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Maturity and Spawning of Snailfish, *Liparis ochotensis* (Schmidt), in the East Sea

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Maturity and spawning of the snailfish, *Liparis ochotensis* was investigated based on samples collected by gill net in the East Sea of Korea from December 2008 to December 2009. The average total length of *L. ochotensis* was 63.4 cm and 64.1 cm for females and males, respectively. Gonadosomatic index (GSI) began to increase in July, reaching a maximum in November, then began to decrease in December. Monthly changes in the GSI values of *L. ochotensis* were similar for females and males. Annual reproductive cycle of this species can be divided into five successive stages in females: early growing stage (May), late growing stage (June to July), mature stage (August to September), ripe and spent stage (September to December), and recovery and resting stage (January to April). Males show four successive stages; growing stage (May to July), mature stage (August to September), ripe and spent stage (September to December), and recovery and resting stage (January to April). Relationship between total length (TL) and fecundity (F) was $F=0.00003TL^{4.002}$ ($R^2=0.703$), and F increased with TL. Total length at 50% group maturity was estimated to be 55.8 cm. Our findings suggest that the spawning period takes place from September to December, with the main spawning period occurring from October to December.

Key words: East Sea, Maturity, *Liparis ochotensis*, Snailfish, Spawning

Introduction

Liparis ochotensis is a fish species belonging to the family Liparidae (Nakabo, 2002; Kim et al., 2005a) and inhabits the East Sea around Hokkaido, Japan, as well as the Sea of Okhotsk and the Kuril Islands (Kim et al., 2005b). *L. ochotensis* is mostly found between 50 and 200 m depths. Morphologically, its body is small and translucent, with round spots covering the head and body; males are dark purple, and females are yellowish brown (Kim et al., 2005b). Hatched eggs of *L. ochotensis* are entangled together, forming a lump, known as demersal eggs (Kim et al., 1986a). This species is easily confounded with *L. tanakai*, and, to date, it has been the focus of only a few taxonomy and ecology studies.

L. ochotensis is caught mainly in the East Sea with coastal gill nets by the coastal complex and eastern sea trawl fishery. Its commercial value is very high, but the sustainability of the catch remains uncertain. To sustainably harvest *L. ochotensis*, efficient resource management is required through scientific stock assessment, which requires ecological assessments of *L. ochotensis*.

Many studies have examined the Liparidae family of fish, including a brief record on several juvenile fishes species (Matsubara and Iwi, 1954), a study examining selection and adaption in the life cycle of *Liparis tanakai* (Kawasaki et al., 1980), an examination of the larva and juvenile fishes of the *L. tanakai* family in the northwest sea of Japan (Sokolvoskii and

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Sokolovskaya, 2003), domestic studies of spawning and hatched juveniles of *L. ochotensis* (Kim et al., 1986a) and *L. tanakai* (Kim et al., 1986b), and studies of the prey habits of *L. tanakai* (Hun, 1997) and the larva of *L. tanakai* inhabiting the seagrass beds of Gwangyang Bay (Kwak and Huh, 2003). However there is currently no published ecological examination of the maturity and spawning of *L. ochotensis*.

Thus, we examined the maturity and spawning of *L. ochotensis* in the East Sea of Korea, focusing on the gonad development process, monthly variation in the gonadosomatic index, oocyte diameter, number of eggs, and length at 50% group maturity from December 2008 to December 2009. This is the first report of the reproductive characteristics of *L. ochotensis*, which will help provide an understanding of its population structure. .

Materials and Methods

L. ochotensis were collected monthly from the East Sea from December 2008 to December 2009 (Fig. 1). Collected samples were sorted into males and females and measured for total length (TL) to the nearest 0.1 cm, body wet weight (BW) to the nearest 0.1 g, and gonad wet weight (GW) to the nearest 0.01 g.

To investigate the gonad development process of *L. ochotensis*, gonad maturity was checked by visual observation for gonad size, color, transparency of eggs, and egg size. The gonad maturity process was divided into the following four stages: early and late growing, mature, ripe, and spent.

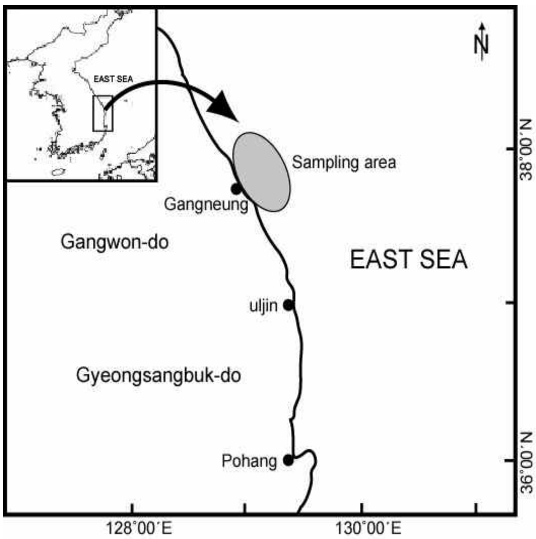

Fig. 1. Sampling area for *L. ochotensis* caught by gill net in the East Sea from December 2008 to December 2009.

The gonadosomatic index (GSI) was determined as follows:

$$GSI = \frac{GW}{BW} \times 10^3,$$

where GW is gonad wet weight (g), and BW is body wet weight (g).

The internal structure of gonads and histological changes throughout development were observed after extraction from collected samples. A portion of the testis and ovary was fixed in Bouin's fluid, embedded in paraffin wax, sectioned at 4-6 μm, and stained with Mayer's hematoxylin, followed by 0.5% eosin counter-staining. Specimens were then enclosed with marinol following the dewatering process and examined by light microscopy (DE/Axio image D1, Zeiss, Germany).

Oocyte diameter was measured at the major axis with a profile projector (Nicon, V-16E) after the egg block had been separated from the ovary in the improved Gilson solution (Love and Westphal, 1981).

Fecundity (*F*) was calculated as follows using the wet weight method after selection of mature eggs that had not been fully laid by observation with a microscope, separation from the individual by fine needle, and counting using a dissecting microscope (Bagenal and Braum, 1978):

$$F = \frac{A - B}{C'} \times e$$

where *F* is eggs per ovary, *A* is ovary weight, *B* is ovary shell weight, *C* is the weight of the section of the ovary, and *e* is the spawn number in *C*.

Length at 50% group maturity was calculated as the ratio of mature individuals by considering the over-mature individuals during the spawning period as the number of potential spawners in a year, and was estimated using a logistic formula (Zhang, 1991) as follows:

$$P_i = \frac{1}{1 + e^{(b_1 - b_2 TL_i)}}$$

where, P_i is group maturity ratio at the i body-length level, TL_i is total length at the *i* body-length level, and b_1 and b_2 are constants.

Results

Length-frequency distribution

Total number of *L. ochotensis* examined was 534, including 304 females and 230 males. The range of total length was 42.0-83.0 cm for females and

33.4-90.7 cm for males, and the average total length was 63.4 cm and 64.1 cm for females and males, respectively (Fig. 2).

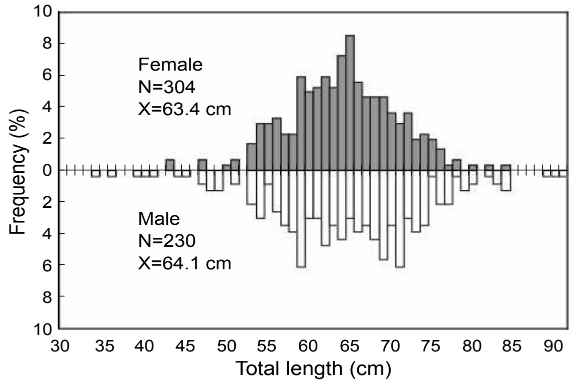

Fig. 2. Length-frequency distribution of *L. ochotensis* in the East Sea from December 2008 to December 2009.

Estimation of spawning period

Monthly changes in maturity

Visual analysis of the four stages of ovary maturity revealed that the ovary was dwarfish in the early and late growing stage, with milky white eggs that were difficult to see. In the mature stage, the ovary size gradually increased, and both light pink and milky white eggs were seen. The ovary reached its maximum size during the ripe stage, and the eggs were light yellow, >1 mm diameter, and easily separated. During the spent stage, the ovary shrank and hung low, and small milky white eggs were observed.

Observation of the monthly changes in maturity of *L. ochotensis* (Fig. 3) revealed that 9.1-100% of early- and late-growing-stage individuals were found between December 2008 and August 2009, whereas mature individuals accounted for 4.2-90.9% of individuals

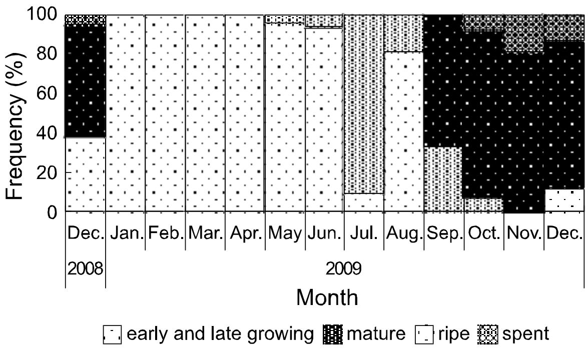

Fig. 3. Monthly distribution for the different maturity stages of female *L. ochotensis* in the East Sea from December 2008 to December 2009.

from May to October 2009 and 6.7-84.6% of individuals from September to December 2009. Spent individuals accounted for 7.7-12.5% of those caught in December 2008 and from October to December 2009.

Monthly changes in gonadosomatic index

GSI of female *L. ochotensis* (Fig. 4A) showed a high value of 7.9 in December 2008, which rapidly decreased in January 2009, ranging from 1.2-2.6 till June. It showed a slight increase to 6.1 in July 2009, then rapidly increased to 14.0 in September 2009, maintaining a level of 9.2-15.7 from October to December 2009.

GSI of male *L. ochotensis* (Fig. 4B) was 2.0 in December 2008, which rapidly decreased to less than 1.0 in January 2009, a level that was maintained until May. It started to increase and was 1.1-1.3 from June to August 2009 and 1.8-2.4 from September to November 2009, then decreased again to less than 1.0 in December. Thus, monthly changes in GSI values of *L. ochotensis* were similar for females and males.

Given the observed gonad growth process and monthly changes in gonadosomatic index, the general spawning period of *L. ochotensis* was estimated to occur from September to December, with the main

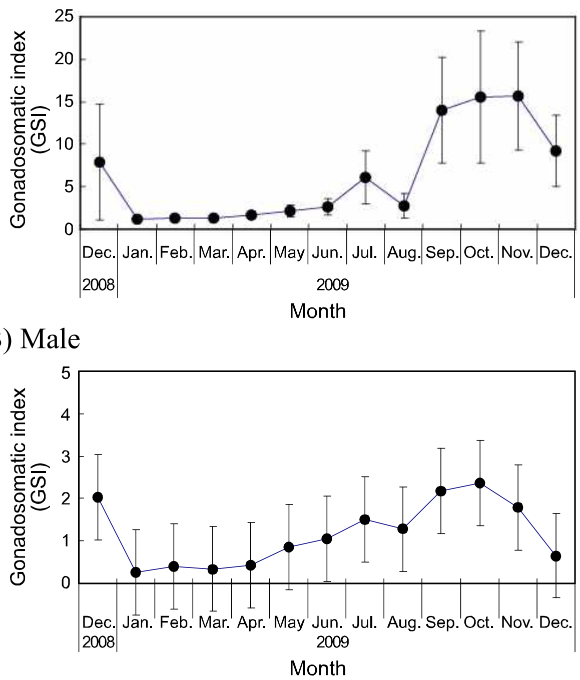

Fig. 4. Monthly changes in gonadosomatic indices (GSI) of female and male *L. ochotensis* in the East Sea from December 2008 to December 2009.

spawning period taking place from October to

December.

Histological variation of gonad development

Ovary

Early growing stage

Initial ovary activation occurred in May, with chromatin-nucleolus oocytes and peri-nucleolus oocytes 20-150 μm in diameter appearing in the ovarian lobules during the early growing stage (Fig. 5A).

Late growing stage

From June to July, most individuals exhibited late-growing-stage ovaries, with oocytes 150-800 μm in diameter. Yolk and yolk granules occupied the lobules of the ovaries (Fig. 5B, C).

Mature stage

Ovaries showed rapid growth from August to September and contained yolk-filled oocytes 500-1,000 μm in diameter. Ovaries showing migratory nucleus stage were observed (Fig. 5D, E).

Ripe and spent stages

Ripe ovaries were observed from September to December, when mature eggs approximately 1,100-1,700 μm in diameter filled the ovarian lobules of females (Fig. 5F). Ovaries showing residual follicles were also found (Fig. 5G).

Recovery and resting stage

Non-discharged oocytes and remaining follicles were recovered and absorbed in the ovarian lobules of female individuals that had finished spawning. After December, ovarian lobules were shrunken. Individuals within the recovery and resting stage were observed until April (Fig. 5H).

Testis

Growing stage

Spermatogonia were actively dividing and proliferating in the epithelium of fat testicular lobules, and spermatocytes were found from May to July (Fig. 6A).

Mature stage

From August to September, spermatocytes in maturation division, spermatids in transformation, and sperm as well as some spermatogonia were found in the testicular lobules (Fig. 6B).

Ripe and spent stage

Spermatozoa after transformation were densely aggregated in the testicular lobules and vasa efferentia, and sperm were spent from September to December (Fig. 6C).

Recovery and resting stage

Following the release of sperm, individuals recovered while sperm were absorbed and the gonads became shrunken. Some spermatogonia were arrayed on the epithelium of the testicular lobules. Individuals in the resting stage were found from January to April (Fig. 6D).

Oocyte diameter

Monthly changes in oocyte diameter (Fig. 7) were such that, after the spent stage, oocytes ranged from 0 to 500 μm during the growing stage until July and from 500 to 1,000 μm in the mature stage from July to September. The oocyte diameter range during the ripe stage was 1,100-1,700 μm, indicating that oocyte diameter increased during ovary maturation, following a unimodal normal distribution.

Fecundity

Fecundity was analyzed by following 35 mature individuals from September to November (Fig. 8). Fecundity ranged from 463,250 to 1,351,482 eggs. Fecundity increased with total body length with the following relationship: $F=0.00003TL^{4.002}$ ($R^2=0.703$).

Length at 50% group maturity

To determine total body lengths of individuals participating in reproduction, 114 mature females collected between August and December were examined (Fig. 9). In total, 25% of individuals ≤53 cm long were found to be mature, whereas all individuals ≥63 cm in length were mature. After applying the appearance ratio of mature individuals by body size grade to the logistic formula, the body length of *L. ochotensis* females at 50% group maturity was estimated to be 55.8 cm (TL).

Discussion

We assessed the maturity and spawning of *L. ochotensis* caught in the East Sea from December 2008 to December 2009. *L. ochotensis* belongs to the family Liparidae and exhibits similar morphological characteristics to *L. tanakai*. The two species are distinguished by the existence of a ridge on the pectoral fin base of *L. ochotensis* (Kim et al., 2005b). Additionally, it has been suggested that *L. ochotensis* and *L. ingens* are the same species (Kido, 1988). *L. ochotensis* only inhabit the East Sea (Kim et al.,

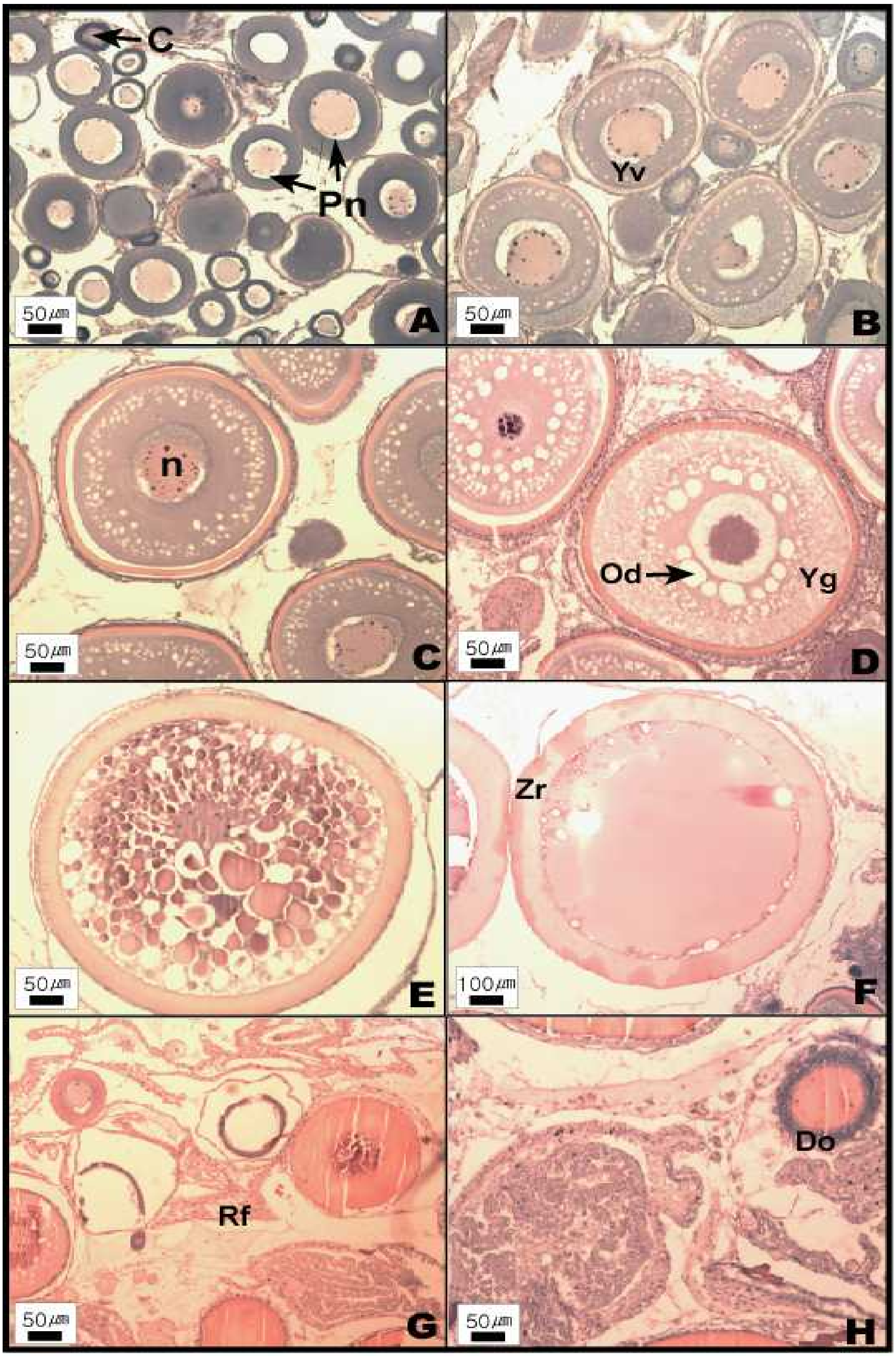

Fig. 5. Photomicrographs of ovarian developmental phases of *L. ochotensis*. A: Early growing stage, B-C: Late growing stage, D-E: Mature stage, F: Ripe stage, G: Spent stage, H: Recovery and resting stage. C: chromatin, n: nucleus, Pn: perinucleolus, Yv: yolk vesicle, Od: oil droplet, Yg: yolk globules, Zr: zona radiata, Rf: residual follicle, Do: degenerating oocyte.

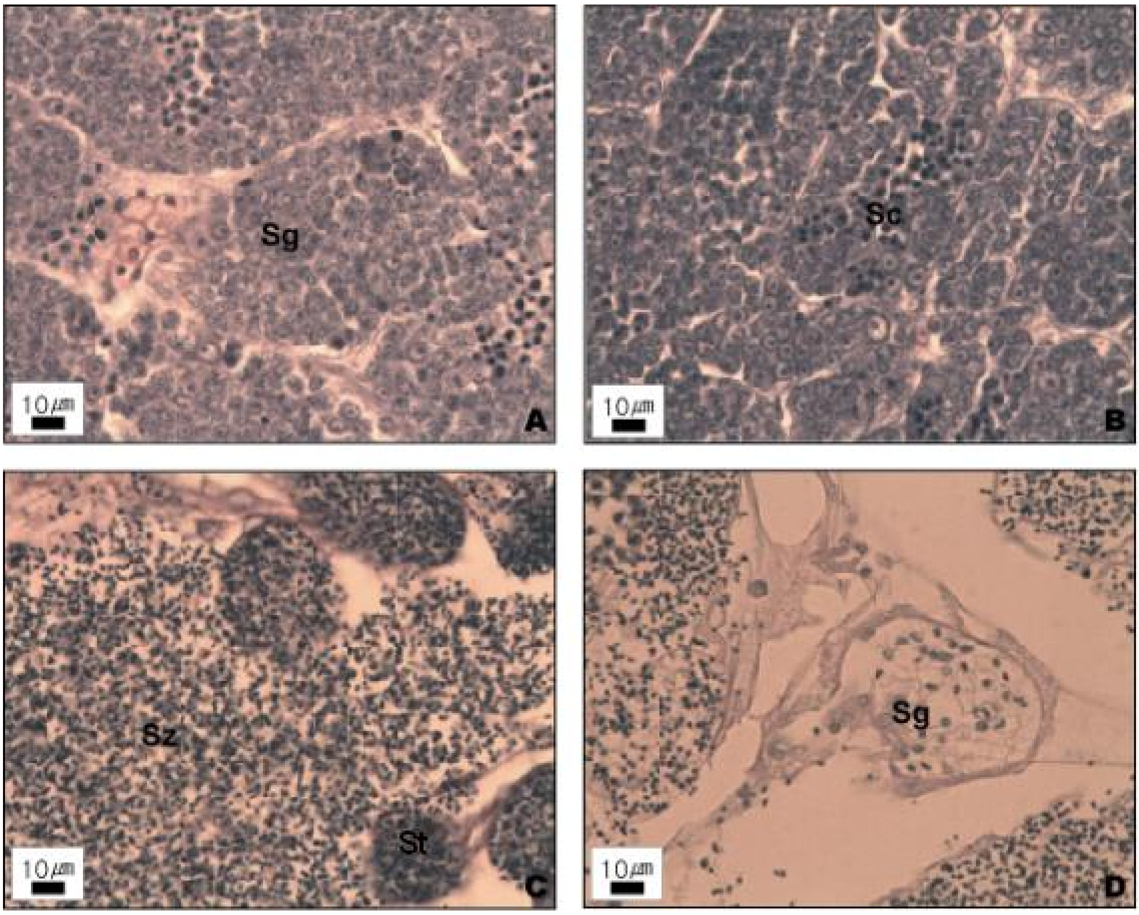

Fig. 6. Photomicrographs to testicular developmental phases of *L. ochotensis*. A: Growing stage, B: Mature stage, C: Ripe and spent stage, D: Recovery and resting stage. Sg: spermatogonium, Sc: spermatocyte, St: spermatid, Sz: spermatozoon.

2005b), whereas *L. tanakai* are distributed throughout the coasts of Korea (Kim et al., 2001; NFRDI, 2004). Mature eggs of *L. ochotensis* were light yellow and filled the gonads. Kim et al. (1986a) reported that yolk of discharged eggs of *L. ochotensis* was straw yellow or light purple in color, which is in agreement with our observations. Ripe individuals were observed from September to November, and the gonadosomatic indices (GSI) started to increase in September and maximized in October. Thus, the main spawning period of *L. ochotensis* was estimated to occur from October to December.

Kim et al. (1986a) reported that mature *L. ochotensis* were also collected in January, suggesting that the spawning period extends one month longer than we observed. Generally, fish maturity and spawning occur when the environment is suitable, and the growth of internal gonadal structures shows periodicity based on the spawning period. Such periodic changes tend to be dominated by external factors such as water temperature and photoperiod, which change the endocrine activities in the fish body, directly effecting reproductive phenomena (De Vlaming, 1972; Nishi, 1979; Shimizu and Hanya, 1983). Thus, we propose that the differences in spawning period between our study and that of Kim et al. (1986a) reflect differences in the environmental conditions between the two study periods.

Previous reports of the spawning period of Liparidae species, which are similar to *L. ochotensis*, suggest that *L. tanakai* spawn from December to February (Kim et al., 2005b), and *L. choanus* spawn from winter to spring (Kim et al., 2005b). Kim et al. (1986b) reported catching spawning *L. tanakai* in December, and another report suggests that mature *L. tanakai* individuals occurred from October to November (Huh, 1997). Therefore, it appears that the spawning period of *L. ochotensis* is separated from that of *L. tanakai* or *L. choanus* by a 1-3 month gap.

Aida (1991) divided the reproduction periods of

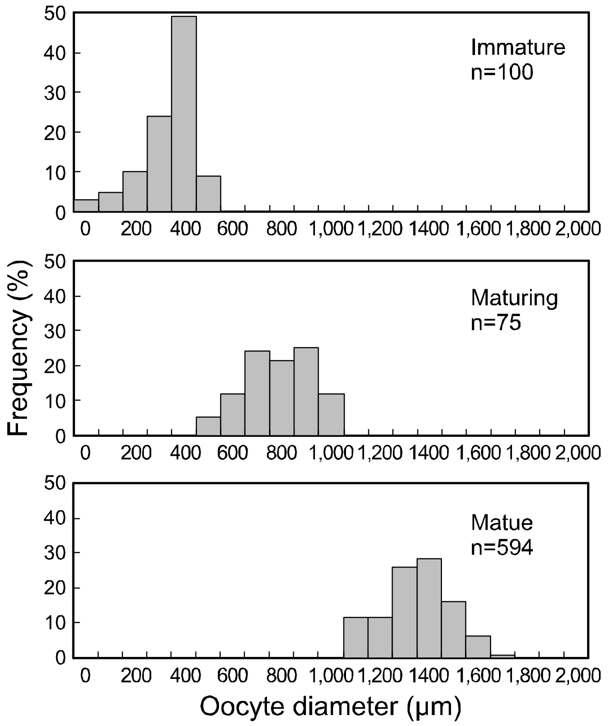

Fig. 7. Size frequency distributions of oocyte diameter of *L. ochotensis* in the East Sea.

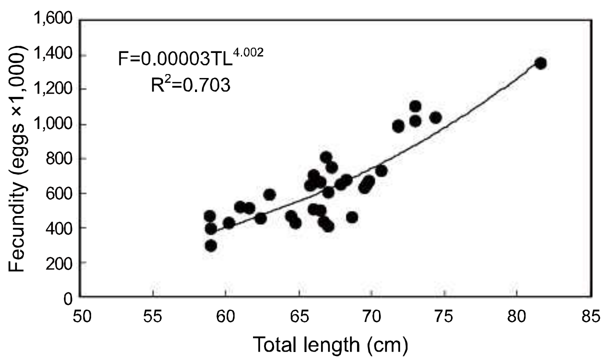

Fig. 8. Relationship between total length and fecunddity of *L. ochotensis* in the East Sea from December 2008 to December 2009.

fishes into the following six types: spring spawner, spring-to-summer spawner, summer spawner, spring-to-autumn spawner, autumn spawner, and winter spawner, and suggested that these types are related to environmental factors such as water temperature and photoperiod. Given that the main spawning period of *L. ochotensis* in the East Sea occurred between October and December, this species appears to be an autumn spawner.

We found that oocyte diameter of ripe *L. ochotensis* from September to November was 1,100-

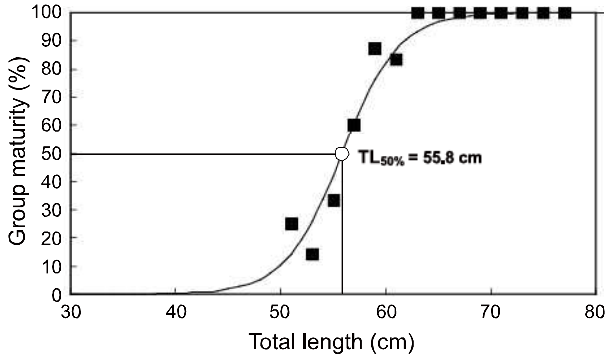

Fig. 9. Relationship between total length and group maturity of female *L. ochotensis* in the East Sea from December 2008 to December 2009.

1,700 μm, whereas Kim et al. (1986a) reported it to be 1,550-1,650 μm. Thus, oocyte diameter increases to more than 1500 μm immediately before spawning. The reported oocyte diameter for *L. tanakai* was 1,680-1,780 μm (Kim et al., 1986b), demonstrating that the oocyte diameter of *L. ochotensis* is smaller than that of *L. tanakai*. Additionally, the progression of oocyte diameter can be predicted by the spawning frequency during the spawning period (Kim, 1993). Specifically, *L. ochotensis* appears to be a one-time spawner, as oocyte diameter showed a unimodal normal distribution during the spawning period.

Fecundity of *L. ochotensis* ranged from 463,250-1,351,482 eggs, and increased with total body length. Osteichthyes fish are generally known to have a tendency toward increased fecundity as body length increases (Kim and Zhang, 1994); *L. ochotensis* appears to have followed this pattern.

Length at 50% group maturity of *L. ochotensis* was estimated to be 55.8 cm in females; however, it was not possible to assess the age of the mature group.

Until now, the available ecological information on *L. ochotensis* was insufficient relative to its com-mercial value. Based on the results of this research, the resource status of *L. ochotensis* must be established for effective, sustainable management, and subsequent examinations of age and growth are required.

Acknowledgements

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