Dias and Hislop, 1996) females attain sexual maturity at a larger size than males. It is likely that differential growth rates and size at sexual maturity for both sexes are common sexual dimorphisms of lophiidae fishes.

Only one report on age and growth of *L. setigerus* indicates that anglerfish reach approximately 150, 230 and 300 mm *TL* at 2, 3 and 4 years old, respectively (Tokimura et al.[1]) However, my result using vertebral centra differs from the previous study, which used length-frequency distribution data for combined sexes. My length frequency distributions showed that most of the larger individuals (> 300 mm *TL*) were females. Furthermore, the spawning season of *L. setigerus* (Yoneda et al., 1997a) in the East China Sea occurs over a long period from May to November; and the present study suggests that females grow faster and live longer than males. These findings clearly suggest that cohorts would not occur in the length-frequency distribution from data combining both anglerfish sexes. This difference in data treatment is the likely reason for the large difference between the results of the earlier studies and those reported here.

CHAPTER II REPRODUCTION OF *LOPHIOMUS SETIGERUS*

Introduction

As mentioned in the preface, all female members of Lophiiformes, except for *Antennarius caudimaculatus* (Pietsch and Grobecker, 1980), are thought to extrude gelatinous egg masses (Fulton, 1898; Gill, 1908; Connolly, 1920; Dahlgren, 1928; Berril, 1929; Breder, 1949; Bigelow and Schroeder, 1953; Mosher, 1954; Rasquin, 1958; Ray, 1961; Mito, 1963; Feinberg, 1984; Armstrong et al., 1992; Afonso-Dias and Hislop, 1996; Yoneda et al., 1997a). The ovarian structure of Lophiiformes is quite different from that of many teleost fishes (Fulton, 1898; Rasquin, 1958; Armstrong et al., 1992; Afonso-Dias and Hislop, 1996; Yoneda et al., 1997a). More recently, Yoneda et al. (1997a) reported on the ovarian structure and spawning season of *L. setigerus* and presented evidence of multiple spawning and batch fecundity. These studies, however, were mainly focused on females and little attention has been given to reproduction of males. There has only been two reports on the testicular histology of *Lophius americanus* (Armstrong et al., 1992) and one on *Lophius piscatorius* (Afonso-Dias and Hislop, 1996). This chapter investigates the gonadal structure and specialized spermatogenesis so as to provide on understanding of their reproductive biology. Secondary, the annual reproductive cycle described as it relates to lunar activity, and the size and age at sexual maturity for both sexes and batch fecundity are examined.

Materials and methods

The anglerfish were collected from the commercial trawl fishery and from three trawl surveys conducted by the Seikai National Fisheries Research Institute, National Fisheries University and Nagasaki University during the period from March 1991 to February 1996 in the East China Sea (Fig. 10). All specimens were measured to the nearest millimeter in total length (*TL*) and to the nearest gram in body weight (*BW*) and visceral weight (*VW*), and to the nearest 0.1 g in gonad weight (*GW*) and liver weight (*LW*). The gonads were preserved in Bouin's solution for histological observations or 10% formalin for measurement of oocytes.

Specimens were sexed by examination of olfactory organs. Caruso (1975) has reported that the olfactory organs of male Lophiidae fishes, including *L. setigerus*, are larger in size than those of similar sized females. My preliminary examination showed that this size difference occurred in sexually immature (100-150 mm *TL*) as well as sexually mature individuals (*TL* ≥ 300 mm). Males had organs 5-7 times larger than those of similar sized females.

For histological preparation, gonads were embedded in paraffin, celloidin or methacrylate polymer resin (Technovit, Kulzer). Paraffin sections of 5-10 μm were stained with Mayer's haematoxylin- eosin (H&E), and with periodic acid-Schiff (PAS) for identification of yolk vesicles. Celloidin sections of 12-15 μm were stained with H&E. Methacrylate polymer resin sections of 2-3 μm were stained with H&E or with a 1% solution of toluidine blue. For identification of oil droplets, ovarian fragments were embedded in gelatin, and sections of 20-25 μm were stained with sudan III. Developmental stages of oocytes were categorized according to Yamamoto (1956)

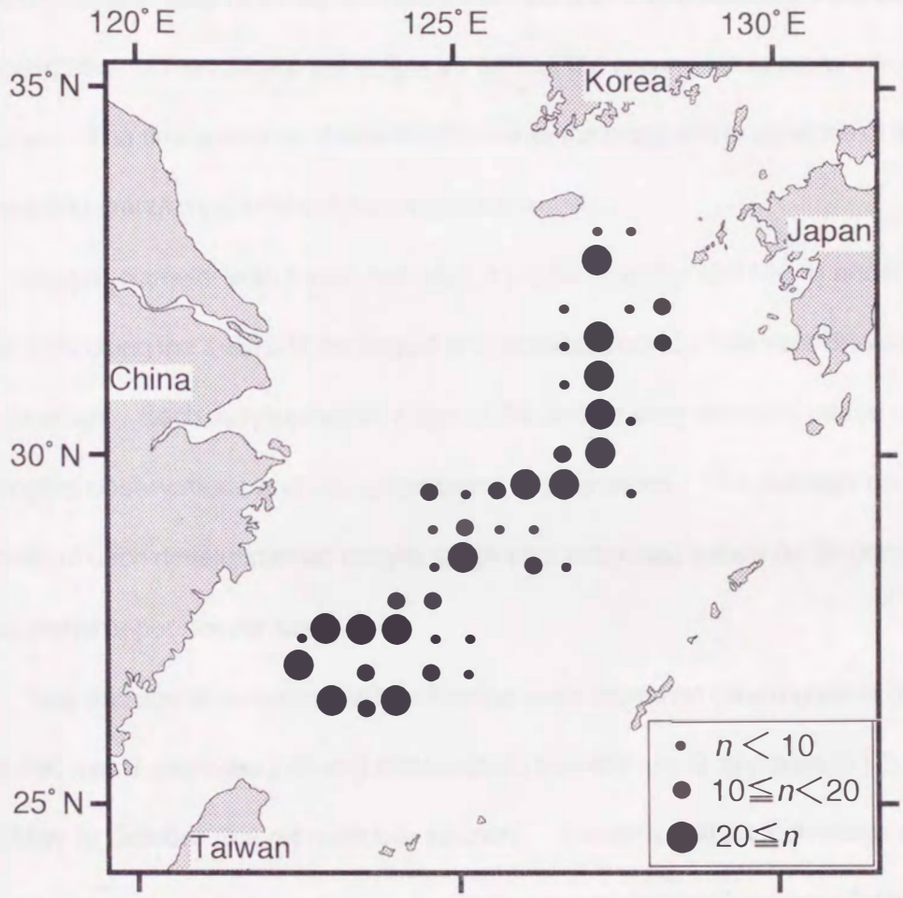


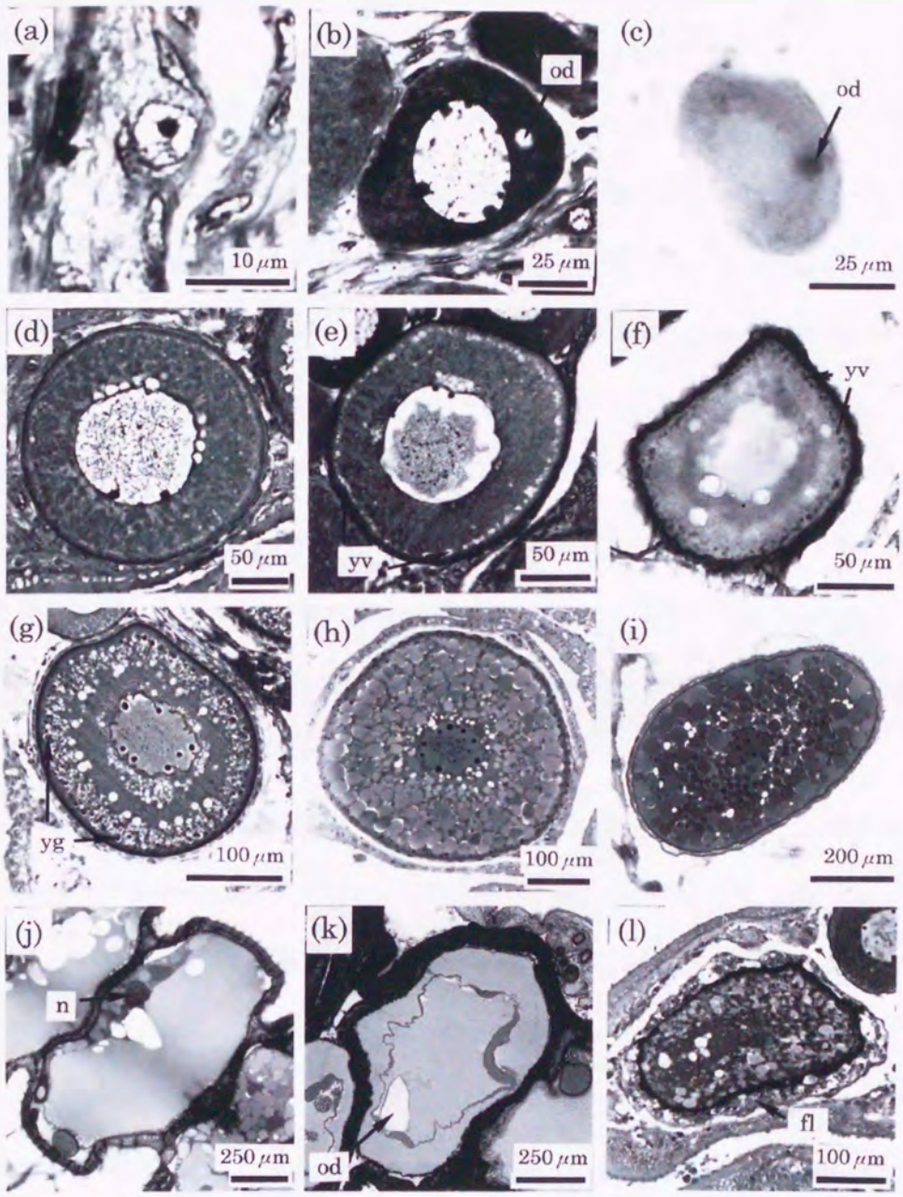
Fig. 10. Geographical distribution of specimens of *L. setigerus* collected in the East China Sea. *n*, number of fish examined.

(Fig. 11). Histological classification of atretic oocytes and postovulatory follicles followed Hunter and Macewicz (1985). The atretic stages of the oocytes were classified into early [Fig. 11(I)] (corresponding to alpha (α) stage (Hunter and Macewicz, 1985)) or late stage (corresponding to beta (β)or subsequent atretic stages) characterized by the degree of disorganization of the follicular cell layers as well as the occurrence of many intercellular vacuoles. The final criteria for characterization was the presence or absence of flocculent material and granular pigments in the ovigerous lamella.

Oocyte diameter was measured using a profile projector (20-100 ×) and the range determined using the means of the largest and smallest oocytes from each developmental oocyte stage. Each developmental stage of the oocyte was identified based on histological observations and microprojectional appearances. The average oocyte diameter at each developmental oocyte stage was examined based on 50 oocyte measurements per oocyte stage.

Size and age at sexual maturity estimates were based on examination of 234 males (104-350 mm *TL* and ages 2-9) and 406 females (104-428 mm *TL* and ages 2-12) collected from May to October (the reproductive season). Sexually mature individuals were defined as those with testis in the late spermatogenesis and mature stages for males and in the mid-developmental (secondary yolk stage of oocyte) on more advanced stages of the ovary for females (see Results). Ages of individual fish were determined based on counts of annual ring marks on the surface of the vertebral centrum (see Chapter I). To estimate the mean total length (L_{50}) and age at sexual maturity for males and females, the fraction of mature fish per 10 mm length or age interval was fit to a logistic function by using the Marquardt method (Draper and Smith, 1966).

Fig. 11. Photomicrographs of oocytes at different developmental stages in *L. setigerus*. (a) Chromatin nucleus stage (less than 20 μm in diameter). (b) Early perinucleolus stage (15-100 μm), multiple nucleoli are seen toward the periphery of the nucleus, and an oil droplet is located near the nucleus. (c) same as (b) but stained with sudan III and haematoxylin. (d) Late perinucleolus stage (65-190 μm), oil droplets around the nucleus have increased in number and follicle cells surrounding the oocyte have formed a narrow layer. (e) Yolk vesicle stage (140-220 μm), positively small vesicles, indicating yolk vesicles, appear in the peripheral region of the cytoplasm. (f) same as (e) except stained with PAS. (g) Primary yolk stage (170-280 μm), yolk globules appear and increase in number between the yolk vesicles, both granulosa and thecal cell layers are clearly observed. (h) Secondary yolk stage (250-470 μm), oocytes are larger and yolk globules occupy the cytoplasm. (i) Tertiary yolk stage (350-760 μm), yolk accumulation progressed rapidly, which resulted in a marked increase in the size of oocytes. (j) Migratory nucleus stage (680-970 μm), yolk globules began to fuse with one another, oil droplets fuse to form larger ones. (k) Mature stage (830-1100 μm), after germinal vesicle breakdown, yolk globules form a single mass and oil droplets coalesce to form larger ones. (l) Atretic stage, characterized by the disintegration of the nucleus and yolk globules, and by the hypertrophy of the follicle layer. od, oil droplet; yv, yolk vesicle; yg, yolk globule; n, nucleus; fl, follicle layer.


(a)
10 μm
(b)
od
25 μm
(c)
od
25 μm
(d)
50 μm
(e)
yv
50 μm
(f)
yv
50 μm
(g)
yg
100 μm
(h)
100 μm
(i)
200 μm
(j)
n
250 μm
(k)
od
250 μm
(l)
fl
100 μm

The gonadosomatic index (*GSI*) and hepatosomatic index (*HSI*) were calculated as followed:

$$GSI = (GW / (BW - VW)) \times 100$$

$$HSI = (LW / (BW - VW)) \times 100.$$

All mature specimens ($TL \geq L_{50}$) of each sex were used for the examination of monthly changes in *GSI* and *HSI*. The Mann-Whitney U-test or one way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test was performed to test for significant deferences between groups of fish in their *GSI* and *HSI* values.

Ovarian stage was defined by the developmental stage of the most advanced oocyte within an ovary. Fifty to eighty random ovigerous lamellae samples from each ovarian stage were examined to determine the oocyte stage composition within each ovarian stage. Oocytes less advanced than the yolk vesicle stage oocytes [Fig. 11(e)] were excluded from these calculations.

Batch fecundity for each female was calculated as the product of the number of secondary yolk stage oocytes per unit weight (of each of the six sampling sites) times the total ovarian weight. Linear regression analysis was used to examine the relationship between the natural logarithms of batch fecundity and fish total length (mm).

The morphology of the ovaries of anglerfish was flattened with the marginal portion coiled (see Results). In order to determine whether secondary yolk stage oocytes were randomly distributed throughout the ovary, the density (no. oocytes/ g ovary wt.) of secondary yolk stage oocytes from 18 ovary locations within the ovaries of five fish was compared. Sampling locations within each ovarian lobe (left and right) were defined in terms of longitude (anterior, middle, posterior) and cross-section (inside and outside of lobe

near the marginal portion, center). A two-way ANOVA was performed to test for the effects on oocyte density of sample location within each ovary as well as between samples from ovaries of individual fish.

Results

Structure of the testis and spermatogenesis

The pairs of testicular lobes were located in the posterior portion of the abdominal cavity and were suspended by the mesorchium. The longitudinal main sperm ducts, covered with layers of thick connective tissue, were located beneath the testicular groove (hilus) of each testis [Fig. 12(a)]. These ducts fused near the posterior end of each testicular lobe to form a common sperm duct which led to a genital pore. Seminal lobules radiated toward and terminated blindly at the testicular periphery of the main sperm duct. Spermatogonia with a prominent nucleolus were distributed randomly along the seminal lobules. Spermatocytes were oval or spherical and had nuclei with abundant, irregularly condensed chromatin. Each germinal cyst containing spermatogonia or developing spermatocytes was arranged in a single layer on the wall of the seminal lobules [Fig. 12(b)]. In the testis, spermatids were released from the germinal cysts into the lumina of the seminal lobules, where each was transformed into spermatozoa. The spermatids at an early stage had large round nuclei which later became indented and condensed. The late-stage spermatids became roundish with condensed nuclei, considerable cytoplasm and apparent vacant areas. Both spermatids and spermatozoa with oval heads existed in the lumina of the seminal lobules and sperm ducts [Fig. 12(c) and (d)].

Structure of the ovary

The right and left ovarian lobes of *L. setigerus* were connected to each other at their posterior ends, forming a single organ. The marginal portion of the ovary was coiled dorsoventrally in the abdominal cavity [Fig. 13(a)]. The ovarian wall consisted of a

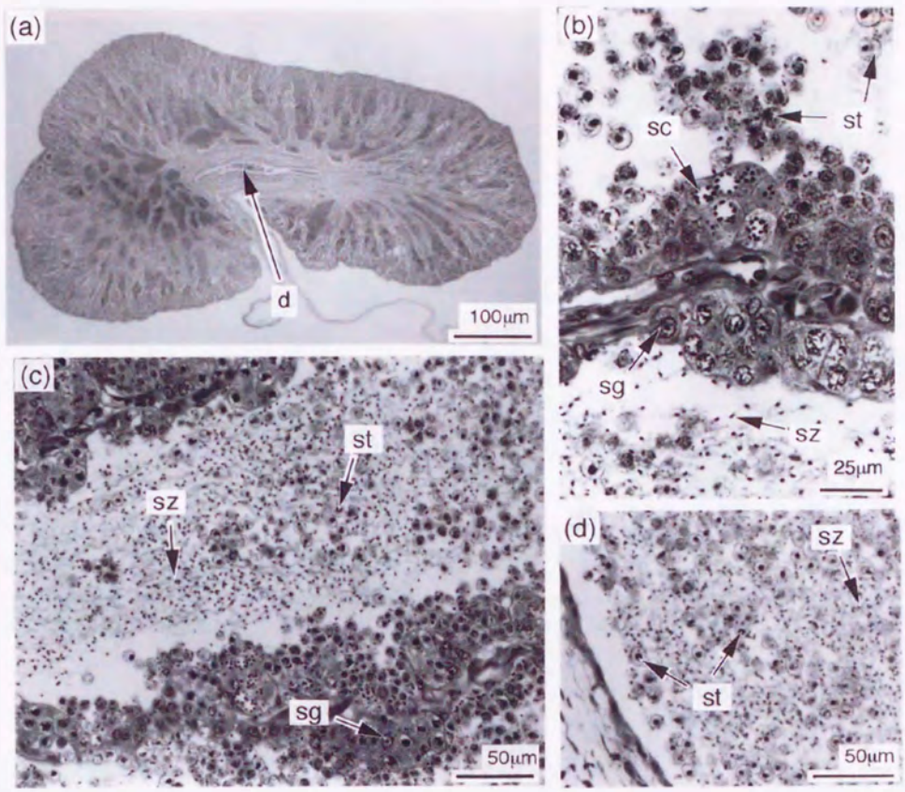


Fig. 12. Photomicrographs of sections of the testis of *L. setigerus*. (a) Transverse section of the testis. (b) and (C) Transverse sections of the seminal lobule during spermatogenesis; (b) shows that spermatids are released into the lumen of the seminal lobule and (c) shows that both spermatids and spermatozoa exist in the lumen of the seminal lobule. (d) Transverse section of the main sperm duct during spermatogenesis; both spermatids and spermatozoa exist in the main sperm duct. d, main sperm duct; sg, spermatogonia; sc, spermatocyte; st spermatid; sz, spermatozoon.

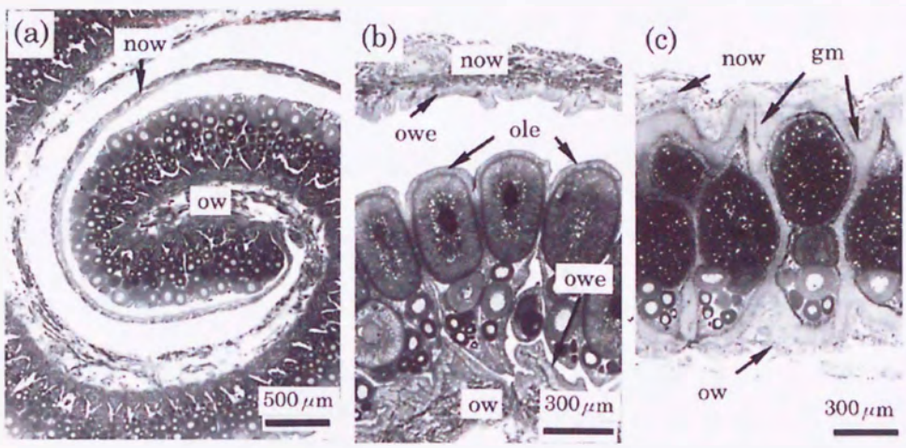


Fig. 13. Photomicrographs of sections of the ovaries of *L. setigerus*. (a) Longitudinal section of the marginal portion of the ovary. (b) Transverse section of the ovary. (c) Transverse section of the ovary during the gelatinous material production. now, nonovigerous ovarian wall; ow, ovarian wall; ole, ovigerous lamella epithelium; owe, ovarian wall epithelium; gm, gelatinous material.

squamous epithelium layer, a connective tissue layer, a smooth muscle layer and a single layer of epithelium (ovarian wall epithelium) facing the ovarian lumen. Differences in the ovarian wall could be recognized between the ovigerous side and the nonovigerous side [Fig. 13(b) and (c)]. Stalk-like ovigerous lamellae protruded from the ovarian wall and were covered by a single cell layer of epithelium (ovigerous lamella epithelium). Hence, the ovarian lumen was lined with both ovarian wall epithelium and ovigerous lamella epithelium.

The ovigerous lamella contained many oocytes at different stages of development. In a reproductively active ovary, one or two of the most advanced oocytes were located at the terminal portion of each ovigerous lamella. Previtellogenic oocytes were located near the basal portion of the ovigerous lamella throughout the year [Fig. 13(b) and (c)].

Changes in the ovigerous lamellae epithelia and ovarian wall epithelia during ovarian development

During the previtellogenic stage, corresponding to the perinucleolus and yolk vesicle stages, the ovigerous lamella epithelium was squamous in shape, less than 5 μm in thickness, and contained a small nucleus. The ovarian wall epithelium was cuboidal in shape, 7-13 μm in thickness, and its nucleus was located centrally [Fig. 14(a) and (b)].

When the oocytes reached the secondary yolk stage, there was secretion of gelatinous material from the numerous villi that had developed along the apical surface of the epithelia of both the ovigerous lamellae and ovarian wall. The ovigerous lamella epithelium became hypertrophied, reaching 5-20 μm in thickness, and had a large centrally located nucleus. The ovarian wall epithelium was columnar, 30-50 μm in

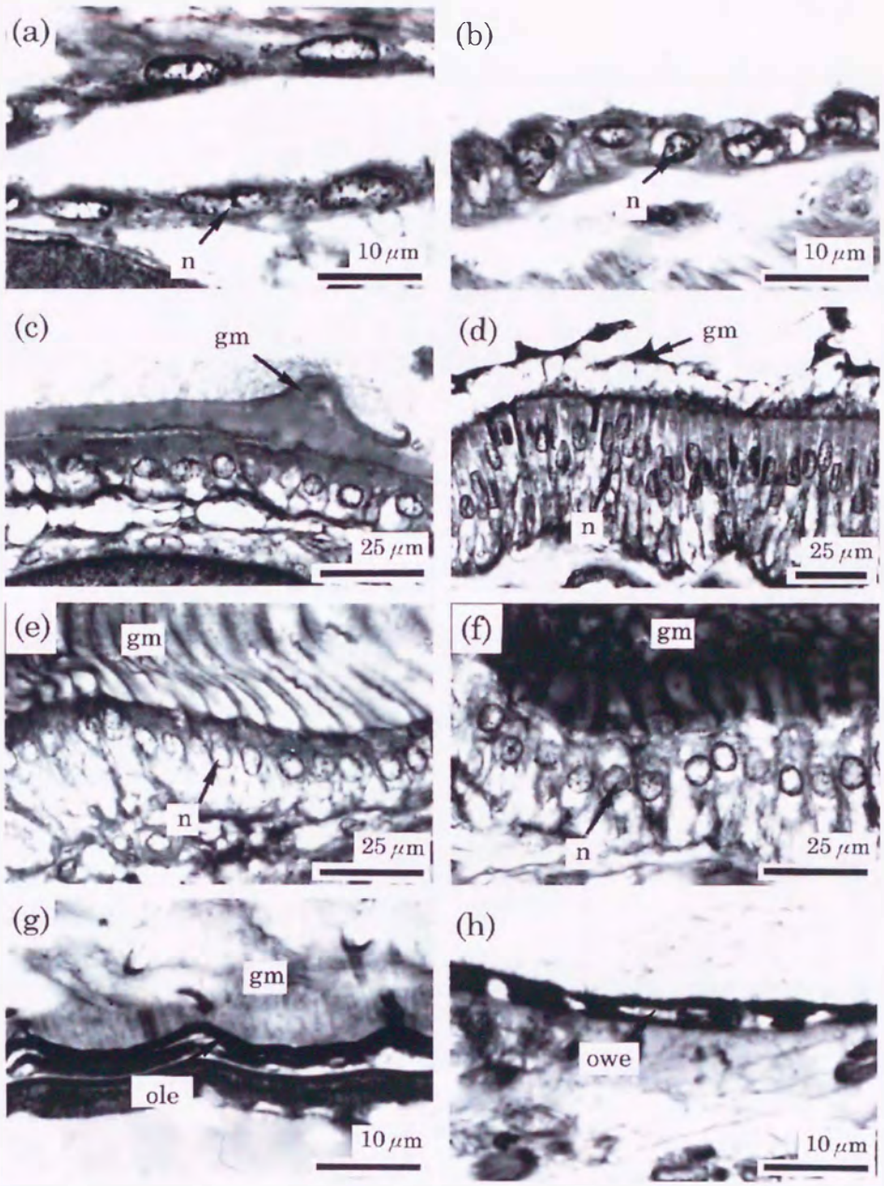


Fig. 14. Photomicrographs of the ovigerous lamella epithelium and ovarian wall epithelium at various ovarian phases of maturation in *L. setigerus*. (a) Ovigerous lamella epithelium at the previtellogenic stage. (b) Ovarian wall epithelium at the previtellogenic stage. (c) Ovigerous lamella epithelium at the secondary yolk stage. (d) Ovarian wall epithelium at the secondary yolk stage. (e) Ovigerous lamella epithelium at the tertiary yolk stage. (f) Ovarian wall epithelium at the tertiary yolk stage. (g) Ovigerous lamella epithelium (ole) at maturation. (h) Ovarian wall epithelium (owe) at maturation. n, nucleus; gm, gelatinous material.

thickness, with a nucleus located in the apical side of the cell [Fig. 14(c) and (d)]. At the tertiary yolk stage, active secretion of gelatinous material was found at the apical surfaces of the epithelia of both the ovigerous lamellae and ovarian wall. The ovigerous lamella epithelium was columnar, 10-30 μm in thickness, and its nucleus was peripherally located. The ovarian wall epithelium became slightly roundish, 20-40 μm in thickness and the nucleus was located centrally [Fig. 14(e) and (f)].

During the migratory nucleus and mature ovary stages, the epithelia of both the ovigerous lamellae and ovarian wall were reduced in size and became squamous in shape, 1-4 μm in thickness for the ovigerous lamella epithelium and 1-3 μm for the ovarian wall epithelium. Secretion of gelatinous material was reduced and finally stopped at ovulation [Fig. 14(g) and (h)].

Degeneration of postovulatory follicles

Postovulatory follicles were located between the terminal and central portion of the ovigerous lamella. The degeneration of postovulatory follicles can be divided into three stages based on their histological characteristics.

Stage I [Fig. 15(a), (b) and (c)]. Newly-formed postovulatory follicles showed a very convoluted shape with many folds, and contained a very small follicular lumen. The granulosa cells were columnar or cuboidal and were arranged in an orderly manner along the thecal cells with blood capillaries. The nuclei were located in the basal portion of the granulosa cells. The postovulatory follicles at this stage were mainly found during or just after ovulation.

Stage II [Fig. 15(d), (e) and (f)]. The postovulatory follicles were greatly reduced

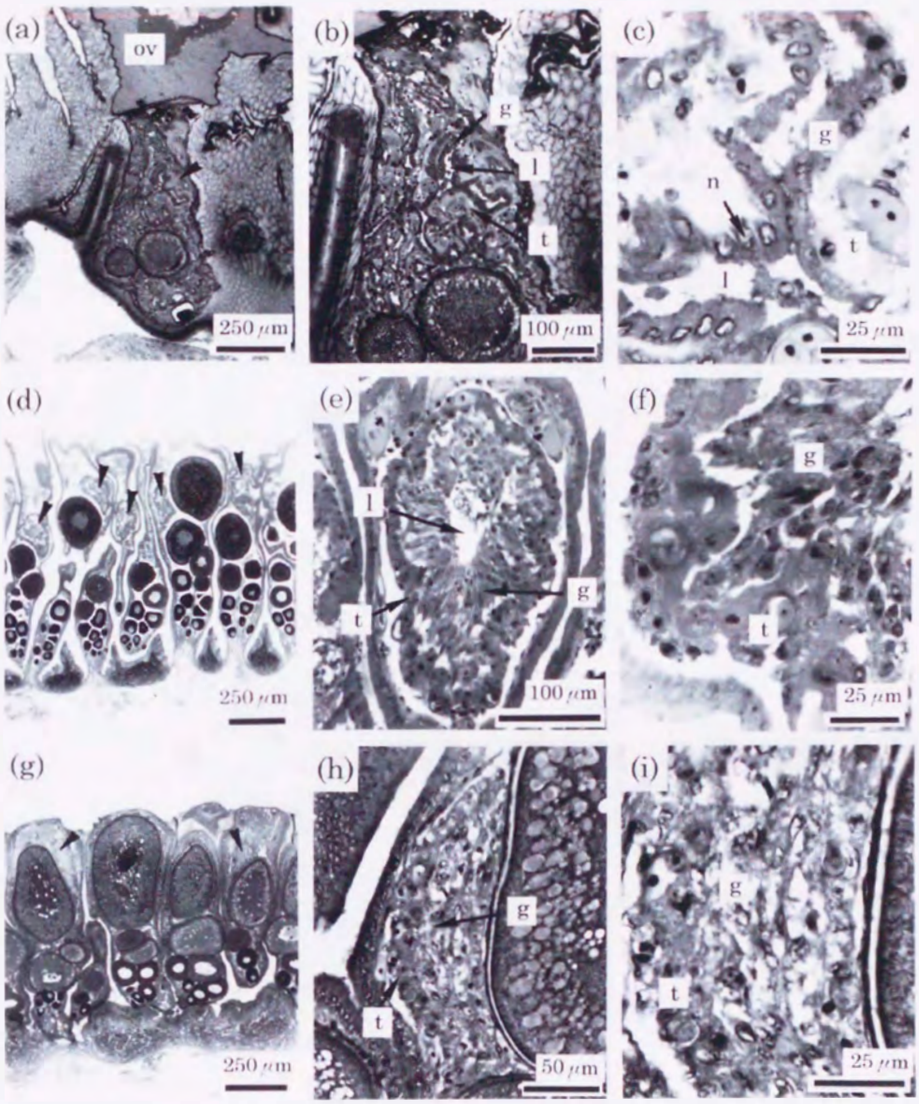


Fig. 15. Photomicrographs of degenerative stages of the postovulatory follicles of *L. setigerus*. Arrowheads in left panels indicate the postovulatory follicles shown higher magnifications in middle and right panels. (a), (b) and (c) Stage I. (d), (e) and (f) Stage II. (g), (h) and (i) Stage III. ov, ovulated oocyte; g, granulosa cell layer; t, thecal cell layer; l, follicular lumen; n, nucleus.

in size and had fewer folds. The follicular lumen was still observed but narrowed, and became less distinct with time. The single layer of hypertrophied thecal cells contained some vacuoles while the granulosa cells had irregular shapes and pycnotic nuclei. The granulosa cell layer had collapsed into the follicular lumen.

Stage III [Fig. 15(g), (h) and (i)]. The postovulatory follicles were much smaller in size than in the previous stage and the follicular lumen continued to decline in size or were absent. The granulosa cell layer was indistinct and the thecal cell layer was much regressed.

Mature stages of testes

Immature stage [Fig. 16(a)and (b)]. Germinal cysts containing spermatogonia and spermatocytes were observed along the wall of the seminal lobules, and spermatids were present in the lumina of the seminal tubules. Spermatids and a few spermatozoa were found in the small main sperm duct. All specimens having the testis at this stage were ≤ 120 mm *TL*.

Early spermatogenesis stage [Fig. 16(c)]. The testis increased in size compared with the previous stage. Germ cells at all stages of spermatogenesis were present. An increase in the number of spermatocytes and spermatids were observed. In the lumina of the seminal lobules and main sperm duct, spermatids were mixed with relatively few spermatozoa.

Late spermatogenesis stage [Fig. 16(d)]. Active spermatogenesis occurred in the testis. Spermatids and spermatozoa were more abundant in the lumina of the seminal lobules and main sperm duct than in the previous stage.

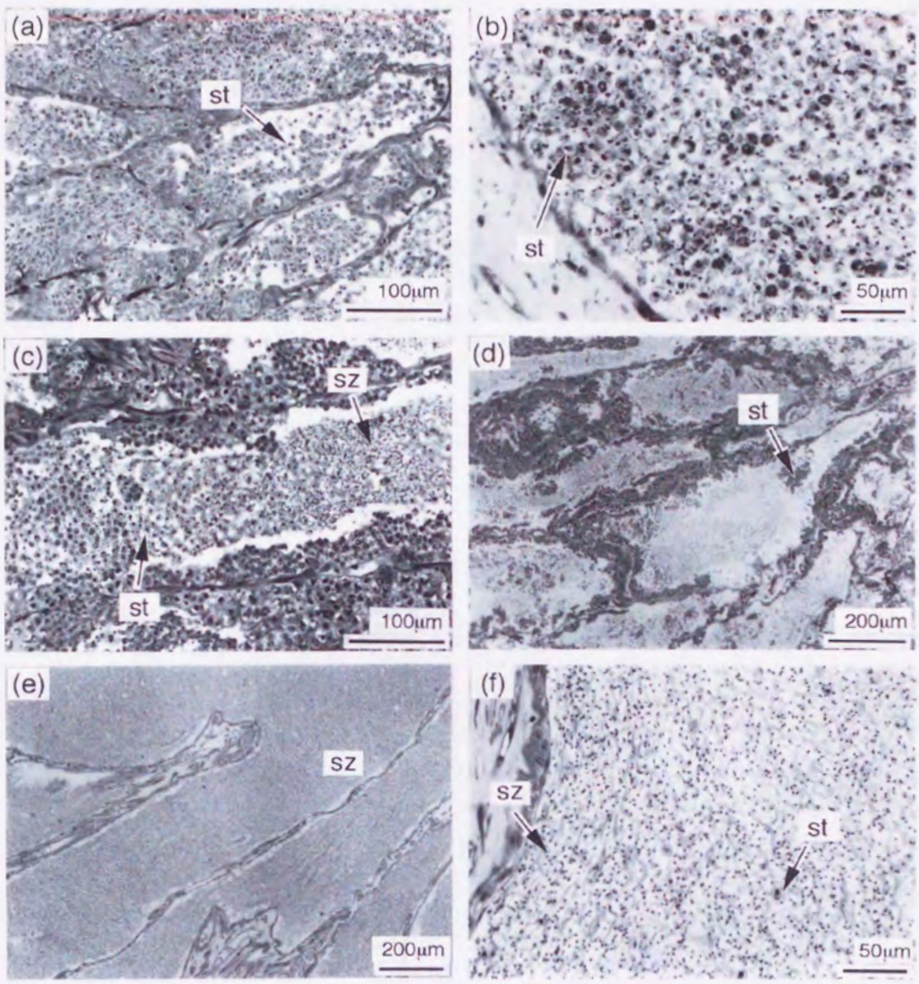


Fig. 16. Photomicrographs of testis at different maturity stages in *L. setigerus*. (a) and (b) Immature stage, (b) shows the main sperm duct. (c) Early spermatogenesis stage. (d) Late spermatogenesis stage. (e) and (f) Mature stage, (f) shows the main sperm duct. st, spermatid; sz, spermatozoon.

Mature stage [Fig. 16(e)and (f)]. Large quantities of spermatozoa and a few spermatids were present in the lumina of the seminal lobules and main sperm duct. In the seminal lobules, spermatogonial divisions and further spermatogenesis occurred, though germinal cysts containing spermatogonia and spermatocytes were few or absent found around the main sperm duct.

Mature stages of ovaries

Immature stage [Fig. 17(a)]. Only previtellogenic oocytes were present and the epithelia of both the ovigerous lamellae and ovarian wall were thin.

Developing stage [Fig. 17(b)]. Most advanced oocytes had reached the primary to tertiary yolk stages. At the tertiary yolk stage gelatinous material occupied the ovarian lumen.

Mature stage [Fig. 17(c)]. The most advanced oocytes were classified as in the migratory nucleus or mature stages. Just before spawning the ovulated oocytes were found in the gelatinous material forming within the ovarian lumen.

Spawning stage [Fig. 17(d)]. Vitellogenic oocytes (mainly in the primary and secondary yolk stages of the oocyte) and postovulatory follicles were present.

Spent stage [Fig. 17(e)]. Vitellogenic oocytes were degenerating (early atretic stage) and the postovulatory follicles were often observed.

Resting stage [Fig. 17(f)]. Late atretic stage oocytes and previtellogenic oocytes were present and the epithelia of both the ovigerous lamellae and ovarian wall were thin.

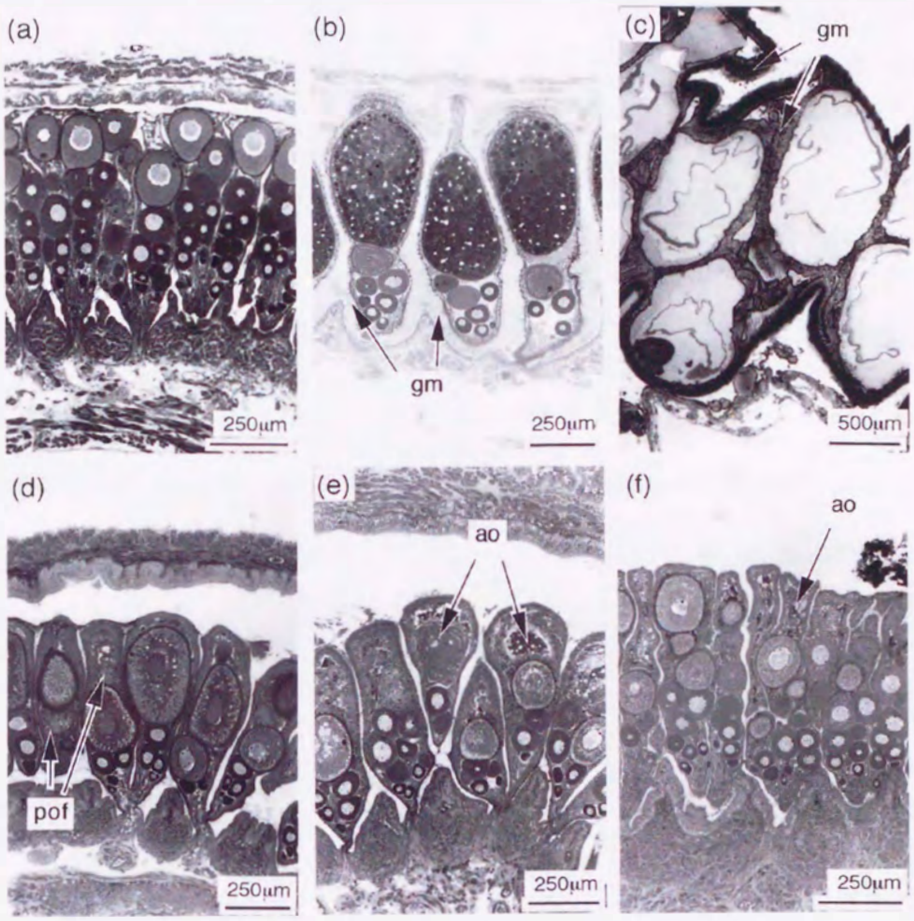


Fig. 17. Photomicrographs of ovaries at different maturity stages in *L. setigerus*. (a) Immature stage. (b) Developing stage. (c) Mature stage. (d) Spawning stage. (e) Spent stage. (f) Resting stage. gm, gelatinous material; pof, postovulatory follicle; ao, atretic oocyte.

Annual reproductive cycle

Males in a mature condition were found over a longer period of the year than were females (Fig. 18). Spermatogenesis occurred and males with mature testis were collected throughout the year. Vitellogenesis started in January and continued until November. Females at the mature and spawning stages were collected during the period from May to November. Females having spent ovaries occurred between August and December and these having resting ovaries from December through April.

Occurrence of females with postovulatory follicles

The ovarian stage of 65 females having both postovulatory follicles and developing vitellogenic oocytes was examined (Table 5). Stage I postovulatory follicles only occurred in the primary and secondary yolk stage ovaries. Stage II and III postovulatory follicles occurred in all yolk stage ovaries, but mainly in the secondary yolk stage ovaries. The ovaries at the migratory nucleus and mature stages had none of the stages of postovulatory follicles.

Lunar spawning cycle

The *GSI* values of mature males and females collected throughout the lunar cycle and during the spawning season (May-November) are shown in Fig. 19. The variability in index values for males during the late spermatogenesis and mature stages of the testis did not appear to change throughout the lunar cycle. Females having low *GSI* values (< 25), corresponding to the developing or spawning stage of the ovary, were collected throughout the lunar cycle. Females with the highest indices (> 50), which were in the

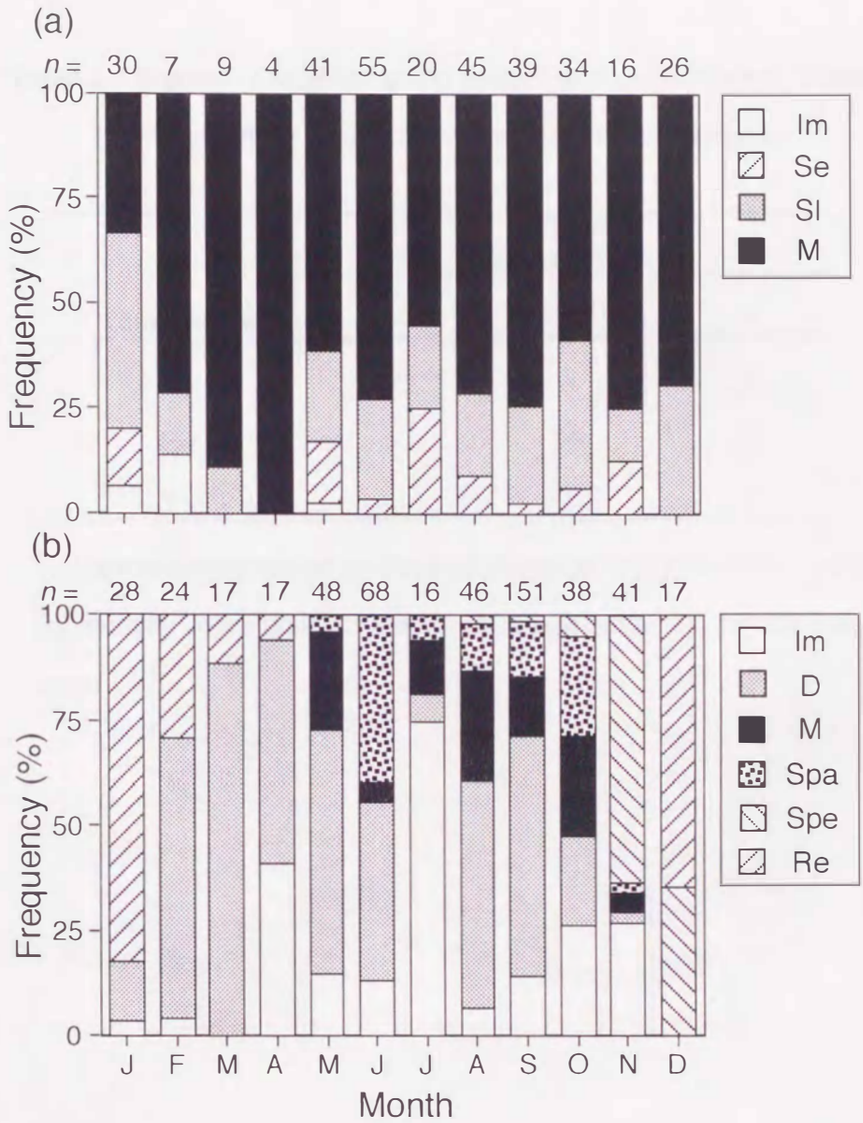


Fig. 18. Monthly changes in the frequency of occurrence of the various maturity stages of gonads for male (a) and female (b) *L. setigerus* in the East China Sea. *n*, number of fish examined; Im, immature stage; Se, early spermatogenesis stage; Sl, late spermatogenesis stage; M, mature stage; D, developing stage; Spa, spawning stage; Spe, spent stage; Re, resting stage.

Table 5. Number of females having ovaries with postovulatory follicles (POF) at various stages of ovarian maturity in *L. setigerus*

	Stage of POF		
Ovarian stage *	I	II	III
Py	2	4	3
Sy	2	28	21
Ty		1	4

* Ovarian stage based on the most advanced oocytes in the ovary. Py, primary yolk stage; Sy, secondary yolk stage; Ty, tertiary yolk stage.

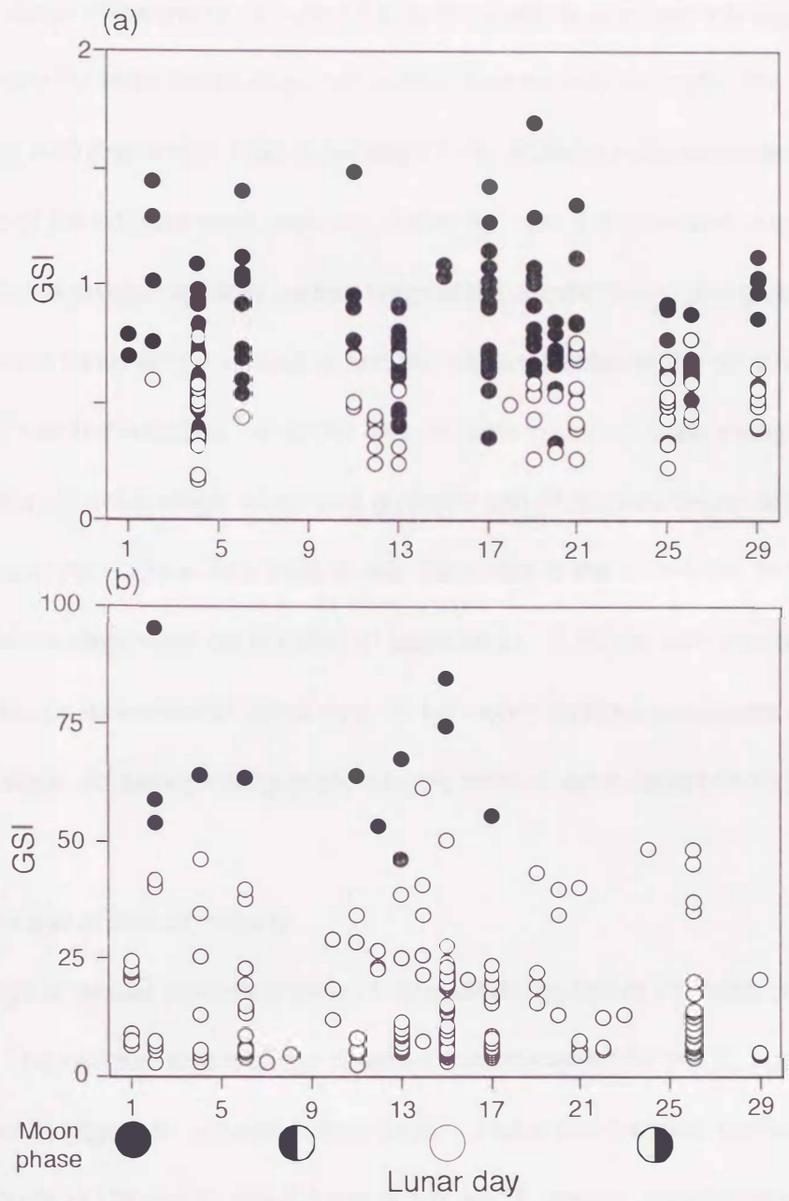


Fig. 19. Gonadsomatic index (*GSI*) of mature males (a, $n = 224$) and females (b, $n = 191$) *L. setigerus* by lunar day during the spawning season (May-November). Closed circles represent indices for mature males and for late mature stage (mature stage of oocyte) females. Open circles represent indices for the late spermatogenesis stage males and the developing, early mature (migratory nucleus stage of oocyte) and spawning stages of females.

late mature stage of the ovary, occurred during two periods: one from 1-6 days after the new moon and the other from 5 days before the full moon to 2 days after the full moon.

During 1-10 September 1995 (lunar days 7-16), females in the secondary to tertiary yolk stages of the oocytes were captured (Table 6). On 4 September, a female in the early mature stage (late migratory nucleus stage of the oocyte having yolk globules and oil droplets almost fused with one another and the nucleus located at the periphery of the cytoplasm) was first collected. After this date, females in various mature stages (from the early migratory nucleus stage, when yolk globules and oil droplets began to fuse with each other and the nucleus was located near the center of the cytoplasm, to the mature stage of the oocytes) were caught until 10 September. A female with ovulated oocytes was collected on 9 September (lunar day 15, full moon) and two specimens at the spawning stage, containing newly postovulatory follicles, were caught on 10 September.

Size and age at sexual maturity

Size and age at sexual maturity showed a clear difference between males and females (Fig. 20). The minimum size and age at sexual maturity were 159 mm *TL*, age 3 for males and 268 mm *TL*, age 5 for females, respectively. Males and females reached mean sexual maturity at 178 mm *TL*, age 3.3 and at 303 mm *TL*, age 6.1, respectively. All males ≥ 230 mm *TL* and age 6 and females ≥ 350 mm *TL* and age 9 were mature.

Monthly changes in GSI and HSI

The mean *HSI* for males was highly variable throughout the year, but exhibited two peaks, one in April and the other in September [Fig. 21(a)]. The mean *GSI* for males

Table 6. Occurrence of females *L. setigerus* at the developing, mature and spawning stages of the ovary collected during the period of 1-10 September 1995

Sampling date in period	1-3	4	5	6	7	8	9	10
Lunar day	7-9	10	11	12	13	14	15 *	16
Most advanced oocyte								
Sy or Ty	4	7	14	10	5	11	14	7
Mn (e)			1		1	5		
Mn (l)		1	1					
Ma			1	1	1			1
Ma (ov)							1	
Py (n - POF)								2

Sy, secondary yolk stage; Ty, tertiary yolk stage; Mn (e), early migratory nucleus stage; Mn (l), late migratory nucleus stage; Ma, mature stage; ov, ovulation; Py, primary yolk stage; n-POF, newly postovulatory follicles.

*, full moon.

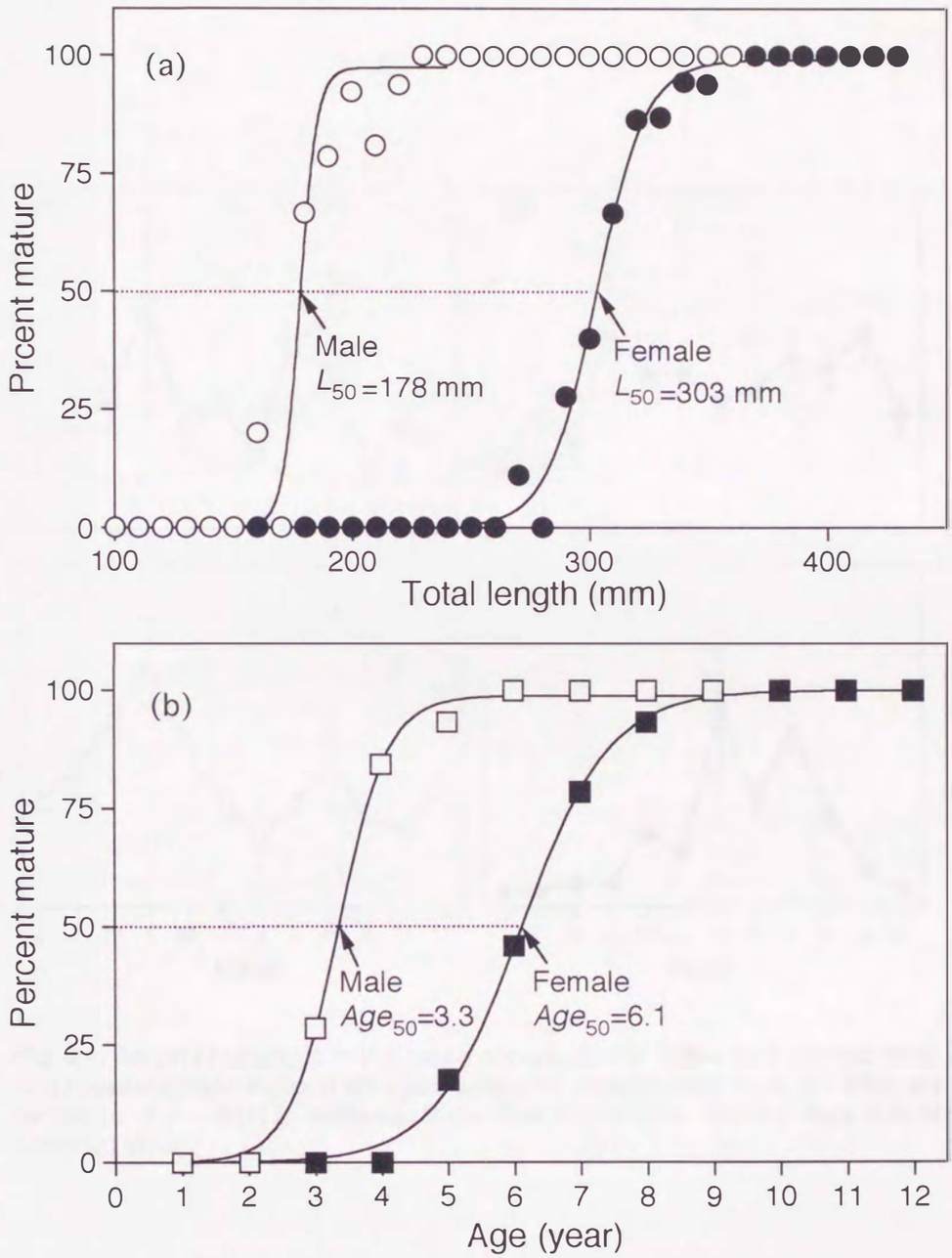


Fig. 20. Percent mature by 10 mm length intervals (a) and age (b) fitted to a logistic function of male (open circle, $n = 234$) and female (closed circle, $n = 364$) *L. setigerus* collected from May through October. Arrows indicate the mean total length (L_{50}) and age (Age_{50}) at sexual maturity.

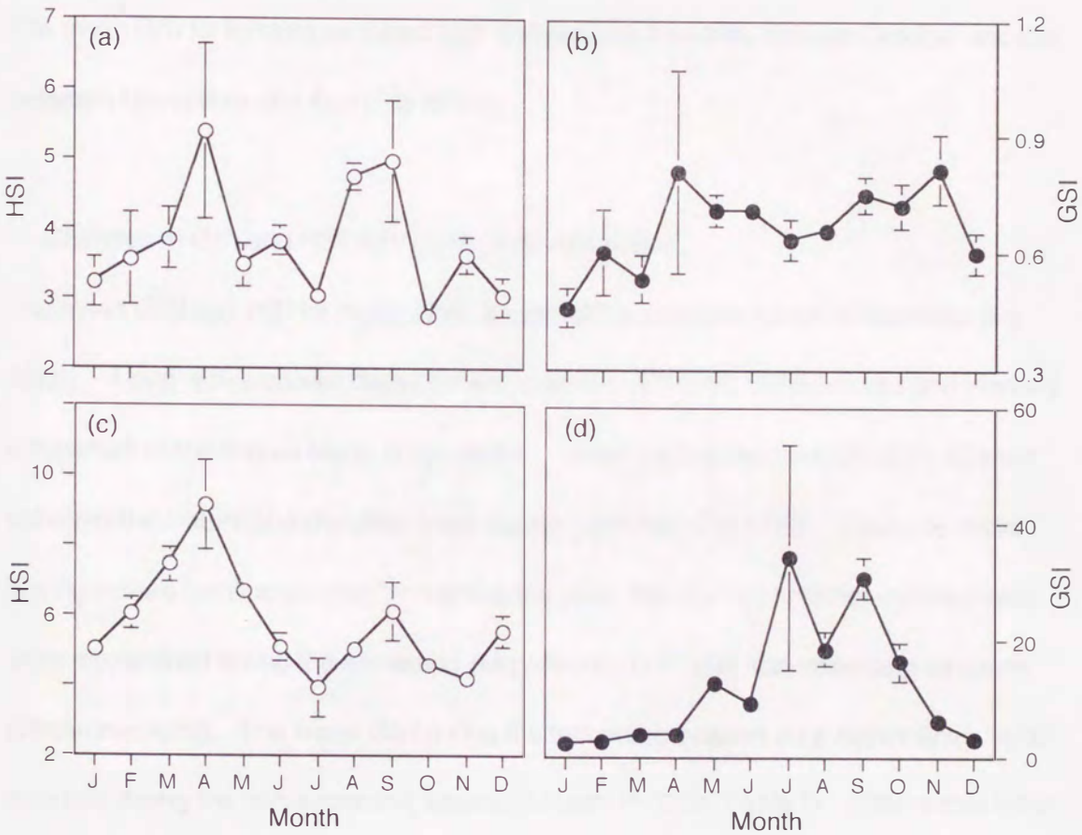


Fig. 21. Monthly changes in the mean gonadsomatic index (*GSI*, closed circle) and hepatosomatic index (*HSI*, open circle) for mature male (a, b, $n = 250$) and female (c, d, $n = 401$) *L. setigerus* in the East China Sea. Vertical lines indicate standard error.

increased in April and remained relatively high until November and they decreased gradually in December [Fig. 21(b)]. The mean *HSI* for females increased from December and peaked in April, just prior to the spawning season [Fig. 21(c)]. After April, the mean *HSI* decreased and remained low throughout the May to November spawning season. The mean *GSI* for females remained high and variable from May through October and low between November and April [Fig. 21(d)].

Changes in GSI and HSI with gonadal development

The mean *GSI* and *HSI* for males were lowest at the immature stage of the testis [Fig. 22(a)]. Along with testicular development, both the *GSI* and *HSI* increased and reached a maximum at the mature stage of the testis. These indices were significantly different between the mature and the other three stages (ANOVA, $P < 0.05$). Because males having mature testis appeared throughout the year, the *GSI* and *HSI* for mature males were reexamined during the spawning (May-November) and non-spawning seasons (December-April). The mean *GSI* during the spawning season was significantly higher than that during the non-spawning season (U-test, $P < 0.01$; Table 7). Differences in the mean *HSI* during the spawning and non-spawning seasons were insignificant ($P > 0.05$).

The mean *GSI* and *HSI* for females were also lowest at the immature stage of the ovary [Fig. 22(b)]. The mean *HSI* for females increased rapidly until it reached a maximum at the mid-developmental stage of the ovary and then decreased until it reached a minimum at the late mature stage (mature stage of oocyte) of the ovary. The mean *HSI* during the developing stage of the ovary was significantly higher than at any other ovarian stage (ANOVA, $P < 0.001$). The mean *GSI* for females gradually

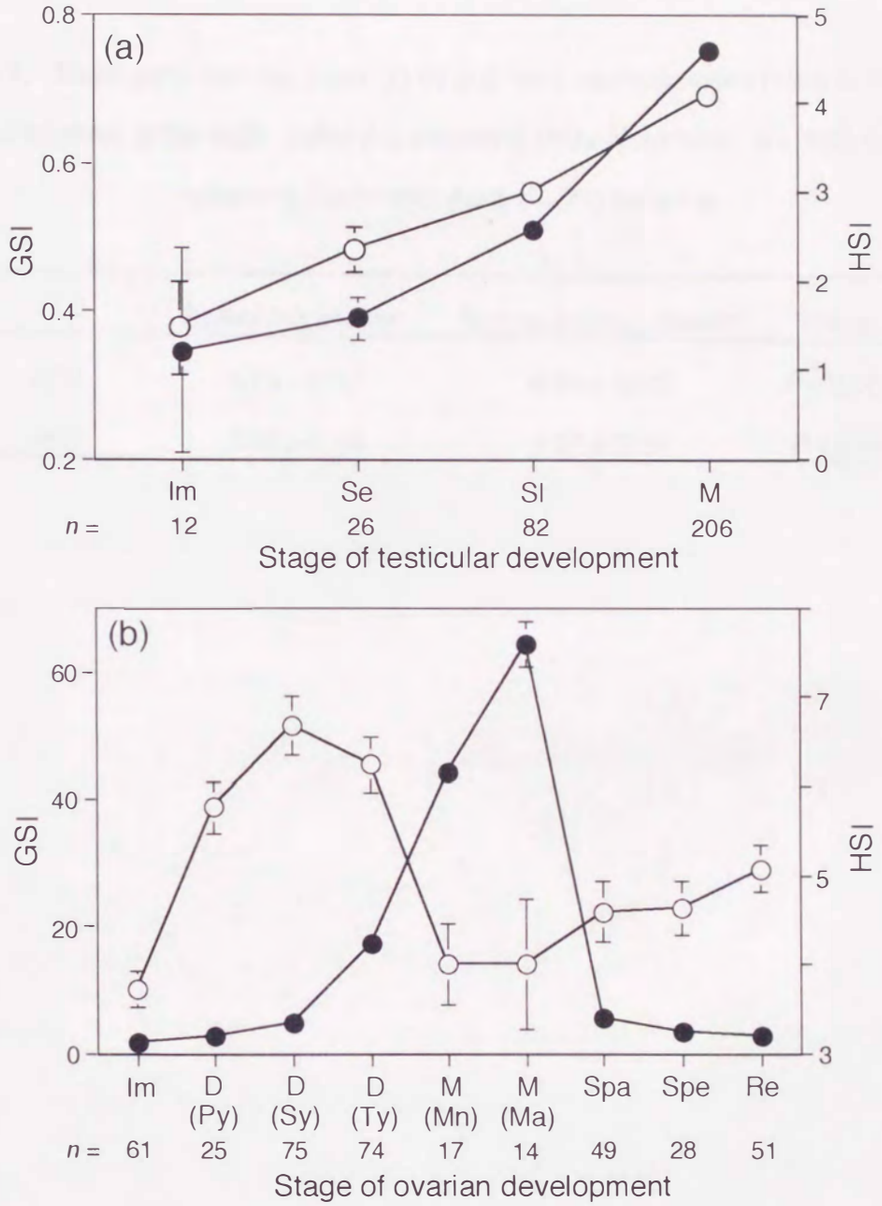


Fig. 22. Mean gonadsomatic index (*GSI*, closed circle) and hepatosomatic index (*HSI*, open circle) at each stage of maturity for male (a) and female (b) *L. setigerus*. Vertical bars indicate standard error. *n*, number of fish examined; Im, immature stage; Se, early spermatogenesis stage; SI, late spermatogenesis stage; M, mature stage; D, developing stage; Py, primary yolk stage of oocyte; Sy, secondary yolk stage of oocyte; Ty, tertiary yolk stage of oocyte; Mn, migratory nucleus stage of oocyte; Ma, mature stage of oocyte; Spa, spawning stage; Spe, spent stage; Re, resting stage.

Table 7. Mean gonadsomatic index (*GSI*) and hepatosomatic index (*HSI*) (± S. E.) at the mature stage of the testis during the spawning (May-November, $n = 135$) and non-spawning (December-April, $n = 71$) seasons

	Spawning season	Non-spawning season	U-test
GSI	0.79 ± 0.02	0.65 ± 0.03	$P < 0.001$
HSI	3.92 ± 0.13	4.37 ± 0.24	$P > 0.05$

increased until the mid-developmental stage of the ovary and then increased markedly and peaked at the late mature stage of the ovary. The mean *GSI* was significantly higher at the mature stage of the ovary than at all other ovarian stages ($P < 0.001$). The mean *GSI* at the spawning, spent and resting stages of the ovary were not found to be statistically different ($P > 0.05$).

Oocyte composition during ovarian development

A total of 34 ovaries with no postovulatory follicles were used in this examination (Fig. 23). Oocytes at the yolk vesicle stage occurred in the ovigerous lamellae at all times. When a group of oocytes attained the secondary yolk stage, they formed an advanced batch which separated from the adjacent group of smaller oocytes. Between the secondary yolk and mature ovary stages, the smaller oocyte group stayed at the primary yolk stage.

Batch fecundity

Batch fecundity was estimated from 20 specimens collected from May to August 1993. The relationship between batch fecundity(*BF*) and total length (*TL*, mm) was described by the equation: $BF = 556.2 \times TL^{1.157}$ ($300 \leq TL \leq 396$; $r^2 = 0.22$; $P < 0.05$; Fig. 24). Batch fecundity ranged from 330×10^3 eggs in a fish 350 mm *TL*, to 610×10^3 eggs in a fish 380 mm *TL*.

The location of oocytes within the ovaries revealed no significant effect on oocyte density (Table 8). Advanced yolked (secondary yolk stage) oocytes were randomly distributed within the ovary; samples could be taken from any location without bias.

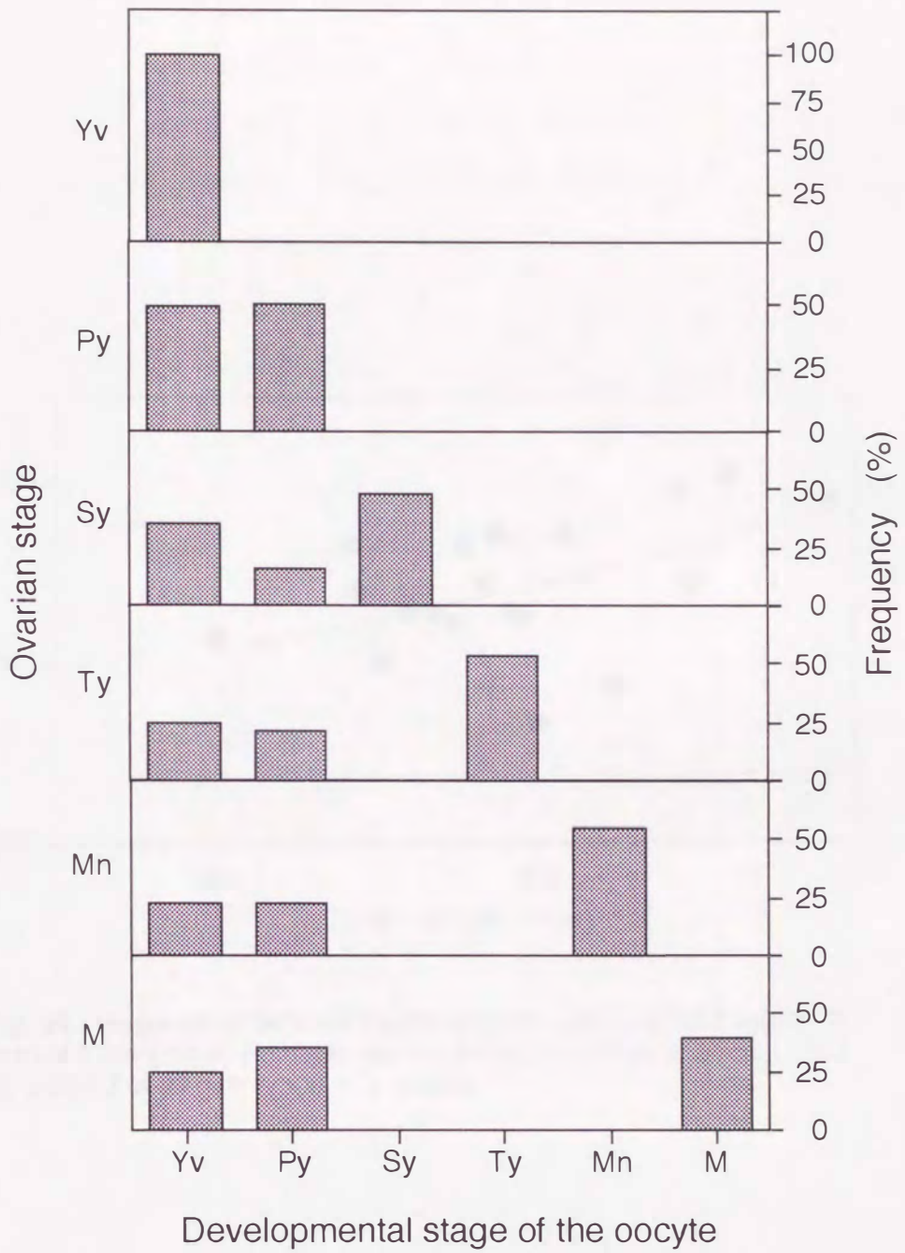


Fig. 23. Percent frequency of occurrence of developmental stages of the oocytes in the ovigerous lamellae at each ovarian stage in *L. setigerus* (Ovarian stages are based on the most advanced oocytes in the ovary). Chromatin nucleus and perinucleolus stage oocytes were not examined. Yv, yolk vesicle stage; Py, primary yolk stage; Sy, secondary yolk stage; Ty, tertiary yolk stage; Mn, migratory nucleus stage; M, mature stage.

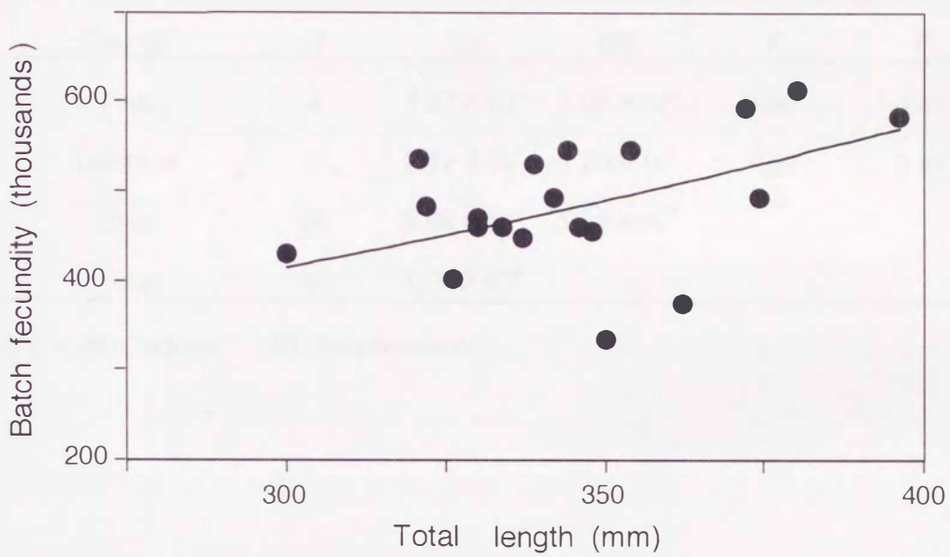


Fig. 24. Relationship between batch fecundity (*BF*) and total length (*TL*, mm) of *L. setigerus*. Females were collected from May to August 1993. $BF = 556.2 \times TL^{1.157}$ ($300 \leq TL \leq 396$).

Table 8. Results of analysis of variance testing for the effect of tissue sampling location within the ovary of *L. setigerus* on secondary yolk stage oocyte density (number of oocytes per unit of sample weight (g))

Two-way analysis of variance

Source	df	SS	MS	*F*	*P*
Fish	4	4.07×10^{7}	1.02×10^{7}	0.86	0.49
Location	17	2.12×10^{8}	1.25×10^{7}	1.05	0.41
Error	68	8.03×10^{8}	1.18×10^{7}		
Total	89	1.06×10^{9}			

SS, sum of square; MS, mean square.

Discussion

The testicular structure of *L. setigerus* was similar to other teleosts in having unrestricted spermatogonial (Grier et al., 1980; Grier, 1981) or lobular type testis (Billard et al., 1982; Billard, 1986). Although the process of spermatogenesis conformed to that of other teleosts, it was not completed within the germinal cysts. Rather, spermatids were released into the lumina of the seminal lobules. This specialized spermatogenesis was also found in *Lepadogaster lepadogaster* (Mattei and Mattei, 1978), *Neoceratidae* fishes (Jespersen, 1984), blenniid fishes (Lahnsteiner and Patzner, 1990a, b; Lahnsteiner et al., 1990) and *Ophidion* sp. (Mattei et al., 1993). In these fishes, various developing germ cells from spermatocytes to spermatids are released from the germinal cysts into lobules or tubules of the lumina and these released germ cells do not have synchronous differentiation. Mattei et al. (1993) concluded that this specific type of spermatogenesis should be termed 'semi-cystic' type and distinguished from the 'cystic' type which applies to most teleosts. Afonso-Dias and Hislop (1996) observed that in mature male *L. piscatorius*, the lumina of the seminiferous tubules are often packed with many spermatozoa and some spermatids. However, these authors as well as Armstrong et al. (1992), reporting on reproduction of male *L. americanus* did not classify spermatogenesis in these species as the 'semi-cystic' type. The present study is the first to do this for Lophiidae.

The ovarian structure of *L. setigerus* was similar to those reported for other Lophiiformes, *L. americanus* (Armstrong et al., 1992), *L. piscatorius* (Fulton, 1898; Afonso-Dias and Hislop, 1996), *Antennarius scaber*, *Histrio histrio* and *Ogcocephalus vespertilio* (Rasquin, 1958): confluence of the two ovarian lobes at their posterior ends,

and stalk-like ovigerous lamellae within which arrangement of oocytes show a gradation in developmental stages. In the case of *H. histrio* (Rasquin, 1958), the ovigerous lamella shows subdivisions branching into the ovarian lumen, which is different from that of *L. setigerus*, as well as other Lophiiformes species. As described earlier, females of the Lophiiformes, except for *A. caudimaculatus* (Pietsch and Grobecker, 1980), spawn gelatinous egg masses (Fulton, 1898; Gill, 1908; Connolly, 1920; Dahlgren, 1928; Berril, 1929; Breder, 1949; Bigelow and Schroeder, 1953; Mosher, 1954; Rasquin, 1958; Ray, 1961; Mito, 1963; Feinberg, 1984; Armstrong et al., 1992; Afonso-Dias and Hislop, 1996; Yoneda et al., 1997a). In the present study, the epithelium of both the ovigerous lamellae and ovarian wall, from which gelatinous material was secreted showed morphological changes associated with the ovarian maturation cycle. This was also the case for *H. histrio* (Rasquin, 1958) and *L. americanus* (Armstrong et al., 1992). In *L. piscatorius* (Fulton, 1898), however, the ovarian wall epithelia on the nonovigerous side of the ovarian wall did not secrete gelatinous material.

Some Scorpaeniformes, such as *Dendrochirus brachypterus* (Fishelson, 1977,1978), *Sebastolobus alascanus* (Erickson and Pikitch, 1993) and *Sebastolobus macrochir* (Koya and Matsubara, 1995; Koya et al., 1995), also spawn floating gelatinous egg masses and have specialized ovarian structures including the stalk-like ovigerous lamellae and the secretory epithelia like Lophiiformes. Ultrastructural and histochemical studies in *S. macrochir* revealed that the inner layer of the gelatinous material was synthesized in the ovigerous lamella epithelium and secreted by exocytosis, while the outer layer of the gelatinous material was synthesized in the ovarian wall epithelium and released as a macroapocrine secretion (Koya and Matsubara, 1995). Further studies are

needed to clarify this process in Lophiiform fish.

Indirect evidence for multiple spawning of individual females of *L. setigerus* was provided by the occurrence of ovaries with postovulatory follicles and developing vitellogenic oocytes between May and November. This suggests that females that had previously spawned at least once that year probably matured and spawned again. The postovulatory follicles continued to disappear from the ovaries until the developing oocytes attained the tertiary yolk stage when a large amount of gelatinous material was secreted from the epithelia of both the ovigerous lamellae and ovarian wall. In general, ovaries of multiple spawners have both postovulatory follicles and vitellogenic oocytes simultaneously, after which the postovulatory follicles gradually disappear as the vitellogenic oocytes develop (Hunter and Goldberg, 1980; Hunter and Macewicz, 1985; Wright, 1992; Murayama et al., 1994; Schaefer, 1996). In *Histrio* and *Antennarius*, females repeatedly release an egg mass at intervals of approximately 3-10 days (Breder, 1949; Mosher, 1954; Rasquin, 1958; Ray, 1961). On the other hand, Afonso-Dias and Hislop (1996) suggest that female *L. piscatorius* seem unlikely to spawn more frequently than once a year; and Feinberg (1984) describes (preliminary published comment) female *L. americanus* spawning a gelatinous ribbon of eggs once a year. My study is the first to report on multiple spawning per season in Lophiidae.

Throughout the sampling year (from May to November), females were collected having ovaries with mature stage of the ovaries - as well as with spawning stage of the ovary. Furthermore, the fact that females with postovulatory follicles and degenerating vitellogenic oocytes (spent stage of the ovary) were collected primarily in November can be used as a key histological marker for the cessation of spawning (Hunter and Macewicz,

1985). The results reported here confirm that the spawning season of *L. setigerus* in the East China Sea extends from May to November. The extended seven month spawning season also suggests multiple spawnings in this species.

The seasonal ovarian cycle of *L. setigerus* is most similar to that of multiple spawning fishes, e.g. *Engraulis mordax* (Hunter and Macewicz, 1985), *Micropogonias undulatus* (Barbieri et al., 1994) and *Cynoscion regalis* (Lowerre-Barbieri et al., 1996). The one exception is that gelatinous material is secreted from the epithelia lining of the ovarian cavity from the latter developing to mature stages of the ovary of *L. setigerus.* Vitellogenesis occurred throughout most of the year and females in the mature and spawning stages appeared in seven months of the year while males with testis full of spermatozoa occurred throughout the year. Yamada (1986) reported that the spawning season of *L. setigerus* occurs from April to May in the East China Sea. Minami (1988) found that the larvae of *L. setigerus* appeared in the East China Sea and the southwest Japan Sea mainly in the autumn. These findings on the timing of the spawning season are not compatible and suggest a restricted spawning period. My result clearly shows that anglerfish have a long spawning season extending from May to November.

The present study indicated that females with developing ovaries or ovaries in the spawning stage were collected throughout the lunar period while females in the late mature stage appeared around the time of the new or full moon. Furthermore, the onset of final maturation was found to coincide with the approach of the full moon. This suggests that female anglerfish have a weak semilunar spawning periodicity. For many fishes with a semilunar reproductive periodicity, it is believed that spring tides provide eggs with protection from predators and maximize their dispersion (Johannes, 1978; Ross, 1983;

Taylor, 1984; Thresher, 1984). In Lophiiformes fishes, the egg veil is considered an excellent device for broadcasting large numbers of eggs over great geographical distances and for providing them with protection from predators (Pietsch and Grobecker, 1980; Armstrong et al., 1992; Afonso-Dias and Hislop, 1996). The spawning of *L. setigerus* around the spring tides may make the egg veil an even more effective advantage.

The present study also revealed that female *L. setigerus* reached mean sexual maturity at a larger size and at an greater age than males. In *L. litulon* (Kosaka, 1966; Yamada, 1986), *L. americanus* (Armstrong et al., 1992) and *L. piscatorius* (Afonso-Dias and Hislop, 1996) females also mature at a larger size and/or a greater age than males. Size at sexual maturity for both sexes of these latter species are much greater than that of *L. setigerus*. These species are also larger reaching maximum lengths of 1 m *TL* or more (Tsimenidis and Ondrias, 1980; Armstrong et al., 1992; Afonso-Dias and Hislop, 1996; Yoneda et al., 1997b), whereas most *L. setigerus* are less than 400 mm *TL* and grow slower (see Chapter I).

The present study indicated that the seasonal cycle of the *GSI* for females was inversely related to that of the *HSI*. There was a significant inverse correlation between the development of the ovary and the reduction in weight of the fish liver (*HSI*). The rapidly rising *GSI* from the middle developing to mature stage of the ovary is due to the accumulation of a large amount of gelatinous material and the increasing oocyte volume. Conversely, the mean *HSI* of females decreased from the middle developing stage of the ovary and reached a minimum at the late mature stage of the ovary. In teleosts, as in most other vertebrates, the site of synthesis of the precursor protein of yolk (vitellogenin)

is the liver, and the secreted vitellogenin is selectively taken up from the bloodstream by developing oocytes (Wallace and Selman, 1981; Nagahama, 1987). This suggests that the rapid accumulation of yolk may be one of the reasons for the decrease in the fish liver-weight (*HSI*). The interaction between gelatinous material and liver is unknown, but it is likely that the liver plays an important role in the synthesis and secretion of gelatinous material. The fact that the average *HSI* of females reaches a maximum in April, just before the spawning season, is due to the high occurrence of females with middle developing stage ovaries, whereas the lower *HSI* of females in the spawning season is caused by repeated spawnings.

The seasonal cycles in the *GSI* and *HSI*, as found in females, did not appear in males. This resulted from the consistently high occurrence of mature testis throughout the year, at which stage both the mean *GSI* and *HSI* were at a maximum for males. Nevertheless, the mean *GSI* at the mature stage of the testis was significantly higher during the spawning season than during the non-spawning season. In salmonid fishes, including the rainbow trout *Oncorhynchus mykiss* and the Arctic charr *Salvelinus alpinus* (Lahnsteiner et al., 1993) and cyprinid fishes including the bleak *Alburnus alburnus* and the chub *Leuciscus cephalus* (Lahnsteiner et al., 1994), the seminal fluid is actively secreted at the epithelia of the sperm ducts during the spawning season. It is likely that the statistical difference in the *GSI* between non-spawning and spawning seasons in *L. setigerus* may be due to the secretion of seminal fluid.

Hunter and Goldberg (1980) first described the use of hydrated oocytes for estimation of batch fecundity in a multiple spawning fish, *E. mordax*. In *L. setigerus*, during and after the tertiary yolk stage, a large amount of gelatinous material was rapidly

secreted which accumulated in the ovarian lumen. Hence, counts of advanced oocytes from a small portion of the ovary, when expanded to the total weight of the gelatinous material, may cause a significant overestimate of batch fecundity. However, the oocyte stage-frequency profiles indicated that when the most advanced oocytes attained the secondary yolk stage, they formed a batch that separated from the adjacent group of smaller oocytes. These ovarian characteristics of *L. setigerus* led us to only use the oocytes which had attained the secondary yolk stage for estimates of batch fecundity. My estimates may be high, if some oocytes of this most advanced batch degenerate between the tertiary yolk and mature stages. However, this seems unlikely because degenerating oocytes were never observed in the 132 females collected from May to August which lacked postovulatory follicles and whose ovary had progressed past the secondary yolk stage.

The size range (300-396 mm *TL*) of specimens examined for estimation of batch fecundity was relatively narrow. However, the present study indicates that female *L. setigerus* reach sexual maturity at 303 mm *TL*, and large numbers of mature specimens (92 % of 428 individuals) were found in the size range of 300-400 mm *TL*. This suggests that the range of individuals examined for batch fecundity estimation encompasses most of the mature females in the population.

The number of oocytes found in two of the females examined for batch fecundity was extremely low: the batch size for the 350 and 362 mm *TL* females was calculated as 330×10^3 and 370×10^3, respectively. This is the primary cause for the high variation ($r^2 = 0.22$) in batch fecundity / total length relationships. No significant difference in oocyte density between these two and the other eighteen females was found (ANOVA, $P>0.05$).

The total ovarian weight of the two females, however, was the lowest of all of the specimens examined for estimation of batch fecundity. Eliminating these two females, the batch fecundity of each individual correlates moderately well with total length ($n = 18$; $BF = 251.0 \times TL^{1.303}$; $r^2 = 0.51$; $P < 0.001$).

CHAPTER III AGE AND GROWTH OF *LOPHIUS LITULON*

Introduction

The anglerfish *Lophius litulon* is distributed in Japan from Hokkaido to Kyushu, in the Gulf of Po-Hai, the East China and Yellow Seas (Caruso, 1983; Yamada, 1986; Yoneda et al., 1997b). Anglerfish are landed by commercial fisheries around Japan, and are mainly consumed during the winter season when they command a high market price. As mentioned in the preface, the larvae of anglerfish are planktonic before settling on the bottom, as other Lophiidae fishes (Connolly, 1920; Dahlgren, 1928; Berril, 1929; Mito, 1963; Matsuura and Yoneda, 1986; Minami, 1988; Watson, 1996), and they attain about 1.5 m in total length (Mishima, 1956; Ochiai and Tanaka, 1986; Hori, 1995). The spawning season of *L. litulon* in the East China and Yellow Seas occurs from February to June (Yamada, 1986).

Commercial anglerfish landings reported in various Japanese waters fluctuated widely (Tominaga, 1991; Hori, 1995). In the coastal waters of western Hokkaido, the annual landing of *L. litulon* between 1985 and 1988 ranged from 15-48 *t*, but in 1989 markedly increased to 156 *t* and thereafter has gradually decreased. Tominaga (1991) suggested that the marked increase of anglerfish in 1989 may be due to the occurrence of a strong year-class. However, little information is available for the assessment and management of the fishery. Previous studies of the age and growth of anglerfish based on the analysis of length-frequency distributions have been made in Sendai Bay (Kosaka, 1966) and in the East China and Yellow Seas (Tokimura et al.[1]). These studies, however, grouped the two sexes together and were limited to 1.5 and 3 years old growth, and there was disagreement in their results on the estimated mean growth. Additionally, Suzuuchi[2]

and Hori (1995) described that differences occurred in the maximum size between male and female *L. litulon*. In this chapter, I examined age and growth of anglerfish in the East China and Yellow Seas using vertebral centra.

Materials and methods

The anglerfish were collected during the period from January 1991 to April 1996 from the commercial trawl fishery and from the trawl surveys conducted by the Seikai National Fisheries Research Institute (SNFRI) and by Nagasaki University in the East China and Yellow Seas (Fig. 25). All specimens were measured to the nearest millimeter in total length (*TL*) and to the nearest gram in body weight (*BW*). Gonads and livers were weighed to the nearest 0.1g and gonads preserved in Bouin's solution or 10% formalin for subsequent histological observations and fecundity estimates. Three anglerfish larvae were sampled with a fish larva net (mouth diameter 2 m; length 7.75 m; mesh 1.7 mm + 0.5 mm) in the East China Sea in May 1995. Further in a preliminary survey, a total number of 22 unsexed anglerfish was captured by the bottom trawl net in Tachibana Bay, Nagasaki, in July 1994.

Vertebral centra were chosen as the best hard structure for age determination, based on a preliminary examination, which revealed that each centrum contained concentric bands which appeared to be ring marks (Fig. 26). Otoliths, basihyals, metapterygoids, pelvic girdles and actinosts were also examined; however, these structures were opaque and had extremely irregular outer margins, which made it difficult or impossible to clearly discern ring marks.

Vertebra number 9 was used for age determination, but number 8-10 were used if number 9 was damaged in preparation. After vertebra number 8-10 were soaked in hot water and cleaned, the vertebrae were cut mid-frontally and their dorsal parts were used for examination. Ring marks on the anterior face of the centrum were counted and measured under a profile projector at 5 × magnification using reflected light. A broad, opaque band and a narrow, translucent band appeared alternately on the centrum surface. Any

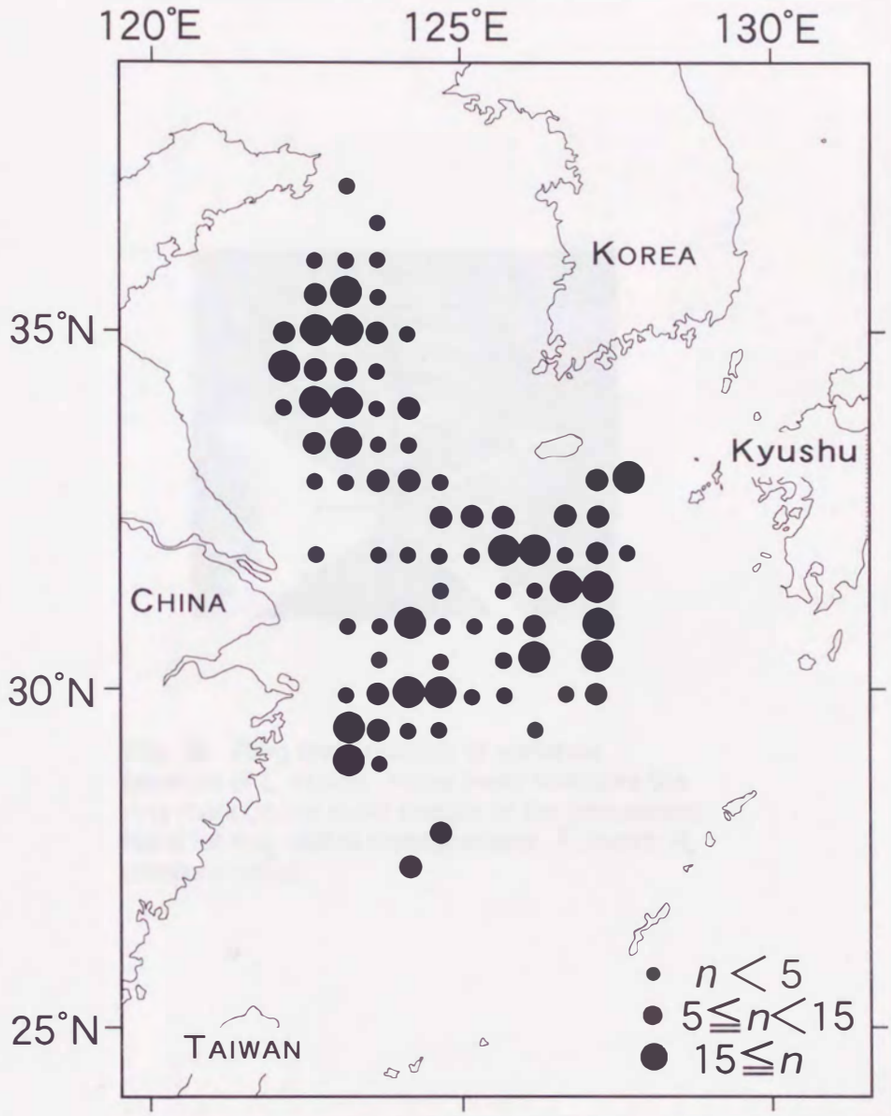


Fig. 25. Geographical distribution of specimens of *L. litulon* collected in the East China and Yellow Seas. *n*, number of fish examined.

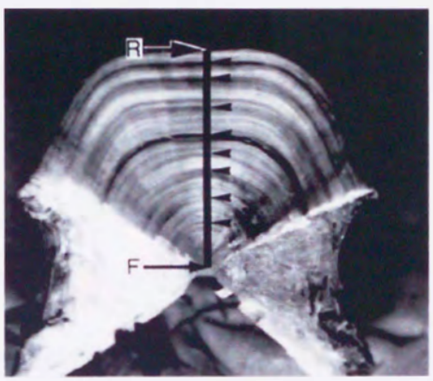


Fig. 26. Ring mark reading of vertebral centrum of *L. litulon*. Arrow head indicates the ring mark on the outer margin of the translucent band for ring radius measurement. F, focus; R, centrum radius.

translucent band which ran round the whole of the centrum surface was a 'true band' and was counted, and one that did not do this was a 'false band' and was not counted. Distances from the focus (F) to the outer margin of the translucent band of each ring mark (ring radius, r_n) and the centrum radius (R) were measured on a transverse plane along a straight line through the focus (Fig. 26).

Marginal increment of centrum (MI) was examined on the basis of all specimens possessing 1-15 ring marks by the following equation:

$$MI = (R - r_{max}) / (r_{max} - r_{max-1})$$

where R is the centrum radius (mm) and r_{max} is the distance (mm) between the focus and the outer margin of the last translucent band.

Specimens of the same length were found to show different sized centrum radii. In order to remove this variation of centrum radii, each ring radius was standardized by the following equation (Mio, 1961):

$$\hat{r}_n = (\hat{R}/R) \times r_n \qquad (1)$$

where $\hat{r}_n$ is the standardized ring radius at the time of n-th ring formation and $\hat{R}$ is an estimate derived from putting the measured TL in the TL-R regression.

To evaluate growth of males and females, mean back-calculated TLs were fitted to the von Bertalanffy growth equation using the Marquardt method (Draper and Smith, 1966; Akamine, 1995).

Females (n = 329) were significantly larger in TL than males (n =492; Mann-Whitney U-test, $P < 0.001$). Length-frequency distributions showed large numbers of both males and females 300 to 450 mm TL, whereas most of the larger individuals (> 600 mm TL) were females (Fig. 27). Maximum size of males was 650 mm TL and females 1013 mm TL.

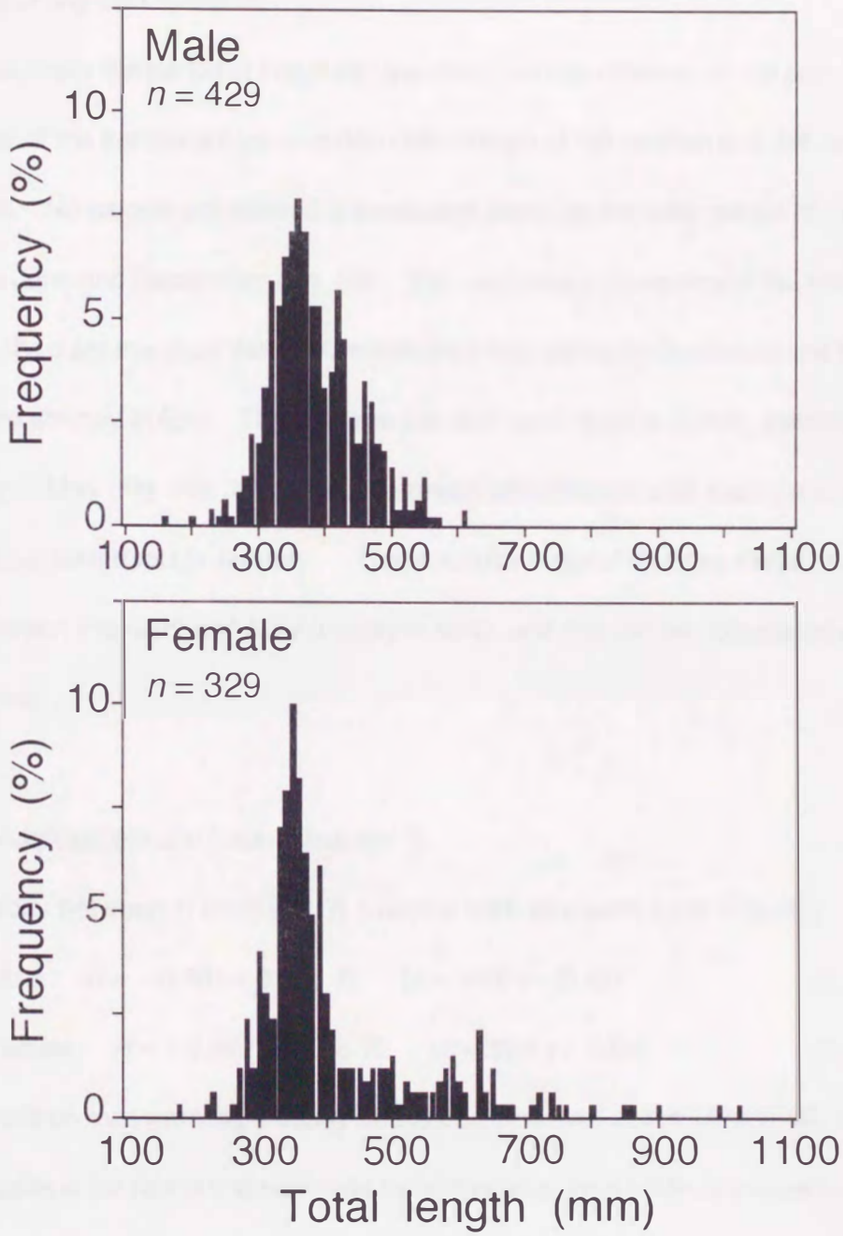


Fig. 27. Length-frequency distribution of *L. litulon* sampled from the East China and Yellow Seas. *n*, number of fish examined.

Results

Period of ring mark formation

In order to clarify the period of ring mark formation, monthly changes of the percentage frequency of the translucent band on the outer margin of the centrum and *MI*s were examined. No sample possessed a translucent band on the outer margin of the centrum between June and September (Fig. 28). The percentage frequency of the translucent band on the outer margin of the centrum remained high between December and March and decreased sharply in April. The minimum *MI*s with new opaque bands occurred from February to May (Fig. 29). After May, the mean *MI* increased until reaching a maximum during November through January. These results suggest that ring marks form once a year between February and May (mostly in April), and this can be considered as an annual mark.

R-TL relationships and back-calculated TL

Regressions between *R* (mm) and *TL* (mm) for each sex were linear (Fig. 30) :

Male : $R = -0.701 + 0.015\ TL$ ($n = 492$; $r = 0.95$) (2)

Female : $R = -0.692 + 0.015\ TL$ ($n = 329$; $r = 0.98$) (3)

The regression lines were significantly different between the sexes (ANCOVA, $P < 0.001$). Total lengths at the time of ring mark (age mark) formation were back-calculated for each sex using equations (2) and (3), and the standardized ring radii by equation (1). The mean back-calculated *TL*s at each age were shown in Tables 9 and 10. The oldest male and female specimens were age 8 and 15, respectively. The coefficients of variation at each age showed that there was a high variability in size at the same age and this was found in both sexes. At age 1, the mean back-calculated *TL*s for males and females were 119 and

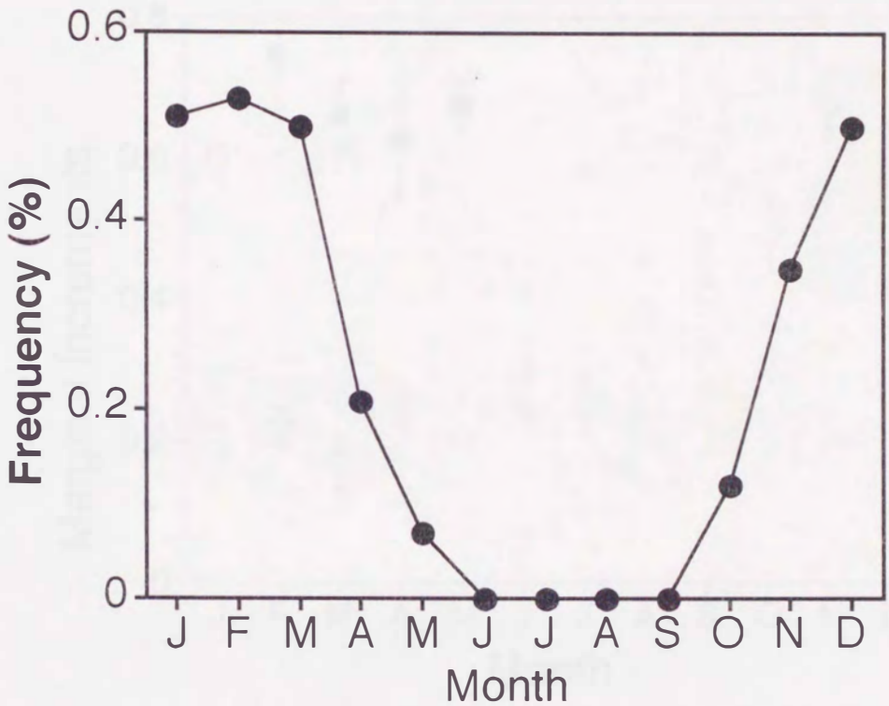


Fig. 28. Monthly changes in the percentage frequency of the translucent band on the outer margin of the centrum for *L. litulon*.

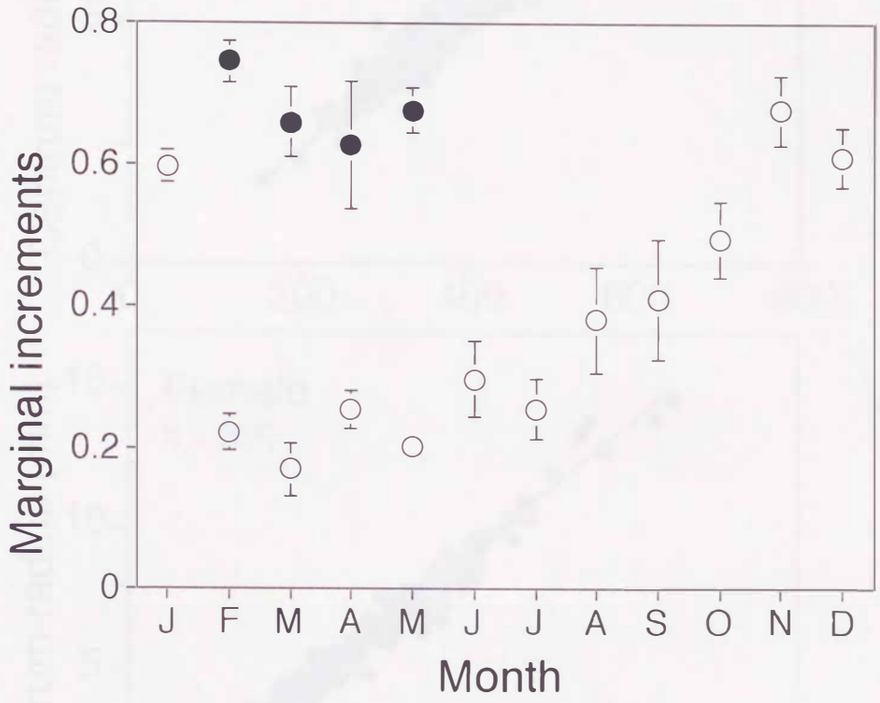


Fig. 29. Monthly changes in the marginal increment of the centrum of *L. litulon*. Vertical bars indicate standard errors. Closed circles indicated the mean marginal increments of centra without new opaque bands between February and May.

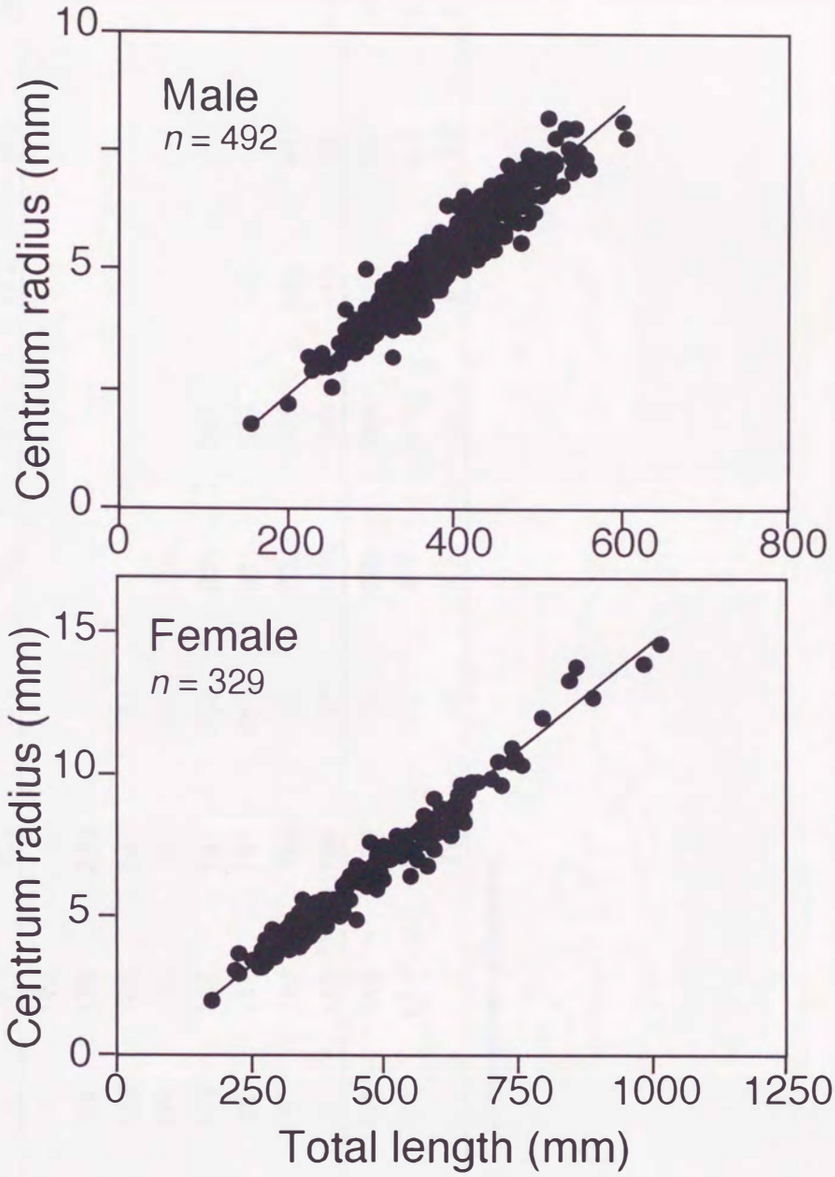


Fig. 30. Relationship between the centrum radius and total length for male and female *L. litulon*. *n*, number of fish examined.

Table 9. Mean back-calculated total length (mm) at each age for male *L. litulon*

Age (year)	*n*	TL_1	TL_2	TL_3	TL_4	TL_5	TL_6	TL_7	TL_8
1	3	122							
2	14	130	209						
3	148	128	207	281					
4	141	120	197	269	334				
5	129	108	187	262	331	393			
6	45	111	187	262	331	398	455		
7	9	118	199	264	331	395	455	501	
8	3	113	188	256	320	389	449	501	552
Mean (weighted)	492	119	197	270	332	394	455	501	552
C. V. (%)		17.7	11.9	9.7	8.1	8.1	6.9	5.2	3.0
Growth increments*1			78	73	62	62	61	47	50

n, number of fish examined; *C.V.*, coefficients of variation.

*1 $\overline{TL}_n - \overline{TL}_{n-1}$ (mm).

Table 10. Mean back-calculated total length (mm) at each age for female *L. litulon*

Age (year)	*n*	TL_1	TL_2	TL_3	TL_4	TL_5	TL_6	TL_7	TL_8	TL_9	TL_{10}	TL_{11}	TL_{12}	TL_{13}	TL_{14}	TL_{15}
1	1	116														
2	31	149	232													
3	143	133	214	290												
4	69	128	208	280	346											
5	38	119	204	288	368	441										
6	20	117	205	298	382	468	542									
7	9	137	228	306	381	460	538	599								
8	7	140	224	315	387	461	533	611	661							
9	5	134	212	296	361	424	495	568	623	680						
10 - 13	4 *1	145	221	282	349	433	501	570	627	679	725	778	829	836		
14 - 15	2 *2	127	207	272	349	422	494	571	632	689	748	815	866	914	973	1009
Mean (weighted)	329	131	213	289	361	449	529	590	640	681	733	793	844	888	973*3	1009*3
C. V. (%)		15.3	11.2	10.3	10.4	9.4	8.2	6.4	6.9	5.9	3.5	3.2	3.1	4.3	0.7	
Growth increments*4			82	75	73	88	80	60	50	41	52	60	51	44	85	36

n, number of fish examined; *C.V.*, coefficients of variation.

*1 One fish at age 10; none of fish at age 11; two fishes at age 12; one fish at age 13.

*2 One fish at age 14; one fish at age 15.

*3 Not to be used for growth analysis.

*4 $\overline{TL}_n - \overline{TL}_{n-1}$ (mm).

131 mm *TL*, respectively, showing a significant difference between sexes at the first standardized ring radius (Mann-Whitney U-test, $P < 0.001$). The annual growth increments indicated that females grew faster than males. Both males and females grew rapidly until an age 6 with growth gradual slowing in older specimens except for females age 14 in which growth increment was estimated at > 80 mm. The values of back-calculated *TL*s at age 14 and 15 may, however, be required correction by accumulation of specimens of the largest size group, because those *TL*s were calculated only from two specimens; one was 1018 mm *TL* at age 15 and the other 981 mm *TL* at age 14. Finally, growth equations were calculated using the mean back-calculated *TL*s for age 1-8 in males and for age 1-13 in females.

Growth

In order to indirectly verify my age to length estimation, I examined the initial growth trend by tracing the observed *TL* for each ring mark group, based on monthly collected young fish of sexed and unsexed fishes (Fig. 31). In May, 3 larval fishes (9-21 mm *TL*) were collected by the fish larva net and 7 young fishes (8-101 mm *TL*) by the trawl net. These can be classified into two groups of 0 and 1 year of age, as *L. litulon* spawn between February and June in the East China and Yellow Seas (Yamada, 1986). Among the young fish collected, 26 fishes: 22 fishes (74-161 mm *TL*) in July, one fish (174 mm *TL*) in October and 3 fishes (157-228 mm *TL*) in February were identified as one ring mark group, based on ring mark reading. In addition, 9 fishes: 2 fishes (199 and 225 mm *TL*) in August, 2 fishes (219 and 226 mm *TL*) in December and 5 fishes (226-250 mm *TL*) in February were of two ring mark group. These data on the growth trend of young fish were compatible with the mean back-calculated *TL* for males and females at the first and second year of age and therefore

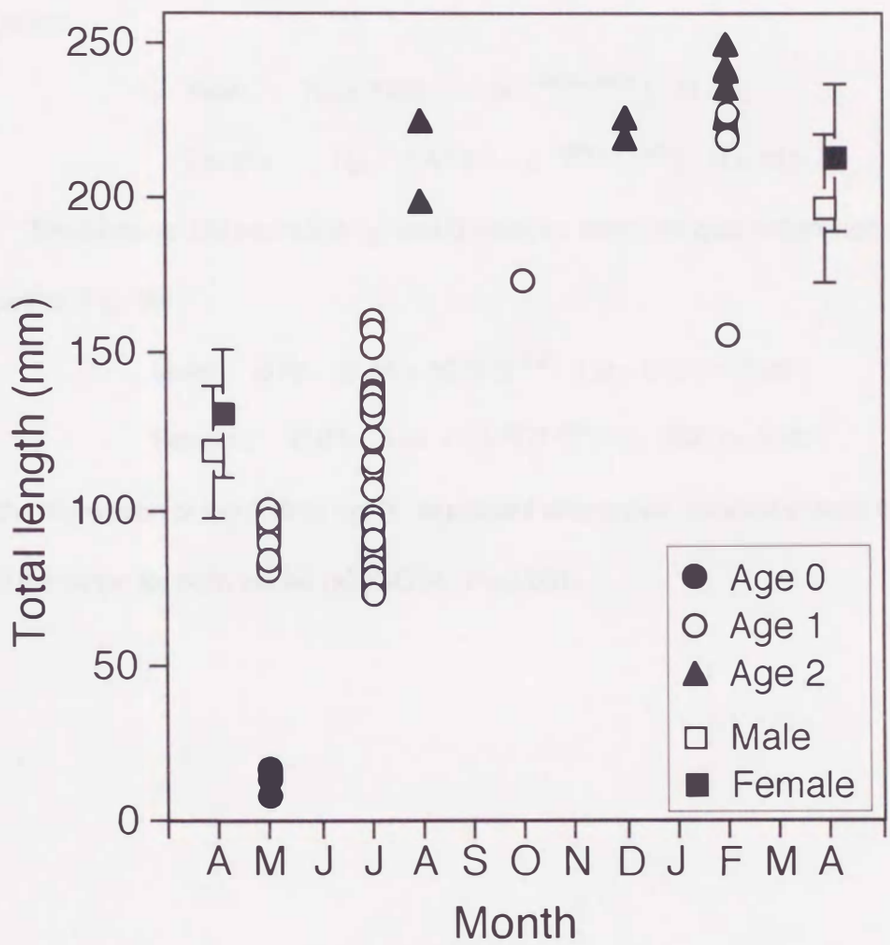


Fig. 31. Monthly changes of total lengths observed for young *L. litulon* (≤ 250 mm *TL*, circles and triangle marks), and comparable mean total lengths (square marks) back-calculated from the first and the second ring radii. Vertical bars indicate standard deviation.

it is considered that the back-calculated *TL* for each ring mark using the centrum reasonably reflect the fish *TL* at each age.

The mean back-calculated *TL*s for each sex were fitted to the von Bertalanffy growth equation:

$$\text{Male}: \quad TL_t = 1130\,(1 - e^{-0.080(t + 0.401)}) \quad (t \le 8)$$

$$\text{Female}: \quad TL_t = 1547\,(1 - e^{-0.064(t + 0.345)}) \quad (t \le 13)$$

Relationship between *BW* (g) and *TL* (mm) for each sex was determined as following equation (Fig. 32) :

$$\text{Male}: \quad BW = (3.44 \times 10^{-4})\,TL^{2.47} \quad (n = 512;\ r = 0.96)$$

$$\text{Female}: \quad BW = (4.01 \times 10^{-5})\,TL^{2.85} \quad (n = 302;\ r = 0.98)$$

In the regression of log *BW* to log *TL*, significant differences occurred in both the intercept and the slope for both sexes (ANCOVA, $P < 0.001$).

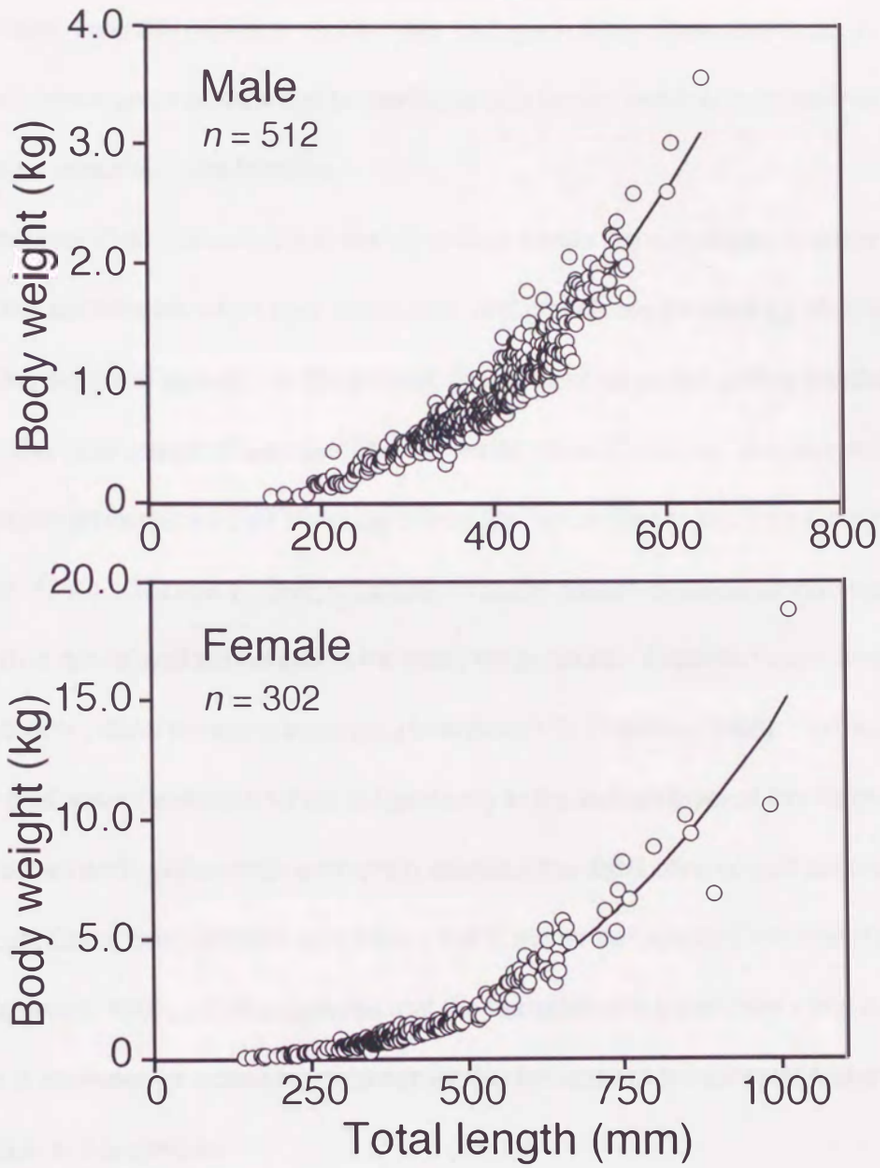


Fig. 32. Relationship between body weight and total length for male and female *L. litulon*. *n*, number of fish examined.

Discussion

This is the first report on age and growth of male and female anglerfish in the East China and Yellow Seas using the vertebral centra. My findings indicate differences occur in the growth increment and maximum age between the two sexes, which is reflected in that most of the larger specimens are females.

Peabody (1961) reported that annual growth zones are developed in some ectothermal vertebrates when their metabolism and growth are arrested by strongly contrasting seasonal cycles. In the present study, most anglerfish with a translucent band on the outer margin of the centrum occurred between December and March, the time when anglerfish increased their distribution from the Yellow Sea to the East China Sea (Yamada, 1986). Tokimura (1992) suggested that the reason for the seasonal migration of anglerfish is due to cyclical changes in the water temperature. Anglerfish are mainly caught in waters with a water temperature ranging from 6 to 13 °C (Yamada, 1986). In the summer season cold water (less than 13°C) is found only in the bottom layer of the Yellow Sea. On the other hand , in the winter and spring seasons the distribution of cold water extends to the East China Sea, which is affected by the Continental Coastal Cold Water (Kondo, 1985; Tokimura, 1992). It is suggested that the formation of a translucent band in the centrum is attributed to increasing prey availability because of the extension of anglerfish distribution in this periods.

My findings indicate that the estimated *TL* at each age for females are larger than those for males, and observed longevity for females is greater than males, which is consistent with the sex-specific length-frequency distributions. This may contribute to the differences in size at sexual maturity. In the East China and Yellow Seas, male and female anglerfish sexually mature at about 350 and 500 mm in body length (*BL*),

respectively (Yamada, 1986). Histological observations of the gonad of *L. litulon* suggest that sexual maturity occurs in males and females at about 340 to 550 mm in *TL* and at 5 and 6 years of age (see Chapter IV).

The back-calculated *TL*s of four *Lophius* spp.: *L. budegassa*, *L. piscatorius* (Tsimenidis and Ondrias, 1980), *L. americanus* (Armstrong et al., 1992) and *L. litulon* are shown in Fig. 33. In females, three *Lophius* spp. (except *L. budegassa*) reach a size larger than 1 m *TL*, and *L. litulon* grows the slowest of the three. The growth differences between sexes of *L. americanus*, *L. budegassa* and *L. piscatorius* are smaller than that of *L. litulon*. All species, however, appear to have a longer lifespan for females.

My result from the East China and Yellow Seas using vertebral centra is quite different from the previous studies which analyzed using the length-frequency distribution data for combined sexes. Kosaka's study from Sendai Bay showed that *L. litulon* reach about 250 mm *BL* in the first year of the life and about 400 mm *BL* at 1.5 years old (Kosaka, 1966). Tokimura et al.[1] reported that *L. litulon* from the East China and Yellow Seas reach about 100, 370 and 540 mm *TL* at 1, 2 and 3 years old, respectively. However, they may be over estimated, as my estimations for age 1 and 2 closely agreed with field observations on the occurrence of young fish 1 and 2 years age groups. My findings suggest that two factors may cause difficulty in the estimation of age and growth of anglerfish by analysis of the length-frequencies for combined sexes. First, smaller fishes, corresponding to age 0-2, are rare in the samples although most of their gonads may be undifferentiated macroscopically. Most specimens of both males and females that I sampled ranged from 300 to 450 mm in *TL*, and their modes were similar, 360 mm *TL* for males and 350 mm *TL* for females, instead of any clear difference in the sex-specific length-frequency distributions. This was also found from landings of the bottom trawl

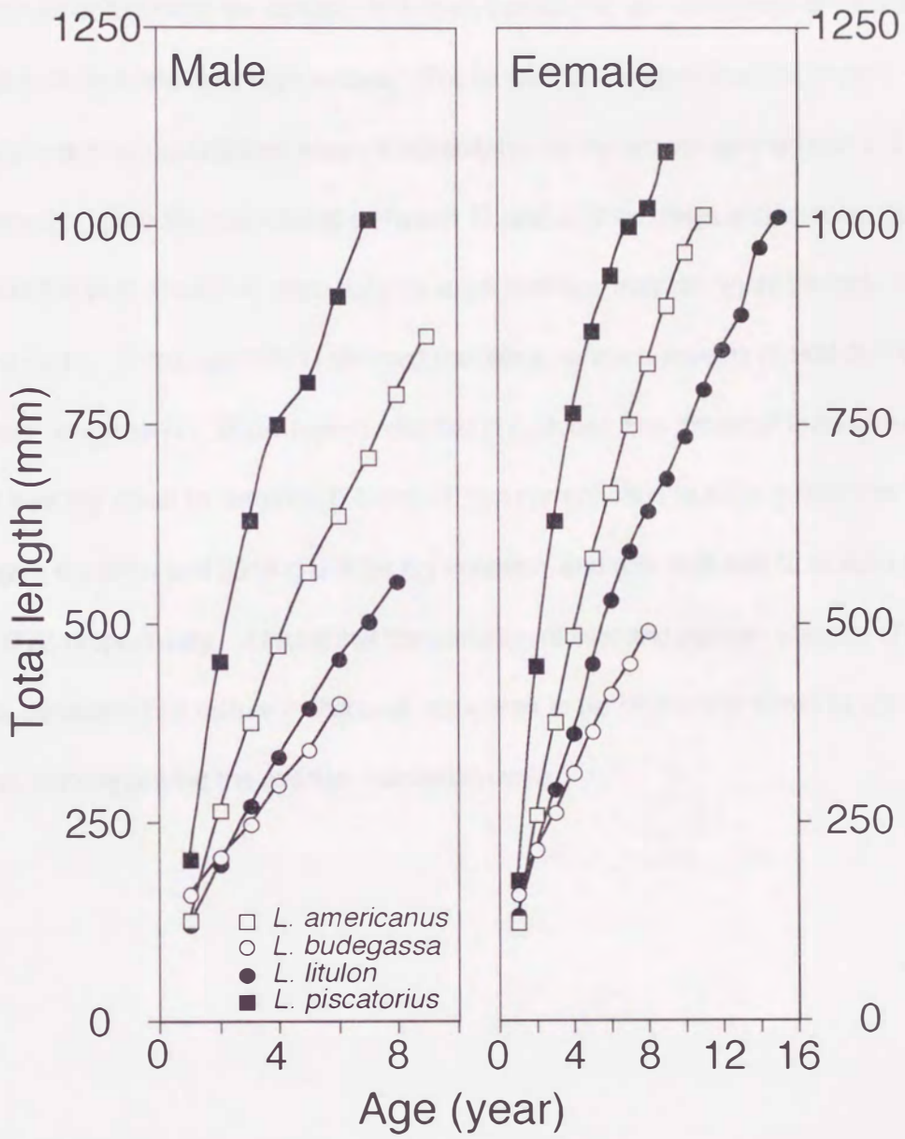


Fig. 33. Mean back-calculated total lengths at each age for four species of *Lophius*. Data for *L. litulon* from the present study, data for *L. americanus* from Armstrong et al., data for *L. budegassa* and *L. piscatorius* from Tsimenidis and Ondrias.

survey by SNFRI between January and February in 1995 and 1996. Secondary, my findings suggest that females grow faster and live longer than males, and growth increments for both sexes are variable between individuals, as coefficients of variation at each age indicated relatively high values. These findings suggest that the length-frequency analysis for combined sexes is not suitable for the growth estimation of *L. litulon*.

Although I give the relationship between *TL* and *BW* for males and females taken throughout the year, their *BW*, especially for larger females, may be highly variable over a seasonal cycle. Tominaga (1991) showed the relationships between *TL* and *BW* for individuals (sex was not differentiated) collected in October, and his result is considerably different from my result for females: 600 mm *TL* fish corresponds to 3739 g *BW* from Tominaga's equation and 3318 g *BW* for my equation, and with 800 mm *TL* to 9551 g and 7533 g *BW*, respectively. I found that the variation of liver and ovarian weights of larger females, considered as mature individuals, appeared to be high in the winter to spring seasons, accompanying the ovarian maturation cycle.