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Daily growth increments in otoliths of milkfish, *Chanos chanos* (Forsskål), larvae

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The formation of daily growth increments of otoliths was studied in the reared larvae of milkfish. The first growth increment was formed during the yolk-sac reabsorption period *c*.2 days after hatching, and increment formation continued on a daily schedule regardless of growth rate. The initial incremental zone was of amorphous structure, and the subsequent incremental zone was of needle-shaped crystalline structure; the former structure was formed in the yolk-sac reabsorption period of the larva and the latter in the exogenous feeding period. Accordingly, ageing of milkfish larvae is possible by counting growth increments, and timings within the developmental stage of the larvae can be understood by examining the otolith microstructure.

I. INTRODUCTION

Milkfish, *Chanos chanos* (Forsskål), is one of the most important brackish-water cultured species in Taiwan. The demand of milkfish fry for culture is estimated at approximately 200 million individuals per year. Induced spawnings of milkfish were attempted in the Philippines, Taiwan and Hawaii, but very limited quantities of fry were produced, and the supply of milkfish fry is still largely dependent on the naturally recruited stock. The supply of wild-captured fry fluctuates and is insufficient to meet demands, so that progress in development of the milkfish culture industry has been hampered for a long time. Increased knowledge of the early life history of milkfish is considered to be important for improving the catch efficiency of wild fry and for helping in artificial propagation and larval rearing (Villaluz *et al.*, 1982; Juario *et al.*, 1984; Lam, 1984).

Since the daily formation of growth increments in fish otoliths was reported by Pannella (1971), ageing larval and juvenile fish by counting daily growth increments has become a widely used technique in studying the early life history of the fish. Thus, the research on the timing of spawning and hatching (Ralston, 1976; Struhsaker & Uchiyama, 1976), growth rate (Townsend & Graham, 1981; Methot, 1981; Bolz & Lough, 1983) and of life history transitions (Radtke & Dean, 1982; Campana, 1984; Neilson *et al.*, 1985) have often involved examination of otolith microstructure.

This paper aims to validate the daily growth increments in otoliths of reared milkfish larvae, from which the ageing technique can be applied to the wild populations.

II. MATERIALS AND METHODS

The milkfish larvae used in this study were hatched from naturally spawned eggs in captivity at Tung-shing Aquatic Hatchery, Pingtung Hsien, Taiwan in April–August 1986.

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TABLE I. Water temperature at 1 m depth in milkfish fry rearing pond, measured at 06.00 and 15.00 hours in April and August 1986

Date	No. of measurement	Water temperature (°C)			
		06.00 hours		15.00 hours	
		Mean	S.D.	Mean	S.D.
April 12–19	11	26·15	0·50	29·48	0·48
April 26–29	11	28·20	0·28	31·46	1·27
August 4–6	9	29·04	0·31	33·11	0·33

They were fed with *Chlorella,* rotifers and artificial food (Lin, 1984). The water temperature of the rearing pond was 26–29° C and 29–33° C in April and August, respectively (Table I).

Five to ten larvae were collected from the rearing pond at about 06.00 hours daily over the entire larval period up to 17 days after hatching, at which time the fry were released for stocking. The larvae collected were preserved immediately in absolute ethyl alcohol. The total length of the larvae was measured to the nearest 0·1 mm after 1-week fixation. Both sagittal otoliths were removed and mounted with Permount on a microscope slide with proximal surface down. Otolith measurements and increment counts were made with a Video-Microscope-IBM/PC image analysis system (VISION, VID-512) at magnification ×400–1000. Resolution ability of image analysis was 512 × 512 pixels, and the pixel intensity was 64 gray level. Polarized light source was used to improve the visibility of rings.

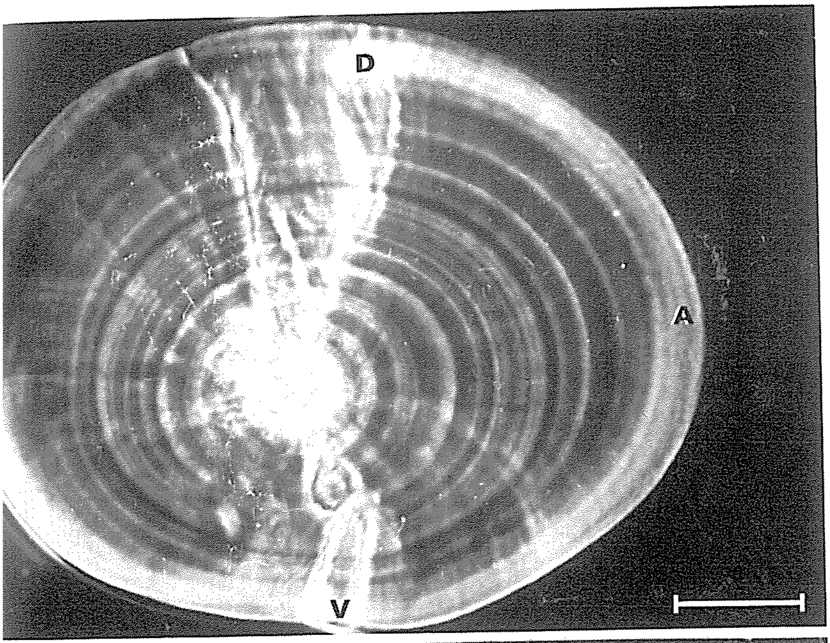
A growth increment is a bipartite structure composed of incremental and discontinuity zones. These two zones have different light transmittance and are easily discernible by the gray level of the image when the otolith is examined under the image analysis system. Both maximal and minimal radii from primordium to edge of the otolith along the anterior–posterior and dorsal–ventral axes of the otolith, respectively, (Fig. 1) were measured to the nearest μm. In order to examine the microstructure of the incremental zone, selected otoliths were photographed using a Zeiss Universal large research phase microscope.

The fish length/age and otolith radius/age relationships were fitted with an exponential growth equation, and the otolith radius/fish length relationship with linear regression. These relationships between April and August were compared by covariance analysis (Snedecor & Cochran, 1969).

III. RESULTS

OTOLITH GROWTH INCREMENT

When viewed under the phase-contrast microscope with transmitted light, the otoliths of milkfish larvae showed a series of alternate dark and light bands which corresponded to the heavily calcified incremental zones and organic-rich discontinuity zones (Fig. 1). At a high magnification (Fig. 2) the first incremental zone was seen as a thick amorphous structure, while subsequent incremental zones were of needle-shaped crystalline structure. A single growth increment comprising an incremental zone plus a discontinuity zone is generally assumed to represent 1 day. An otolith at age 6 days is shown in Fig. 2. The yolk sac of the milkfish was absorbed 2 days after hatching and feeding began from this time on. Comparing the number of growth increments in the otolith with the age of the larvae, it appears that the first growth increment was deposited during the yolk sac absorption period and the subsequent increments were deposited during the exogenous feeding period.



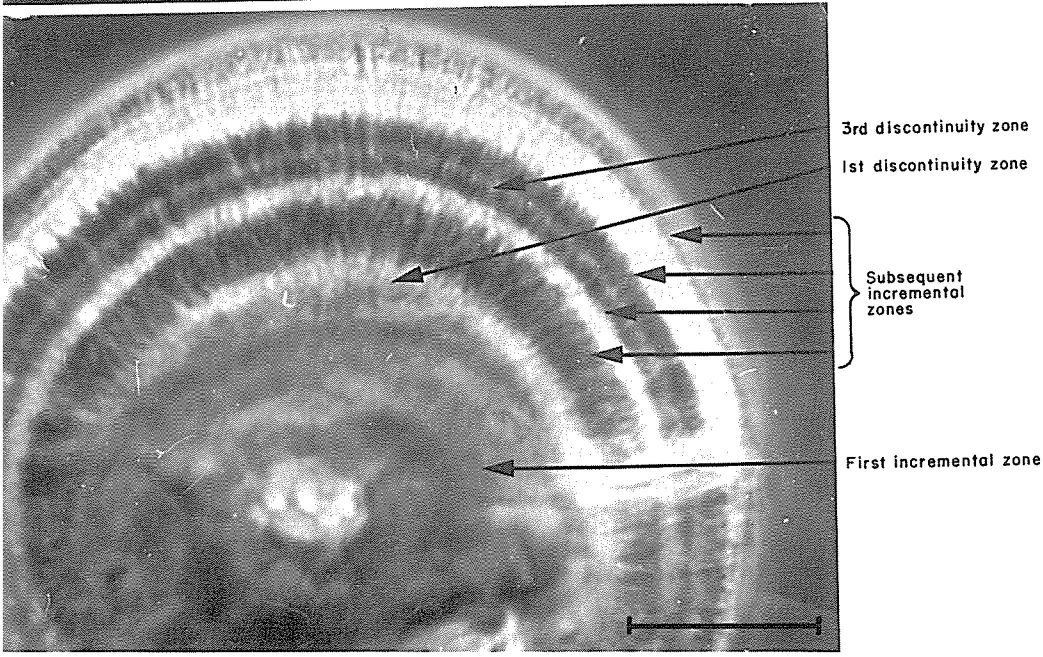


FIG. 1. Sagittal otolith from right sacculus of a 13-day-old milkfish larva, 8·4 mm T.L. (× 1600). Scale bar = 20 μm. A, anterior; P, posterior; D, dorsal; V, ventral. Dark band is incremental zone; light band is discontinuity zone.

FIG. 2. Sagittal otolith of a 6-day-old milkfish larva, 5·4 mm T.L. (× 5100). Scale bar = 10 μm. The first incremental zone is a wide amorphous structure and the subsequent four incremental zones (dark bands) are needle-shaped crystalline structures. The latter are separated by discontinuity zones (light bands). The third discontinuity zone is very narrow.

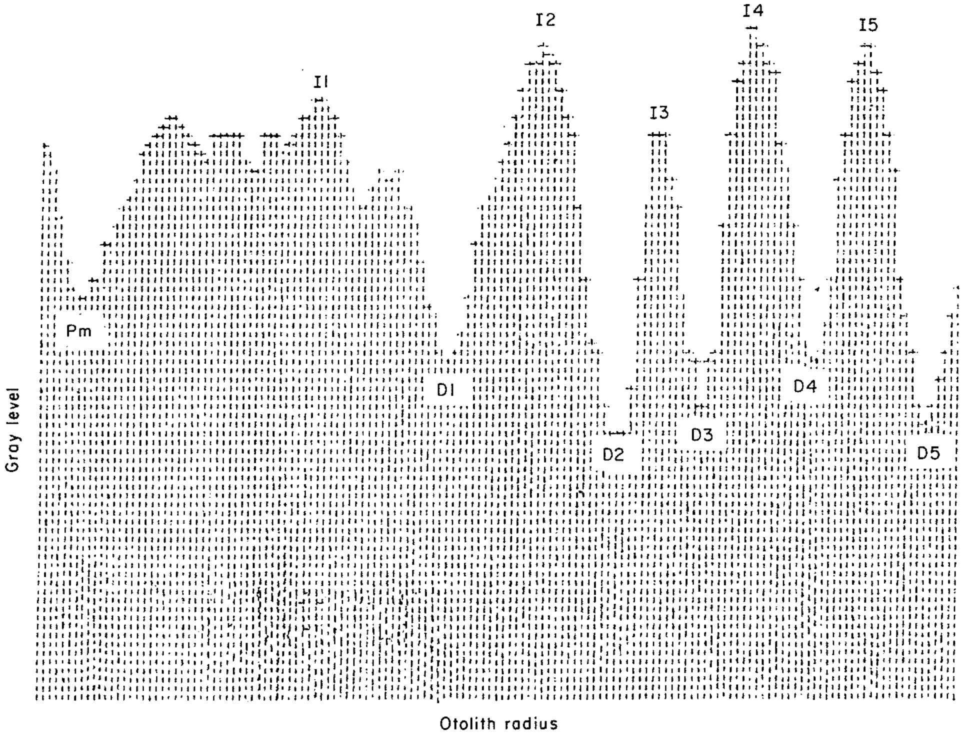


FIG. 3. Digitized image diagram scanned from primordium to edge of the otolith of Fig. 2. Crests (I1–5) and troughs (D1–5) of the diagram represent the incremental and discontinuity zones of the otolith, respectively. Pm, primordium.

The number of growth increments in an otolith could be easily measured by using the Video-Microscope-IBM/PC image analysis system, in which the microscope-viewed incremental and discontinuity zones were converted into a digitized image diagram. The digitized image diagram scanned from the otolith of Fig. 2 is shown in Fig. 3. The crests and troughs from left to right in Fig. 3 corresponded to the incremental zones (dark bands) and discontinuity zones (light bands) of the otolith from the primordium to the edge shown in Fig. 2. The third discontinuity zone in Fig. 2 may be overlooked by the naked eye because it is narrow and unclear; however, the corresponding third trough in Fig. 3 distinguished it. The method of image processing is, therefore, considered a good auxilliary tool in reading the growth increments of the otoliths of larvae.

FISH GROWTH

Total length (range 3·0–12·0 mm) was plotted against age (range 1–17 days) separately for the milkfish larvae collected in April and August (Fig. 4). An exponential growth curve fitted well to represent their growth:

$$\text{April; } Y = 3{\cdot}419\, e^{0{\cdot}071X} (n = 69, r = 0{\cdot}951)$$

$$\text{August; } Y = 3{\cdot}985\, e^{0{\cdot}057X} (n = 40, r = 0{\cdot}958)$$

where Y is the total length in mm and X is the age in days after hatching.

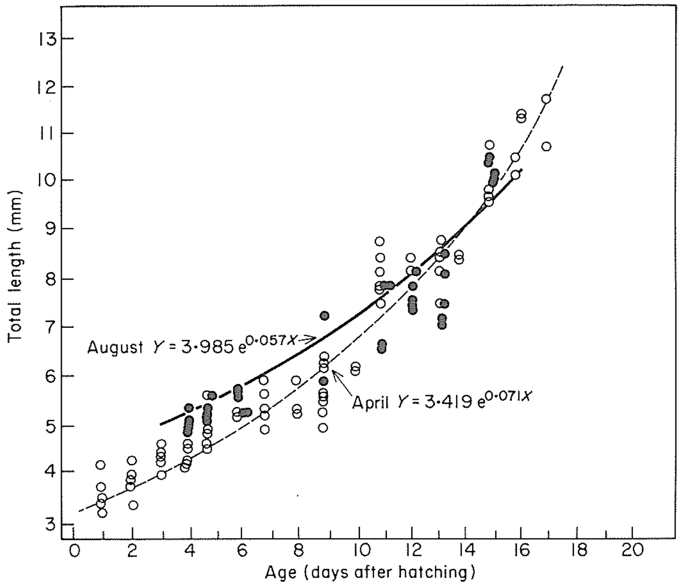


FIG. 4. Plot of total length *v.* age in days after hatching for milkfish larvae collected in April, ○ -- ○, and August, ●——●, 1986, with fitted exponential growth equation.

The mean total length of newly-hatched larvae was 3·6 mm (range 3·2–4·3 mm) and the growth was exponential. A significant difference in the growth between larvae collected in April and those in August was detected using the covariance analysis method (F_o, F_a, N.S.; F_b, $P<0{\cdot}01$; where F_o, F_a and F_b indicate the F-test difference between residual mean squares, adjusted means and slopes of regression lines, respectively).

OTOLITH GROWTH

The sagittal otolith at hatching is essentially spherical; as it grows it elongates along the anterior–posterior axis to become oval in shape. It generally has a slightly convex distal side and a flat proximal side with three or four furrows radiating from a central primordium. A slight protuberance is apparent on the anterior edge of the otolith, and the posterior edge is rounded (Fig. 1).

The relationships between the otolith radii (both the maximum radius along the anterior–posterior axis and the minimum radius along the dorsal–ventral axis) and the total length in the months of April and August are plotted in Fig. 5. The following linear regressions were fitted:

$$\begin{array}{lll} \text{April:} & \text{Max} & Y=-34{\cdot}897+12{\cdot}132X\,(n=81, r=0{\cdot}951) \\ & \text{Min} & Y=-13{\cdot}424+6{\cdot}548X\,(n=81, r=0{\cdot}928) \\ \text{August:} & \text{Max} & Y=-33{\cdot}163+10{\cdot}626X\,(n=71, r=0{\cdot}918) \\ & \text{Min} & Y=-11{\cdot}852+5{\cdot}451X\,(n=71, r=0{\cdot}938) \end{array}$$

where Y is the radius of otolith in μm and X the total length of fish in mm. The growth rate of the maximum radius was approximately twice that of the minimum radius of otoliths collected in April and in August. Meanwhile, a significant

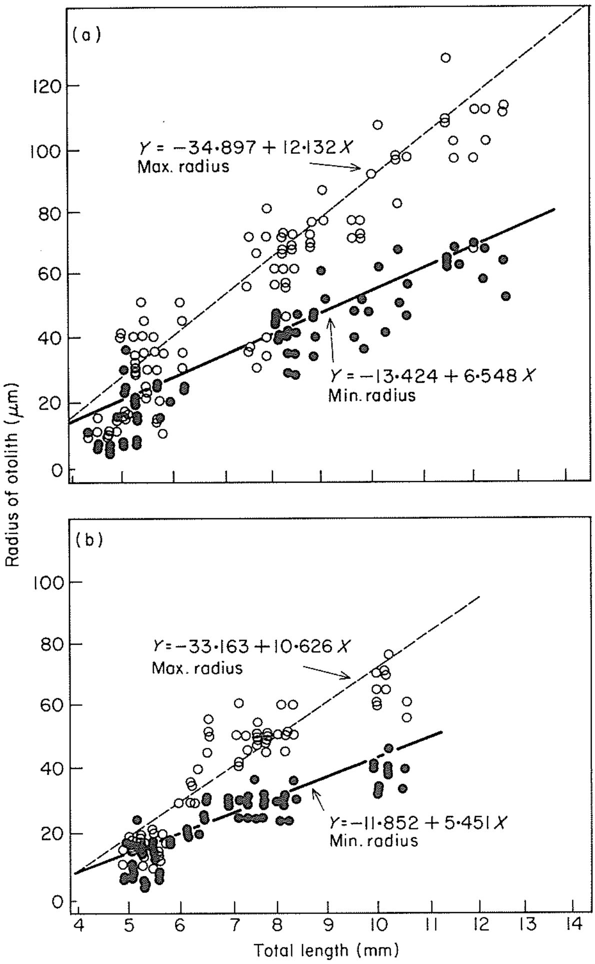


FIG. 5. Plots of maximum and minimum otolith radii *v.* total length for milkfish larvae hatched in (a) April and (b) August 1986, with fitted linear regression lines.

difference in the maximum otolith radius/fish length relationships between the milkfish larvae collected in April and those in August was indicated from the covariance analysis of regression lines (F_0, N.S.; F_b, $0{\cdot}01 < P < 0{\cdot}05$; F_a, $P < 0{\cdot}01$).

The maximum otolith radius/age relationships for milkfish larvae collected in April and August (Fig. 6), were fitted to an exponential growth curve as follows:

$$\text{April; } Y = 8{\cdot}947\, e^{0{\cdot}155X} (n = 81, r = 0{\cdot}965)$$

$$\text{August; } Y = 10{\cdot}141\, e^{0{\cdot}138X} (n = 71, r = 0{\cdot}967)$$

where Y is the maximum radius of sagittal otolith in μm and X the age in days after hatching. The sagittal otolith of milkfish at the time of hatching was 9–10 μm in radius, and it grew exponentially. Covariance analysis indicated that there was a significant difference in otolith radius/age relationships between the milkfish larvae collected in April and those in August (F_0, N.S.; F_b, F_a, $0{\cdot}01 < P < 0{\cdot}05$).

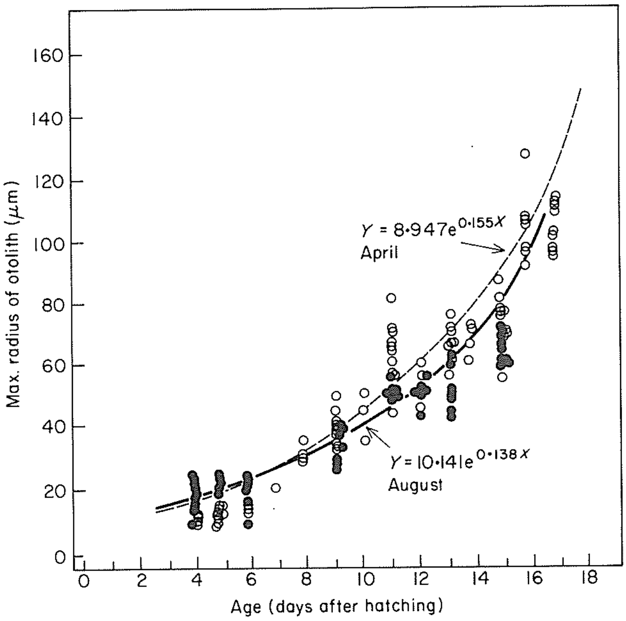


FIG. 6. Plot of otolith maximum radius *v*. age in days after hatching for milkfish larvae collected in April, ○ – – ○, and August, ●——●, 1986, with fitted exponential growth equation.

RELATION BETWEEN THE NUMBER OF OTOLITH GROWTH INCREMENTS AND AGE IN DAYS AFTER HATCHING

The number of otolith growth increments *v*. age in days after hatching for milkfish larvae collected both in April and in August are shown in Fig. 7. Linear regressions were fitted as follows:

$$\text{April; } Y = -0{\cdot}930 + 1{\cdot}076X\ (n = 80,\ r = 0{\cdot}979)$$
$$\text{August; } Y = -0{\cdot}565 + 1{\cdot}024X\ (n = 67,\ r = 0{\cdot}984)$$

where Y is the number of growth increment in otolith of milkfish larvae and X is the age in days after hatching. The slopes of these regressions were not significantly different from 1 in either case (t-test, $P > 0{\cdot}05$), but the intercepts were significantly different from 0 for April ($P < 0{\cdot}01$) and August ($0{\cdot}01 < P < 0{\cdot}05$) and not significantly different from -1 in both months ($P > 0{\cdot}05$). Furthermore, the regressions for April and August were not significantly different by covariance analysis (F_o, F_b and F_a all N.S., $P > 0{\cdot}05$).

Accordingly, the relationship between the number of growth increments in the otolith (N) and the age of milkfish in days after hatching (D) for larvae collected both in April and in August can be simplified as $N = D - 1$.

Otoliths formed their initial growth increment at about 2 days after hatching, and the increment formation continued on a daily schedule.

IV. DISCUSSION AND CONCLUSION

The relationship between the number of growth increments in the otolith of milkfish and age in days after hatching, $N = D - 1$, indicates that growth increments

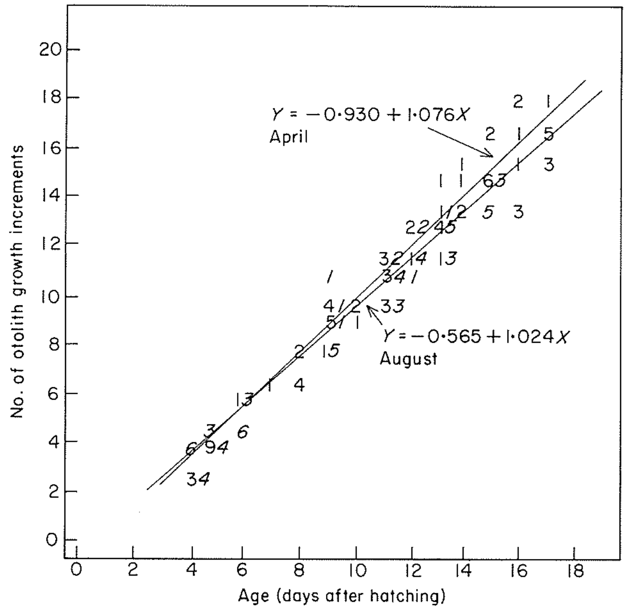


FIG. 7. Plot of otolith increments *v.* age in days after hatching for milkfish larvae hatched in April and August 1986, with fitted linear regression line (figures indicate the number of observations at each age: upright figures, April; italic figures, August).

are formed daily, because the slope equals unity. The intercept −1 implies that initial daily growth increment is formed on the second day after hatching. In milkfish larvae the yolk sac is completely absorbed in approximately 50 h (Lin, 1984); a similar time of about 2 days was observed in the present study. Therefore, it is considered that initial daily growth increments begin at the time of completion of the yolk absorption. This was also found in cod (Radtke & Waiwood, 1980), Atlantic herring (Lough *et al.,* 1982), Pacific herring (McGurk, 1984) and Japanese anchovy (Tsuji & Aoyama, 1984). The structural pattern of the first incremental zone and the following incremental zones are different. The former is an amorphous structure, while the latter is a needle-shaped crystalline structure (Fig. 2). The formation times of these structures correspond to the yolk-sac period and exogenous feeding period, respectively. The utilization of the yolk sac by larvae is directly related to water temperature (Blaxter, 1956; Blaxter & Hempel, 1966) and variations in water temperature at hatch could reduce or extend the time to first feeding and consequently otolith increment formation. The water temperature in August was about 3° C higher than that in April during the egg incubation period (Table I). However, the first growth increment was not formed earlier in the milkfish hatched in August than that in those hatched in April, because the intercept was not different from −1 for either month (Fig. 7).

Comparison of the regression lines of otolith radius *v.* fish length and of otolith radius and fish length *v.* age for the milkfish larvae between April and August shows that the growth rate of milkfish larvae varies with season. Environmental and genetic factors both influence the growth of milkfish larvae to a great extent; these include water temperature, salinity, feeding conditions, stocking density, health conditions of larvae, genetic make-up of breeders, and other environmental factors (Lin, 1984; Campana & Neilson, 1985). The milkfish larvae used in this study were collected in two different months in which the water temperature was obviously different (Table I), and the larvae came from different spawners. The effect of genetic make-up on the growth rate of the progeny cannot be evaluated in this study. Nevertheless, the number of otolith growth increments was still highly positively correlated with age in days after hatching and there was no difference in the regression lines of otolith growth increment on age between April and August (Fig. 7). The increment formation is independent of growth rate, but is age-dependent; thus, ageing of milkfish larvae is possible by counting the growth increments in otoliths.

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