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# Identification of Seasonal Populations of Japanese Anchovy, *Engraulis japonicus* in Kagoshima Bay, Japan by Examination of Otolith Micro-rings

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**Abstract:** Standard lengths of adult Japanese anchovy *Engraulis japonicus*, collected mainly twice a month during 1997-98 in Kagoshima Bay, southern Japan showing one or two length groups. Otoliths from each group were ground thinly, and widths of first 10 and next 10 micro-rings were measured. The widths of first 10 micro-rings from each length group were clearly distinguished into either narrower or wider width groups. Gonadosomatic indices of the narrower group were high in autumn and those of the wider group in spring, indicating the former group being autumn population and the latter spring one. Each two cohorts in the narrower and wider groups, recognized in the frequency distribution of standard length showed that the narrower cohorts grew better than the wider ones.

**Key words:** Seasonal population, Japanese anchovy, otolith, micro-ring, GSI, growth

## Introduction

Japanese anchovy, *Engraulis japonicus*, katakuchi-iwashi in Japanese, is one of the most important commercial fish in Kagoshima Prefecture and Kagoshima Bay as well as in Japan. Its yield occupied 3.90% (233,113 tons) of the total marine fish catch of Japan in 1997 (Suisansha, Japan, 1999), and 7.98% (7,286 tons) of that of Kagoshima Prefecture in 1998 (Statistical Yearbook, 1998). In Kagoshima Bay, it is exploited mainly as bait fish for skipjack tuna and its catch occupied 39.09% (2,201 tons) of the total bay catch in 1998 (Statistical Yearbook, 1998). In addition to its commercial importance, the anchovy is of significant importance in production of marine ecosystem as a major source of food for large piscivorous fish (Hayashi, 1961).

Japanese anchovy comprises four (Hayashi, 1961) or seven local (Takeshita and Tsukahara, 1971), and two seasonal, spring and autumn, populations (Hayashi, 1961; Takeshita and Tsukahara, 1971). Seasonal populations are distinguished on the basis of number of vertebrae and size of annuli on scales (Takeshita and Tsukahara, 1971). According to ten years (1983-93) ichthyoplankton collections in Kagoshima Bay, the anchovy showed two peaks, spring and autumn, in abundance of the larvae (Ozawa *et al.*, unpubl. data). Prior to investigation on spawning ecology of the anchovy in Kagoshima Bay, the present authors tried to distinguish seasonal populations based on number of vertebrae and size of first annual ring, but failed eventually: average numbers of vertebrae were not different significantly between most of the collections (45.04-45.46 in 22 collections) and specimens with clearly discernible annuli on scales were very few in frequency (17.82% in average of 22 collections) (Ahmed, 1997).

Recently, micro-rings of otolith usually called daily rings are widely used for study of fish ecology, especially in early stages as an indicator of growth and duration of a particular stage (Ozawa and Sato, 1993). The growth of Japanese anchovy larvae is known to be different between seasonal populations, with spring population growing faster than autumn one (Hayashi and Kondo, 1957). This difference may be printed on otoliths as difference of widths between micro-rings. In fact, micro-rings of otoliths indicated that the anchovy larvae in Suruga Bay, Japan grew faster in spring than in autumn (Kurokawa, pers. comm.).

In this study using adult specimens collected from the bay, micro-rings near focus of otoliths were examined to distinguish seasonal populations.

## Materials and Methods

Fish were collected by commercial catch in Kagoshima Bay, southern Japan. The sampling was made twice a month from June 1997 to May 1998, except October and December 1997 of once a month collection. Of 22 collections, 18 were at the inner part and four at the middle part just south of Sakurajima (Fig. 1 and Table 1). At each collection, 150 fish except on 26 February 1998 (100 fish) were collected randomly soon after

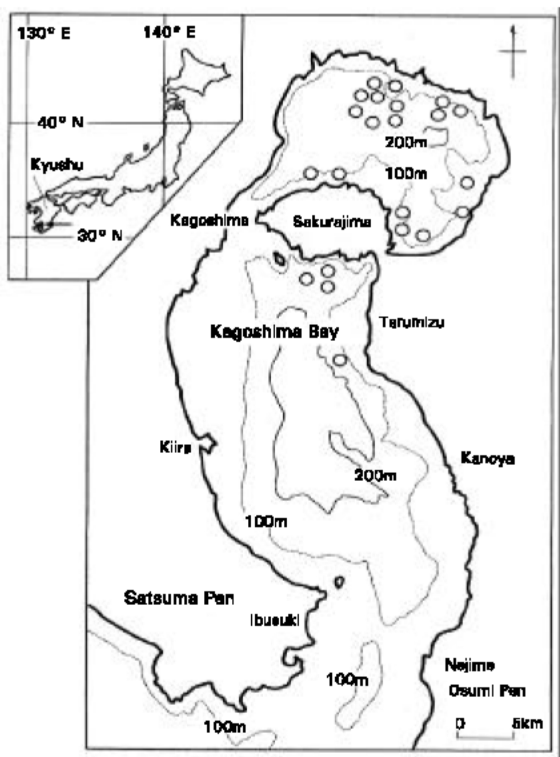


Fig. 1: Study area in Kagoshima Bay with sampling sites (Open circles)

Table 1: Collection record of anchovy samples from Kagoshima Bay for the study

| Sample No. | Collection date | Location | Number of fish | Size range | |
|---|---|---|---|---|---|
| | | | | SL (mm) | BW (g) |
| 1 | 1 June 1997 | M | 150 | 94-124 | 8.73-22.63 |
| 2 | 17 June | M | 150 | 70-119 | 3.97-20.56 |
| 3 | 1 July | I | 150 | 60-102 | 2.12-12.66 |
| 4 | 15 July | I | 150 | 67-101 | 2.33-12.16 |
| 5 | 2 August | I | 150 | 69-101 | 3.30-11.81 |
| 6 | 26 August | I | 150 | 71-112 | 4.04-13.25 |
| 7 | 10 September | I | 150 | 67-100 | 3.21-10.97 |
| 8 | 27 September | I | 150 | 74-103 | 4.56-10.64 |
| 9 | 11 October | I | 150 | 81-108 | 5.85-14.75 |
| 10 | 10 November | I | 150 | 87-112 | 7.87-15.16 |
| 11 | 30 November | I | 150 | 93-113 | 9.26-17.10 |
| 12 | 6 December | I | 150 | 99-114 | 10.66-17.17 |
| 13 | 10 January 1998 | I | 150 | 100-123 | 10.84-19.14 |
| 14 | 22 January | I | 150 | 98-118 | 8.55-18.64 |
| 15 | 26 February | M | 150 | 79-101 | 4.48-10.85 |
| 16 | 28 February | M | 150 | 85-104 | 6.20-12.30 |
| 17 | 24 March | I | 150 | 84-114 | 7.94-17.00 |
| 18 | 27 March | I | 150 | 90-111 | 7.49-16.09 |
| 19 | 5 April | I | 150 | 90-110 | 6.99-13.86 |
| 20 | 21 April | I | 150 | 86-104 | 6.13-12.41 |
| 21 | 26 May | I | 150 | 70-106 | 3.23-12.50 |
| 22 | 31 May | I | 150 | 90-109 | 8.26-14.67 |

I, inner part; M, middle part of the bay; SL, standard length BW, body weight

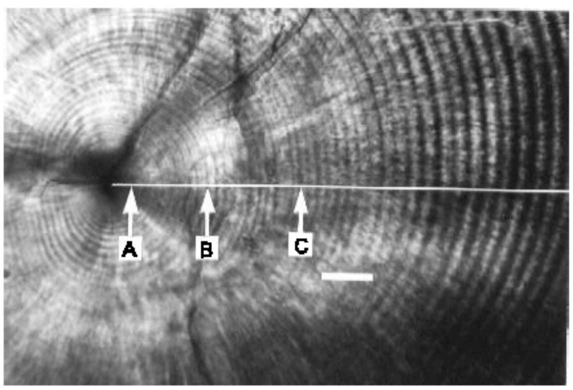


Fig. 2: Photograph (light microscope) of a "narrower width group" otolith micro-rings of *Engraulis japonicus* (71 mm SL caught on 17 June 1997). A, focus margin; A-B, first 10 micro-rings; B-C, next 10 micro-rings; white line, measured axis; scale bar= 20 μm. For "narrower width group" refer to the text and Fig. 4.

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 to 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. After defrosting, SL and BW were measured, and both left and right sagittae, the largest of three pairs of otoliths were extracted, rinsed with deionized water and dried at room temperature. Standard length frequency distributions with 5 mm interval were prepared for each sample. The existence of length groups in each length frequency distribution was checked by a computer analysis based on Hasselblad's maximum likelihood method (Hasselblad, 1966). This analysis revealed number of length groups, and on each group distinguished, provided mean and standard deviation of *SL* and proportion of number of individuals. Extracted otoliths of ten fish from uni-length group samples and those of five fish from each length group in multi-length group samples were chosen randomly to make sections. In multi-length group samples, otoliths from fish, overlapping each other in length frequency distribution were excluded. Right otoliths were embedded in cyanoacrylate resin (Konishi Kabushiki Co.) on glass slides with their distal side up and left overnight for hardening. They were ground up to focus with 2000 grade dry carbon carbide sand paper. Ground surfaces were rinsed with deionized water and dried at room temperature.

Ground otoliths were observed on annuli and micro-rings under a compound microscope with transmitted light at magnification of x400. Measurement axis was taken as a straight line from center of focus to the tip of posterior edge. Since the widths between successive micro-rings were too narrow to get reliable data, the widths of first 10 (from focus margin to 10th micro-ring) and of next 10 micro-rings were measured along the axis (Fig. 2) and were divided respectively by 10 for a particular specimen. Each of those widths of, a particular specimen in a length group was summed and divided by the number of specimens to get average width of micro-ring.

Using ovaries, gonadosomatic index (GSI) was calculated as GSI = (GW/BW) x 100. In multi-length group samples, GSIs from fish overlapping each other in length frequency distribution were excluded from analysis.

## Results

A total of 3,250 specimens from 22 samples were collected for study, with their standard length ranging from 60 to 124 mm and body weight from 2.12 to 22.63 g (Table 1). The maximum two length groups were present in frequency distribution of standard length (Fig. 3). Each of two groups from 1st July 1997 to 26th August 1997 seemed to compose the same cohort respectively. The smaller length group on 10 September 1997 seemed to be succeeded by the uni- length groups from 27 September 1997 to 22 January 1998. The uni-length groups from 26 February 1998 to 31 May and the larger group on 26 May 1998 seemed to compose the same cohort.

Any distinct annulus-like marks could not be observed on sectioned otoliths. Therefore, ages could not be assigned to

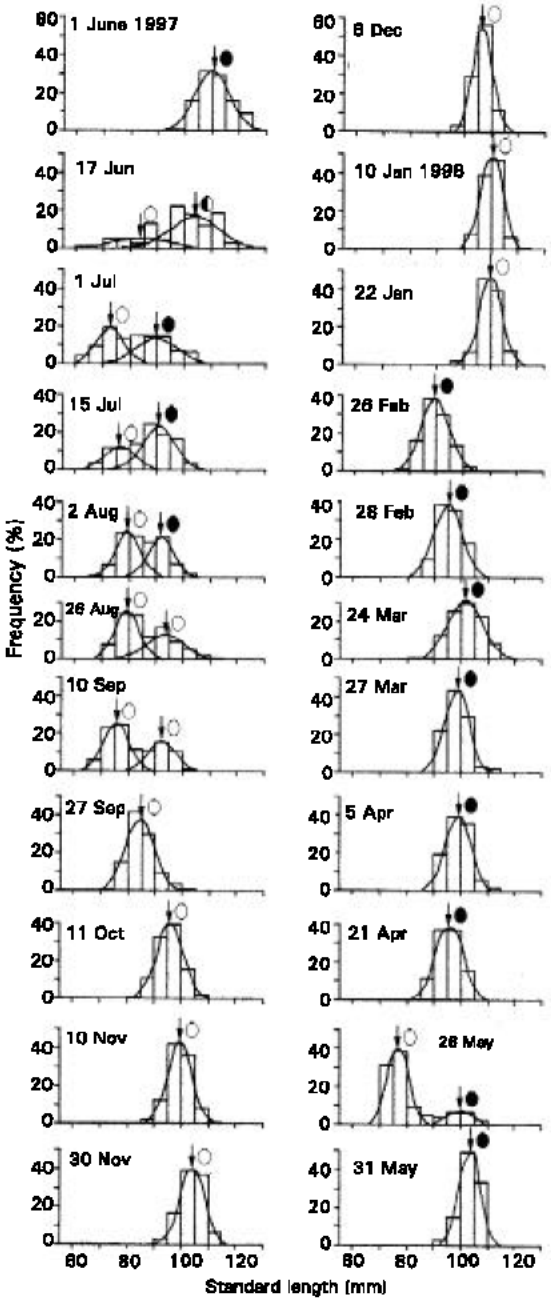


Fig. 3: Standard length frequenc distribution of Engraulis japonicus. Arrows indicate the mean of length group distinguished (see the text). For open, closed and half closed circles, refer to the text and explanation of Fig. 4.

any specimens. The average and range of width of the first 10 micro-rings are shown in Fig. 4. From 17 June to 2 August 1997, length groups distinguished on each collection date showed different average widths of micro-rings.

Except the one of larger groups on 17 June, the widths of each length group inclusive of uni-length group on 1st June 1997 were clearly separated into either wider (averaging from 4.25 to 4.50 $\mu m$ and ranging from 3.85 to 5.51 $\mu m$) or narrower (averages from 3.37 to 3.46 $\mu m$ and ranges from 2.48 to 4.04 $\mu m$) groups (Fig. 4). All four length groups, each two on 26 August and 10 September showed similar average widths (3.41- 3.56 $\mu m$) and ranges (2.53-4.17 $\mu m$), which apparently belonged to the narrower group.

The averages (3.40-3.60 $\mu m$) and ranges (2.58-4.47 $\mu m$) in seven uni-length groups from 27 September to 22 January were very similar to each other, belonging apparently to the narrower groups. The averages (4.61- 4.77 $\mu m$) and ranges (3.82-6.20 $\mu m$) in the next seven uni-length groups from 26 February 1998 to 31 May and in the larger length group on 26 May 1998 were also very similar to each other, and apparently wider than those of the narrower group. The averages and ranges were larger than those of the wider group from June to August (4.25-4.50 ($\mu m$ in average and 3.85-5.51 $\mu m$ in range), however, since the differences in both were small, they were considered the same wider group (Fig. 4). Except the larger length group on 17 June 1997 and the smaller one on 26 May 1998, all length groups were separated into either the narrower or wider groups, showing the existence of two distinct populations in Kagoshima Bay. Therefore, the smaller length group on 26 May 1998 having an average of 2.93 ($\mu m$ with a range of 2.53-3.23 $\mu m$ was included in the narrower group. However, the larger length group on 17 June 1997 having average of 3.93 $\mu m$ with a range of 3.10-4.79 $\mu m$ could not be assigned to any group since both its average and range were intermediate between those of narrower and wider groups.

The widths of next 10 micro-rings are shown in Fig. 4. As a whole, they (averages 3.63-5.61 $\mu m$ and ranges 3.32-6.45 $\mu m$) were a little wider than those of first 10 micro-rings (averages 2.93-4.77 $\mu m$ and ranges 2.48- 6.20 $\mu m$). On the other hand, most of each ranges of average widths both in wider and narrower groups were larger as compared to those of first 10 micro-rings, making large overlap between wider and narrower groups. The averages (4.85-5.19 $\mu m$) and ranges (3.97-5.81 $\mu m$) of the first half four wider groups were smaller than those (averages 5.21-5.61 $\mu m$ and ranges 4.47-6.45 $\mu m$) of latter half eight wider ones as in the first 10 micro-rings. Those (averages 4.15-4.80 $\mu m$ and ranges 3.32-5.76 $\mu m$) of narrower groups except on 26 May 1998 were nearly constant, but a few of them, e.g., 1st July 1997 and 10th January 1998, were very close to those of first half four wider groups, especially in average. Excluding these narrower-groups, the same two groups as in the first 10 micro-rings seemed to be distinguished. The larger length group on 17 June 1997 unassigned to any group with the first 10 micro-rings was also undistinguishable with the next 10 micro-rings since its average (4.56 $\mu m$) and range (3.92-5.56 $\mu m$) were nearly the same with those of narrower groups excluding above.

From above, the Japanese anchovy in Kagoshima Bay was distinguished into two (wider and narrower) groups based on the first 10 and, though incomplete, next 10 micro-rings on ground otoliths. GSIs are shown in Fig. 5 according to the results of first 10 micro-rings. Those of narrower groups were low from June to 2 August 1997, then increasing on 26 August remained high until 6 December, thereafter remained low until 26 May 1998. Those of wider groups decreased from 1st June to 2nd August 1997, were low in February 1998, remained high from March to 31 May except 26 May of low value. The undistinguished larger length group on 17 June 1997 seemed to belong to the wider group, if based on GSL. The narrower group showed high GSI in autumn (August to December), and the wider one in spring (March to July).

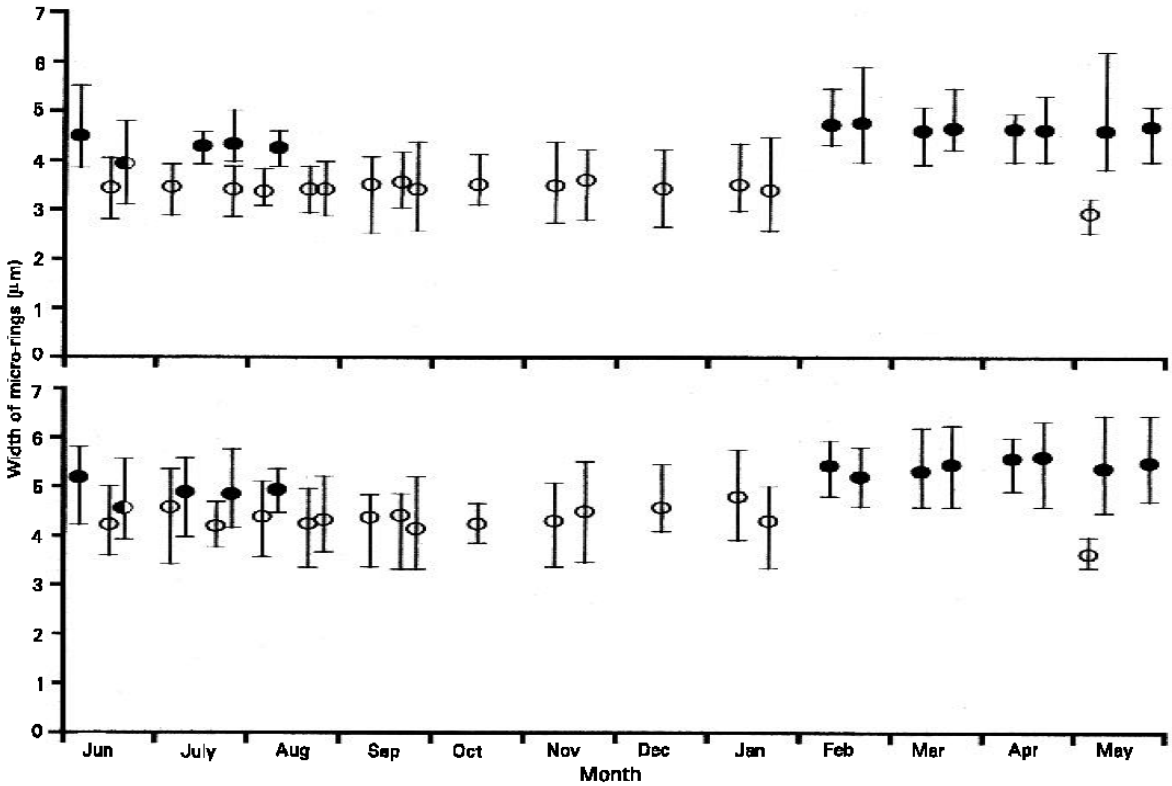


Fig. 4: Average (circles) and ranges (vertical bars) of width of first 10 (above) and next 10 (below) otolith micro-rings. Open and closed circles indicate respectively narrower and wider groups of the width of first micro-rings with a half closed one undistinguished to any group.

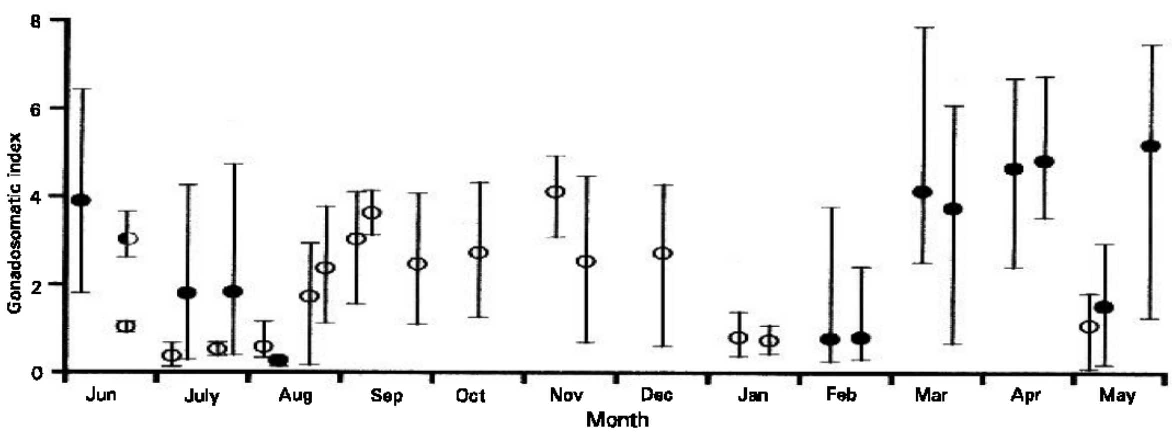


Fig. 5: Averages (circles) and ranges (vertical bars) of GSI (GW/BW X100). For open, closed and half closed circles, refer to the text and explanation of Fig. 4.

Therefore, it can be said that the former was autumn population, and the latter was spring one. The average and standard deviation of standard length are shown in Fig. 6, based on the results of first 10 micro-rings. Referring to Fig. 3, four cohorts seem present: each two in the narrower and wider groups: the narrower group from 1 July to 26 August (the smaller length group) 1997 (N-I), and from 10 September (the smaller length group) 1997 to 22 January 1998 (N-2); and the wider group from 1st July to 2nd August 1997 (W-I), and from 26 February to 31 May 1998 (W-2). N-I grew from 72.60 to 79.40 mm SL in average within about two months, and N-2 from 75.70 to 109.70 mm SL in average within five months. W-I grew from 89.75 to 91.45 mm SL in average within one month, and W-2 from 89.70 to 103.25 mm SL in average within four months. Thus, the two narrower group cohorts, especially N-2, grew better as compared with the

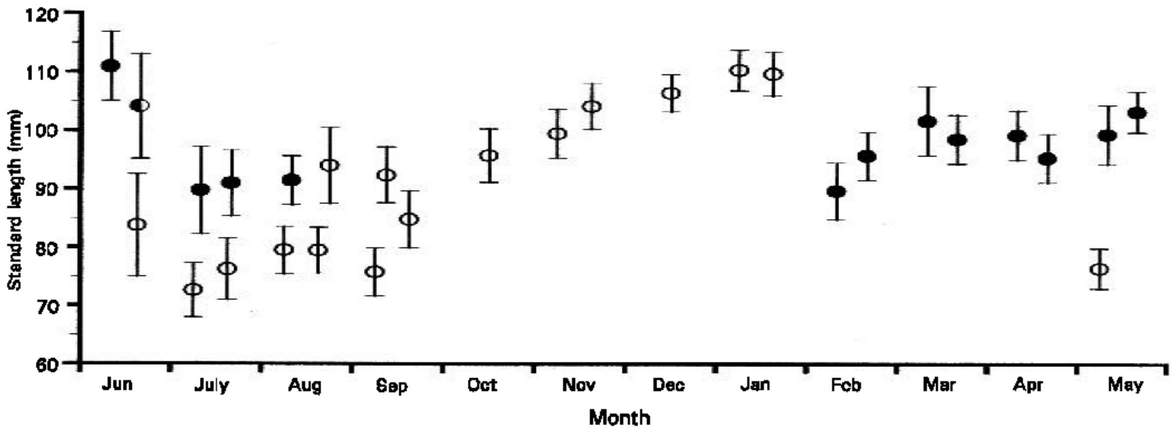


Fig. 6: Mean (circles) and standard deviation (vertical bars) of standard length frequency distribution shown in Fig .3. For open, closed and half closed circles, refer to the text and explanation of Fig. 4.

two wider group cohorts, especially W-2 showing no growth from March to May.

## Discussion

To distinguish local and seasonal populations, various methods and techniques have been used in fisheries biology as follows: morphological methods; ecological methods including mark and recapture, and fisheries data; and biochemical and genetic methods (Nose *et al.*, 1988). The seasonal populations of Japanese anchovy were successfully identified based on the number of vertebrae and the size of first annual ring (Takeshita and Tsukahara, 1971). However, the anchovy in Kagoshima Bay showed no significant difference in number of vertebrae and no significant frequency of specimens with clearly discernible annuli on scales (Ahmed, 1997). Also in this study, they did not show any annulus-like marks either on intact or sectioned otoliths.

Recently, otolith micro-rings have been used for the estimation of early life of fish such as birth dates, growth and duration of particular developmental stage (Tsukamoto *et al.*, 1998; Kawakami *et al.*, 1998; Methot and Kramer, 1979; Palomera *et al.*, 1988; Ozawa and Penaflor, 1990). There seems no study having distinguished directly the populations with otolith micro-rings. However, the temporal and spatial difference of growth in larval stages has been estimated in several studies with micro-rings, for example, in Norwegian herring (Moksness and Fossum, 1992; Fossum and Moksness, 1993), Japanese anchovy (Kim and Kim, 1986; Tominaga, 1996), Japanese sardine (Ohshimo *et al.*, 1997), etc. Those studies were the suggestion of the present study which was able to distinguish the seasonal populations of Japanese anchovy in Kagoshima Bay with the widths of micro-rings. When no techniques are available, the examination of micro-rings seems useful for distinguishing the populations.

As suggested above, width of micro-rings is a mirror of growth. In this study on Japanese anchovy in Kagoshima Bay, the spring population had wider width of micro-rings than the autumn one, suggesting that the former population grew faster than the latter just after hatch. Growth of fish is influenced by a number of exogenous and endogenous factors such as food abundance and quality (Hofer *et al.*, 1985), temperature (Goolish and Adelman, 1984), oxygen availability (Weber and Kramer, 1983), salinity (McKay and Gjerd, 1985), light (Nelson and Geen, 1982), and egg size at hatching (Coleman and Galvani, 1998). Those factors alone or together can control the growth of fish. Water temperature seems to have significant effect on the growth of Japanese anchovy larvae since those spawned in April to June and July to September took 24 to 48 days and 23 to 34 days, respectively, to grow to about 20 mm in total length (Tominaga, 1996). According to ten years, 1983-93, survey in Kagoshima Bay, surface temperature followed sine curve with the lowest temperature in February or March and highest one in August (Watanabe, 1996). The high GSI lasted from March to June in spring population, and from September to December in autumn one (Fig. 5). Therefore, the temperatures are higher, *i.e.*, better, for the larval growth of autumn population than spring one. On the other hand, water temperature seems to act reversibly on the growth of Japanese anchovy larvae. Egg diameters of Japanese anchovy were negatively correlated with surface water temperature (Imai and Tanaka, 1987), being larger in spring population (about 1.4 mm in diameter) than autumn one (about 1.3 mm) (Ochiai and Tanaka, 1986). Larvae hatched from larger eggs may grow faster than those from smaller ones.

In Kagoshima Bay, copepods, the main diets of anchovy larvae (Uotani *et al.*, 1978) showed two seasonal peaks, with spring one higher than autumn (Nozawa and Saisho, 1980), suggesting that the larvae of spring population may grow faster than those of autumn one. As far as the factors examined, egg size and food abundance seem to be dominant factors controlling the growth of the anchovy larvae in Kagoshima Bay.

The growth of Japanese anchovy through life is difficult to study, and the results are not conclusive (Hayashi, 1961). In this study, the autumn population cohorts grew better than the spring ones (Fig. 6). Recently, the growth of Japanese anchovy seems rarely studied, therefore results comparable with the present one are absent.

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