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著者	SATO Hajimu, KAWASAKI Tsuyoshi
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## Ovarian Maturation and Oviposition in Kinubari, *Pterogobius elapoides*

Hajimu SATO and Tsuyoshi KAWASAKI

Department of Fisheries, Faculty of Agriculture,  
Tohoku University, Sendai, Japan

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### Summary

A number of a coastal marine goby, *Pterogobius elapoides*, were reared under two different food conditions. They spawned 2–7 times during 22–82 days. There was no obvious relation between the number of batches and the ration level. Although the mean batch size and weight of low-ration fish were smaller than those of high ones, the ration level had no effect on the mean dry weight of eggs. Frequency distribution of oocyte diameter of *P. elapoides* was “bimodal” and the sizes of consecutive batches were much the same. This pattern of maturation and oviposition is termed “bimode-multiple batch spawning”.

There are many patterns of ovarian maturation and oviposition in the teleosts as far as we know. Kawasaki (1) classified patterns of ovarian maturation and oviposition in the oviparous marine teleosts into five broad types. However, since the number of species examined in this line has been too small to systematize patterns of maturation and oviposition completely, more intensive study is requested in the future. This report presents an observation in a goby which shows a different pattern from Kawasaki's five types. It was confirmed from a rearing experiment by Dotsu and Tsutsumi (2) that kinubari, *Pterogobius elapoides*, is an intermittent spawner and eggs laid are protected by their fathers until hatching. We reared kinubari in troughs to study the pattern of ovarian maturation and oviposition and the change in size of the oocyte diameter in successive stages of maturity, in a bid to discuss their adaptive strategy for the regulation of fecundity under different food conditions.

### Terminology and Definition

Patterns of ovarian maturation and oviposition are designated by the number of modes in frequency distribution of the follicle (or oocyte) diameter and spawning times during a reproductive cycle according to Matsuura (3).

We define terms as follows: fecundity (FC) is the number of yolked oocytes (including ripe eggs) in an ovary just prior to the first spawning in a reproductive

cycle, fertility (FT) being the number of eggs deposited during a reproductive cycle, and potential annual fecundity (PAF) is the total number of oocytes which form yolk in a year; this is an expansion of Macer (4)'s definition. Batch is a group of oocytes which have been or are to be spawned (or ovulated) simultaneously.

### Material and Method

Specimens were collected from Onagawa Bay located on the Pacific coast of northern Honshu, Japan, in June and July, 1981. They were kept in eight troughs holding some 45 l of running sea water situated in the Onagawa Fisheries Laboratory of Tohoku University. Although experimental fish were fed by three different levels, 6:3:1, specific feeding rates for particular fish can be grouped into two levels (high ration: 91-42, low ration: 16-5) (Table 1). Food used was compound feed pellet prepared for common carp. All fish were weighed and measured just before and after spawning period. The diameter of residual eggs from spent ovaries was measured to get frequency distribution.

Developmental stages of oocytes were distinguished by external appearance with pervious light under a microscope following Matsuura (3). We reorganized developmental stages described by Yamamoto (5) into a simpler form as follows:

*Resting stage* Very small, translucent, non-yolked; corresponding to chromatin nucleolus and peripheral nucleolus stages.

*Growing stage* Partially or totally opaque, vitellogenic; yolk vesicle and yolk globule stages.

*Maturing stage* Large oocyte prior to ovulation, the second meiotic division in progress; migrating nucleus, pre-maturation, and maturation stages.

TABLE 1. *Initial and Final Measurements, Growth, and*

Fish	Days in captivity $D$	Food taken $F$ (g)	Feeding rate $R^1$ (g/day)	Specific feeding rate $R/W_i$	Initial measurements	
					Body length $L_i$ (mm)	Body weight $W_i$ (g)
A1	33	9.7	0.29	42	84.6	6.9
A2	58	14.5	0.25	54	73.6	4.6
B1	81	27.7	0.34	61	79.4	5.6
B2	98	28.9	0.29	91	69.0	3.2
C3	94	33.5	0.36	55	84.5	6.5
C4	94	33.5	0.36	58	80.2	6.2
D2	84	14.2	0.17	63	67.6	2.7
E1	61	10.9	0.18	10	115.6	18.2
F3	38	3.9	0.10	16	81.5	6.3
F4	33	2.6	0.08	12	77.5	5.9
G1	118	7.1	0.06	14	76.3	4.3
G2	35	1.5	0.04	15	69.6	2.7
H1	118	7.1	0.06	5	104.4	11.5
H2	43	2.3	0.05	7	85.4	7.6

1)  $R=10^3F/D$     2)  $GSI=10^2G/W_f$

*Ripe egg* Oocyte posterior to ovulation.

Eggs laid had been collected just after spawning and preserved in a 4 per cent formal saline solution. After several months, the number of eggs of each batch were counted and each batch was weighed after drying at 60°C for 24 hours.

**Results**

*Change of the Distribution of Oocyte Diameter*

In Figure 1 frequency distributions of the oocyte diameter are arranged in a progressing order. In an ovary just after spawning, Oocyte Group (OG) I seemingly to be spawned next time proceeds to the growing stage, although gonadosomatic index (GSI, gonad weight as a percentage of body weight) of this fish remains at about 1 per cent (H2). OG I continues to grow (B1) and when its mode has reached about 0.65 mm, a part of OG II, another Oocyte Group, begins to grow and the GSI rises to 7 per cent (E1). When the mode of OG I has further progressed, all oocytes of OG II complete development to growing stage and the GSI increases to some 11 per cent (A2). At the same time OG I proceeds to the maturing stage and becomes ripe with eggs 0.9–1.5 mm across, being ovulated thereafter.

The GSI rises rapidly from 15 to 18 per cent probably owing to hydration. Growth of OG II follows spawning of OG I. Since the number of oocytes in a complete Oocyte Group in Figure 1 resembles the mean batch size actually produced in Table 2, it becomes evident that the batch in Figure 1 is deposited at one time and this process recurs several times.

*Food Conditions of Experimental Females*

Final measurements			GSI <sup>2)</sup>	Growth rate		Specific growth rate	
Body length <i>L<sub>f</sub></i> (mm)	Body weight <i>W<sub>f</sub></i> (g)	Gonad weight <i>G</i> (g)		BL $\Delta L^3)$ ( $\mu\text{m}/\text{day}$ )	BW $\Delta W^4)$ (mg/day)	BL $\Delta L/L_i$	BW $\Delta W/W_i$
79.6	4.66	0.51	10.9	103	1.7	1.40	0.37
84.2	5.89	0.44	7.5	59.3	3.7	0.747	0.66
76.3	3.97	0.06	1.5	74.5	8.2	1.08	2.6
89.4	5.39	0.38	7.1	52.1	-12	0.617	-1.8
90.2	6.25	0.49	7.8	106	1.1	1.32	0.18
77.0				112		1.66	
116.2	13.42	0.88	6.6	9.84	-79	0.00851	-4.3
75.1	2.63	0.02	0.8	-10.2	-14	-0.134	-3.3
104.4	7.85	0.46	5.9	0	-31	0	-2.7
84.3	3.81	0.04	1.0	-25.6	-88	-0.300	-11.6

3)  $\Delta L = 10^3(L_f - L_i)/D$     4)  $\Delta W = 10^3(W_f - W_i)/D$

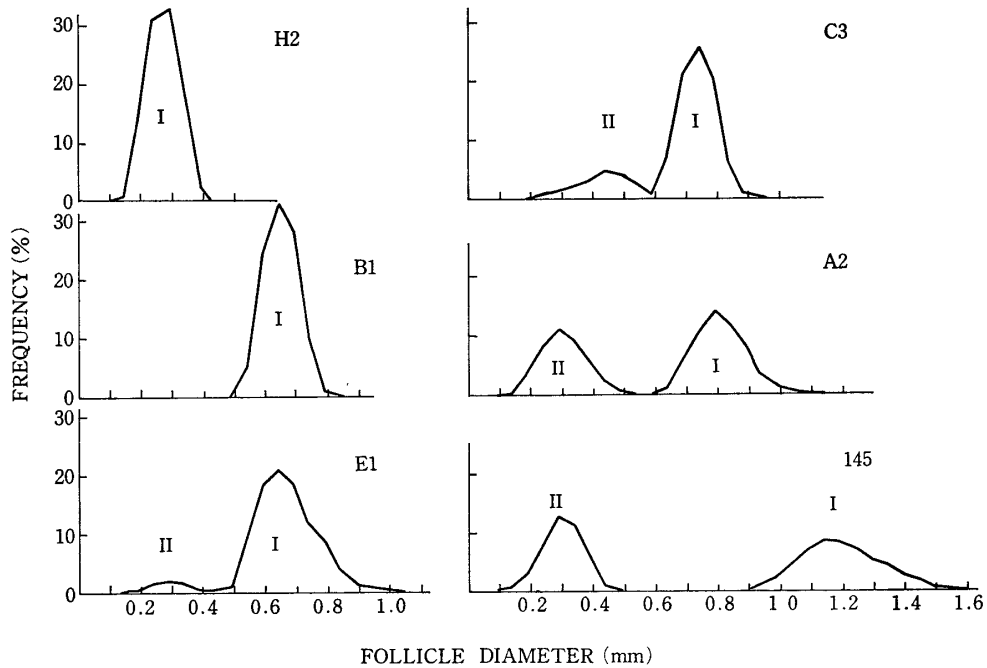


FIG. 1. Frequency distribution of follicle (holding yolked oocyte) diameter arranged in progressing order. H2, B1, etc. denote fish in captivity identified in Table 1. 145 is a fish which had been caught on August 8, 1980, and was examined thereafter. I and II denote a predecessor batch and a subsequent one respectively. Data are grouped at every 0.05 mm interval.

TABLE 2. Egg Production by Experimental Female Goby

Fish	No. batches produced	Mean interspawning interval (days)	Fertility (Total egg number)	Mean batch size	Total dry weight of eggs (mg)	Mean dry weight of a batch (mg)	Dry weight of $10^4$ eggs ( $\mu\text{g}$ )
A1 <sup>c</sup>	2	23.0	3350 <sup>b)</sup>		360.0 <sup>b)</sup>		107.5
A2 <sup>d)</sup>	3	11.0	9417 <sup>b)</sup>		786.5 <sup>b)</sup>		83.5
B1 <sup>d)</sup>	7	11.2	18289	2613	1669.6	238.5	91.3
B2	2	25	1286	643	114.4	57.2	89.0
C3	3		8619	2873	791.2	263.7	91.8
C4	5	13.3	15212	3042	1339.5	267.9	88.1
D2 <sup>d)</sup>	6 (5) <sup>a)</sup>	11.6	7260	1452	634.4	126.9	87.4
E1 <sup>d)</sup>	4 (3) <sup>a)</sup>		15522	5174	1103.8	367.9	71.1
F3 <sup>c)</sup>	3	12.5	6610	2203	583.0	194.3	88.2
F4 <sup>c)</sup>	2	19	3941	1971	358.3	179.2	90.9
G1	4 (3) <sup>a)</sup>	20	3392	1131	300.8	100.3	88.7
G2 <sup>c)</sup>	1	—	1454	1454	117.2	117.2	80.6
H1	6	16.8	15527	2588	1556.5	259.4	100.2
H2 <sup>d)</sup>	4	12.3	5404	1801	496.9	165.6	92.0

- a) Numeral in parentheses denotes the number of batches actually collected.  
 b) partially collected.  
 c) fish escaped during experiment.  
 d) fish died during experiment.

#### Observations on the Spawning of Fish in Captivity

##### (a) The Duration and Number of Times of Spawning

All 14 females in captivity spawned. During 17–25 July, 1981, eight females

spawned their initial batch and the final spawning occurred on 16 October. The water temperature during spawning was 17–23°C which was a peak for temperature cycle in Onagawa Bay. Duration of spawning of the females was 22–82 days and the numbers of batches were 2–7. There was no obvious relation between the number of batches and the ration or body length of their mothers.

Intervals between batch layings were 10–13 days in the former half of the reproductive period, irrespective of ration size, exclusive of Fish A2, B1 and F4 which had been subordinated by dominant ones in the troughs. While this interval remained unchanged for fish fed high ration throughout the reproductive period, it undoubtedly became long, 15–24 days, for fish fed low ration, especially for Fish H1 which continued to spawn for a long time, in the latter half of the reproductive period.

(b) *Relations between the Body Size and the Batch Size, Batch Weight or Dry Egg Weight*

Both the size and weight of a batch produced are positively correlated with the body length\* of spawning females, and the correlation coefficients between batch size and body length and that between weight and length are 0.704 and 0.674 respectively (Figures 2 and 3). The relation between the body length and mean

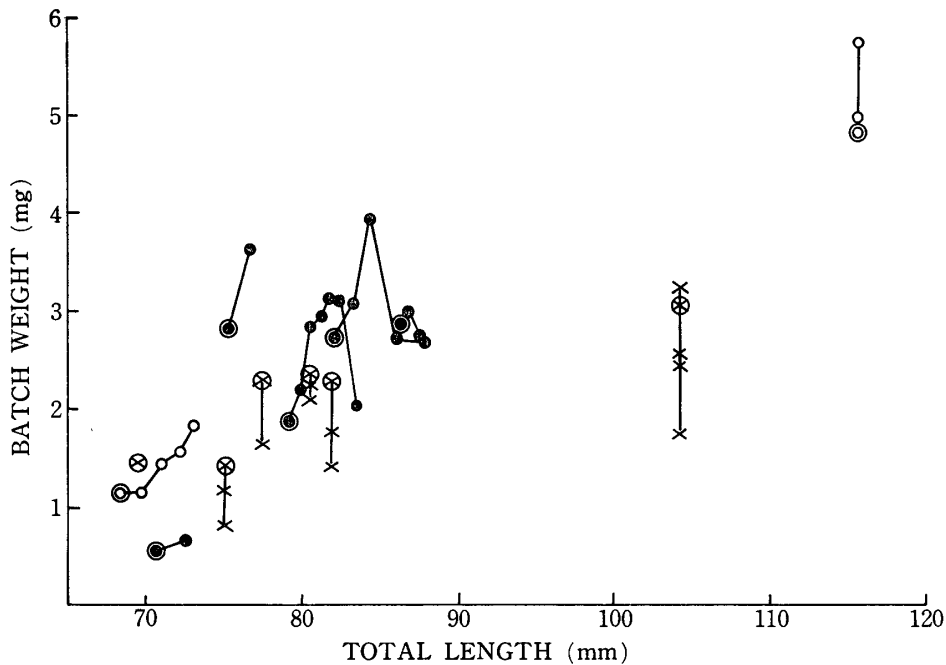


FIG. 2. Relation between batch size and total length estimated. Symbols are connected for the same female and a batch collected initially is circled, ● high ration; ○ medium ration; x low ration.

\* Body length is the distance from the tip of the snout to the hindmost margin of the caudal fin.

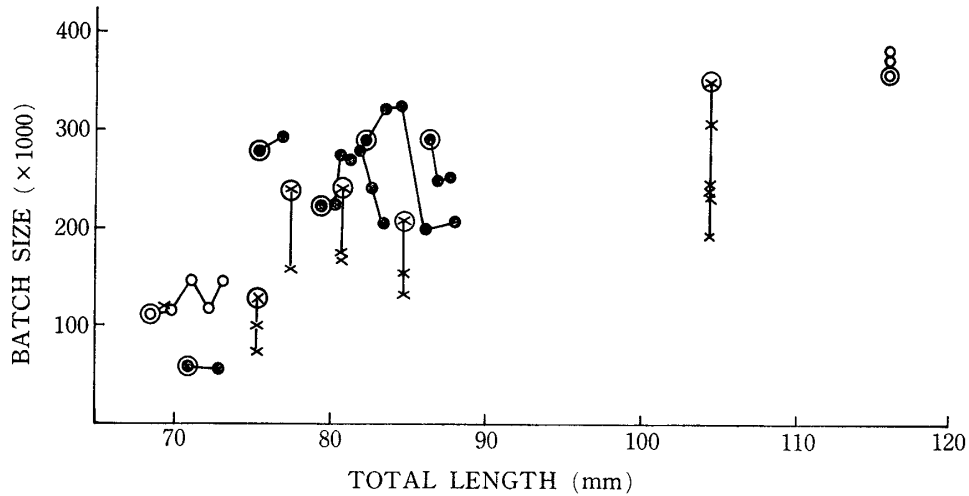


FIG. 3. Relation between batch weight and total length estimated. For explanation, see FIG. 2.

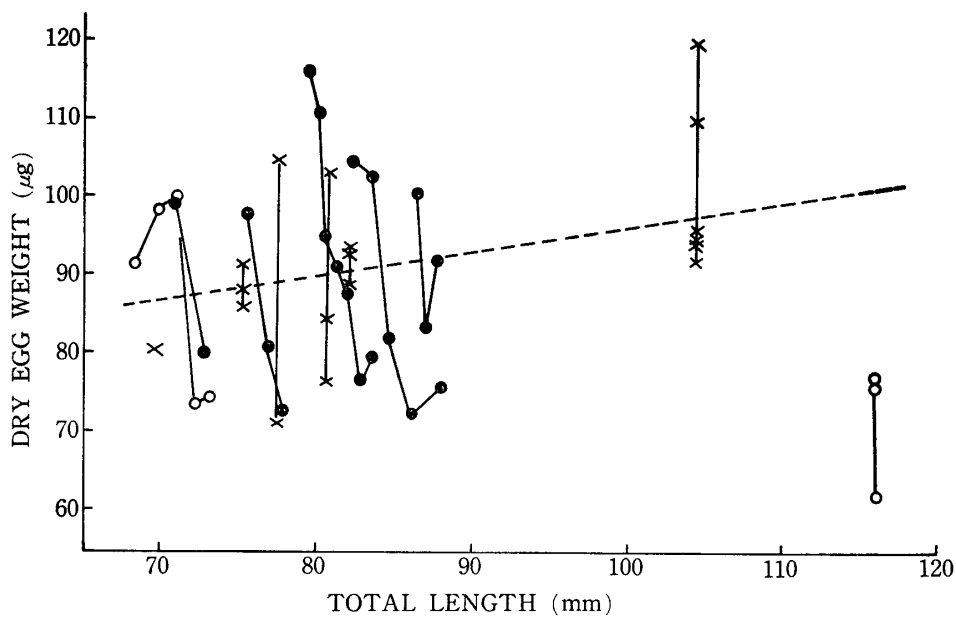


FIG. 4. Relation between mean dry weight of an egg of a batch and estimated total length of fish at the time when the batch is released. A dashed line is a regression of average value of mean dry weight of an egg on mean total length for a fish, calculated exclusive of BL 116 mm fish. For other explanations, see FIG. 2.

dry egg weight in a particular batch produced is not statistically significant with  $r$  of 0.282 (Figure 4). However, a high correlation coefficient of 0.837 is observed between the mean dry egg weight produced by a female through a spawning cycle and the mean body length in Figure 4, except for E1 with very light eggs.

(c) *Effects of the Ration Level on Reproduction and Growth*

All females fed with high ration increased in length at a rate of 0.083 mm a

day during the experimental period, but such an increase did not occur in weight. Those fed with low ration lost weight and their length remained unchanged. Specific growth rate of body length per day was  $1.14 \times 10^{-3}$  for fish fed with high ration and  $-0.009 \times 10^{-3}$  for low ration. In body weight, these were 0.40 and  $-5.48 \times 10^{-3}$  for high and low rations respectively (Table 1).

Although the size and weight of a batch produced from high-ration fish increased with increasing fish size as spawning progressed, the mean dry egg weight decreased. For low-ration fish, however, the size and weight of a batch decreased with decreasing fish size. While the mean batch size and weight of low-ration fish were smaller than those of high ones, no significant difference was found between mean dry egg weights of the two groups.

### Patterns of Ovarian Maturation and Oviposition

Frequency distribution of oocyte diameter of kinubari is "bimodal" and only a slight difference occurs among the sizes of consecutive batches. This goby is a reiterative spawner laying several batches in a reproductive cycle. For example, a repeated spawning of 4 times was observed in an experiment by Dotsu and Tsutsumi (2) and a maximal 7 time spawning occurred in the present study. Spawning interval was 10 to 24 days. We term this pattern *bimode-multiple batch spawning*.

It has been reported that some species of Gobiidae have the same pattern of the frequency distribution of oocyte diameter as kinubari; for example, rock goby, *Gobius paganellus* (6), sand goby, *G. minutus* (7), Fries' goby, *Lesueurigobius friesii* (8), and Norway goby, *Pomatoschistus norvegicus* (9). These goby species seem to spawn at least twice in a reproductive cycle according to the authors. Coastal gobies of Japan, for example *Mugilogobius abei* (10), observed in captivity were reported to spawn more than once at an interval of several days. Although there are no marine gobies for which both the mode of maturation and that of spawning are known, many seem to be *bimode-multiple batch spawners* like kinubari.

Wallace and Selman (11) suggested that the subsequent batch of oocytes begins vitellogenesis while the predecessor is undergoing the second meiotic division in two sticklebacks, *Gasterosteus aculeatus* and *Apeltes quadracus*, which are *unimode-multiple batch spawners* (1), and this phenomenon is caused by an endocrine action. In this goby, the mechanism of endocrine action seems to differ from that of sticklebacks, since the subsequent batch (OG II) proceeds to a growing stage prior to the second meiosis of the predecessor (OG I) (Figure 1).

The mechanism determining fecundity in the two patterns, *unimode-multiple batch spawning* and *bimode-multiple batch spawning*, could be the same, in that the batch size is regulated by body size of the spawner at the time when a batch in the ovary enters the growing stage and the fertility is controlled by the duration under the favorable conditions for reproduction and the number of batches. In



these cases, the fertility is equal to the potential annual fecundity and more numerous by far than the fecundity.

Wootton (12) observed effects of the feeding level on reproduction for a stickleback, *Gasterosteus aculeatus*, which were kept at three different ration levels like the present experiment. He found that the body weight of female at maturation, mean batch size and the total number of eggs produced (fertility) increased, while interspawning interval decreased with increasing ration. The size of egg and frequency of spawning (batches) were mostly invariable. The results obtained with kinubari coincide to those by Wootton (Table 2). For *unimode-multiple batch* and *bimode-multiple batch spawners*, we can conclude that the food conditions during the reproductive period directly controls the reproductive characters, while those in the remaining period in a year only affect the body size, which regulates the batch size in spawning.

Kinubari seems to allocate a large proportion of the matter taken from outside to the reproductive activity, in order to repeat rapid vitellogenesis, as exemplified by the fact that the GSI rises from one to over ten per cent during a short period of ten days or more.

It is characteristic of *bimode-multiple batch spawners* that stable food supply and higher temperature are able to make efficient vitellogenesis occur at short intervals. According to Takagi (13), the gobies originated from the coastal waters in the tropical and subtropical areas, where larval and juvenile fishes seem to live under the severe conditions in terms of being preyed upon. We consider that kinubari, a species of Gobiidae, has selected this pattern of ovarian maturation and oviposition along with parental care and compensated for the fecundity and egg size restricted by such a small body.

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