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Maturation and Spawning Frequency of *Lestrolepis japonica* (Aulopiformes: Paralepididae) in Kagoshima Bay, Southern Japan

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Abstract

The maturation and spawning frequency of a paralepidid fish *Lestrolepis japonica* were studied in 270 specimens collected in Kagoshima Bay, southern Japan, from April to December 2002 (excluding November). Monthly changes in frequency of mature ovaries and gonadosomatic indices indicated that spawning started in May, peaked in July to September, and was completed in December. From the appearance of age 0-day postovulatory follicles (POF0) and age determination, spawning months lasted from July to October in age 0→1 fish (arrow indicates the addition of age in September), June to October in age 1→2 fish, May to October in age 2→3 fish, and May to August (the month of life span) in age 3 fish. Spawning frequencies were estimated from the fraction of POF0. The age 0→1 fish spawned 49 times, age 1→2 fish 62, age 2→3 fish 73, and age 3 fish 51, totaling 234 times in life. Reliable batch fecundity could not be obtained because of the scarcity of specimens with hydrated oocytes.

Mesopelagic, synchronously hermaphroditic fishes of the family Paralepididae, characterized by a compressed, moderately to extremely elongated body and sharply pointed jaws, are distributed widely from continental slopes to mid-ocean waters in tropical to cold waters, some species being distributed circumglobally. Ten genera and about 60 species are recognized in the family[1], the largest in the suborder Alepisauroidei.

In spite of such a wide occurrence and speciose composition, the biology of paralepidids has been poorly studied because of their being infrequently collected. However, moderate collections of *Lestrolepis japonica* have been made in Kagoshima Bay, southern Japan, allowing age and growth of that species to be reported, probably being the first such account for the family[2]. In this paper, the maturation and spawning frequency of *L. japonica* are studied.

Materials and Methods

Kagoshima Bay, southern Kyushu, Japan, is latitudinally elongate, opening to the south, and is divided into southern (maximum depth 230 m) and northern (210 m) parts by the narrow, shallow West Sakurajima Channel. The specimens used in this study were caught in the bay from April to December 2002 (except November) by a commercial anchovy purse seine operator, working at night for about 20 days a month before and after the new moon. Specimens and associated collection data were primarily supplied by the purse seine operator, the specimens being kept on ice until arrival at the laboratory. From April to December 2002 (except November, when *L. japonica* was not collected), 270 specimens were obtained, mainly from

Table 1 Catch data of *Lestrolepis japonica* used in this study

Month/Year	No. of specimens	SL, mm
Apr./ '02	14	134.3 - 159.0
May	41	135.6 - 170.5
June	39	148.6 - 184.1
July	8	155.6 - 175.3
Aug.	45	155.0 - 182.5
Sep.	66	157.8 - 193.0
Oct.	46	147.5 - 172.2
Dec.	11	135.8 - 177.2
Total	270	

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the southern part of the bay (Table 1).

In the laboratory, after measurements of standard length (*SL*, mm) and body weight (*BW*, g), sagittal otoliths and gonads were extracted. Gonads were weighed to the nearest 0.01 g (*GW*, g) after removal of surface water with tissue paper and preserved with 10% formalin. Gonadosomatic Index (*GSI*) was calculated as *GSI*=(*GW*/*BW*)x100. Otoliths were cleaned and stored dry in vials for age determination.

External features of gonads were observed under a microscope with reflected light at low magnification (x40). Four phases of maturity were recognized.

Recovering or immature phase: gonads thin, colorless, all oocytes transparent;

Premature phase: gonads thickened, pale yellow, a few medium-sized, opaque oocytes present among many small, transparent oocytes;

Mature phase: gonads increasingly thickened, pale yellow, medium-sized opaque oocytes more numerous than small transparent oocytes, large hydrated oocytes recognizable in a few specimens;

Spent phase: gonads less thickened, rounded in cross section, colorless, all oocytes transparent.

Mid-gonad sections of some 5 mm length were embedded in paraffin wax, cut at 6-9 μm thickness with a rotary microtome, and stained following Harris' hematoxylin and eosin stain method, for observation under light microscope with transmitted light (x200). The development of ovarian oocytes was classified into the following seven stages; chromatin-nucleolus, perinucleolus, yolk vesicle, primary and secondary yolk globule, migratory nucleus, and mature stages, *i.e.*, hydrated oocytes. Postovulatory follicles (POF) and atretic oocytes were also observed.

Observations of ovarian oocytes indicated that hydrated oocytes were present in very few specimens, compared with POF in many specimens during the spawning season. Therefore, the postovulatory follicle method, based on the fraction of age 0-day postovulatory follicles POF0[3], was adopted here so as to estimate spawning frequency. Since aging of POF was not done in this study, the definition of POF0 given by Hunter and Macewicz[3] for northern anchovy, *Engraulis mordax*, was followed.

To determine the onset of spawning month by age (in year) group, ages were examined from otoliths of selected specimens according to Harada and Ozawa[2]. Since the month of adding age was set at September as in Harada and Ozawa[2], fish got one year older during the collection period, and the addition of age is indicated by arrows (*e.g.*, age 0→1).

Using specimens of age groups under spawning, the fraction of spawning (*fm* in month *m*) was calculated as number of specimens with POF0/number of specimens examined. The frequency of spawning (*Fi*) by age group *i*, was calculated as the sum of *fm* · *dm* between months *s* and *e*, where *dm*, days in month *m*; *s*, month at start of spawning; *e*, month at end of spawning. *Fi* was summed so as to obtain the lifetime frequency of spawning, *F*.

Batch fecundity, *i.e.*, number of oocytes shed in a single spawning session, was examined using mature ovaries as follows. The posterior half of each gonad was weighed to the nearest 0.01 g (*gw*) after removal of surface formalin with tissue paper, and put into a petri dish with a small amount of water. All oocytes were separated from each other, those with axis length greater than 0.15 mm being measured to the nearest 0.05 mm under an optical projector at x20 magnification. Oocytes less than 0.15 mm length occurred in very high numbers and were not measured. A frequency distribution of oocyte sizes (0.05 mm intervals) was constructed. Only when the largest mode was well separated from others, was the number of oocytes in the former (*b*) counted and batch fecundity (*BF*) calculated, using the formula, *BF*=*GW*(*b*/*gw*).

Results and Discussion

The appearance of maturity phases of ovaries and frequency distributions of *GSI* by month are shown in Fig. 1. Recovering or immature ovaries were dominant in April, May and December, their *GSI*'s (0.21-1.05) being the lowest of the four phases. Premature ovaries appeared in very low frequencies in May and June; *GSI*'s were low in May (0.42-1.01) but increased in June (1.19-2.65). Mature ovaries were seen in a few specimens in May, in almost all specimens in June, in all specimens in July, August and September, and in almost all specimens in October; *GSI*'s gradually increased from 0.98-2.68 in May to 1.58-4.64 in July, August and September, subsequently decreasing to 1.24-2.93 in October. Low frequencies of spent ovaries were apparent in October and December;

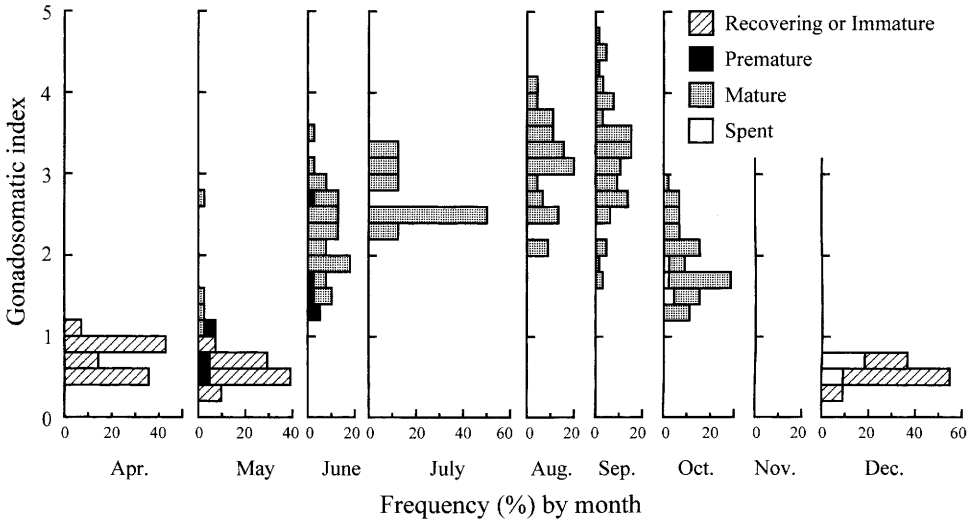

Fig. 1 Phases of ovarian maturity and frequency (%) of gonadosomatic index by month in *Lestrolepis japonica*.

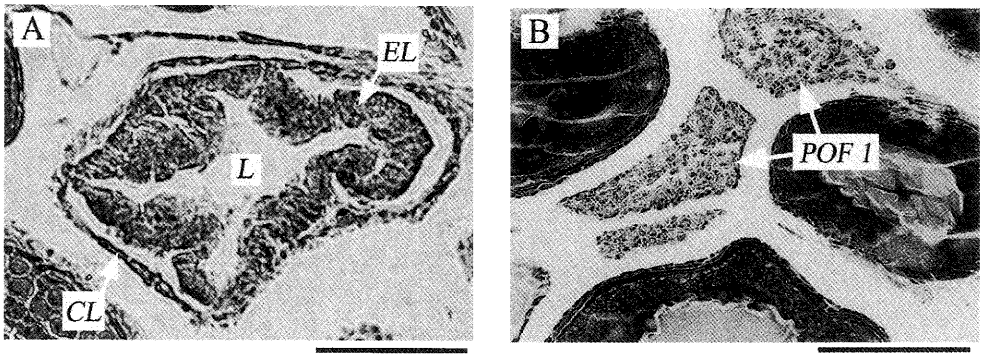

Fig. 2 Photograph of postovulatory follicle (POF) of *Lestrolepis japonica*. A, Age 0-day (POF0) and B, older than age 1-day (POF 1). EL, epithelial layer of granulosa cells; L, lumen of POF; CL, connective tissue layer of thecal cells. Bars 0.1 mm.

GSI's in October were moderately high (1.42-1.82) but decreased in December to 0.53-0.64, similar to those of recovering or immature ovaries. In all of the histological sections of spent ovaries, all of the oocytes, except those in the chromatin-nucleolus and perinucleolus stages, were atretic, apparently indicating the completion of spawning.

The appearance of mature ovaries and changes of *GSI* (Fig. 1) were consistent with the appearance of POF0 (see below), thereby indicating the spawning state of *L. japonica*. Spawning occurred in some individuals in May, in almost all individuals in June, in all in July, August and September, and was completed in some individuals in October and in all in December. Judging from the highest *GSI* levels, July, August and September were considered the main spawning months.

Postovulatory follicles (POF) are composed of the innermost lumen after ovulation, an inner epithelial layer of granulosa cells and an outer connective tissue layer of thecal cells[4]. Two clearly distinct types of POF were observed (Fig. 2), one being large (about 250-290 μm in long axis) and convoluted with several small to large folds (Fig. 2A). Other characteristics included: a large, wide multi-branched lumen containing eosinophilic granular material; an epithelial layer comprising several rows of nearly oval, almost irregularly arranged granulosa cells with prominent nuclei at their bases, some cells being slightly hypertrophied; epithelial and connective tissue layers clearly separated from each other; no apparent degeneration of the follicle. The other POF was smaller (about 70-150 μm in long axis) and rarely convoluted (prominent folds absent) (Fig. 2B). Other characteristics included: lumen greatly reduced or absent; poor differen-

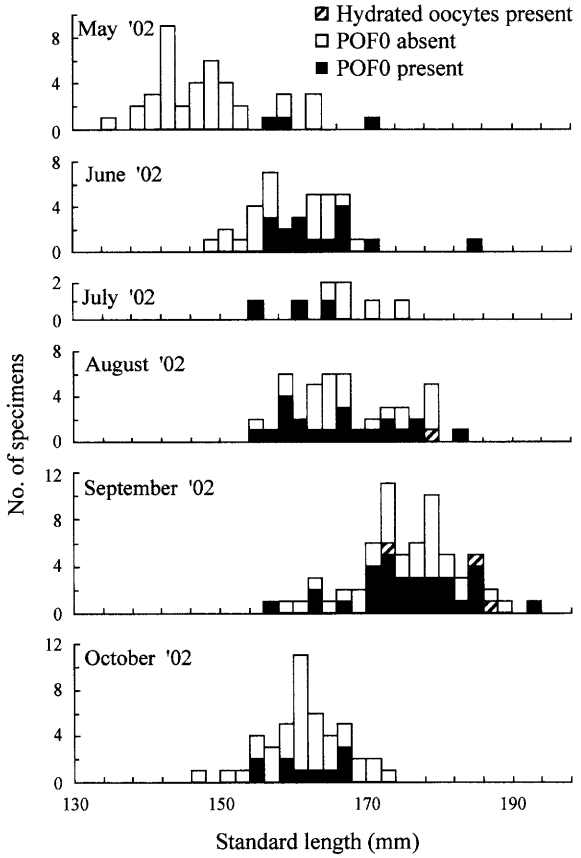

Fig. 3 Frequency distribution of number of specimens, and appearance of POF0 and hydrated oocytes against standard length in May to October in *Lestrolepis japonica*.

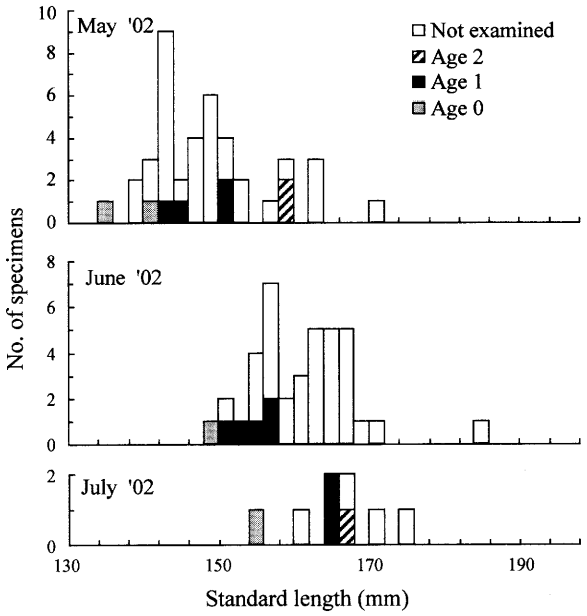

Fig. 4 Age determination in May to July in *Lestrolepis japonica*.

tiation of epithelial and connective tissue layers, the cells and nuclei of those layers being poorly stained or absent, with vacuoles common. The former POF was very similar to the POF of age 0-day (POF0) and the second to the POF of older than 1-day (POF1) of northern anchovy, *Engraulis mordax*[3], similarly in skipjack tuna, *Katsuwonus pelamis*[5], bigeye tuna, *Thunnus obesus*[6] and Japanese anchovy, *Engraulis japonicus*[7]. Therefore, the former POF was regarded as POF0 in this study.

POF0 appeared from May to October, during which time fish sizes increased gradually toward September and decreased in October (Fig. 3). In May, fish showing two modes of SL were collected, POF0 appearing only in the larger mode. In June, POF0 appeared in all except the smaller SL fish, appearing in the latter in July. In August and September, POF0 were apparent in all fish sizes. In October, the size of fish decreased, POF0 appearing around the mode of SL.

Ovaries with hydrated oocytes but without POF0 were observed very rarely: one specimen in August and three in September (Fig. 3). Such hydrated oocytes may be considered due either to spawning failure or awaiting the next spawning opportunity. Because of their rarity, they were regarded as resulting from spawning failure in this study.

Ages were determined from the otoliths of selected specimens to determine the onset of spawning by different age groups (Fig. 4). In May, the smaller mode lacking POF0 (Fig. 3) was composed of age 0 and 1 fish, the larger mode having POF0 being age 2. Therefore, age 2 fish spawned from May. Although not confirmed by the age determination, three years old fish exist[2] and they were considered to spawn from May. In June, smaller sized fish were examined, the smallest lacking POF0 being age 0, the others having POF0 being age 1. Therefore, age 1 fish spawned from June. In July, the smallest specimen having POF0 was age 0, indicating that age 0 fish spawned from July. Since spawning persisted until October, age 0→1 fish spawned from July to October, age 1→2 fish from June to October, age 2→3 fish from May to October, and age 3 fish from May to August which is the month of life span[2].

Spawning fraction *fm* was obtained for age 2→3 and age

3 fish in May. On the basis of their comprising the larger mode (Fig. 3), *fm* was calculated as 0.375 (specimens having POF0 3/specimens examined 8). In June, excluding age 0→1 fish, *fm* was calculated as 0.421 (specimens having POF0 16/specimens examined 38) for age 1→2, 2→3, and 3 fish. In July, fish of all ages spawned, the *fm* of 0.375 (specimens having POF0 3/specimens examined 8) being assigned to all age groups. Similarly, the following *fm* values were calculated and assigned to all age groups present: August, 0.489, indicating the spawning every two days (specimens having POF0 22/specimens examined 45); September, 0.515, indicating spawning every two days (specimens having POF0 34/specimens examined 66); October, 0.217 (specimens having POF0 10/specimens examined 46). (Specimens with hydrated oocytes in August and September were included in counts of those with POF0.)

The *fm* values were highest in August and September, medium in May to July, and lowest in October, near the end of spawning (Figs. 1 and 5).

Spawning frequency was obtained separately by age group (Table 2). Age 0→1 fish spawned 49 times during July and October, age 1→2 fish 62 times during June and October, age 2→3 fish 73 times during May and October, and age 3 fish 51 times during May to August, totaling 234 times over the three year life span.

During a ten year (1983-93) period of fish larvae collection by plankton net in Kagoshima Bay, larvae of *L. japonica* appeared from May to December (Ozawa *et al.*, unpublished). The numbers of *L. japonica* larvae collected in November accounted for less than 0.1% of the total, those collected in December being negligible or zero each year. Therefore, the lack of collection in November in this study is unlikely to have had any significant effect on the spawning frequencies calculated above.

The frequency distribution of oocyte diameter in single specimen collected in August and three in September, with hydrated oocytes but lacking POF0 (Fig. 3), is shown Fig. 6. Two modes were present, larger one being distinctly separated from the smaller mode and composed only of hydrated oocytes. Since absence of POF0 is an indication

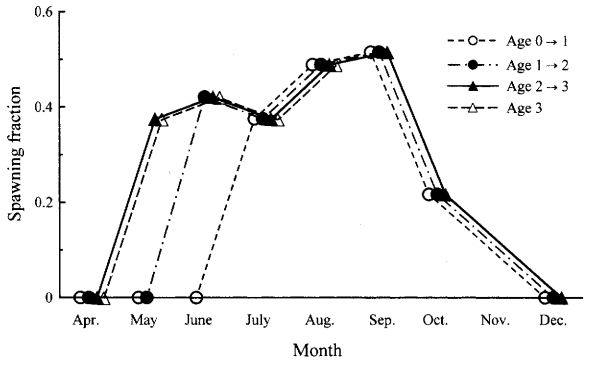

Fig. 5 Spawning fraction by month and age groups of *Lestrolepis japonica*. Arrows indicate the addition of age in September (see text).

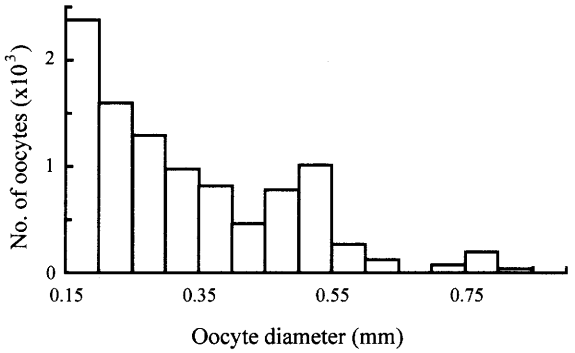

Fig. 6 Frequency distribution of oocyte diameter of a specimen of *Lestrolepis japonica* (187.8 mm *SL* caught on September, 2002) with hydrated oocytes but lacking POF0.

Table 2 Spawning frequency by age groups of *Lestrolepis japonica*. Arrows indicate the additon of age in September (see text)

Month	Spawning fraction				Duration (day)	Spawning frequency				
	Age 0→1	Age 1→2	Age 2→3	Age 3		Age 0→1	Age 1→2	Age 2→3	Age 3	
May	0	0	0.375	0.375	31	0	0	11.438	11.438	
June	0	0.421	0.421	0.421	30	0	12.632	12.632	12.632	
July	0.375	0.375	0.375	0.375	31	11.438	11.438	11.438	11.438	
Aug.	0.489	0.489	0.489	0.489	31	15.159	15.159	15.159	15.159	
Sep.	0.515	0.515	0.515	—*	30	15.708	15.708	15.708	—	
Oct.	0.217	0.217	0.217		31	6.739	6.739	6.739		
Total						49.043	61.675	73.112	50.666	234.495

*No collection due to the life span (see Harada and Ozawa[2])

that spawning had not occurred immediately before collection, the number of hydrated oocytes present in each fish was considered to represent batch fecundity, *BF*. The lower limit of the smaller mode overlapped the upper limit of oocytes characterized by smaller diameters, being composed of secondary yolk globule and migratory nucleus stage oocytes, and comprising 5.68-14.28 times the number of oocytes in the larger mode, such oocyte numbers being unable to be used for estimation of batch fecundity. Therefore, batch fecundity was obtained only from the hydrated oocytes of the above four specimens, as follows: 164 (172.9 mm *SL* specimen), 176 (179.8 mm *SL*), 347 (184.8 mm *SL*) and 312 (187.8 mm *SL*). Due to the small number of specimens, the correlation of BF=12.379 SL- 1994.9 (R^2=0.747) calculated from the above data was unrealistic, *BF*, for example, being -14 at 160 mm *SL*. Nevertheless, the *BF* of *L. japonica* can be considered as low as 300-350 in individuals of 190 mm *SL*.

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