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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 5(6): 728-732 2002

Annual Egg Production of Seasonal Populations of Japanese Anchovy, (*Engraulis japonicus)* in Kagoshima Bay, Southern Japan

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Abstract: Annual egg production of Japanese anchovy, *(Engraulis japonicus)* was studied using specimens collected in Kagoshima Bay, Japan from June 1997 to May 1998 and separated into spring and autumn populations. External features and gonadosomatic index of ovaries indicated that the spawning season of spring population lasted from February to July, and that of autumn one from the latter half of August to December. Spawning frequencies were estimated with the fraction of females having age 0-day postovulatory follicles, and batch fecundity was correlated with standard length. The spring population spawned, though underestimate, a total of 16.7x10^4 eggs in every 2.1 days in average within 80.5 days, and autumn one 15.9x10^4 eggs in every 2.8 days within 96 days.

Key words: Japanese anchovy, seasonal population, postovulatory follicle, batch fecundity, spawning frequency, egg production

Introduction

Fish, specially marine ones, are very fertile. In small fish such as whitings (Kumai and Nakamura, 1977), dragonets (Zhu *et al.*, 1991) and anchovies (Clarke, 1987), such fertility comes from long duration of spawning season and high frequency of spawning. The long spawning seasons have been well known from the past, on the other hand, the high frequencies have been assessed recently based on observation in captivity (Kumai and Nakamura, 1977; Zhu *et al.*, 1991) and histological examination on hydrated eggs or postovulatory follicles (POF) (Hunter and Macewicz, 1985).

Japanese anchovy is one of the important commercial fish around Japan. Its spawning season is long (Hayashi, 1961), and its frequency of spawning is high (Tsuruta, 1987). Stocks of Japanese anchovy are composed of local and seasonal populations (Hayashi, 1961; Takeshita and Tsukahara, 1971). Therefore, annual egg production should be estimated separately by those populations. Tsuruta (1992) estimated annual egg production of seasonal populations in Sagami Bay: 13.2x10^4 eggs for spring population, 19.5x10^4 for summer one, and 2.6x10^4 for autumn one. Those populations were distinguished based on catch months, but not on biological characters. Japanese anchovy is also one of the important fishes in Kagoshima Bay, southern Japan. The present authors (Ahmed *et al.*, 2001) distinguished two seasonal, spring and autumn, populations of the anchovy in Kagoshima Bay based on otolith micro-rings. In this study, the annual egg production of those populations was estimated.

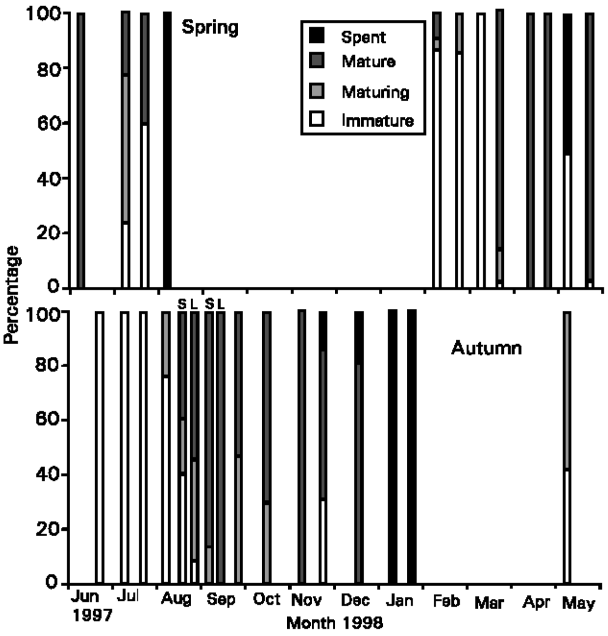

Fig. 1: Proportion of maturity stages of ovaries by seasonal populations.

Materials and Methods

Fish were collected from commercial purse seine catch in Kagoshima Bay, southern Japan, twice a month from June 1997 to May 1998 except October and December 1997 (once a month collection) totaling 22 collections. At each collection, 150 fish except on 26 February 1998 (100 fish) were collected randomly soon after catch, preserved with ice aboard, and transported to the laboratory. For examination of maturity, 50 fish were randomly chosen at each collection, standard length (SL, mm) and body weight (BW, g) were measured to the nearest 1.0 mm and 0.01 g respectively, and gonads were dissected, weighed to the nearest 0.001 g (GW, g) and preserved with 10% formalin. The other specimens were stored in freezer at -40°C and defrosted to measure SL and BW. Using ovaries, gonadosomatic index (GSI) was calculated as GSI = (GW/BW) x 100. Details of sampling data and ranges of SL and BW are shown in Fig. 1 and Table 1 of Ahmed *et al.* (2001). In Japanese anchovy, left gonad is larger than right one both in length and height. External features of both ovaries were observed by naked eye and under microscope at low magnification (x20) to define maturity which was separated into four stages: immature, maturing, mature and spent. For the estimation of annual egg production F, spawning frequency I and batch fecundity B were obtained as follows. Postovulatory follicle method (Hunter and Macewicz, 1985) was adopted to estimate the spawning frequency in this study. A segment of about 3 mm long taken from middle portion of left ovary was embedded in paraffin wax and cut in 8 μm thickness with a rotary microtome. The sections were stained according to Harris' hematoxylin and eosin stain method and observed under light microscope (x200) for postovulatory follicles (POF) which are composed of innermost lumen after ovulation, an inner epithelial layer of granulosa cells

Table 1: Data of females used for histological study by seasonal populations

Date	No$_{1)}$	SL (mm)$_{2)}$	BW (g)$_{3)}$	GSI$_{4)}$
Spring				
1 June	20	94-124	8.73-21.22	1.80-6.43
1 July	14	88-101	6.69-10.95	0.26-4.26
15 July	10	91-100	6.31-10.08	0.40-4.74
2 August	3	92-98	7.79-8.72	0.14-0.36
26 February	20	80-96	5.00-8.57	0.40-2.66
28 February	20	88-103	6.26-11.91	0.58-2.40
24 March	16	99-112	10.17-16.31	2.52-7.88
27 March	20	92-110	9.47-14.79	0.68-6.10
5 April	20	94-110	8.21-13.86	2.41-6.72
21 April	20	87-104	6.7-11.40	3.55-6.76
26 May	11	85-106	6.39-12.42	0.19-2.96
31 May	20	99-109	9.17-13.42	1.26-7.49
Total	194			
Autumn				
17 June	1	77	4.19	1.03
1 July	5	62-74	2.73-3.88	0.15-0.68
15 July	2	76	4.29-4.34	0.37-0.68
2 August	4	74-78	3.52-4.77	0.34-1.16
26 August (S)^{5}	5	74-78	4.35-5.33	0.17-2.94
26 August (L)^{6}	11	93-106	7.05-10.63	1.13-3.78
10 September (S)	13	67-76	3.34-5.00	1.56-4.11
10 September (L)	6	91-97	7.62-8.88	3.13-4.15
27 September	20	75-99	4.56-9.58	1.10-4.09
11 October	20	88-106	7.68-12.70	1.26-4.33
10 November	20	91-112	8.66-15.16	3.09-4.93
30 November	20	93-110	9.26-15.15	0.69-4.48
6 December	20	100-114	10.66-15.98	0.61-4.28
10 January	20	104-116	11.05-15.90	0.53-1.38
22 January	20	101-118	10.16-17.78	0.61-0.96
26 May	14	71-84	3.72-6.01	0.40-1.83
Total	201			

1), number of specimens; 2) standard length; 3) body weight
4), gonadosomatic index; 5), smaller length group (see the text)
6), larger length group (see the text)

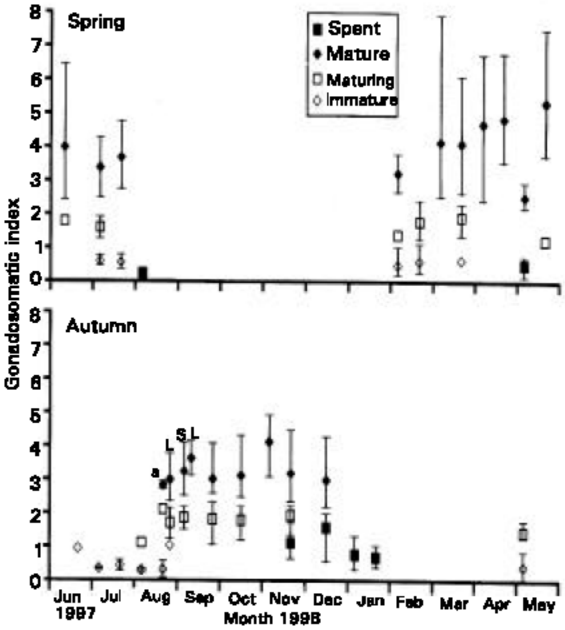

Fig. 2: Average (symbols) and ranges (vertical bars) of gonadosomatic index in females > 67 mm SL by seasonal populations. For S and L, See the text.

an outer connective tissue layer of thecal cells (Hunter and Goldberg, 1980). Since aging POF was not made in this study, the following definition by Hunter and Macewicz (1985) for northern anchovy (*Engraulis mordax*) was referred.

Postovulatory follicle, age 0-day (POF0): The new POF showing no sign of degeneration are relatively large, irregular in shape, with an irregular lumen which contains eosinophilic granules of uncertain origin. The follicle cell layers form loose folds or loops. The granulosa cells are columnar or cuboidal and in some cases have hypertrophied slightly; these cells are arranged orderly along the edge of the lumen with prominent nuclei at either the apex or base of the cell. The thecal cell layer contains blood capillaries. Elapsed time from spawning <24 h.

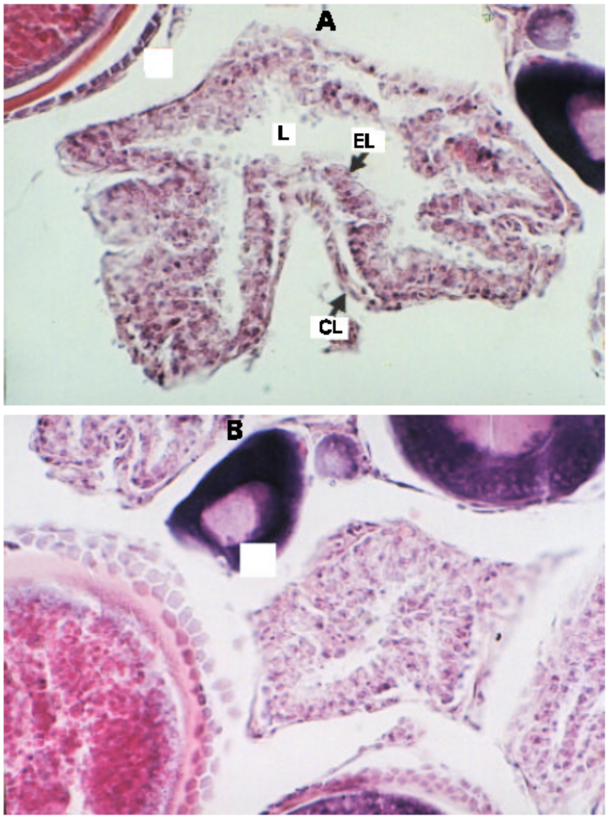

Fig. 3: Photographs of postovulatory follicle. A) Age 0-day (POF0) and b) Older than age 1-day POF (POF1). EL, epithelial layer of granulose cells; CL, connective tissue layer of thecal cells; L, lumen of follicle; scale bar = 40 μm.

Postovulatory follicle, older than 1-day (POF1): Degeneration is pronounced in POF of about 24 h after spawning which is greatly shrunken and shows less irregular form with fewer folds than POF0. The lumen is much reduced with some granules. The granulosa cells are not linearly arranged, vacuoles are common, and only few of the cell walls are intact. The underlying layer of thecal cells is present but indistinct. Elapsed time from spawning >24 h. The batch fecundity, i.e., number of eggs being shed in a single spawning, was estimated with count of eggs in largest mode in ovaries as follows. A section about 3 mm long was cut from middle part of left ovary, weighed to nearest 0.001 g (gw, g) after removal of formalin from surface with tissue paper, and put into a petridish with a small amount of water. All eggs were separated from each other, and their longest axis of more than 0.10 mm long was measured to the nearest 0.05 mm under an optical projector at x20 magnification. Eggs less than 0.10 mm length were too many to measure. A frequency distribution of egg size by 0.05 mm interval was constructed, and the number of eggs in the largest mode group, b, was summed. Batch fecundity, B, was calculated using the formula, B = GW x (b/gw).

Table 2: Details of egg population by seasonal populations

Data	Spawning Fraction	Duration (day)	Spawning frequency	Mean SL (Mm)	Batch fecundity	Egg production
Spring						
1 June 1997	0.60	15.5	9.300	110.90	6381	59347
1 July "	0.14	14	1.960	89.75	3218	6307
24 March 1998	0.31	13.5	4.185	101.70	4822	20179
27 March "	0.10	6.0	0.600	98.55	4355	2613
5 April "	0.45	12.5	5.625	99.25	4456	25065
21 April "	0.65	25.5	16.575	95.35	3914	64872
26 May "	0.50	20.0	10.000	99.35	4470	44705
31 May "	0.60	3.0	1.800	103.25	5064	9115
Total		80.5[1]	38.785[1]			166549[1]
Autumn						
10 September 1997, (L)[2]	0.50	16.0	8.00	92.35	3739	29912
27 September "	0.10	15.5	1.550	84.75	2945	4564
10 October "	0.35	22.0	7.700	95.70	4128	31789
10 November "	0.75	25.0	18.750	99.45	4594	86141
30 November "	0.20	13.0	2.600	104.15	5224	13582
6 December "	0.20	20.5	4.100	106.40	5544	22729
Total		96[3]	34.700[3]			158805[3]

[1] Data of 1st June and July were not included (see the text); [2] Larger length group (see the text);
[3] Data of September 10 were not included (see the text)

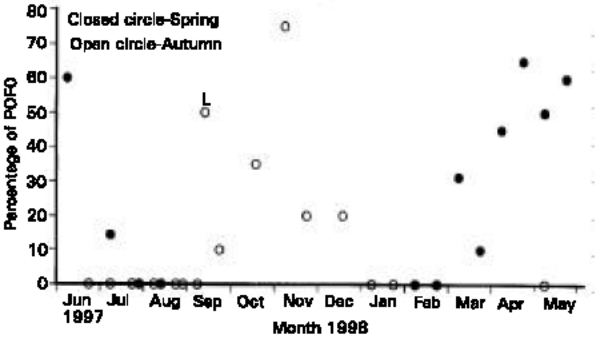

Fig. 4: Proportion of POFO by seasonal populations. For S and L, see the text.

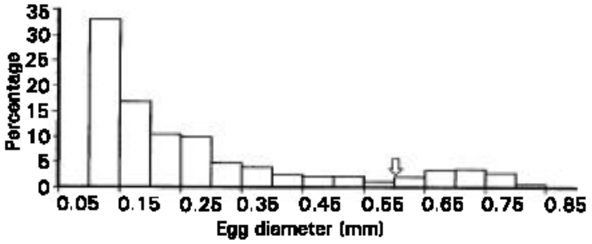

Fig. 5: Frequency distribution (%) of egg diameter of a female belonging, to spring population (100 mm SL caught on 5 April, 1998)

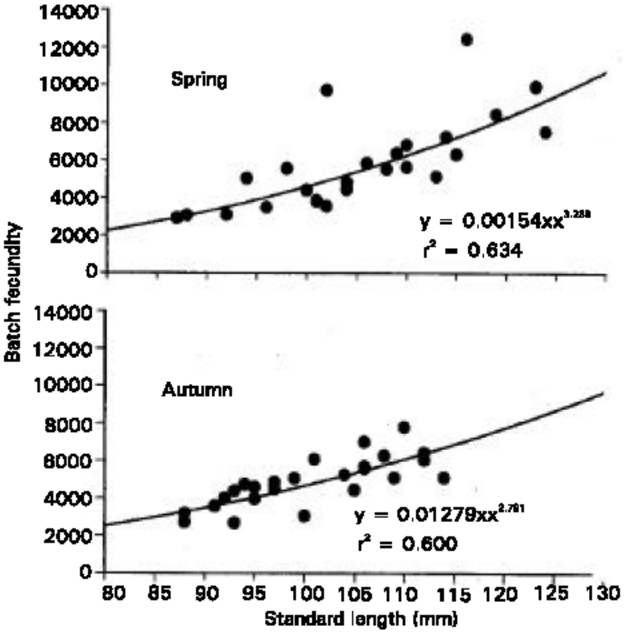

Fig. 6: Relationship between batch fecundity (y) and standard length (x) of seasonal populations.

In the previous study, the present authors (Ahmed *et at.*, 2001) recognized one or two length groups in SL at each collection, and separated them into either spring or autumn population except the larger length group on 17 June 1997 which had not been identified to any population and was not used in this study. In all of the study subjects above, fishes overlapping between two length groups in length frequency distribution were not used as in GSI of Ahmed *et al.* (2001). Except the batch fecundity separated into the seasonal populations, the results below were studied according to the length group separated into the seasonal populations. Number of specimens used was different among the length groups, and if necessary, is mentioned prior to the description of results.

Batch fecundity of a particular length group with a mean SL of I (Ahmed *et al.*, 2001), BI, was obtained substituting I into a relationship between batch fecundity and SL in each seasonal population. Spawning frequency of a particular length group I, FI, was calculated as the product of a ratio of specimens with POFO and a duration in day of the ratio which was the half duration between previous and next collection dates of a particular collection. Therefore, the egg production of a particular length group I, FI, was the product of BI and FI, and if a particular length group consisted of a spawning cohort, the annual egg production F was obtained as follows:

∑FI = ∑BI x FI

Results

Maturity by external features was not different between right and left ovaries. The proportions of maturity stages are shown in Fig. 1 separately by the spring and autumn populations, in which the autumn population in August and September was composed of two modal, smaller and larger, length groups (Ahmed *et al.*, 2001). In spring population, the immature stage dominated in February,

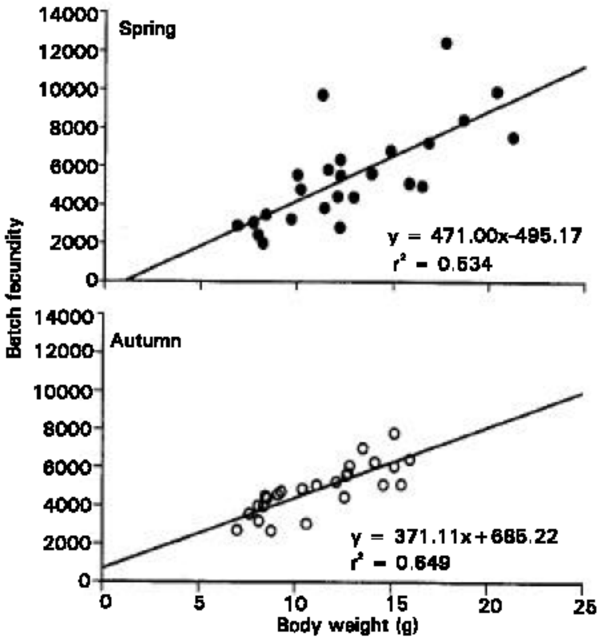

Fig. 7: Relationship between batch fecundity (y) and body weight (x) of seasonal populations

then the mature one from March to June with about 50% of the spent stage in the first half of May. In July, the immature and maturing stages dominated, and the spent one in August. In spring population, the mature stage dominated successively from March to June. In autumn population, ovaries matured gradually with the progress in season. The immature stage appeared from June to August and in May in the next year, and dominated from June to the first half of August. The maturing stage appeared from August to the latter half of November and in May of the next year, and never dominated except in May of the next year.

The mature stage appeared from the latter half of August to December, and dominated except the smaller length group of the latter half of August. The spent ovaries appeared from the latter half of November to January, showing dominance in January. In autumn population, the mature stage dominated successively from at least September to December.

Females having mature ovaries are considered those spawning or near to spawn, and females having spent ones those ending or ended spawning. GSI is shown in Fig. 2. GSI of immature stage ranged from 0.10 to 1.17, of maturing one from 1.10 to 2.42, of mature one from 2.18 to 7.88, and of spent one from 0.14 to 2.03 in both seasons. GSI overlapped largely between the immature and spent stages, and separated obscurely between the maturing and the other three stages. GSI of mature stage was clearly higher than those of other stages, indicating, together with their proportions in Fig. 1, the spawning season lasted from February to July in spring population, and from the latter half of August to December in the autumn population.

A total of 395 ovaries, 194 of the spring population and 201 of the autumn one, was sectioned histologically for POF observation (Table 1). Maximum 20 ovaries were chosen randomly for each length group but all ovaries for length groups having less than 20 females. The number of specimens was only three on 2 August 1997 of spring population, and less than five from 17 June to 2 August 1997 of autumn population. However, these were all out of the spawning seasons. Since separated into two modal length groups within the spawning season of autumn population, the specimens were only five for the smaller length group on 26 August and six for the larger length group on 10 September 1997. In this study, two distinct types of POF (Fig. 3) were observed. The one was large in size (about 200-250μm) in long axis) and convoluted with several small to large folds. A large, wide lumen having several branches and containing granular materials was present inside POF. An epithelial layer was composed of several lines of granulosa cells. The granulosa cells were nearly oval and arranged almost irregularly with prominent nuclei at their bases. Some of them showed a slight hypertrophy. The connective tissue layer composed of a line of rectangular thecal cells with prominent nuclei was separated partly from the epithelial layer by slender lumens. Blood capillaries in the thecal layer were not observed. No degeneration of the follicle was apparent (Fig. 3a). The other POF was smaller in size (about 50-130 μm in long axis) and rarely convoluted without prominent folds. The lumen was greatly reduced or absent. The epithelial layer and the connective tissue layer were not differentiable, and cells and nuclei of those layers were poorly stained or absent, with vacuoles being common (Fig. 3b). The first POF was very similar to POF0 of Hunter and Macewicz (1985), and clearly distinct from the second POF. Therefore, the first POF was regarded as POF0.

The frequencies of POF0, *i.e.*, spawning fraction are shown in Fig. 4 separately by the seasonal populations. In the spring population, the frequencies increased from March to May and June, then decreased to July. The highest frequency of 65% occurred in the latter half of April. In autumn population, positive frequencies appeared from the first half of September (the larger length group) to December, showing irregular change and the highest frequency of 75% in the first half of November. Typical frequency distribution of egg diameter from a specimen of 100 mm SL caught on 5 April 1998 belonging to the spring population is shown in Fig. 5. All the mature ovaries showed a mode of larger size eggs separable from smaller ones. As shown by an arrow in Fig. 5, number of eggs larger than the interval having the least number of eggs was counted. Batch fecundity was correlated with SL (Fig. 6), and the following relationships were calculated.

$B = 0.00154 \times SL^{3.236}$ ($r^2 = 0.634$) for the spring population, and
$B = 0.01279 \times SL^{2.761}$ ($r^2 = 0.600$) for the autumn population.

According to analysis of co-variance (ANCOVA), there was no statistical difference between them, since $F_0 = 0.405$ for the slopes and 0.007 for the adjusted means. Batch fecundity was also correlated with BW (Fig. 7), and the following relationships were obtained.

$B = 471.00\,BW - 495.17$ ($r^2 = 0.534$)

for the spring population and $B = 371.11\,BW + 685.22$ ($r^2 = 0.649$) for the autumn population. They were also not different statistically.

Egg production of a particular length group I, FI, was calculated as the product of BI (batch fecundity) and FI (spawning frequency) in Table 2, in which BI is a batch fecundity at a mean SL, and FI the product of a spawning fraction and a duration. The spring population from 28th March to 31st May 1998 was considered to consist of the same cohort (Ahmed *et al.*, 2001). Its spawning duration was 80.5 days, spawning frequency 38.79, and annual egg production 16.7x10^4. These values were underestimates, since the cohort might spawn in latter months as shown by the specimens on 1 June and 1 July 1997. Except 10 September 1997 (larger length group), all the other specimens from 27 September to 6 December 1997 were considered to consist of the same autumn population (Ahmed *et al.*, 2001). Its spawning duration was 96 days, spawning frequency 34.50, and annual egg production 15.9x10^4.

Although the underestimates in the spring population, the main spring population in Kagoshima Bay spawned a total of 16.7x10^4 eggs in every 2.1 days in average within 80.5 days, and the main autumn one 15.9x10^4 eggs in every 2.8 days within 96 days. Thus, the annual egg production was a little higher in spring population than in the autumn one. This difference seems mainly due to the difference in spawning interval, *i.e.*, spawning fraction (Table 2), between the populations since the average batch fecundity, 4513.5 for the spring population and 4487.0 for the autumn one, was nearly the same and the spawning duration was longer in the autumn population than the spring one.

Discussion

The present POF0 was not absolutely the same with that of Hunter and Macewicz (1985) of northern anchovy, (*Engraulis mordax*), especially about blood capillaries of thecal cell layer: they were not observed in this study. The present POF_0 shared, except blood capillaries, the characteristics with six species referred to by Hunter and Macewicz (1985), did not show any sign of degeneration such as vacuoles commonly seen in northern anchovy, and was distinct from the present POF1. Therefore, there seems no problem for the definition of POF0 in this study. Tsuruta and Hirose (1989) and Tsuruta (1992) observed POF for the estimation of interspawning interval of Japanese anchovy, but did not describe. Thus, it is desirable to confirm the deterioration and resorptive process of POF of Japanese anchovy.

Spawning intervals, *i.e.*, the reciprocal of spawning fraction, were studied on several anchovies. Although variable with water temperature (Imai *et al.*, 1998), food condition (Kawaguchi *et al.*, 1990; Tsuruta and Hirose, 1989; Tsuruta, 1992), and population size in captivity (Tsuruta, 1992), they were very short: 1.3-4 days in *Anchoa mitchilli* (Luo and Musick, 1991), 7-11 days in *Engraulis capensis* (Melo, 1994), 6-8 days in *Engraulis mordax* (Hunter and Goldberg, 1980), 2-16.7 days in *Encrasicholina heteroloba* (Wright, 1992), 2 days in *Encrasicholina purpureus* (Clarke, 1987), 1.4-4.3 days in Japanese anchovy (Tsuruta and Hirose, 1989; Tsuruta, 1992), and 1.3-10.0 in Japanese anchovy (Table 2). These results indicate that anchovies spawn frequently at the longest in every 10 days, and Japanese anchovy is one of the most frequent spawners. The other two parameters of this study for the estimation of annual egg production of Japanese anchovy are well within their ranges ever reported: the batch fecundity of 2,500-13,000 eggs, or about 4,500 eggs in average in this study vs 2,000-60,000 by Usami and Sugiyama (1962), 1,468-5,088 by Takeshita and Tsukahara (1971), 3,834-10,548 by Tsuruta (1992); the duration of spawning of at least 80 to 96 days in this study vs 31 (Takao, 1990) to 210 (Tsuruta, 1992) from the rearing experiments.

One of the contributions of this study to the spawning ecology of Japanese anchovy is that they were obtained based on the seasonal populations separated with otolith micro-rings (Ahmed *et al.*, 2001). It is not easy to distinguish seasonal populations of Japanese anchovy. For example, Takeshita and Tsukahara (1971), one of the most precise studies on seasonal populations, could not identify 0-25% of specimens to any seasonal populations (Takeshita and Tsukahara, 1971). Therefore, the annual egg production of seasonal populations of Japanese anchovy was not estimated in the past. Although Tsuruta (1992) reported the annual egg production of three seasonal populations of Japanese anchovy in Suruga Bay, those populations were separated based on catch months, but not on biological evidence. In Japanese anchovy, the total eggs spawned in seas seem changeable according to the biomass level of seasonal populations: when the biomass is high, the total eggs are more in spring than in autumn (Zenitani and Asano, 1996). Considering the present results that the annual egg production of individuals was only 800 eggs more in the spring population than in the autumn one, such change may be due to the increase in biomass, but not in spawning activity of each individual.

Acknowledgments

We express our sincere thanks to Mr. Shigeaki Iwamoto, Hamaichi Suisan, Hayato, Kagoshima for help in collecting the specimens, and Dr. Kaworu Nakamura, Faculty of Fisheries, Kagoshima University for help in observing histological sections.

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