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Change in Egg Size of Japanese Flounder during One Spawning Season*

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Spawning of Japanese flounder *Paralichthys olivaceus* in captivity was studied at ambient temperature and photo period in one spawning season. The broodstock consisted of five groups of one female and two males each. The spawning lasted 113 days from February 18 to June 10 during which temperature ranged from 9.1 to 19.8°C. The spawning period and the date of first spawning differed among females. The proportion of viable eggs collected during the spawning season varied among females from 6.6 to 29.3%. Mean egg diameter ranged from 0.757 to 0.986 mm and tended to decline during the spawning season. Egg diameter was negatively correlated with the time from the commencement of spawning and the water temperature at spawning.

INTRODUCTION

The Japanese flounder *Paralichthys olivaceus* is found in a wide area from the Kuril Islands and Karafuto to Hongkong. It lives in rather deep waters during the winter time, but from February to June it migrates to shallower waters, about 20 m deep, for spawning (Okada, 1966). Among pleuronectiformes, this flounder is the most highly prized food fish in Japan and has a commercial value almost equivalent to that of red sea bream *Pagrus major*.

Hatchery reared flounder have been reported to spawn in captivity from the age of 2 years with manipulation of water temperature and/or photo period (Ijima et al., 1986; Min, 1988; Tsujigado *et al.,* 1989) as well as at ambient temperature and photo period (Tsujigado *et al*., 1989). Spawning without hormonal induction or manipulation of temperature and photo period may start from late January to February at a temperature around 10°C and generally ceases in April to May when the temperature rises above 20°C, in Kyushu.

Several authors have related egg size of marine fish to the size of the newly hatched larvae and the growth and activity of the larvae until the start of exogenous feeding (Blaxter and Hempel, 1963; Hunter, 1981; Knutsen and Tilseth, 1985). Most published works regarding spawning of flounder in captivity include data on spawning period, egg production and egg quality. Few published data exist on the spawning of individual flounder at ambient temperature and photo period (Hirano and Yamamoto, 1992). No data are available on the variability in the size of eggs of individual females throughout the spawning season.

The purpose of the present study was to determine the spawning period and

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spawning periodicity of individual females; to monitor the egg size of different batches and to ascertain if individual flounder show a seasonal variation in egg-size; to investigate the egg quality.

MATERIALS AND METHODS

This study was carried out at the Fishery Research Laboratory of Kyushu University situated in the northern part of Kyushu Island, Japan, in 1993. The fish used were hatched and raised at the same laboratory and belonged to a group of about 40 individuals, 4 or 5 years old. The group was maintained in an outdoor circular concrete tank of 20 m^3 water capacity, under ambient conditions of photo period and temperature. From the beginning of February daily observations were made on the tank to identify eggs liberated by females. On 17 February, when the first eggs were found, five females and ten males were selected, measured and weighed. Females had distinct signs of ovarian swelling and males were examined for normal milt release by stripping. Five groups (named A, B, C, D and E), consisting of one female and two males each, were separated and transferred into outdoor concrete circular tanks. Groups A, B and C were put into tanks of 3 m^3 water capacity (diameter = 1.8 m; water depth = 1.2 m) and groups D and E into tanks of 2 m^3 capacity (diameter: 1.6 m; water depth: 1.0 m). Direct sunlight was intercepted by a roof situated about 3.0 m above the surface of the tanks. A continuous flow of sand-filtered sea-water (ca. 32 in salinity) was supplied to the tanks at a rate of about 40 *l*/min. Vigorous aeration was provided through diffuser stones. Moist pellets whose main stuff were brown fish meal and raw mackerel were offered ad libitum once daily. Water temperature was monitored daily to the nearest 0.2°C.

To collect the eggs released daily, an egg-collecting net was fixed under the outlet pipe outside the spawning tanks. The net was fixed every day in the evening at 16:00 and was removed the next day between 10:00 and 11:00 as long as fish continued to spawn eggs. The amounts of buoyant and sunken eggs were estimated volumetrically using the mean number per aliquot. A sample of an average 100 buoyant eggs was used to estimate the fertilization rate and the proportion of eggs that appeared to be developed abnormally. The eggs were classed as viable if they were buoyant, fertilized and appeared to be developing normally. Egg size was determined by measuring the diameter of 50 buoyant eggs to the nearest 0.001mm by using a microscope with a calibrated eye-piece. The mean egg diameters obtained from each batch of all the females recorded during the spawning period were tested for a statistical difference using the student t - test.

RESULTS

Duration and periodicity of spawning

The commencement of spawning occurred at 12.2%. Spawning season lasted 113 days from February 18 to June 10. During this period the water temperature fluctuated from 9.1 to 19.8°C (Fig. 1). Spawning ceased when temperature had risen to 21.0°C.

All the females produced eggs and their spawning data are summarized in Table 1.

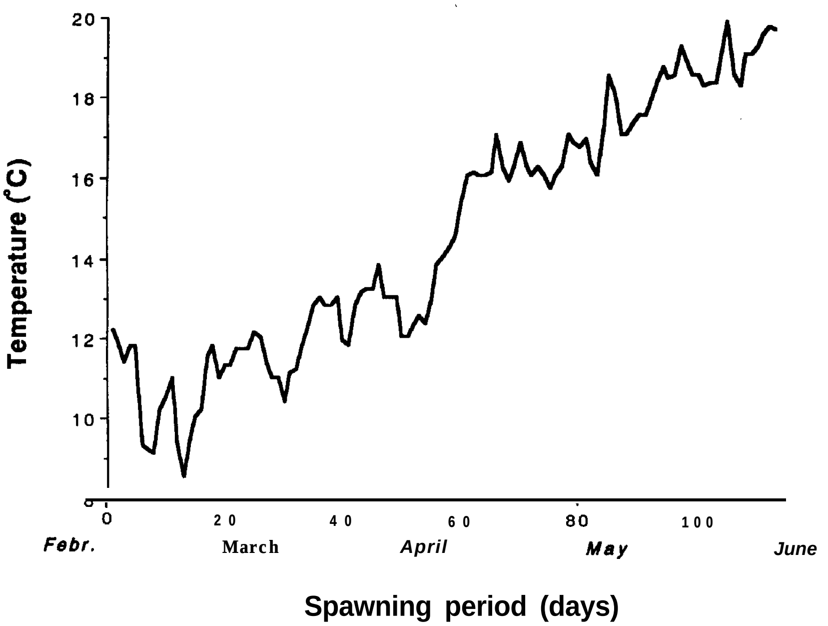

Fig. 1. Water temperature during the spawning season

Table 1. Summary of spawning data for five female P. *olivaceus* in captivity.

	Female				
	A	B	C	D	E
Body weight (g)	1380	1610	1540	1540	1260
Total length (cm)	48.0	50.0	48.2	50.5	47.0
Date of first spawning	11 April	18 Feb.	24 Feb	28 March	20 Feb.
Date of last spawning	10 June	21 April	29 May	24 April	16 April
Spawning period (days)	61	63	95	28	56
Number of batches	53	26	23	19	16
Rate of buoyant eggs (%)*	29.9	18.9	6.8	21.7	27.5
Rate of viable eggs (%)*	29.3	18.5	6.6	21.5	27.0

* Percentage from 100 eggs

Individual spawning period varied considerably between females from 28 to 95 days. There was little difference in the spawning periods of females B and E. Females B, C and E began to spawn in the latter half of February while female D began to spawn more than a month later. Female A had the latest onset of spawning on April 11 (53 days later than the onset of B) and released 53 batches of eggs during a 61-day spawning period. The rest of the females released a considerably smaller number of batches varying from 16 to 26.

Egg quality

The fertilization rate of the buoyant eggs of all the females was almost 100% with exception of the first 2-4 batches of females B, E and C which had low rates varying from 3 to 9%. The percentage of the buoyant normally developed eggs was high, fluctuating between 96 to 100%. The proportion of buoyant eggs collected during the spawning season varied among females from 6.8 to 29.9% while the proportion of viable eggs varied from 6.6 to 29.3% (Table 1).

Changes in the size of eggs

Mean egg diameters of individual females were negatively correlated with the time from the commencement of spawning (Fig. 2). There was a tendency for the size of the

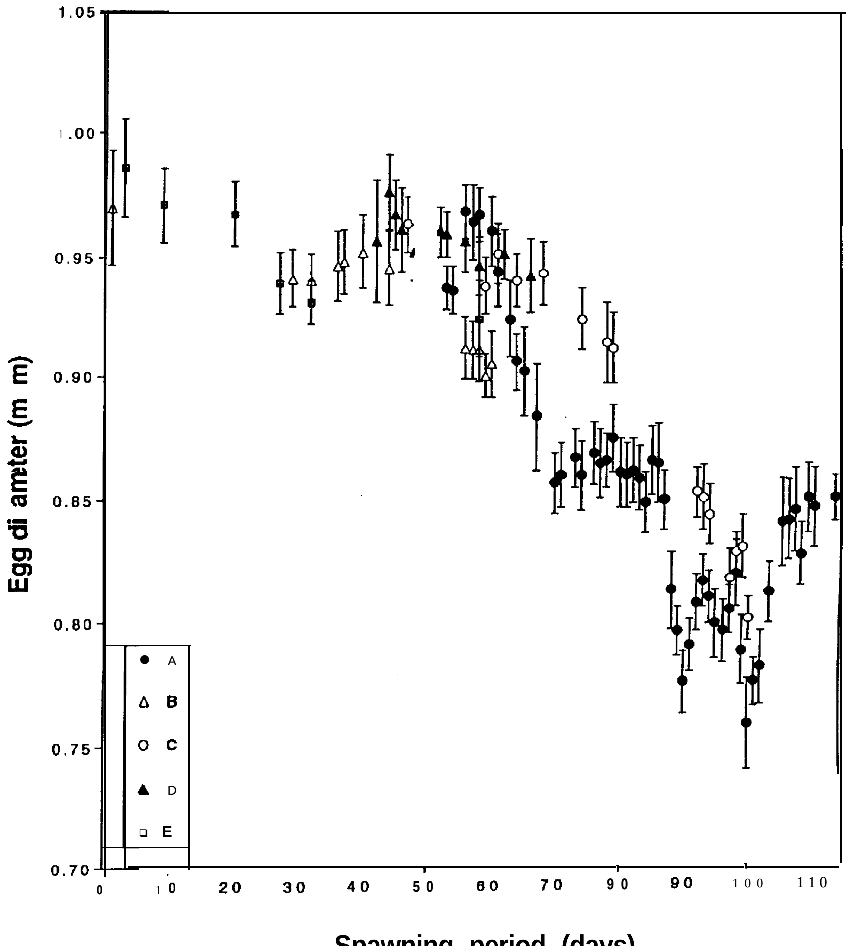

Fig. 2. Relationship between mean egg diameters and time in days from the commencement of spawning.

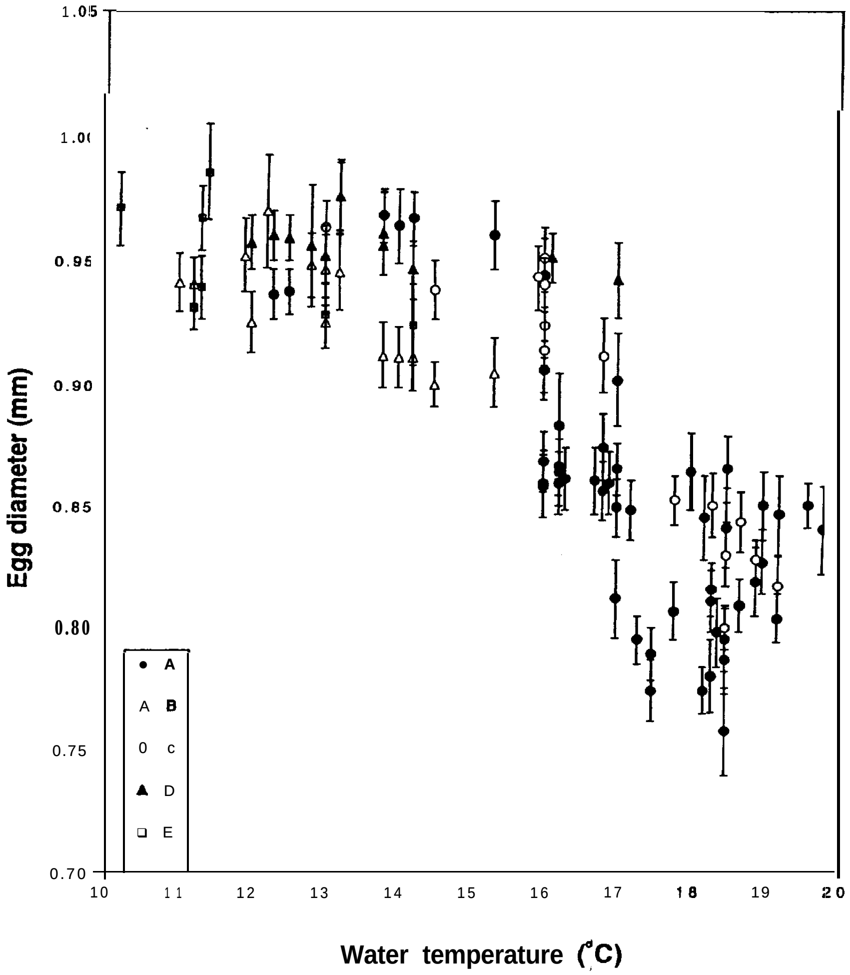

Fig. 3. Relationship between mean egg diameter and water temperature at spawning.

Table 2. Summary of egg diameters for five female *P. olivaceus* spawned in one spawning season and their level of significance.

Female	Egg diameter (mm)*		
	maxium	minimum	
A	0.968±0.011	0.757±0.018	$p<0.001$
B	0.970±0.023	0.900±0.009	$p<0.001$
C	0.963±0.011	0.800±0.009	$p<0.001$
D	0.976±0.015	0.942±0.015	$p<0.001$
E	0.986±0.020	0.924±0.016	$p<0.001$

* Mean diameter of 50 eggs obtained from each batch. Mean±SD

eggs to decrease as the spawning season progressed. Since water temperature increased during the spawning period, there was also a negative correlation between the egg size and temperature at spawning (Fig. 3). The mean egg diameter produced over the whole spawning season among females ranged from 0.854 to 0.954 mm. On the other hand, maximum value in diameter ranged from 0.963 to 0.986 mm and minimum value in diameter ranged from 0.757 to 0.942 mm. The maximum and minimum values of egg diameters from all the females were significantly different ($p < 0.001$, Table 2). Female A showed the greatest decline in egg diameter (21.8%).

DISCUSSION

The results of this study indicate that under natural fluctuations of water temperature and photo period the spawning period of individual flounder may vary within a single season, and a female can have a prolonged spawning lasting up to three months.

The percentage of the sinking eggs over the whole spawning period varied among females from 70.1 to 93.2%. Tsujigado *et al.* (1989) observed that from two groups of spawners the rates of buoyant eggs were 29.7% and 32.2%. Also, Hirano and Yamamoto (1992) reported that from five groups of spawners, each consisting of one female and three males, the rates of buoyant eggs varied from 16.2 to 32.9% and the fertilization rates of the buoyant eggs were law, varying from 29.3 to 53.1% respectively. These proportions of buoyant eggs are generally of the same level as found in the present study (with exception female C). Over-ripening of eggs inside the ovary has been recognized as a possible major cause of poor quality egg batches in captive broodstocks (Nomura *et al.*, 1974; Sakai *et al.*, 1975; McEvoy, 1984). Bromley et *al*. (1986) suggested that stress, sperm quality and damage to the eggs during collection might contribute to the production of non-viable eggs. The nutritional quality of broodstock diets has been also proved to be an important factor (Watanabe et al., 1984).

The present study confirms that average egg diameter of individual flounder females changes from batch to batch and the egg size may differ considerably with spawning period. In addition, egg size generally declines as the spawning season progresses along with a rising of water temperature. The decline of egg size over a period in which the temperature is rising has been also observed in individual marine fish spawned "naturally" in captivity such as in red sea bream (Matsuura *et al.*, 1988) and in silver sea bream, *Spar-us sarba* (Lin *et al.*, 1990), as well as in hand-stripped fish such as in turbot, *Scophthalmus maximus* (Howell and Scott, 1989). The reason for this size decline is still unclear. Bromley *et al*. (1986) suggested that temperature at the time of spawning may have a direct influence on egg size and Devauchelle *et al. (1987)* that the variation in egg diameter should be a consequence of events closer to oviposition. McEvoy and McEvoy (1991) studied the size fluctuation in the eggs of captive turbot, under constant temperature prior to and throughout their spawning season, and suggested that the observed decline of the egg size would have to be genetically controlled.

Further information on the fecundity and spawning periodicity of individual flounder are needed, by studying a large number of females. It would also be of great interest to investigate the effects of egg size on larval size and the survival of larvae during the transition period from yolk-sac to exogenous feeding.

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