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Effects of starvation on the formation of daily growth increments in the otoliths of milkfish, *Chanos chanos* (Forsskål), larvae

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The effects of starvation on daily growth and increment formation in the otolith were examined using a double oxytetracycline-labelling method on larval milkfish, *Chanos chanos* (Forsskål), reared under different feeding regimes. The results indicated that the differences in body and otolith growth between the larvae fed once and three times a day were not significant, and that the otolith growth increment was deposited daily in both groups of fed larvae. In contrast, the starved larvae grew at a slower rate than fed larvae in body length and otolith dimensions, and the otolith growth increment in the starved larvae was not deposited on a daily basis. After undergoing starvation, the larvae were unable to recover their normal growth either in otolith increment deposition or in body and otolith growth even though they were fed. Therefore, the application of ageing techniques based on counting otolith growth increments seems to be inaccurate for starved larvae.

Key words: *Chanos chanos*; starvation effects; growth; otolith; daily growth increment.

I. INTRODUCTION

Milkfish, *Chanos chanos* (Forsskål), one of the most important fish species cultured in the southeast Asian region, have been cultured for over 300 years in Taiwan. For cultivation purposes, large numbers of milkfish fry are caught in the surf zone of coastal waters during the period April to September (Chen, 1952). Although much information is available on the ecology of this species, the early life history of milkfish is still poorly known (Liao *et al.*, 1979; Buri & Kawamura, 1983; Taki *et al.*, 1987; Tzeng & Yu, 1990).

The daily formation of growth increments in the otoliths of milkfish larvae has been validated using hatchery-reared larvae of known age (Tzeng & Yu, 1988) and by examination of the newly deposited increments following marking with oxytetracycline (OTC) (Tzeng & Yu, 1989). Milkfish larvae often experience starvation owing to the fact that food organisms are not sufficiently abundant in the surf zone of coastal waters (Taki *et al.*, 1990).

The object of this paper is to describe the effects of feeding frequency and starvation on the formation of daily growth increments in the otoliths of milkfish larvae using a double OTC-labelling technique to mark the growth margin of otoliths of wild-captured larvae.

II. MATERIALS AND METHODS

EXPERIMENTAL DESIGN

Milkfish larvae used in this study were collected with a skim net in the surf zone in the Tour-Cherng area of northeastern Taiwan on 18 August 1988. The size of the milkfish

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larvae captured ranged from 11·0 to 17·0 mm and averaged 14·2 mm in total length (T.L.). Larvae collected were acclimatized for about 4 days at a density of 6–7 fish per l in 10 ppt sea water, under a natural photoperiod, at a temperature of about 29° C, and fed with formulated elver's feed three times a day until the OTC-labelling experiment was attempted. The larvae were immersed in 400 mg OTC l^{-1} for 24 h to create the first OTC-mark in the otolith. After treatment, the larvae were immediately transferred to 10 ppt natural sea water at a stocking density of 2–3 l^{-1} and fed for 10 days with formulated elver's feed under three different feeding regimes: (1) fed once per 24 h (F1), (2) fed three times per 24 h (F3), (3) fed 1 day (once per 24 h) and starved 4 days (STAV). The time period of starvation for the milkfish larvae was set at 4 days because delayed feeding over a period longer than 4 days causes mass mortality (Taki *et al.*, 1990) and the duration of irreversible starvation for the larvae is 5 days (Liao *et al.*, 1979). The control group was not marked with OTC and was fed once per 24 h. Each feeding regime was duplicated, except for the control group. The larvae were all fed to satiation in the day. After 1 h the remaining food was siphoned from the aquarium. After each 10-day feeding experiment, the larvae were immersed in 400 mg OTC l^{-1} for 24 h to create the second OTC-mark in the otolith and then transferred to 10 ppt natural sea water and fed three times per 24 h with formulated elver's feed for another 10 days. At the end of each experiment, the larvae were immediately preserved in absolute ethyl alcohol. The total lengths of the preserved fish were measured to the nearest 0·1 mm after 1 week fixation.

SPECIMEN AND DATA PROCESSING

The sagittal otoliths were removed from the larvae with an insect needle mounted on a wooden rod under a dissecting microscope. After removal, the otoliths were mounted with permount on a microscope slide with the proximal surface down. A fluorescence microscope (Nikon Optiphot) with incident uv light from a 50-W mercury lamp was used to detect the fluorescent OTC-marked ring on the otolith; transmitted light was used to reveal daily growth increments. The excitation wavelength of the incident uv light was limited by a band-pass filter (400–440 nm) and a long-pass barrier filter (470 nm). A single growth increment, consisting of an incremental zone and a discontinuous zone, was deposited within 24 h (Tzeng & Yu, 1988). The growth increments deposited in the layer between the first and second OTC-bands and in the layer between the second OTC-band and the edge of the otolith were counted, and then the effects of starvation and feeding frequency on the formation of growth increments in the otolith were analysed. Both the counting of the otolith growth increments and the measurement of the radius and increment width of the otolith were made along the longest axis of the otolith using a photograph taken under the combination of reflected UV and transmitted visible light at a magnification of 400 ×. Using a *t*-test, the otolith growth increments, the body growth and the otolith growth were compared between starved and fed larvae (Snedecor & Cochran, 1967).

III. RESULTS

COMPARISON OF MORTALITY RATE BETWEEN FED AND STARVED GROUPS

The mortality rate of milkfish larvae over the 20-day experimental period was 10 and 26% respectively for the two starved groups, compared with 0–6% in fed groups (Table I). The mortality rate of the control group with no OTC treatment was 2%, which was close to that of the experimental fed groups. This indicates that the mortality rate of the larvae was not affected by OTC marking.

MICROSTRUCTURE OF OTOLITH GROWTH INCREMENTS IN FED AND STARVED LARVAE

The otoliths from a starved larva (fed 1 day and starved 4 days) showed a narrower increment width than that of a larva fed three times per day [Fig.

TABLE I. Comparison of mortality rate of milkfish larvae between fed and starved groups. The larvae were fed at a different feeding frequency in the first 10 days after the first OTC marking (period 1), and at same frequency in the second 10 days after the second OTC marking (period 2). Control group with no OTC marking. Starved group (STAV) was fed 1 day (1/24 h) and starved 4 days in period 1

Group	Feeding frequency		Duplicate	Sample size	No. dead			Mortality rate (%)
	Period 1	Period 2			Period 1	Period 2	Total	
Control	1/24 h	3/24 h	1	50	1	0	1	2
F1	1/24 h	3/24 h	1	50	0	3	3	6
			2	50	0	1	1	2
F3	3/24 h	3/24 h	1	50	0	0	0	0
			2	50	0	0	0	0
STAV	Starved	3/24 h	1	50	4	9	13	26
			2	50	2	3	5	10

1(A–D)]. The numbers of otolith growth increments deposited during the first and second 10-day rearing periods were only seven and six rings respectively in the starved larva, compared with 13 and 12 rings deposited in the corresponding rearing periods in the fed larva [Fig. 1(E) and (F)]. This indicates that the growth increments in the otolith of the fed larva were deposited more frequently than one per day in both the first and second 10-day rearing periods. However, no subdaily growth increments similar to those described by Marshall and Parker (1982) in the otolith of sockeye salmon, *Oncorhynchus nerka* (Walbaum) could be discriminated, the contrast and spacing of all growth increments being almost uniform [Fig. 1(E)]. By contrast the otolith of the starved larva appeared to have low-contrast growth increments corresponding to the starvation period, but no particularly narrow or crowded growth increments were found and the spacing of growth increments was similar to that of the fed larva [Fig. 1(F)]. The mean widths of growth increments were *c.* 4·0 and 4·48 μm in otoliths of fed and starved larvae, respectively. The deposition rate of growth increments in the otolith of the larva fed three times a day averaged 1·25 rings per 24 h, and was close to one per day, but only 0·65 ring per 24 h in the starved larva. This indicates that the deposition rate of daily growth increments in otoliths of milkfish larvae might be affected by starvation but not by the number of daily feeds. To get further evidence, the following statistical analyses on fish and otolith growth and growth increments were conducted.

GROWTH OF LARVAE

The mean total length ($\pm$S.D.) of the milkfish larvae was $14{\cdot}2 \pm 0{\cdot}8$ mm at the beginning of the experiment (Fig. 2). After the 20-day rearing period, the mean total length was $19{\cdot}7 \pm 6{\cdot}5$ mm, $19{\cdot}2 \pm 8{\cdot}2$ mm and $17{\cdot}0 \pm 2{\cdot}7$ mm respectively for the two fed groups (F1 and F3) and for the starved larvae. The growth showed no significant difference between the two groups of fed larvae, F1 and F3 ($P > 0{\cdot}05$). In contrast, the growth was significantly less in the starved larvae than in the fed larvae ($P < 0{\cdot}01$).

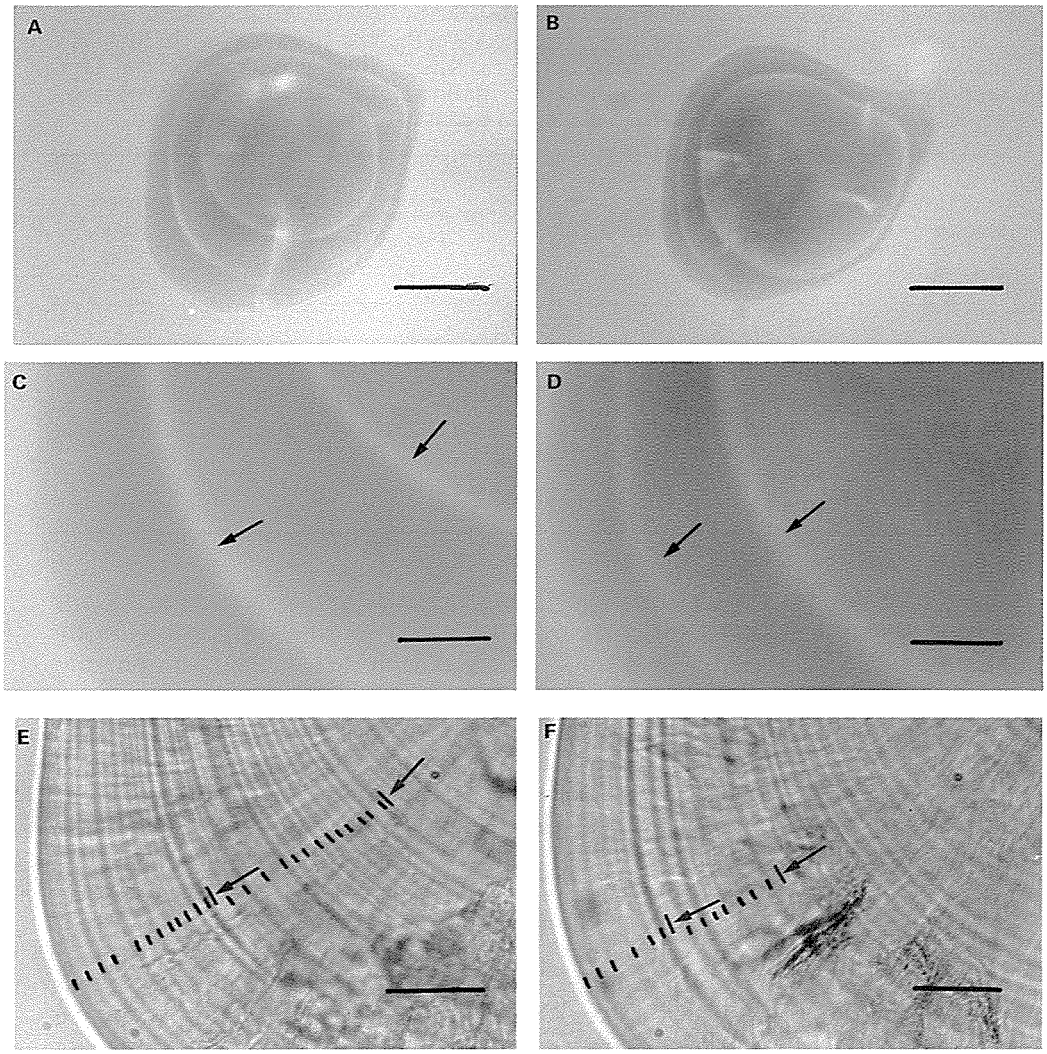

FIG. 1. Fluorophotographs showing OTC marks (A–D) and growth increments (E and F) deposited after OTC marking in otoliths of two milkfish larvae reared under different feeding regimes. (A), (C) and (E) From a 18·7 mm T.L. larva fed three times per 24 h for 10 days, respectively, after the first and second OTC markings. (B) (D) and (F) From a 17·0 mm T.L. larva fed once per 24 h and starved 4 days after the first OTC marking in the first 10 days and fed three times per 24 h after the second OTC marking in the second 10 days. (A)–(D) were photographed with UV light to reveal OTC marks, (E) and (F) with UV and transmitted light to reveal newly-deposited growth increments. (C) and (E) enlarged portion of (A), (D) and (F) from (B). Arrows indicate OTC marks. Bar = 100 μm (A) and (B), 25 μm (C)–(F).

OTOLITH GROWTH

The otolith radius of milkfish larvae before the first OTC-marking period was similar in the F1, F3 and starved groups with a mode at 100–120 μm (Fig. 3). After the first 10-day rearing period, the mean otolith radii of the fed larvae (F1 and F3 groups) were $157{\cdot}6 \pm 3{\cdot}7$ μm and $161{\cdot}5 \pm 4{\cdot}0$ μm respectively, values which are not

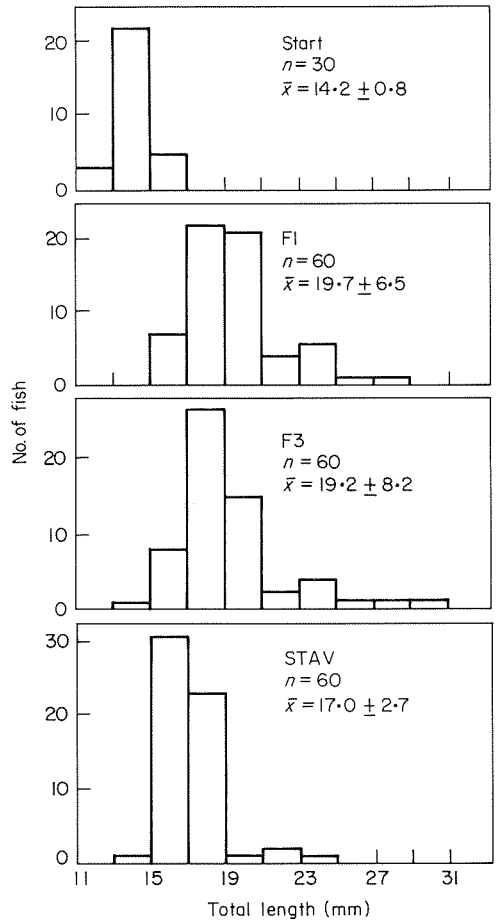

FIG. 2. Length–frequency distribution of milkfish larvae at the beginning of experiment (start) and after 20-days rearing with three different feeding regimes. N=sample size; $\bar{x}$=mean±S.D.; F1, F3 and STAV as in Table I.

significantly different ($P>0{\cdot}05$). In contrast, the mean otolith radius was significantly smaller in the starved larvae (134·7±2·5 μm) than in the fed larvae ($P<0{\cdot}01$) (Fig. 3). After the second 10-day period, the mean otolith radius of the group starved during the first period was still smaller than that of the other two fed groups (STAV, 164·9±4·1 μm; F1, 210·2±6·4 μm; F3, 211·2±6·2 μm) ($P<0{\cdot}01$) (Fig. 3). The increment width from the first to the second OTC band during the first 10-day rearing period (period 1) was also significantly smaller in the starved larvae (26·4±1·5 μm) than in the fed larvae (F1, 43·6±2·5 μm; F3, 51·5±3·5 μm) ($P<0{\cdot}01$) (Fig. 4). Similarly, the mean increment width from the second OTC band to the edge of the otolith deposited during the second 10-day period was also smaller in the starved larvae (30·2±2·4 μm) than in the two fed groups (F1, 52·5±3·5 μm and F3, 50·1±3·4 μm) ($P<0{\cdot}01$) (Fig. 4). These facts indicate that the effect of starvation on increment formation in the otolith of milkfish larvae

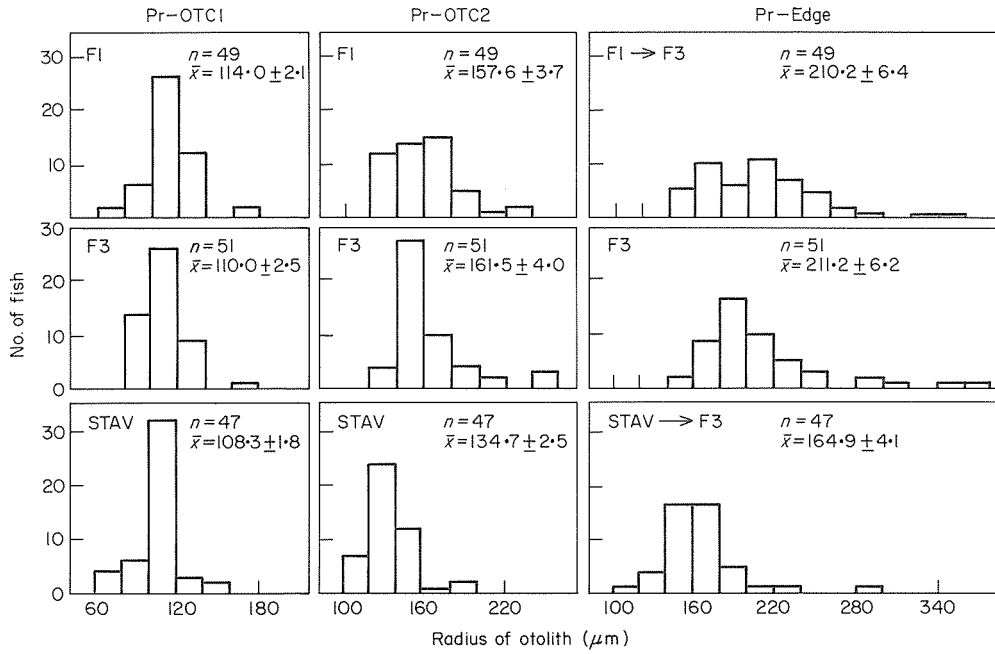

FIG. 3. Frequency distribution of otolith maximum radius measured respectively from primordium to the first OTC (Pr-OTC1), primordium to the second OTC mark (Pr-OTC2) and primordium to the peripheral edge (Pr-Edge) of the otolith of the milkfish larvae reared under different feeding regimes in the first 10 days after first OTC marking and with same feeding regimes in the second 10 days after the second OTC marking. F1, F3 and STAV as in Table I.

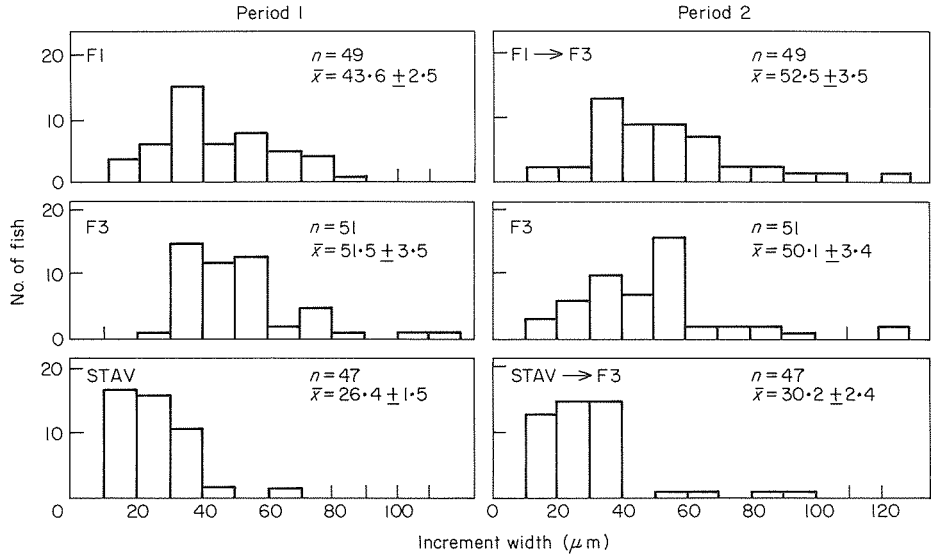

FIG. 4. Frequency distribution of increment width of the otolith of milkfish larvae reared under three different feeding regimes in the first 10 days (period 1) and reared with same feeding regimes in the second 10 days (period 2). F1, F3 and STAV as in Table I.

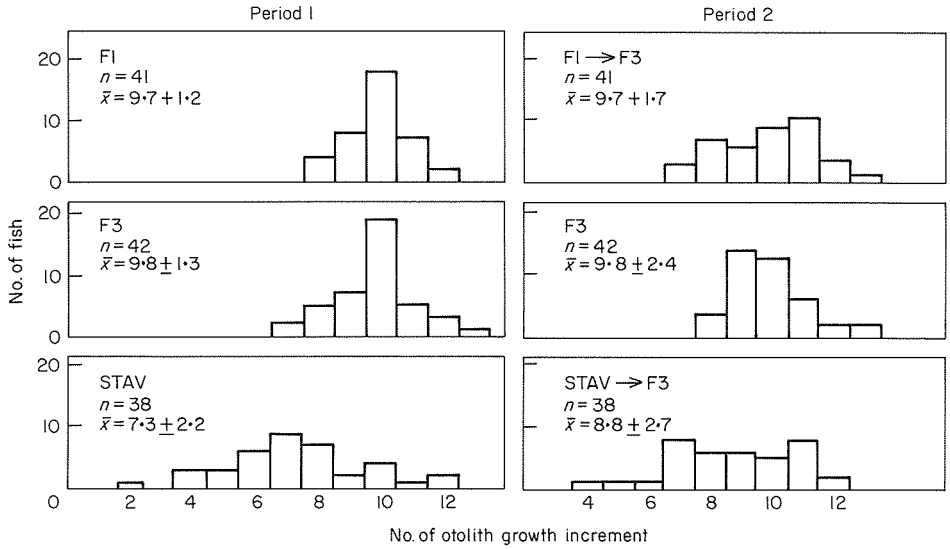

FIG. 5. Frequency distribution of the number of growth increments deposited in the otoliths of milkfish larvae reared under three different feeding regimes in the first 10 days (period 1) and with same feeding regimes in the second 10 days (period 2). F1, F3 and STAV as in Table I.

extended beyond the starvation period when the larvae were given multiple daily feeds.

DAILY GROWTH INCREMENT FORMATION

The mean number of growth increments deposited during the first 10-day rearing period was $7{\cdot}3 \pm 2{\cdot}2$ rings for the starved larvae, which was significantly less than one per day (t-test, $P < 0{\cdot}01$). The numbers of growth increments in otoliths of fed larvae were $9{\cdot}7 \pm 1{\cdot}2$ and $9{\cdot}8 \pm 1{\cdot}3$ rings in the F1 and F3 groups respectively; these were not significantly different from one per day ($P > 0{\cdot}05$). This indicates that the formation of daily growth increments in otoliths of milkfish larvae was affected by starvation but not by feeding frequency within the range tested. On the other hand, although the starved larvae were fed during the second 10-day period, the growth increments deposited in their otoliths ($8{\cdot}8 \pm 2{\cdot}7$) was still less than unity (Fig. 5). This indicates that the effect of starvation on the formation of otolith growth increments extends beyond the period of starvation.

IV. DISCUSSION AND CONCLUSIONS

Otolith growth increments in the starved milkfish larvae were not deposited on a daily basis (Figs 1, 5). Some researchers have considered the possibility that the under-estimation of daily growth increments in otoliths of starved larvae may be due to the poor resolving power of the light microscope, which is unable to discriminate the fine increments deposited during the starvation period (Jones & Brother, 1987). However, the otoliths of starved milkfish larvae did not have particularly narrow or crowded growth increments deposited during starvation episodes; in fact, the mean width of the growth increments in the otolith of the

starved milkfish larva shown in Fig. 1(F) was *c.* 4·48 μm. Indeed, even if the growth increments in the otolith of the starved larva were deposited one per day, the expected mean widths of the growth increments, as estimated from Fig. 4, still has widths of 2·64 and 3·02 μm during the first and second 10-day rearing periods, respectively, which is considerably higher than the resolution limit of the light microscope (0·2 μm) (Campana & Neilson, 1985). Thus, the smaller number of growth increments in the otoliths of starved milkfish larvae seemed not to be an artefact caused by the resolution limit of the light microscope.

The deposition of daily growth increments in the otoliths of fish is regulated by an endogenous circadian rhythm, but factors such as photoperiod, temperature and feeding may act as cyclical entraining influences (Tanaka *et al.*, 1981; Neilson & Geen, 1982, 1985; Campana & Neilson, 1985). The effect of feeding on otolith deposition is more equivocal than is the response to photoperiod and temperature etc. The deposition rate of otolith growth increments between milkfish larvae fed once and three times a day was not significantly different and the otolith growth increments in both groups of fed larvae were deposited approximately on a daily cycle (Fig. 5). This indicates that, so long as the larvae receive one feed per day, daily increment formation is unaffected by the number of daily feeds, which has also been found in other fish species (Taubert & Coble, 1977; Campana, 1983). In contrast, in some species, e.g. juvenile chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), fish fed more than once a day produced significantly more increments than did those fed once daily, but increment number was not directly proportional to feeding frequency (Neilson & Geen, 1982, 1985). A possible explanation for the difference in observations between species in the confusion caused by failing to differentiate between daily and subdaily increments (Campana & Neilson, 1985). In our study we counted all growth increments because daily and subdaily increments in the otoliths of milkfish larvae cannot be differentiated [Fig. 1(E)]. We also found that the number of growth increments in the otoliths of larvae given multiple daily feeds was not proportional to the frequency of daily feeds and was not significantly different from one per day (Fig. 5). Thus, there did not appear to have been an effect of the number of daily feeds on the formation of daily growth increments in the otoliths of milkfish larvae. This point is very important since multiple daily feeding occurs in many fishes in the wild (Keast & Welsh, 1968; Elliott, 1970; Grove *et al.*, 1978). If, on the other hand, the formation of growth increments in otoliths varies with the number of daily feeds, it then becomes impossible to determine the age of the larvae by counting otolith growth increments.

On the other hand, fish larvae subjected to starvation may have their increment deposition disrupted, resulting in apparent non-daily increment formation (Taubert & Cole, 1977). It has been reported that daily growth increments in the otoliths of fish appear not to be affected by food deprivation when body energy reserves are sufficient to maintain limited skeletal growth (Campana, 1983; Volk *et al.*, 1984; Neilson & Geen, 1985). However, such reserves may not have been available to starved northern anchovy, *Engraulis mordax* Girard, larvae, resulting in the cessation of otolith growth (Methot & Kramer, 1979). Similarly, when the somatic growth rate of larval herring, *Clupea harengus* Linnaeus, was below some threshold limit, the otolith growth increments were deposited less than once a day (Geffen, 1982). The otoliths of starved milkfish larvae grew more slowly and produced fewer daily growth increments than those of fed larvae. In addition,

although the duration of starvation of the milkfish larvae in this study was limited to 4 days, i.e. less than the irreversible starvation limit of this species (Liao *et al.*, 1979), the effects of starvation on otolith growth and on the rate of increment formation persisted when multiple daily feeds were resumed (Figs 1–5). Thus, starvation may serve as a masking agent superimposed on the endogenous circadian rhythm of the fish to modify the otolith growth and increment deposition.

In conclusion, starvation affected the rate of otolith increment formation not only during the period when milkfish larvae were subjected to starvation, but also after the starvation period when the larvae had been switched from starvation to multiple daily feeds. The counts of daily growth increments in the otoliths of starved milkfish larvae did not reflect their true age and resulted in inaccurate back calculation of their hatching date and growth rate. In addition, the delayed effect of starvation on the deposition of otolith growth increments might cause errors in estimating the exact timing of the transition in the growth history of larvae relative to changes in environmental conditions. It was reported that approximately 18–36% of natural populations of herring larvae suffered from starvation during the larval stage (McGurk, 1984). Accordingly, we must pay attention to the effects of starvation on otolith growth increment formation when this age determination technique is applied to the ecological study of wild populations.

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