Reproductive cycle, fecundity, and seasonal distribution of the anglerfish *Lophius litulon* in the East China and Yellow seas

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Abstract—The reproductive cycle, sexual maturity, fecundity, and seasonal distribution of the anglerfish *Lophius litulon* were studied from a total of 989 specimens collected in the East China and Yellow seas between March 1991 and July 1997. Males and females reached 50% sexual maturity at 362 mm TL (age 5.4) and at 567 mm TL (age 6.2), respectively. The histology of the gonads showing seasonal changes in both the gonadosomatic index (GSI) and hepatosomatic index (HSI) are described. Mean GSI of females increases rapidly with ovarian development, whereas mean HSI decreases from the middle of vitellogenesis until the ovaries have fully matured. Segregation of oocytes by size within the ovary suggests that *L. litulon* spawns in batches, which was confirmed by observation of a captive specimen. Batch fecundity (BF) in 15 females with secondary yolk stage ovaries was related to total length (TL, mm) by the equation $BF = (-1.64 \cdot 10^6) + 3688.13\ TL$ ($546 \leq TL \leq 846$). In September, most specimens of both sexes were collected in the Yellow Sea. Between November and January their distribution extended from the Yellow Sea into the East China Sea. During the spawning season (February to May), sexually immature individuals were collected throughout the East China and Yellow seas, whereas sexually mature individuals were caught only in the East China Sea and the coastal waters off Kyushu Island, Japan. The spawning grounds of *L. litulon* extend widely from waters of the East China Sea to inshore waters of Kyushu.

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The anglerfish *Lophius litulon* (Jordan) is distributed throughout Japanese waters, in the Gulf of Po-Hai and the East China and Yellow seas (Caruso, 1983; Yamada, 1986; Yoneda et al., 1997). In Japan, this species and a second anglerfish, *Lophiomus setigerus* (Vahl), are consumed as food, and their livers are considered a delicacy. Both species are caught throughout their range by Japanese, Chinese, and Korean trawl fisheries, primarily during winter months when these fish command a high market price. The catch of anglerfish is very small compared to that of other commercially important species in Japan. Anecdotal evidence from fishermen suggests that catches have declined in recent years, although no official landings are reported. The shallow East China and Yellow seas are subject to intense fishing pressure and large anglerfish, especially *L. litulon*, have become particularly scarce.

In spite of their commercial importance, little biological information is available on *L. litulon* and *L. setigerus*. Kosaka (1966) described age, growth, spawning season, and feeding habits of *L. litulon* in Sendai Bay, off the northeast coast of Honshu Island, Japan; Yamada (1986) reported the spawning season and size at maturity for *L. litulon* and the spawning season for *L. setigerus* in the East China and Yellow seas. Recently we reported the reproductive biology of *L. setigerus* (Yoneda et al., 1998a, 1998c) and age and growth of *L. litulon* (Yoneda et al., 1997) and *L. setigerus* (Yoneda et al., 1998b).

According to Yamada (1986) and Tokimura,[1] the distribution of *L. litulon*

[1] Tokimura, M. 1992. Distribution of primary bottom-fishes in the East China and Yellow Seas during the winter of 1991. Seikai Block Sokouo Chosa Kenkyu Kaiho. 3:15–39 [In Japanese.] Seikai National Fisheries Research Institute, Kokubu, Nagasaki 850-0951, Japan.

varies seasonally: in summer it is found mainly in the Yellow Sea, and during winter and spring it extends its range into the East China Sea. Our study was initiated to examine the role of reproductive biology during the seasonal change in anglerfish distribution and to provide scientific background for a national management policy for the anglerfish fishery.

Materials and methods

A total of 989 specimens (males=549, females=412, unknown sex=28), caught between March 1991 and July 1997, were examined (Fig. 1). Of these, 529 specimens were obtained from five trawl surveys conducted by the Seikai National Fisheries Research Institute (SNFRI). A further 425 specimens were bought in fish markets at Shimonoseki, Fukuoka, and Nagasaki, where there are commercial landing sites. The locations and date of capture of these fish were obtained from fishermen. The remainder (n=35) were caught during a benthic trawl survey conducted by Nagasaki University.

Five SNFRI benthic trawl surveys between January and February in 1991, 1995, 1996, and 1997, and in September 1993 were conducted in the East China and Yellow seas, between latitudes 27° and 37°30′N, west of 128°E longitude. The sampling area was divided into five regions by using lines of latitude and longitude, and further divided into 30′ · 30′ (minutes) sampling stations (Fig. 1). Eighty and sixty-nine trawl stations were established in the sampling area in 1991 and 1993, respectively, at depths between 35 and 150 m. In 1995, 1996, and 1997, respective totals of 65, 123, and 78 trawl stations were established in the sampling area at depths between 50 and 200 m. All SNFRI surveys were conducted with the RV *Kaiho Maru*, a 466-ton stern trawler. At each station, a bottom trawl, with a 66-mm codend mesh, was towed for 30 minutes at approximately 3 knots. The net mouth was approximately 20 m wide and 5 m high. Nagasaki University conducted a trawl survey in the East China Sea between 29°and 32°N latitude and 126° and 127°30′E longitude, at depths between 70 and 120 m in May 1995 with the training ship *Nagasaki Maru*, an 842-ton stern trawler. Seventeen sampling stations were established. A bottom trawl net, with a 62-mm codend mesh, was towed for 60 minutes at approximately 3 knots. The net mouth was approximately 17 m wide and 10 m high. Most specimens captured by the commercial fishery were obtained in winter and spring because the fishing areas of commercial vessels vary seasonally owing to seasonal changes in the target fishes. Winter and spring specimens were available from the southern Yellow Sea to the northern East China Sea, between latitudes of approximately 30° and 35°N, west of 128°30′N, at depths between 35 and 200 m, and were supplemented with specimens from the coastal waters of northern Kyushu Island, Japan. Summer and fall specimens were taken from the East China Sea, roughly between latitudes 28° and 33°N, west of 128°30′N, at depths between 35 and 200 m.

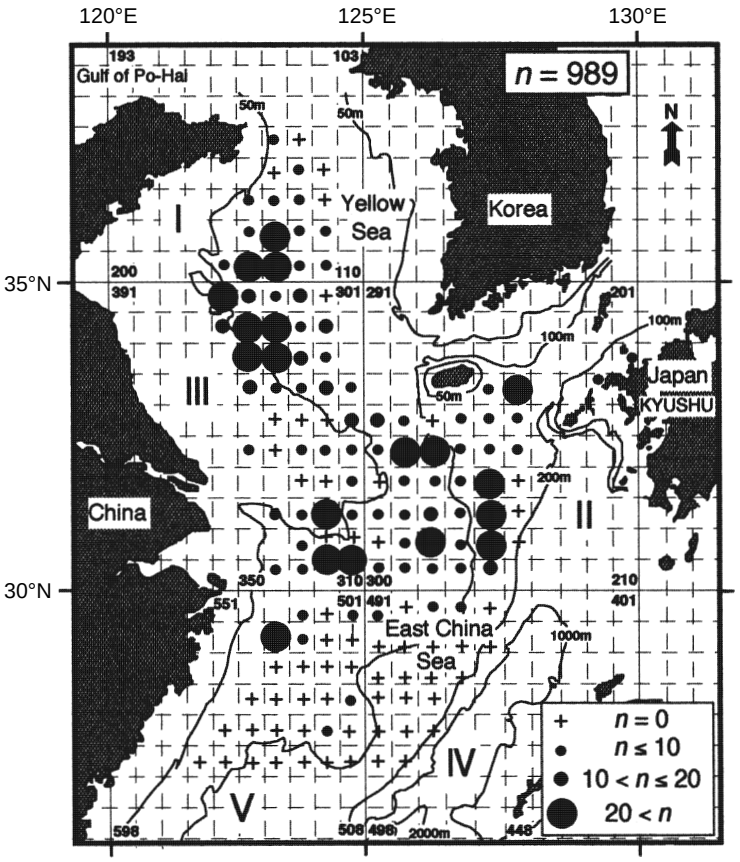

Figure 1

Geographical distribution of specimens of *Lophius litulon* collected in the East China and Yellow seas. Roman numeral (I–V) represents each sampling area. Arabic numeral represents the sampling station number. n = total number of fish examined.

The total length (TL) of all specimens was measured to the nearest millimeter. Body weight (BW) and visceral weight (VW) were determined to the nearest gram, and gonadal weight (GW) and liver weight (LW) were measured to the nearest 0.1 g. From 989 specimens measured during this study, 367 testes and 267 ovaries were collected. For histological examination, the middle portion of the left testicular lobe was excised, as well as three different parts of the ovary (the center of the anterior, middle, and posterior portions of the left ovarian lobe). These tissue samples were fixed in Bouin's fluid for 24 hours and preserved in 70% ethanol. The remainder of each ovary was preserved in 10% formalin to measure oocyte size (diameter, size-frequency distribution, and batch fecundity) because oocytes shrink when fixed in Bouin's fluid.

After dehydration in ethanol, the gonadal tissues were embedded in paraffin wax or methacrylate polymer resin (Technovit, Kulzer). Paraffin sections, 5–10 µm thick, were stained with Mayer's haematoxylin-eosin. Methacrylate polymer resin sections, 2–3 µm thick, were stained with a 1% solution of toluidine blue. Paraffin serial sections

Table 1

Histological characteristics of oocytes at different developmental stages in *Lophius litulon*.

Developmental stage of the oocyte	Oocyte diameter (µm)	Histological characteristics
chromatin nucleus	less than 20	Nucleus has a large nucleolus.
perinucleus	35–160	Multiple nucleoli are seen toward the periphery of the nucleus; oil droplets appear around the nucleus and increase in number; follicle cells surrounding the oocyte have formed a narrow layer.
yolk vesicle	180–250	Yolk vesicles appear in the peripheral region of the cytoplasm.
primary yolk	300–530	Yolk globules appear between the yolk vesicles and increase in number; both granulosa and thecal cell layers are clearly observed.
secondary yolk	480–730	Oocytes are larger and yolk globules fill the cytoplasm.
tertiary yolk	750–1000	Yolk accumulation progresses rapidly, which results in a marked increase in the size of oocytes.
migratory nucleus	950–1300	Yolk globules begin to fuse with one another; oil droplets fuse to form larger ones.
mature	1450–1700	After germinal vesicle breakdown, yolk globules form a single mass and oil droplets coalesce to form larger ones.
atretic	—	Early: disintegration of the nucleus and yolk globules and hypertrophy of the follicular cell layers.
		Late: presence or absence of intercellular vacuoles, flocculent material, and granular pigments.

were mainly used for observations of whole gonadal structure, whereas polymer resin sections were used for detailed observations of oocyte development, spermatogenesis, and the definition of gonadal stages. In our study, polymer resin sections fixed in Bouin's fluid gave the best results. The developmental stages of the oocytes were categorized according to Yamamoto (1956) and Yoneda et al. (1998a) (Table 1). Histological classification of atretic oocytes (Table 1) and postovulatory follicles followed Hunter and Macewicz (1985) and Yoneda et al. (1998a). Only specimens larger than the minimum size at sexual maturity (see "Results" section) were used to examine seasonal changes in gonadal condition.

Oocyte diameter was measured with a profile projector (20–100·), and the range was determined by using the largest and smallest oocytes in the whole projected field from each developmental oocyte stage. Oocyte diameter at each developmental stage was measured in the following manner: the developmental stage of the most advanced oocytes in each ovary was identified from histological sections, and the diameters of 50 formalin-fixed oocytes forming the largest size class in ovaries at each developmental stage were measured (one or two randomly selected specimens).

Because the arrangement of oocytes shows a gradation in developmental stage within each ovigerous lamella (see "Results" section), the composition of oocytes within each ovigerous lamella was considered to be the unit within the ovary. To determine the size-frequency distribution of oocytes within each ovarian stage, 50–80 formalin-fixed ovigerous lamellae samples (corresponding to 300–550 oocytes) from each ovarian stage were examined. All oocytes ≥100 µm in yolk vesicle stage ovaries were measured; in other ovarian stages, only oocytes ≥200 µm were measured. The size-frequency distribution of oocytes at each stage represented the ovary from one randomly selected specimen.

Estimates of size at sexual maturity were based on the examination of males (193–692 mm TL [n=236]) and females (174–1013 mm TL [n=246]) collected during the spawning season between February and May. Of these, 38 females were defined as sexually immature, based on macroscopic observations (Afonso-Dias and Hislop, 1996). The remaining 444 specimens were classified into each gonadal stage on the basis of histological observations. Sexually mature individuals were defined as males with testes in stages 3 and 4, and females with ovaries in mid-stage 2 (oocytes at the secondary yolk stage) or at more advanced stages (see Results). Males with testes in stages 3 and 4 collected in the spawning season were thought to have the potential to spawn because our preliminary observations indicated that milt ran from their genital pores on slight pressure. Females with ovaries with secondary yolk stage oocytes were considered sexually mature because an advanced group of secondary yolk stage oocytes forms an isolated batch and increases in size in tandem with ovarian development. Females with ovaries in stages 2 (late), 3, 4, or 5 were also considered sexually mature, because these individuals were thought to be pre- or postspawning fish. Similarly, to estimate age at sexual maturity, the age of individual fish collected in the spawning season was determined by counting the annual ring marks on the surface of the vertebral centrum (Yoneda et al., 1997). Of 482 spec-

Table 2

Summary of results of oocyte density (the number of secondary yolk stage oocytes per unit sample weight [g]) in the ovaries of five female *Lophius litulon* from various locations and results of two-way analysis of variance. int. = interior; ext. = exterior.

	Position of sample in ovary*					
	Right ovary			Left ovary		
TL (mm)	Anterior (int. or ext.)	Middle (center)	Posterior (int. or ext.)	Anterior (int. or ext.)	Middle (center)	Posterior (int. or ext.)
546	2832^{ext}	2711	2802^{ext}	2768^{int}	2960	3000^{int}
647	3008^{ext}	3113	3067^{int}	3059^{int}	3160	2980^{ext}
662	2917^{ext}	3009	2759^{int}	2913^{int}	2635	3216^{ext}
796	2540^{int}	2771	2691^{ext}	2662^{ext}	2990	2780^{int}
847	2607^{int}	2572	2821^{int}	2680^{ext}	2800	2800^{ext}
Mean	2781	2835	2828	2816	2909	2955

	Two-way analysis of variance			
Source of variation	df	SS	MS	*F*
Right vs. left ovary	1	23130	23130	0.73
Position within ovary	2	105500	52730	1.66
Interaction	2	48140	24070	0.76
Error	24	764700	31860	

imens collected in the spawning season, 410 specimens (187 males and 223 females) were used to estimate age at sexual maturity. The vertebral centra of the other 72 specimens were either damaged in preparation or lost. To estimate the total length (L_{50}) and age at which 50% of males and females are sexually mature, the fraction of mature fish in each interval (10-mm length or year of age) was fitted with a logistic function with the Marquardt method (Draper and Smith, 1966).

All specimens $TL \geq L_{50}$ were used to determine the monthly changes in gonadosomatic index (GSI) and hepatosomatic index (HIS) for adult males and females. The GSI and HSI were calculated in the following manner:

$$GSI = \left(GW / (BW - VW)\right) \cdot 100.$$
$$HSI = \left(LW / (BW - VW)\right) \cdot 100.$$

The Kruskal-Wallis test (one-way analysis of variance, ANOVA) followed by Dunn's multiple comparison test were used to test for significant differences between the GSI and HSI values of gonadal stage groups of fish.

Estimation of batch fecundity followed Yoneda et al. (1998a). Batch fecundity was estimated only from specimens with ovaries containing oocytes in the secondary yolk stage and no gelatinous material. Only 15 females contained such ovaries during our study; these fish were thought to be ready to spawn for the first time during that spawning season because their ovaries contained neither postovulatory follicles nor atretic oocytes. To determine whether secondary yolk stage oocytes were randomly distributed throughout the ovary, densities (no. oocytes/g ovary wt.) of secondary yolk stage oocytes from the six locations within the ovaries of five fish were compared (Table 2): two samples from the center of the right and left ovarian lobes, two samples from the posterior section (either from the interior or exterior) and two samples from the anterior section (either from the interior or exterior). A two-way ANOVA was performed to test for the effect of sample location on oocyte density within each ovary. There was no significant change in oocyte density by location of oocytes within the ovaries (Table 2). Advanced yolked (secondary yolk stage) oocytes were randomly distributed within the ovary and samples could be taken from any location without bias. Fecundity samples were collected from six different parts of the ovary, in the anterior, middle, and posterior portion of each ovarian lobe. Ovarian tissue samples (30–120 mg), each containing approximately 100–350 oocytes, were placed on a slide in water and covered with a cover slip. The most advanced oocytes were counted with a profile projector (50–100·). Batch fecundity for each female was calculated as the product of the number of secondary yolk stage oocytes per unit of weight (of each of the six sampling sites) multiplied by the total ovarian weight. Linear regression analysis was used to examine the relationship between batch fecundity and the total length of the fish.

To examine the seasonal distribution of fish, the numbers of specimens collected at each sampling station during each of the three study periods (September, November–January, and February–May) were compared. The September samples were collected in the 1993 SNFRI trawl survey. Samples for the other two periods came from

the trawl surveys conducted by SNFRI between January and February in 1991, 1995, 1996, and 1997, and from the commercial trawl fishery in 1991–97. Additional samples for February–May were collected in the trawl survey conducted by Nagasaki University in May 1995. Sexually mature individuals collected in September and November–January were defined as those larger than the L_{50} for that sex because most had recrudescent gonads. Between February and May, sexually mature individuals were defined as males with testes in stage 4 and females with ovaries in stages 2 (late), 3, 4, or 5 (see "Results" section). These individuals were thought to be pre- or postspawning fish, possibly collected at or near the spawning grounds. Sexually immature individuals were defined as males with testes in stages 1 or 2 and females with ovaries in stage 1. These individuals were considered to be nonspawning fishes present during the spawning season.

Results

Structure of the testis

The paired testicular lobes are located in the posterior portion of the abdominal cavity. The main longitudinal sperm duct is located ventral to the testicular groove (hilus) in each testis. The seminiferous tubules radiate towards and terminate blindly from the testicular periphery of the main sperm duct. The germinal cysts of spermatogonia, spermatocytes, and young spermatids are arranged randomly on the walls of the seminiferous tubules (Fig. 2A). As young spermatids mature, they are released from their cysts into the lumina of the seminiferous tubules (Fig. 2B). Spermatids and spermatozoa are both found in the lumina of the seminiferous tubules and sperm ducts (Fig. 2, B and C).

Structure of the ovary

The right and left ovarian lobes of *L. litulon* are connected to each other at their posterior ends, forming a single organ. Stalk-like ovigerous lamellae protrude from the ovarian wall and each contains many oocytes at different stages of development. In developing and maturing ovaries, one or two of the most advanced oocytes are located in the terminal portion of each ovigerous lamella, and previtellogenic oocytes are found near the base of the ovigerous lamella throughout the year.

The ovarian lumen is lined with both ovarian wall epithelium and ovigerous lamella epithelium. These epithelia undergo morphological changes accompanying the ovarian maturation cycle (Fig. 3). As ovarian development continues in the secondary and tertiary yolk stages, gelatinous material is secreted from both the ovigerous lamellar epithelium and ovarian wall epithelium, and fills the ovarian lumen.

The early-stage postovulatory follicles are convoluted, with many folds, and contain a follicular lumen (Fig. 4A). The granulosa cells are either columnar or cuboidal and are arranged in a regular manner together with thecal cells (Fig. 4B). The nuclei are located in the basal or middle portion of the granulosa cells. The late-stage postovulatory follicles are much smaller than those in the previous stage, and the follicular lumen continues to decrease

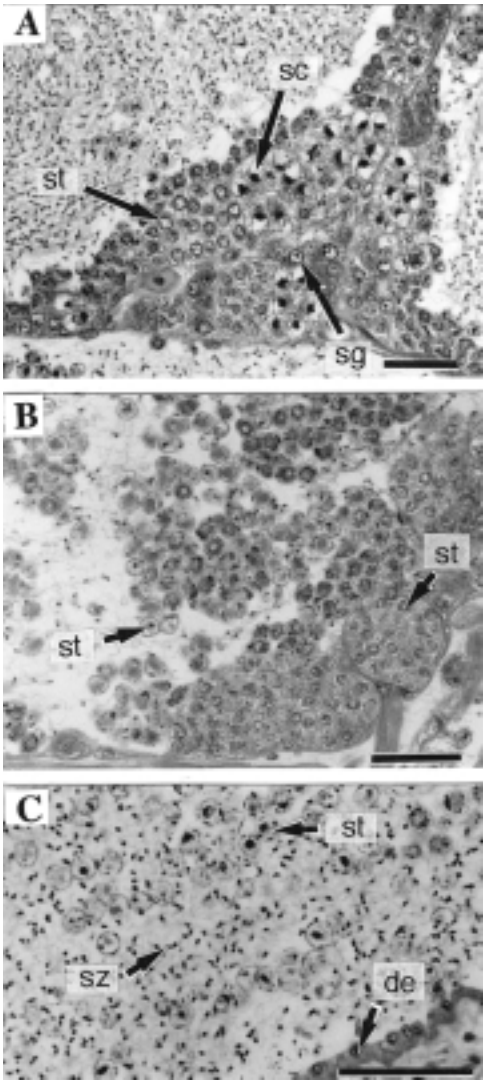

Figure 2

Photomicrographs of sections of the testis of *Lophius litulon*. (**A**) Transverse sections of the seminiferous tubules during spermatogenesis, showing that the germinal cysts are arranged randomly on the walls of the seminiferous tubules. (**B**) Transverse sections of the seminiferous tubules during spermatogenesis, showing that spermatids are released into the lumina of the seminiferous tubules. (**C**) Transverse section of the main sperm duct during spermatogenesis, showing that both spermatids and spermatozoa are present in the main sperm duct. Bar = 25 μm; sg = spermatogonia; sc = spermatocyte; st = spermatid; sz = spermatozoon; de = main duct epithelium.

in size until it disappears (Fig. 4C). Ultimately, the granulosa cell layer becomes indistinct and the thecal cell layer is much regressed.

Maturity stages of testes

The testes can be classified into four stages of maturity according to their histological characteristics. No spent specimens, defined as those in which spermatogenesis has ceased and residual spermatozoa remain in the testis, were found during the study.

Stage 1 immature (Fig. 5A): Germinal cysts containing spermatogonia, spermatocytes, and spermatids are observed along the wall of the seminiferous tubules. Spermatids and spermatozoa are not present in the lumina of the seminiferous tubules and the small main duct. All specimens with testes at this stage were ≤306 mm TL.

Stage 2 early spermatogenesis (Fig. 5B): Germ cells at all stages of spermatogenesis are present. Spermatids and a few spermatozoa are observed in the lumina of the seminiferous tubules and main sperm duct.

Stage 3 late spermatogenesis (Fig. 5C): Active spermatogenesis occurs in the testes. Spermatids and spermatozoa are more abundant in the lumina of the seminiferous tubules and in the main sperm duct than in the previous stage.

Stage 4 mature (Fig. 5D): Large quantities of spermatozoa and a few spermatids are present in the lumina of the seminiferous tubules and main sperm duct. Spermatogenesis and spermatogonial division also occurs in the seminiferous tubules, though few, if any, germinal cysts containing spermatogonia or spermatocytes are found around the main sperm duct.

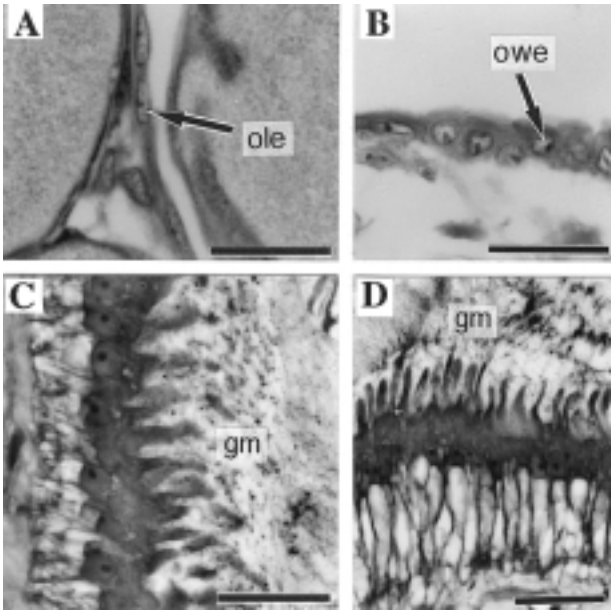

Figure 3

Photomicrographs of the ovigerous lamellar epithelium and ovarian wall epithelium at various stages of ovarian maturation in *Lophius litulon*. (**A**) Ovigerous lamellar epithelium (ole) at the previtellogenic stage. (**B**) Ovarian wall epithelium (owe) at the previtellogenic stage. (A) and (B) show the epithelial cells of both the ovigerous lamella and ovarian wall are squamous or cuboidal in shape and contain a small nucleus. (**C**) Ovigerous lamellar epithelium at the tertiary yolk stage. (**D**) Ovarian wall epithelium at the tertiary yolk stage. (C) and (D) show that gelatinous material is actively secreted from the apical surfaces of the epithelia of both the ovigerous lamellae and ovarian wall. Bar = 25 µm; gm = gelatinous material.

Maturity stages of ovaries

The ovaries can be classified into six stages of maturity according to their histological characteristics and the development of the most advanced oocytes. The classification of stage-4, -5, and -6 ovaries is based on a modification of the atretic states of Hunter and Macewicz (1985).

Stage 1 immature (Fig. 6A): Only previtellogenic oocytes are present and the epithelia of both the ovigerous lamellae and ovarian wall are thin.

Stage 2 developing (Fig. 6B): Most advanced oocytes have reached the primary to tertiary yolk stages. All of the ovigerous lamellae have vitellogenic oocytes. This stage can be subdivided into early and late stages. The early stage is defined by the presence of primary or secondary yolk stage oocytes and the late stage by the presence of tertiary yolk stage oocytes with gelatinous material.

Stage 3 mature (Fig. 6C): The most advanced oocytes are in the migratory nucleus or mature stages. The ovulated oocytes are found in the gelatinous material forming within the ovarian lumen just before spawning. A part of the remaining smaller oocytes contains early yolk stage oocytes.

Stage 4 spawning (Fig. 6D): Vitellogenic oocytes with no sign of atresia and postovulatory follicles are present. Degenerating residual mature oocytes are frequently observed. More than 50% of the ovigerous lamellae have vitellogenic oocytes.

Stage 5 spent (Fig. 6E): Vitellogenic oocytes are degenerating (early atretic stage) and postovulatory follicles are observed. More than 50% of the ovigerous lamellae have only previtellogenic oocytes or atretic oocytes (or both).

Stage 6 resting (Fig. 6F): Late atretic stage oocytes and previtellogenic oocytes are present, and the epithelia of both the ovigerous lamellae and ovarian wall are thin.

To determine how frequently ovigerous lamellae were found with yolked oocytes after spawning, 30–50 ovigerous lamella samples from the ovaries of 18 females with

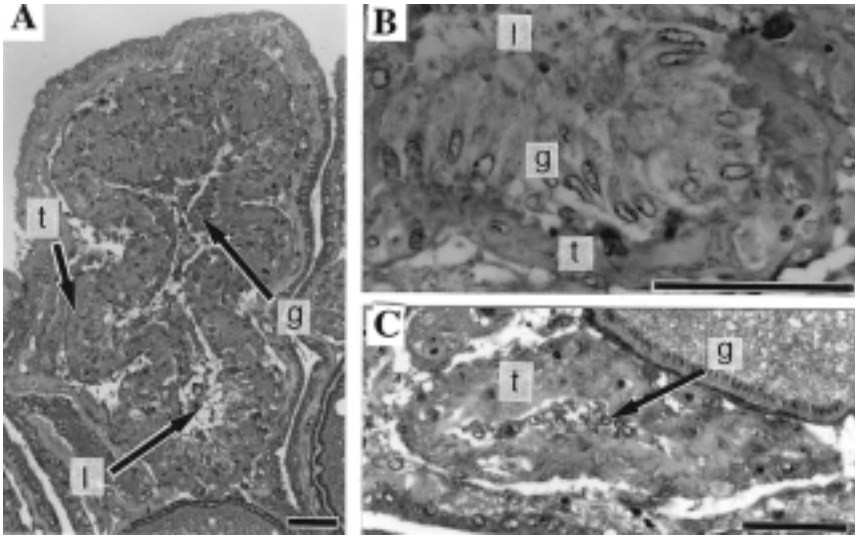

Figure 4

Photomicrographs of atretic postovulatory follicles of *Lophius litulon*. (**A**) and (**B**) Early-stage postovulatory follicles. (B) shows that the granulosa cells are arranged in an orderly manner together with the thecal cells. (**C**) Late-stage postovulatory follicles. Bar = 75 µm; l = follicular lumen; g = granulosa cell layer; t = thecal cell layer.

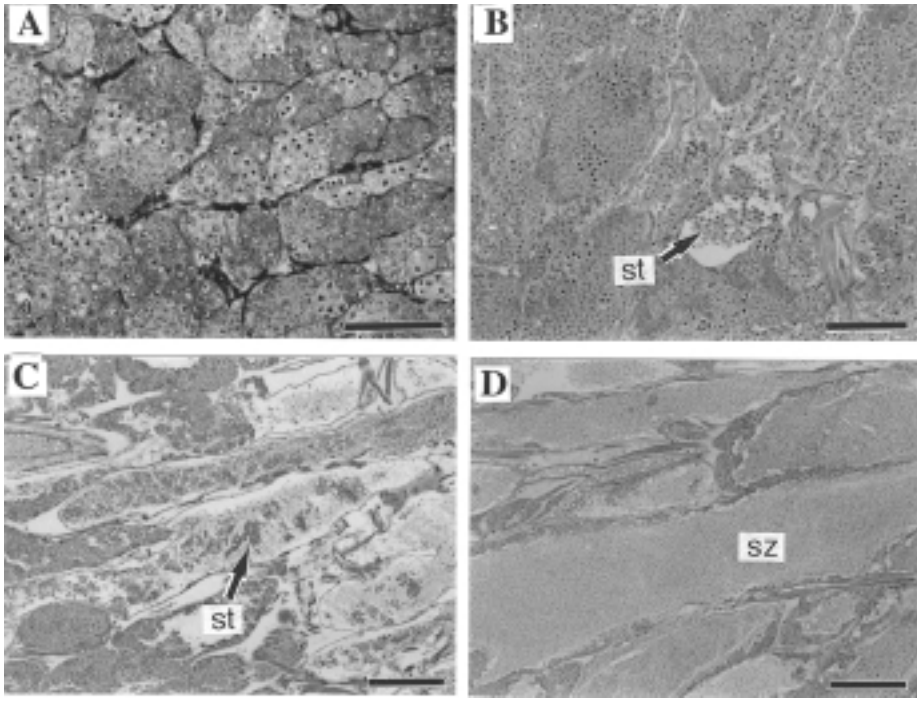

Figure 5

Photomicrographs of testes in the four different stages of maturity in *Lophius litulon*. (**A**) Stage 1 (immature). (**B**) Stage 2 (early spermatogenesis). (**C**) Stage 3 (late spermatogenesis). (**D**) Stage 4 (mature). Bar = 100 µm; st = spermatid; sz = spermatozoon.

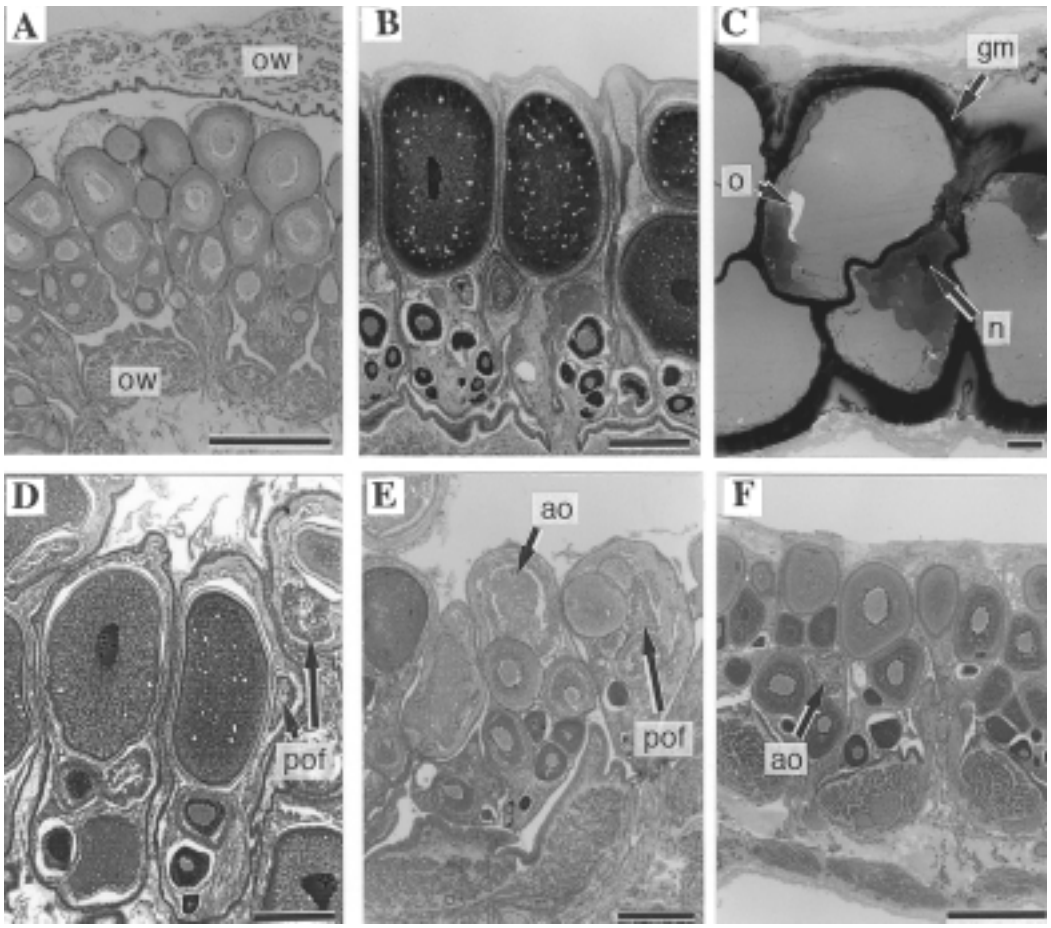

Figure 6

Photomicrographs of ovaries at the six different stages of maturity in *Lophius litulon*. (**A**) Stage 1 (immature). (**B**) Stage 2 (developing). (**C**) Stage 3 (mature). (**D**) Stage 4 (spawning). (**E**) Stage 5 (spent). (**F**) Stage 6 (resting). Bar = 250 µm; ow = ovarian wall; gm = gelatinous material; n = nucleus; o = oil droplet; pof = postovulatory follicle; ao = atretic oocyte.

postovulatory follicles were examined. The frequency of ovigerous lamellae with yolked oocytes present clearly differentiates two ovarian conditions (Table 3). The ovaries of females in which more than 75.0% of the ovigerous lamellae have secondary yolk stage oocytes are in stage 4, regardless of the degenerative stage of the postovulatory follicles. The ovaries of females in which less than 36.4% of the ovigerous lamellae have primary yolk stage oocytes are in stage 5. Some yolked oocytes in the process of becoming atretic are found in specimens with primary yolk oocytes. No females were found with ovaries containing both postovulatory follicles and tertiary yolk or a more advanced stage of oocytes during our study.

Length and age at sexual maturity

There were clear differences between males and females in the size and age at sexual maturity (Fig. 7). The minimum size and age at sexual maturity for males were 325 mm TL and age 4; for females, minimum size and age at sexual maturity were 546 mm TL and age 5. The size and age at 50% sexual maturity for males and females were 362 mm TL and age 5.4, and 567 mm TL and age 6.2, respectively. All males ≥390 mm TL and age 7, and all females ≥630 mm TL and age 8, were mature.

Annual reproductive cycle

Mature males were found over a longer period of the year than mature females (Tables 4 and 5). Spermatogenesis occurs throughout most of the year; therefore males with mature testes are frequently collected. In females, early stages of oocyte development occur between November and March, and the later stages from February through April. Females in stages 3 and 4 were collected from February to May, which is therefore considered the spawning season. Between May and November, most females had stage 1, 5, or 6 ovaries.

Table 3

Results of histological examination of stage-4 and stage-5 ovaries of *Lophius litulon* collected between February and June. Stage-4 ovaries are defined as those in which more than 50% of the ovigerous lamellae have yolked oocytes in females with secondary yolk stage oocytes with no sign of atresia, regardless of the degenerative stage of the postovulatory (POF) follicles. Stage-5 ovaries are defined as those in which less than 50% (or none) of the ovigerous lamellae have yolked oocytes with frequent signs of atresia. GSI = gonadosomatic index.

Date	Total length (mm)	GSI	Oocyte stage[1]	POF stage[2]	Ovigerous lamellae with yolked oocytes (%)	Atretic stage[3]	Ovarian stage
27 Feb 93	594	9.14	sy	l	75.0	—	4
20 Mar 95	634	7.00	sy	l	89.7	—	4
20 Mar 95	738	7.50	sy	l	93.0	—	4[4]
18 Mar 96	622	5.31	py	e	30.4	—	5
18 Mar 96	639	4.50	py	l	17.6	e	5
18 Mar 96	711	6.96	sy	l	97.7	—	4
18 Mar 96	1013	6.02	sy	l	92.1	—	4
17 Mar 97	821	6.81	py	e	20.6	—	5
17 Mar 97	853	5.76	py	e	25.0	—	5
26 Apr 94	645	8.30	py	l	20.0	e	5
14 Apr 95	857	8.60	py	l	16.4	e	5
23 Apr 96	702	6.38	sy	e	92.3	—	4
26 Apr 97	868	8.45	py	l	30.8	e	5
5 May 93	626	6.48	sy	e	77.6	—	4
28 May 94	891	7.70	py	l	18.2	e	5
28 May 94	981	9.20	py	l	36.4	e	5
7 May 97	825	3.47	yv	1	0	e, l	5[4]
7 Jun 97	830	4.20	yv	l	0	e, l	5

[1] Most advanced oocytes are py = primary yolk stage; sy = secondary yolk stage; and yv = yolk vesicle stage.
[2] Postovulatory follicles are e = early stage; l = late stage.
[3] Atretic oocytes are e = early stage; l = late stage.
[4] Photographs of histological sections of stage-4 and stage-5 ovaries are shown in Figure 6 (D and E).

Gonadosomatic and hepatosomatic index

The mean GSI for males increased from September and peaked in January after which it declined (Fig. 8A). The mean HSI for males was highly variable between September and December but gradually decreased from January through July (Fig. 8B). The mean GSI for females increased gradually throughout the early part of the year, peaking sharply in May, after which it dropped rapidly (Fig. 8C). The mean HSI for females started to increase in August and peaked in December (Fig. 8D). It remained low between March and July.

With testicular development, the GSI increased and reached a maximum when the testes were in stage 4 (Table 6). There were significant differences in the value of these indices in the stage-4 testes, compared with those in the other three stages ($P<0.05$). The mean HSI for males peaked in stage-1 testes, but there were no statistical differences in the value of the median HSI between the four stages (ANOVA, $P>0.05$).

In females, the mean GSI gradually increased and peaked in stage-3 ovaries (Table 7), but there were no statistical differences in the value of the median GSI between the early stage-2 and stage-5 ovaries ($P>0.05$). The mean HSI peaked in early stage-2 ovaries and then dropped rapidly with ovarian development. The median HSI during the early and late stage-2 ovaries was significantly higher than in stage-1, stage-3, and stage-5 ovaries ($P<0.05$).

Size-frequency distribution of oocytes

The size of all the oocytes in a group gradually increased in tandem with ovarian development (Fig. 9). When an advanced group of the oocytes reached the secondary yolk stage, they formed an isolated batch that separated almost completely from adjacent groups of smaller oocytes. Between the tertiary yolk and mature ovary stages, only the oocytes in the advanced batch increased in size, and the remainder of the group remained smaller than 550 µm. This finding would suggest that *L. litulon* is a batch spawner (a conclusion which has been supported by aquarium observations—see "Discussion" section).

Batch fecundity

The relationship between batch fecundity (BF) and total length (TL), based on 15 specimens with secondary yolk

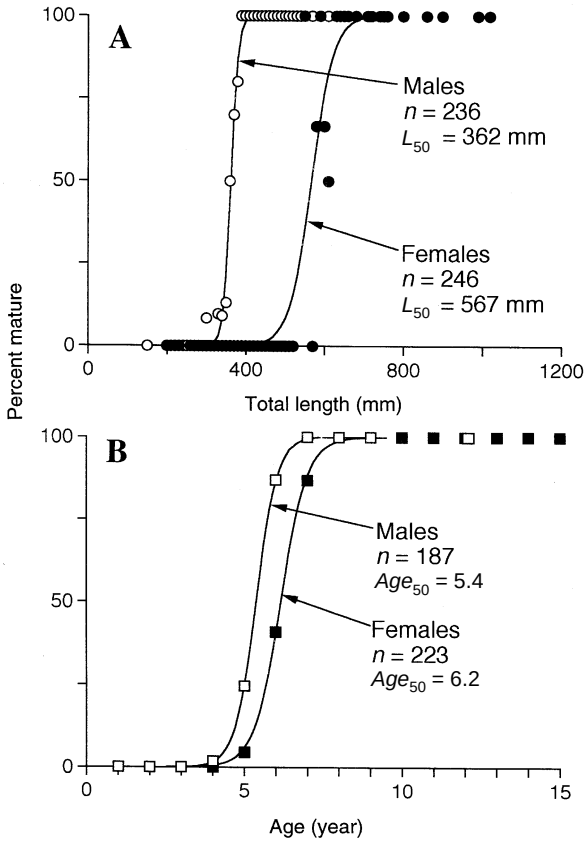

Figure 7

Percent mature fish by size (10-mm length intervals) (**A**) and age (years) (**B**) fitted by a logistic function for male (open circles) and female (solid circles) *Lophius litulon* collected during the spawning season, February through May. n = total number of fish examined; L_{50} = total length at 50% sexual maturity; Age_{50} = age at 50% sexual maturity.

stage ovaries collected between December and February, was described by the equation

$$BF = (-1.64 \cdot 10^6) + 3688.13\ TL$$

for total lengths between 546 and 846 mm (r^2=0.86; P<0.001) (Fig. 10). Batch fecundity ranged from $0.31 \cdot 10^6$ eggs in a 578-mm-TL fish, to $1.54 \cdot 10^6$ eggs in a 796-mm-TL fish.

Seasonal distribution

In September, most of the specimens from both sexes were collected in the Yellow Sea (Fig. 11). Between November and January their distribution extended from the Yellow Sea to the East China Sea. At this time, sampling sites showed a clear difference in the distribution of sexually mature males and females. Males were collected mainly in the East China Sea, whereas females were collected only in the Yellow Sea. During the spawning season, from February throughout May, immature individuals were collected throughout the East China and Yellow seas, whereas mature individuals were caught only in the East China Sea and the coastal waters off Kyushu, and none were caught in the Yellow Sea.

Table 4

Number of males *Lophius litulon* in the East China and Yellow seas at the various maturity stages of the testes by month. Only data from specimens larger than the minimum size at sexual maturity for males (TL=325 mm) have been used in this table. Stage 2 = early spermatogenesis; stage 3 = late spermatogenesis; stage 4 = mature.

	Maturity stage of the testes		
Month	2	3	4
Jan	22	5	12
Feb	40	14	7
Mar	1	4	17
Apr	3	3	5
May	4	24	18
Jun			3
Jul		2	3
Sep		1	3
Oct		8	15
Nov			14
Dec		2	17

Table 5

Number of female *Lophius litulon* in the East China and Yellow seas at the various maturity stages of the ovaries by month. Only data from specimens larger than the minimum size at sexual maturity for females (TL=546 mm) have been used in this table. Stage 1 = immature; stage 2 = developing; stage 3 = mature; stage 4 = spawning; stage 5 = spent; stage 6 = resting.

	Maturity stage of the ovary						
Month	1	2 (early)	2 (late)	3	4	5	6
Jan		5					
Feb	1	8	4		1		
Mar		3	4	1	4	4	
Apr			2	3	1	3	
May					1	3	2
Jun						1	1
Jul	1						2
Aug	1						
Sep	3						
Nov	3	3					
Dec		5					

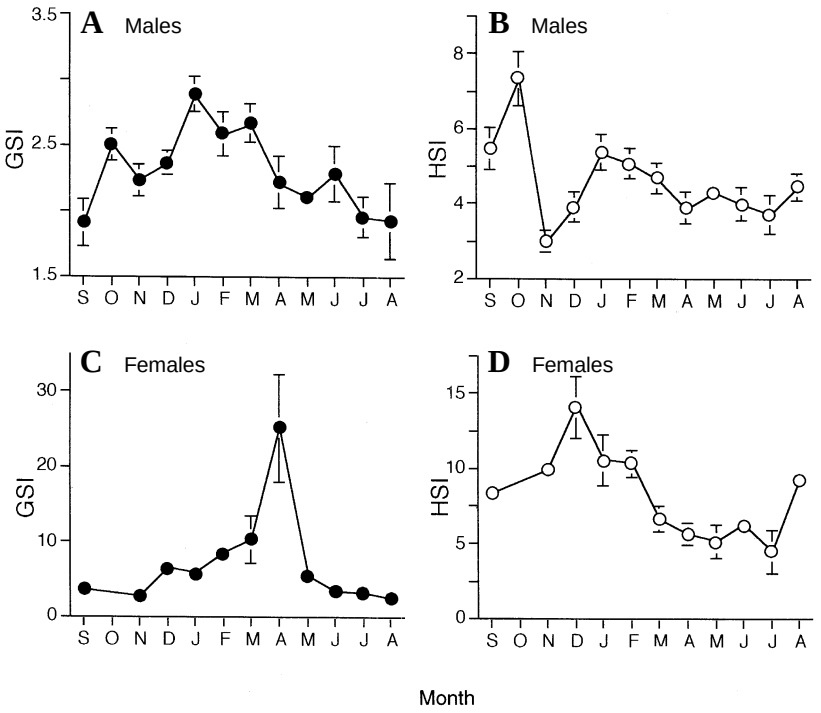

Figure 8

Monthly changes in the mean gonadosomatic index (GSI) and hepatosomatic index (HSI) for mature male (n=306) and female (n=67) *Lophius litulon* in the East China and Yellow seas. Only specimens larger than the size at 50% sexual maturity for males (L_{50}=362 mm) and females (L_{50}=567 mm) were used in our study. Vertical lines indicate standard error.

Discussion

The testicular structure of *L. litulon* is similar to that of other teleosts with unrestricted spermatogonial (Grier et al., 1980; Grier, 1981) or lobular type testes (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 seminiferous tubules and did not differentiate synchronously. This form of spermatogenesis first described in *Lepadogaster lepadogaster* (Mattei and Mattei, 1978), and later termed "semi-cystic" type (Mattei et al., 1993), has subsequently been reported in Neoceratiidae (Jespersen, 1984), blennioid fishes (Lahnsteiner and Patzner, 1990), *Ophidion* sp. (Mattei et al., 1993), *Opistognathus whitehurstii* (Manni and Rasotto, 1997), and *L. setigerus* (Yoneda et al., 1998c).

The ovarian structure of *L. litulon* is similar to that reported in other Lophiiformes such as *L. piscatorius* (Afonso-Dias and Hislop, 1996), *Antennarius scaber*, *Histrio histrio*, and *Ogcocephalus vespertilio* (Rasquin, 1958), *L. americanus* (Armstrong et al., 1992), and *L. setigerus* (Yoneda et al., 1998a). Most female Lophiiformes are thought to spawn gelatinous egg masses, within which individual eggs float in separate chambers (Rasquin, 1958; Armstrong et al., 1992; Afonso-Dias and Hislop, 1996; Yoneda et al., 1998a, 1998c). In *L. litulon*, as in other lophiiform fishes (Rasquin, 1958; Armstrong et al., 1992; Yoneda et al., 1998a, 1998c), gelatinous material was secreted from the epithelium of both the ovigerous lamellae and the ovarian wall. Rasquin (1958) compared the structure of the ovary of *H. histrio* with that of the released egg mass and concluded that the shape of the egg mass was a replica of the internal surface of the ovary. This is expected to be the case in other fishes of the family Lophiiformes. Each stalk-like ovigerous lamella is thought to serve as a "mold," forming a separate chamber within the gelatinous egg mass. The arrangement of oocytes, with the most advanced oocytes at the margins of the ovigerous lamellae, may facilitate the release of mature oocytes into each chamber.

Our examination of the gonadal condition of both sexes indicates that *L. litulon* spawns during the period from February through May. Most females with late-developing, mature, or spawning ovaries are found in March and April. This finding agrees with previous reports. The peak of the spawning season of *L. litulon* occurred between February and March in inshore waters off Kyushu Island (Mito, 1963) and in March and April in the East China and Yellow seas (Yamada, 1986). In Sendai Bay, off the northeast coast of Honshu Island, Japan, the spawning season of *L. litulon* occurs between May and July (Kosaka, 1966).

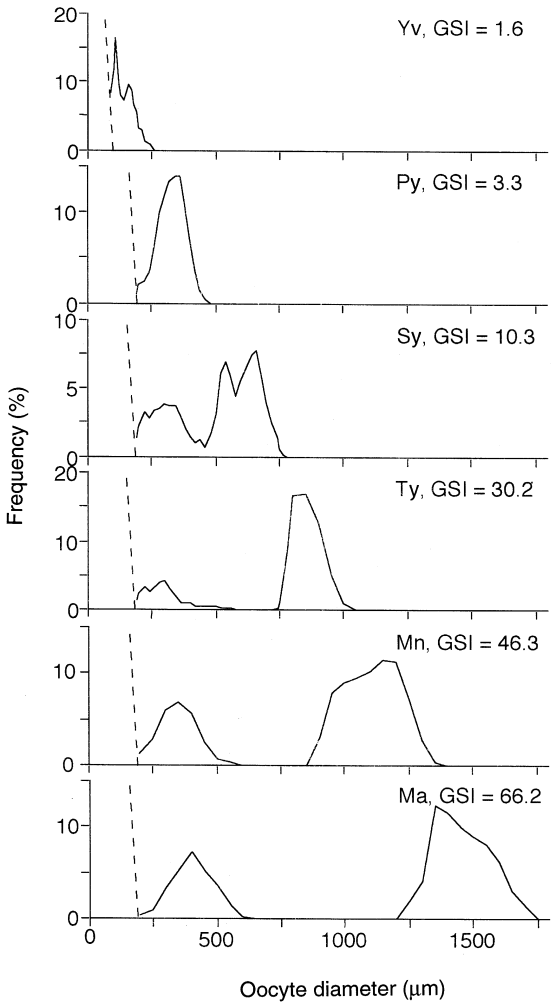

Figure 9

Size-frequency distribution of oocytes at the different stages of maturation in the ovaries of *Lophius litulon*. All oocytes ≥100 µm diameter were measured in yolk vesicle stage ovaries, whereas at all other ovarian stages only oocytes ≥200 µm were measured. GSI = gonadosomatic index; Yv = yolk vesicle stage; Py = primary yolk stage; Sy = secondary yolk stage; Ty = tertiary yolk stage; Mn = migratory nucleus stage; Ma = mature stage.

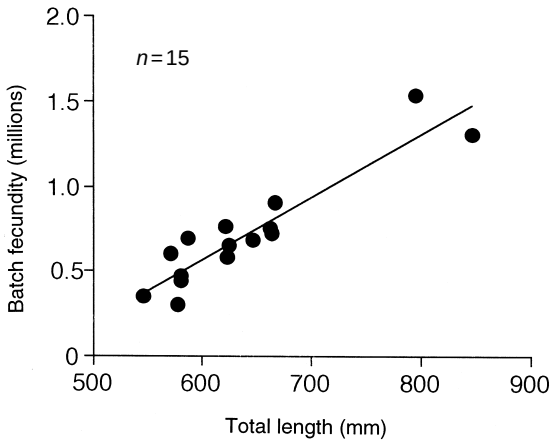

Figure 10

Relationship between batch fecundity and total length for *Lophius litulon*. Females (n=15) with secondary yolk stage ovaries were collected from December through February. $BF = (-1.64 \cdot 10^6) + 3688.13\ TL$ ($546 \leq TL \leq 846$).

Table 6

Gonadosomatic index (GSI, mean ± SE) and hepatosomatic index (HSI, mean ± SE) at each stage of maturity for male and female *Lophius litulon*. Values with different superscripts are significantly different (Dunn's multiple comparison test, $P<0.05$). See tables 5 and 6 for definitions of maturity stages.

Maturity stage of the gonads	*n*	GSI	HSI
Male			
1	47	1.17 ±0.25^a	5.57 ±0.77
2	143	2.00 ±0.22^a	4.70 ±0.44
3	63	2.09 ±0.10^a	4.80 ±0.37
4	114	2.48 ±0.07^b	4.50 ±0.24
Female			
1	206	1.34 ±0.08^a	5.40 ±0.25^a
2 (early)	24	5.73 ±0.42^b	10.38 ±0.70^b
2 (late)	10	15.27 ±2.02^b	11.21 ±1.30^b
3	4	51.78 ±5.49^b	3.58 ±0.23^a
4	7	7.07 ±0.39^b	6.03 ±0.84^{ab}
5	11	6.58 ±0.61^b	4.34 ±0.53^a
6	5	2.85 ±0.37^a	6.37 ±0.94^{ab}

This difference in spawning season depending on latitude would indicate that the spawning season of *L. litulon* in Japanese waters occurs progressively later, with increasing latitude. This also occurs with *L. americanus* (Bigelow and Schroeder, 1953) in American waters and with *L. piscatorius* (Afonso-Dias and Hislop, 1996) in northern European waters.

After spawning, ovaries in two conditions (stages 4 and 5) were observed during our study. Females had postovulatory follicles and yolked oocytes at the primary or secondary yolk oocyte stages. Secondary yolk stage oocytes were more often present in the ovaries of females collected in the first half of the spawning period (February–March). It seems likely that these normally develop into yolked oocytes because no signs of oocyte atresia were observed in histological examination. Specimens with primary yolk oocytes were collected between March and May. However, there was evidence of atresia in half of the fish collected during the latter half of the spawning period (April–May).

A solitary female *L. litulon*, in Oarai Aquarium, released an infertile egg mass on 19 April 1994 and 35 days later extruded another mass.[2] Similarly, another captive female spawned three times, on 15 February, 2 June, and 21 July 1998. These data indicate that *L. litulon* may have the potential to spawn more than once a year, although the five spawnings observed in the aquarium were not accompanied by normal spawning behavior. Recently, we reported a case of repeated spawning in *L. setigerus*, which has a long spawning period from May to November (Yoneda et al., 1998a). In contrast, *L. americanus* (Feinberg, 1984) and *L. piscatorius* (Afonso-Dias and Hislop, 1996) are believed to spawn only once a season. Future field studies are required to determine the spawning frequency for *L. litulon*, which will be important for estimating its reproductive potential.

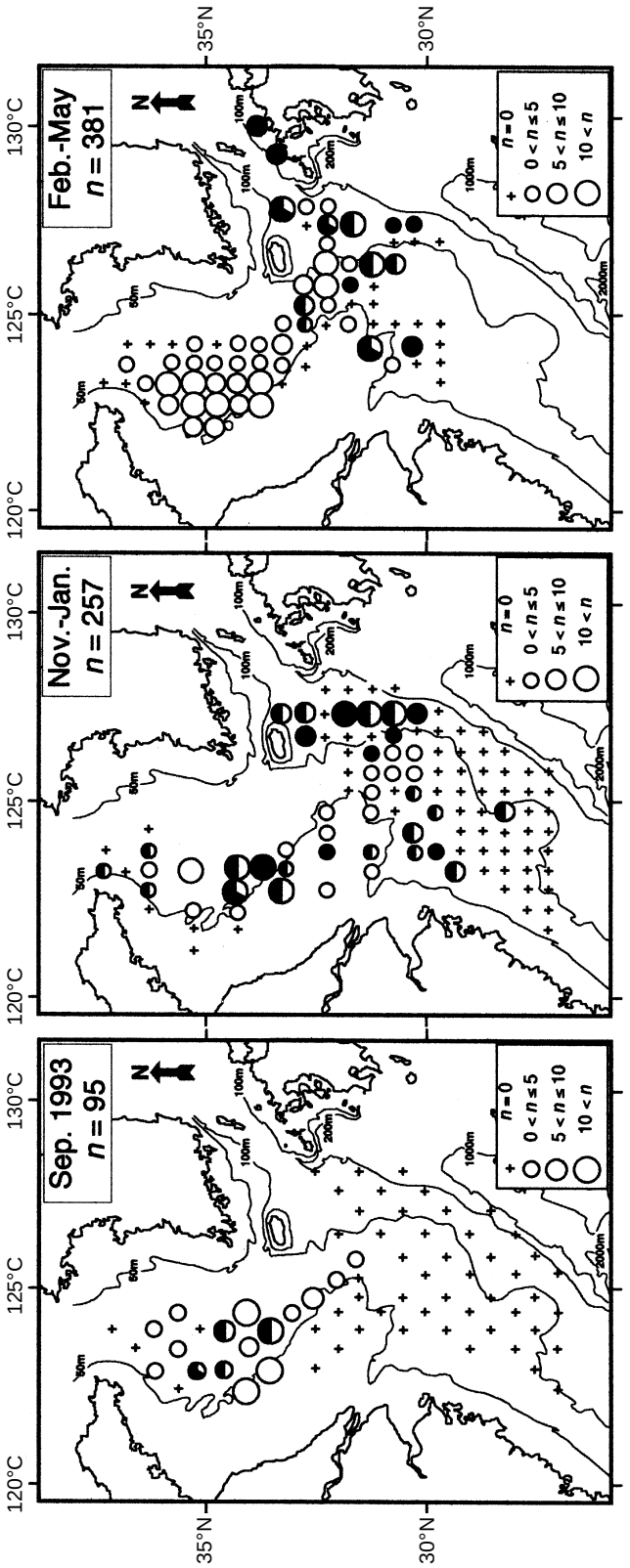

Figure 11

Geographical distribution of specimens of *Lophius litulon* collected in the East China and Yellow seas at three different times of the year. Specimens were identified as sexually immature individuals (open area), sexually mature males (stippled area) and females (solid area). Sexually mature individuals were defined as fish collected in September and November–January, larger than the size at 50% sexual maturity (male=362 mm TL, female=567 mm TL) or fish caught in February–May with stage 4 testes or ovaries that had matured to a least the late-2 (tertiary yolk stage of oocyte) stage. *n* = number of fish examined.

Both sexes of *L. americanus* inhabiting northern waters were larger and a little younger at 50% sexual maturity than fish from more southern waters (Almeida et al., 1995). In *L. litulon,* there also appears to be a size difference at sexual maturity between fish from the East China and Yellow seas (our study) and those from Sendai Bay off northeast Honshu Island (Kosaka, 1966); the minimum size at sexual maturity for males and females was 340 mm body length (BL) and 600 mm BL for Kosaka's report and 325 mm TL and 546 mm TL for our results, respectively. The study from Sendai Bay, however, was carried out about 30 years ago and did not identify the age at sexual maturity; therefore the differences by area for size at sexual maturity may be attributed to different sampling times of the year or to different growth rates. In the East China and Yellow seas, females *L. litulon* reached sexual maturity at larger than 500 mm BL, and the minimum size at sexual maturity was about 350 mm BL in male, as previously reported by Yamada (1986). The size at sexual maturity for female *L. litulon* in the 1980s (Yamada, 1986) is fairly close to our results, whereas that for males is higher than that found in our study. Although the reason for the size difference found in males at sexual maturity is unclear, it may be due to a difference in criteria for maturity. Our histological criteria for maturity in males were based on the characteristics of testicular development with unique spermatogenesis; therefore our determination of sexual maturity is more reliable than macroscopic methods.

There was a noticeable inverse correlation between the development of the ovary and the weight of the liver (HSI). The de-

[2] Kofuji, K. 1998. Personal commun. Oarai Aquarium, Isohama, Oarai, Higashi-Ibaraki, Ibaraki 311-1301, Japan.

crease in HSI that is associated with increasing GSI is probably due to materials that have been stored in the liver becoming mobile and being transferred to the gonads. In teleosts, as in most other vertebrates, the precursor protein of yolk (vitellogenin) is synthesized in the liver. The secreted vitellogenin is selectively removed from the bloodstream by the developing oocytes (Wallace and Selman, 1981; Nagahama, 1987). The rapid accumulation of yolk probably accounts for the decrease in the weight of the liver.

In many fish, batch fecundity is estimated by using migratory nuclei or hydrated oocytes, which can be easily distinguished from the less advanced oocytes: e.g. *Engraulis mordax* (Hunter and Goldberg, 1980; Hunter et al., 1985), *Thunnus albacares* (Schaefer, 1996), and *Rhomboplites aurorubens* (Cuellar et al., 1996). We found that during and after the tertiary yolk stage, a large amount of gelatinous material was rapidly secreted and accumulated in the ovarian lumen. Hence, counts of advanced oocytes from a small portion of the ovary, when extrapolated to the total weight of the gelatinous material, may give more variable estimates. These findings were also evident in *L. setigerus* (Yoneda et al., 1998a). However, the oocyte size-frequency profiles indicated that when the most advanced oocytes reached the secondary yolk stage, they formed a batch that was almost completely separated from the adjacent group of smaller oocytes. These ovarian characteristics of *L. litulon* imply that estimates of batch fecundity can be made only by using oocytes that have attained the secondary yolk stage.

Both the immature and mature distribution of *L. litulon* ranged into the East China and Yellow seas, as previously reported by Yamada (1986) and Tokimura.[1] This species is caught mainly at depths between 50 and 100 m and at temperatures ranging from 6 to 13°C (Yamada, 1986; Tokimura[1]). In the Yellow Sea, the Yellow Sea Central Cold Water, cooler than 10°C, is found throughout the year and there are few seasonal changes in water temperature (±2–3°C). The water of the East China Sea remains lower than 13°C owing to the influence of the Continental Coastal Cold Water in the winter and spring, whereas in summer it increases higher than 20°C (Kondo, 1985; Tokimura[1]). These oceanographic conditions in the East China and Yellow seas may influence the migration of *L. litulon* from area to area throughout the year. A seasonal movement of *L. litulon* has also been reported in Sendai Bay (Kosaka, 1966; Omori, 1979). *Lophius litulon* are most abundant in shallow waters between February and June. From August through October, they disperse toward deeper waters. This seasonal movement in Sendai Bay is observed mainly in immature fish and is thought to be associated with their feeding activities (Kosaka, 1966). In *L. americanus*, a seasonal migration, thought to occur in response to changes in hydrographic conditions, has been observed along the northeastern coast of the United States (Jean, 1965; Almeida et al., 1995).

During the February–May spawning season, mature males and females with ovaries in a condition that suggests they are either about to spawn, or have just spawned, are found in the East China Sea and the coastal waters off Kyushu. In contrast, immature individuals were distributed throughout the East China and Yellow seas during this same period. This indicates that the spawning grounds of *L. litulon* cover a large area, extending from the East China Sea to inshore waters off Kyushu. Furthermore, our study reveals the migratory pattern of both sexes of *L. litulon* in relation to the spawning grounds in the period before the spawning season. In February, with the onset of the spawning season, females collected in the northern East China Sea had developing ovaries (secondary or tertiary yolk stage), whereas those collected in the Yellow Sea had immature or primary yolk stage ovaries. This finding implies that the start of the migration of females seems to be more dependent on ovarian development than oceanographic conditions. Different migratory patterns of the two sexes before spawning have also been reported in European plaice, *Pleuronectes platessa*, in the Straits of Dover off England (Arnold and Metcalfe, 1995). Our study has identified the spawning grounds and migratory pattern of *L. litulon* in broad terms. Further research is needed to identify specific spawning grounds and details of the migratory behavior of this species.

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