INTERACTIVE EFFECTS OF AGE AND ENVIRONMENTAL MODIFIERS ON THE PRODUCTION OF DAILY GROWTH INCREMENTS IN OTOLITHS OF PLAINFIN MIDSHIPMAN, *PORICHTHYS NOTATUS*

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ABSTRACT

Plainfin midshipman,*Porichthys notatus*, were reared in the laboratory under three environmental regimes to determine the influence of certain variables upon otolith growth increment formation. Both larval and juvenile midshipman were used to test diel cycles and constant conditions of light and temperature. Daily growth increments were formed upon hatch unless a diel photoperiod was absent. However, under constant light, an endogenous circadian rhythm became evident after a 2-3 week acclimation period, resulting in daily increment production. With increasing age, the influence of light as a zeitgeber decreased, while daily increments became more prominent in all environments. Temperature fluctuation affected increment appearance, but did not entrain increment deposition.

Daily growth increments in the otoliths of fishes have been observed in a large number of species (Pannella 1971; Brothers et al. 1976; Taubert and Coble 1977; Wilson and Larkin 1980). These concentrically formed increments may be counted or measured to provide a chronological record of past fish growth. Information on hatching date/age (Ralston 1976; Struhsaker and Uchiyama 1976), daily growth rates (Methot 1981), and timing of life history transitions (Pannella 1980; Brothers and McFarland 1981) has been derived from the examination of otolith microstructure. Such data are difficult to obtain from larval and juvenile fishes by other means.

Daily increments are produced through a diel periodicity in the deposition of calcium carbonate on the otolith (Mugiya et al. 1981). However, there is some controversy as to the zeitgeber behind the daily cycle of deposition, if indeed one exists. In a series of experiments upon larval *Lepomis*, Taubert and Coble (1977) determined that a 24-h light-dark cycle was necessary to entrain an endogenous rhythm of increment production. Reversal of the light-dark cycle reversed the daily sequence of increment formation in larval *Tilapia* (Tanaka et al. 1981). However, 36-h "days" and constant light conditions had no effect on daily increment production in juvenile starry flounders, *Platichthys stellatus* (Campana and Neilson 1982). Similarly, constant light or

dark conditions did not inhibit the formation of daily increments in young chinook salmon, *Oncorhynchus tshawytscha* (Neilson and Geen 1982). The contradictory results of the above studies suggest that photoperiod effects on increment production may vary with age or species of fish.

Other environmental variables may influence the daily rhythm of otolith deposition. Diel temperature fluctuation has been implicated as a factor in daily increment production of temperate stream fishes (Brothers 1981), although this suggestion has not been supported by other studies (Campana and Neilson 1982; Neilson and Geen 1982). Feeding frequency may also influence otolith increment production; fish given multiple daily feedings have been reported to produce nondaily increments (Pannella 1980; Neilson and Geen 1982), although recent studies suggest that feeding effects are limited (Tanaka et al. 1981; Marshall and Parker 1982; Campana 1983).

Confidence in the reliability of otolith microstructure examination requires knowledge of those factors that may influence otolith increment production. Conflicting results in the literature suggest that age influences the response of daily increment production to environmental variables such as photoperiod and temperature. This study was undertaken to test that hypothesis. Plainfin midshipman, *Porichthys notatus*, were reared from the egg stage under various light and temperature regimes; constant conditions and diel cycles of each variable were tested. The effect of the regimes on otolith microstructure was noted for both newly hatched and juvenile fish.

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Juveniles were then subdivided and transferred to different regimes, allowing an examination of the interactive influence of greater age and novel environment on increment production.

MATERIALS AND METHODS

Fertilized *Porichthys* eggs were collected intertidally from White Rock, British Columbia, on 9 and 22 June 1982. Yolk-sac larvae remain attached to the rock upon which the eggs were originally deposited (Arora 1948), necessitating the collection of both rocks and egg masses. Upon return to the laboratory, eight separate egg masses (50-250 ova each) were isolated in individual saltwater aquaria and maintained under a diel photoperiod and a temperature of 13°C. Small amounts of methylene blue, streptomycin sulphate, and penicillin G were used to control bacterial and fungal infection. Embryo development varied both among and within egg masses, but the difference appeared to be <2-3 d.

On 1 July, egg masses were exposed to an experimental environment. Environmental regimes were selected to provide a diel periodicity of either photoperiod or temperature. A third regime maintained constant conditions of both variables. In this manner, the influence of both factors on increment formation could be determined for newly hatched larvae. Daily increment production in the constant environment would suggest the presence of an endogenous circadian rhythm. Regimes were as follows:

14L:10D at a constant temperature of 19°C (14L:10D/CT)

24L with 14 h at 21°C and 10 h at 19°C (24L/14T$_1$:10T$_2$)

24L at a constant temperature of 19°C (24L/CT)

Duplicate aquaria, each containing an egg mass (or 2 small masses, if at similar developmental stages), were kept in light-proof, temperature-controlled cubicles under each of the above environments. All lighting was fluorescent (30 μEs/m^2/s). Temperature fluctuations were timer-controlled and conducted parallel to the light cycle. New temperatures were reached 1½ h after initiation. Mean temperatures approximated those of the egg collection site; diel temperature fluctuations were present at the site, but were not recorded. Aquarium water was changed at 7-10 d intervals. Hatching date varied among and within egg masses, beginning between 7 and 11 July. Release from the rock (before completion of yolk-sac

resorption) was more variable, and occurred between 23 July and 9 August. Live adult *Artemia* were first provided as food on 30 July and were consumed by both released and attached larvae. Thereafter, *Artemia* were maintained in all aquaria at all times, with the exception of two 3-d periods when food was not available. Food abundance did not differ among the aquaria. Observations of feeding fish indicated that the accessibility of *Artemia* did not limit growth.

By 10 August, all fish were about 32-d old (posthatch) and had become juveniles (i.e., had assumed the appearance of an adult). To test the effect of an altered photoperiod or temperature cycle on juveniles, one tank from each of the environmental regimes was subdivided (Fig. 1). About 25 fish were transferred from one aquarium ("cohort") to each of the remaining environments, while leaving 25 fish in the original environment as a control. Sagittae were removed from up to 25 of the excess fish to determine the effect of the original environment on newly hatched larvae. In order to remove any intercohort variability of hatching dates, only one of the two available cohorts from each environment was subdivided and sampled. However, low numbers of 14L:10D/CT fish necessitated the transfer of an entire cohort.

For processing, the sagittae were brushed free of tissue and glued sulcus-side up with instant glue on a standard microscope slide. Sagittae were ground and polished with metallurgical lapping film (grit size 30
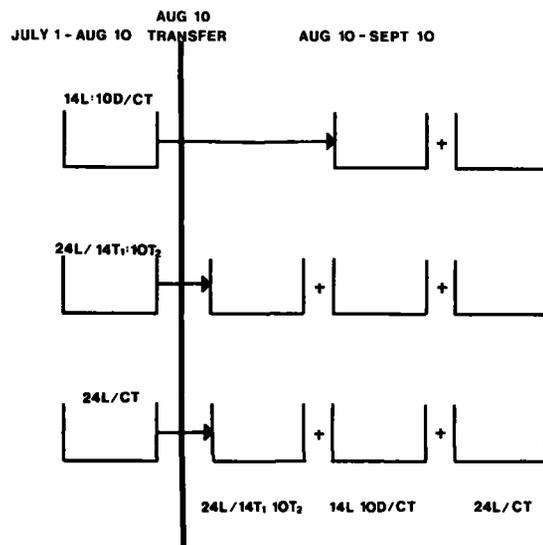

FIGURE 1.—Summary of experimental environmental regimes of plainfin midshipman through time. Fish transferred to new environments on 10 August came from the same egg mass as that sampled on 10 August.

μm to 0.3 μm) until the growth increments in the region of maximal growth were most visible. I defined a growth increment as a bipartite structure, consisting of a narrow opaque band and an adjacent broad translucent region. Growth increments between the otolith periphery and the hatch check were counted at least twice through a compound microscope at a magnification of 400X. Duplicate counts of an otolith never differed by more than 10%. The use of a hand counter eliminated the possibility of a count converging on an expected value. There was little doubt concerning the nature of the hatch check; its radius matched that of radii of otoliths removed at hatch. Growth increments in 14L:10D/CT fish sampled 10 September were counted as above. However, a second series of counts was made from the hatch check to the prominent 10 August check; the second data set served as a substitute for the actual sampling of 14L:10D/CT fish on 10 August.

Increment counts were made from both the left- and right-hand side sagittae. Since the two sides did not differ systematically under any of the environments (paired t-test, $P > 0.05$), the means were used in all data analyses.

Increment widths were measured from photographs with a micrometer. Expected increment widths were calculated from radial measurements (central nucleus to rostral tip) of otoliths from all environments and a variety of sampling dates ($N = 10$ per date). Values for mean increase in radial otolith growth per day were then compared to observed values.

Since individual otoliths often displayed erratic but discernable width trends through time, a measure of the similarity of the widths of two adjacent daily increments was calculated:

$$IR_i = \frac{W_i - W_{i-1}}{(W_i + W_{i-1})/2}$$

where IR_i is the index of increment width regularity for day i, and W_i is the increment width for day i. Such an index gives low values when adjacent increments are similar in width, despite any trends in the data. Index values were calculated for a range of ages in otoliths from a given environment.

RESULTS

Porichthys larvae and juveniles survived and grew under all laboratory environments. Survival exceeded 95% after hatch. Fish sampled about 1 mo after hatch (10 August) did not differ significantly in standard length (ANOVA, $P > 0.05$). By the end of

the study, only those fish maintained in the 24L/14T$_1$:10T$_2$ environment were significantly smaller in length (Scheffe's test $P < 0.01$); the difference was apparently due to unintentional overcrowding from the date of transfer.

Hatching was initiated simultaneously in two of the three initial environments, but started 4 d later in the 24L/CT aquaria. The delay did not appear to be due to the artificial environment, since embryo development among the 24L/CT egg masses lagged behind that of the others at the time of collection. In the aquarium, about 95% of the viable ova hatched within 4 d of hatch initiation. Intratank hatch-date variance would be expected to affect the variance of increment counts. However, the 17-d range of larval release dates (from the rock) was not reflected in the otolith microstructure.

Unground sagittae derived from both pre- and posthatch fish were extremely lobulated in structure. The origin of the numerous lobes was 5-10 "peripheral" nuclei, from which the majority of the growth increments emanated. A central nucleus also had growth increments associated with it, although these were incorporated into the peripheral increments within 10-20 d/increments. A prominent hatch check occurred within 5-10 major increments of the central nucleus. The most prominent check of the older otoliths was that associated with the subdivision/transfer date of 10 August.

Many growth increments were visible in the polished otoliths sampled after hatch. When plotted as a function of time, total increment counts were significantly greater than those expected of daily production ($P < 0.05$) (Fig. 2). Diel light and temperature cycles both produced an increment: age slope of about 3.0, suggesting that numerous subdaily increments were being counted with any daily increments present. Increment clarity, prominence, and width varied substantially within an otolith. However, most increments could be assigned to one of two "levels"—visually prominent/relatively wide and visually faint/relatively narrow. To determine if the first level consisted primarily of daily increments, the expected width of a daily increment was calculated.

	23 July	30 July	9 Aug.	10 Sept.
Mean otolith radius (μm):	270	430	620	875

Daily increments on the order of 12-23 and 5-8 μm wide would be expected in the first and second month posthatch, respectively. These expected increment widths were similar to those observed in the first "level" of growth increments.
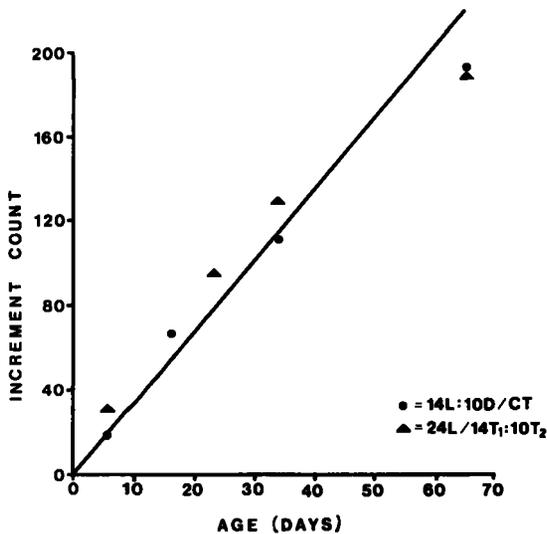
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FIGURE 2.—Total otolith increment count as a function of age for plainfin midshipman from two cyclic experimental environments. A straight line has been fitted to the data, although the relationship is probably curvilinear. $N = 5$ for each data point.

Criteria for distinguishing daily from subdaily increments have been reported previously (Taubert and Coble 1977; Campana and Neilson 1982; Marshall and Parker 1982). Nevertheless, no objective criteria have yet been defined which can be applied to all otoliths. In this study, I have used visual prominence and increment width as guides for differentiating daily and subdaily increments. Increments assigned as daily were 1) of similar visual prominence (contrast) to adjacent daily increments (±30%), 2) of similar increment width to adjacent daily increments (±50%), 3) not merged with adjacent daily increments in the nearest radial groove of the sagitta. Some increments met only some of the criteria and were subjectively assigned as daily or subdaily. The observed widths of daily increments, as classified above, were similar to those expected on the basis of otolith growth calculations (see previous paragraph).

Diel Light Cycle

Otoliths of fish reared under a diel photoperiod and constant temperature (14L:10D/CT) produced clear daily growth increments from the time of hatch. Regression of major increment number against elapsed time produced a slope not significantly different from 1.0 ($P > 0.05$); a slope of 1.0 would indicate that one increment was formed every day.

Increment width varied with location on the otolith and fish age (Fig. 3). Subdaily increments were common at all ages, numbering up to 5 between adjacent daily increments. They were most abundant in the first month after hatch. The distinction between daily and subdaily increments was generally clear; however, increments produced 5-20 d after hatch were the most irregular on the otolith, and were sometimes difficult to interpret. Subdaily increments tended to be prominent in this region, so that distinction was a matter of degree (Fig. 4A).
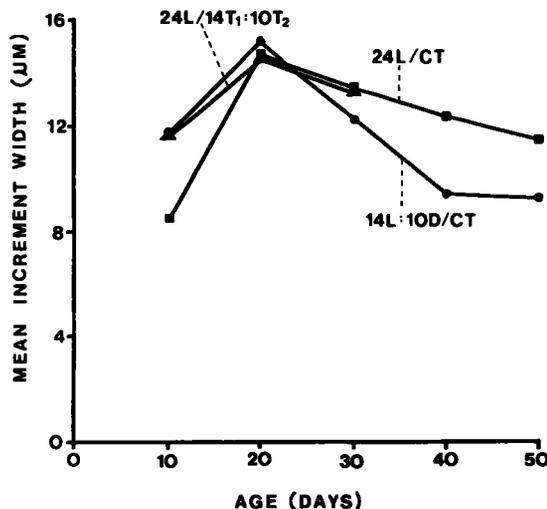

FIGURE 3.—Daily increment width as a function of age for otolith samples of plainfin midshipman from each of the three experimental environments. At a given age, mean widths do not differ significantly among environments, with the exception of values at age 40 d ($P < 0.05$).

Fish transferred to a constant environment (24L/CT) as juveniles produced posttransfer increments that were very different from those produced prior to transfer. Posttransfer increments were visually faint and, in some cases, virtually invisible (Fig. 5A). Subdaily increments were also present. Transfer to a constant environment was not associated with a recognizable lag period during which increments gradually shifted their appearance. Increments produced within 1-2 d of transfer were virtually nonexistent. Nevertheless, posttransfer increments were daily in nature, as indicated by increment counts similar to those expected of daily increment production (Table 1). Daily increments gradually became more prominent after about 15 d posttransfer, their visual contrast improving until the end of the experiment.

TABLE 1.—Growth increment counts in otoliths of plainfin midshipman, *Porichthys notatus*, in relation to elapsed time for various experimental environments. Fish were transferred to new environments (or kept in the original environment as a control) on 10 August.

Environ-ment 1	10 Aug. samples			Environ-ment 2	10 Sept. samples		
	Days after hatch	No. major increments	SE		Days after hatch	No. major increments	SE
14L:10D/CT	34	[1]34.3	0.57	14L:10D/CT	65	66.7	0.80
14L:10D/CT	—	—	—	24L/CT	65	65.1	1.21
24L/14T$_i$:10T$_2$	34	41.1	1.29	24L/CT	65	71.2	0.70
24L/CT	30	49.1	1.33	24L/CT	61	76.9	1.04
24L/CT	—	—	—	14L:10D/CT	61	72.7	1.10
24L/CT	—	—	—	24L/14T$_i$:10T$_2$	61	69.3	0.92

[1]This value was derived from 14L: 10D/CT otoliths sampled 10 September; counts were made from the hatch check to the prominent subdivision/transfer check

Diel Temperature Cycle

Fish hatched under a $24L/14T_1{:}10T_2$ regime deposited growth increments that differed in many respects from those produced under a cyclic photoperiod (14L:10D/CT). Increments produced up to 8 d medial and distal of the peripheral nuclei were characterized by a high incidence of prominent subdaily increments (Fig. 4B), more so than was the case under a cyclic photoperiod. Daily/subdaily similarities are reflected in the data of 10 August (Table 1), where the observed major increment count was significantly different from that expected of daily increments ($P < 0.05$). The high increment count indicates that some subdaily increments were prominent enough to be classified as daily.

Increments produced in the 15-20 d before transfer were generally distinct and regular in appearance. Increment width and the incidence of subdaily increments were similar to those observed in the corresponding region of the cyclic photoperiod otoliths (Fig. 3). However, the appearance of the major increments was unusual in that the opaque portion of each increment was relatively broad and sharply delineated (Fig. 6).

Fish maintained in the $24L/14T_1{:}10T_2$ environment after 10 August were overcrowded and did not grow well. As a result, posttransfer otolith growth was limited, increments were very narrow, and reliable counts could not be made. However, increment counts of representative otoliths suggested that daily increments were deposited after the transfer date.

Juvenile fish transferred from the fluctuating temperature regime to a constant environment (24L/CT) produced posttransfer increments similar to those of fish transferred from 14L:10D/CT to 24L/CT (Fig. 5B). The difference between August and September increment counts corresponds to that expected of daily increment deposition ($P > 0.05$) (Table 1). The first five posttransfer increments were faint and virtually nonexistent; subsequent increments became more distinct and regular with time. Opaque regions within each increment never became as broad and discrete as was observed prior to transfer.

Constant Environment

Otoliths of fish hatched under constant conditions (24L/CT) initially resembled those of the other two environments (with respect to the first 5-8 increments). The subsequent region resembled that of $24L/14T_1{:}10T_2$ fish in that subdaily increments were prominent (Fig. 4C). Although the difference was not significant (Scheffe's test, $P = 0.07$), increment widths tended to be more irregular than those of 14L:10D/CT fish of similar age (Fig. 7). The confusion of daily and subdaily increments in the early larval region resulted in a high variance and a mean increment count that was significantly higher than would be expected of daily increments ($P < 0.05$) (Table 1). After age 10-25 d, daily increments decreased in width (Fig. 3) and became more regular in width (Fig. 7) and appearance, although subdaily increments were still present. Increments with broad, discrete opaque portions were not observed in the 24L/CT fish, as they were in the fluctuating temperature regime. For an unknown reason, otolith growth (but not fish growth) under a 24L/CT regime significantly exceeded that observed under 14L:10D/CT ($P < 0.05$).

Fish remaining in a constant environment after the 10 August transfer date continued to produce distinct increments, although daily and subdaily increments were occasionally difficult to differentiate. Increment width was significantly more irregular than in the posttransfer region of 14L:10D/CT fish (t-test, $P < 0.05$) (Fig. 7). Major increments in the posttransfer region were daily; the regression of increment number against elapsed time resulted in a slope not significantly different from unity ($P > 0.05$).

Posttransfer increments of fish hatched and reared under constant conditions were prominent, although
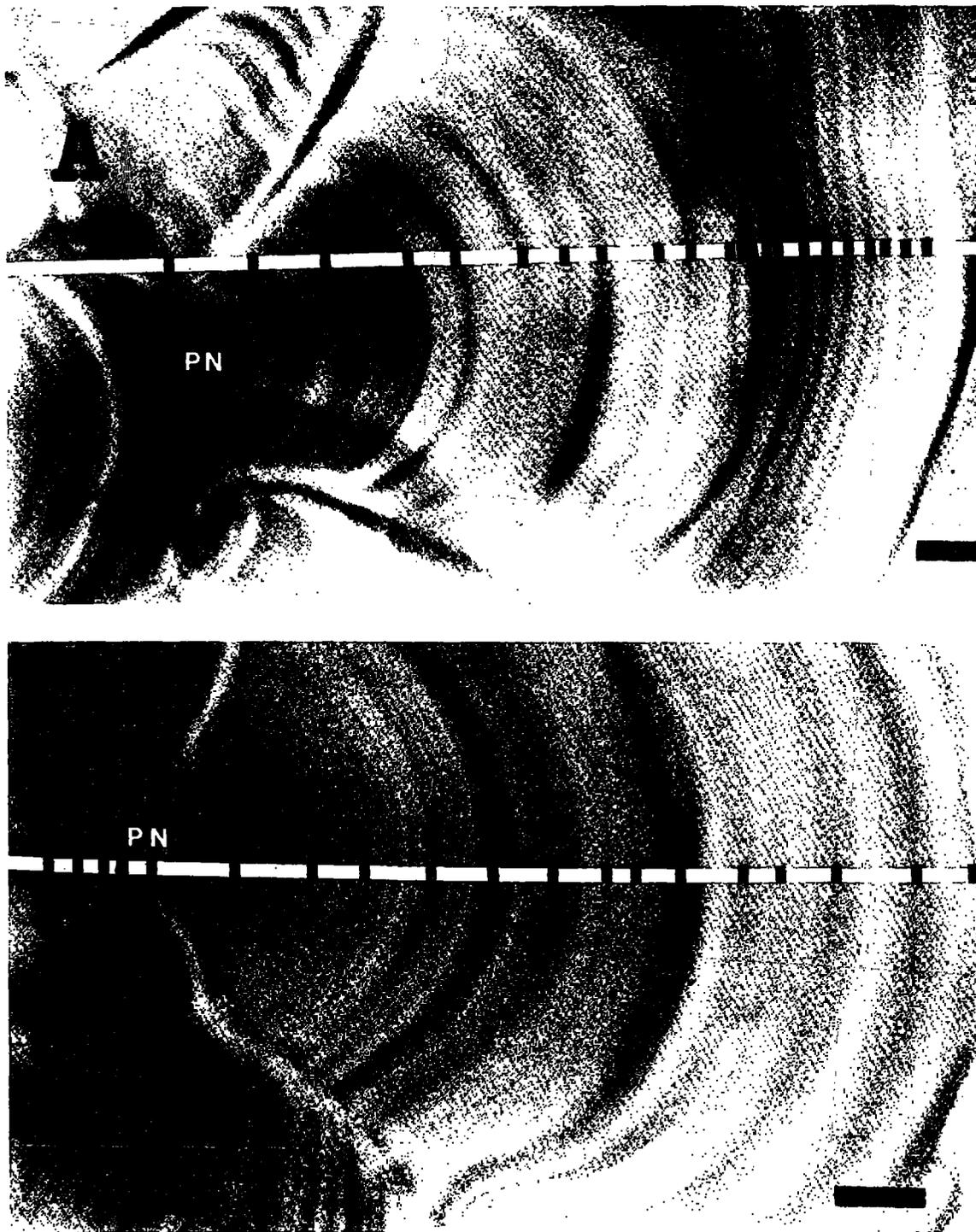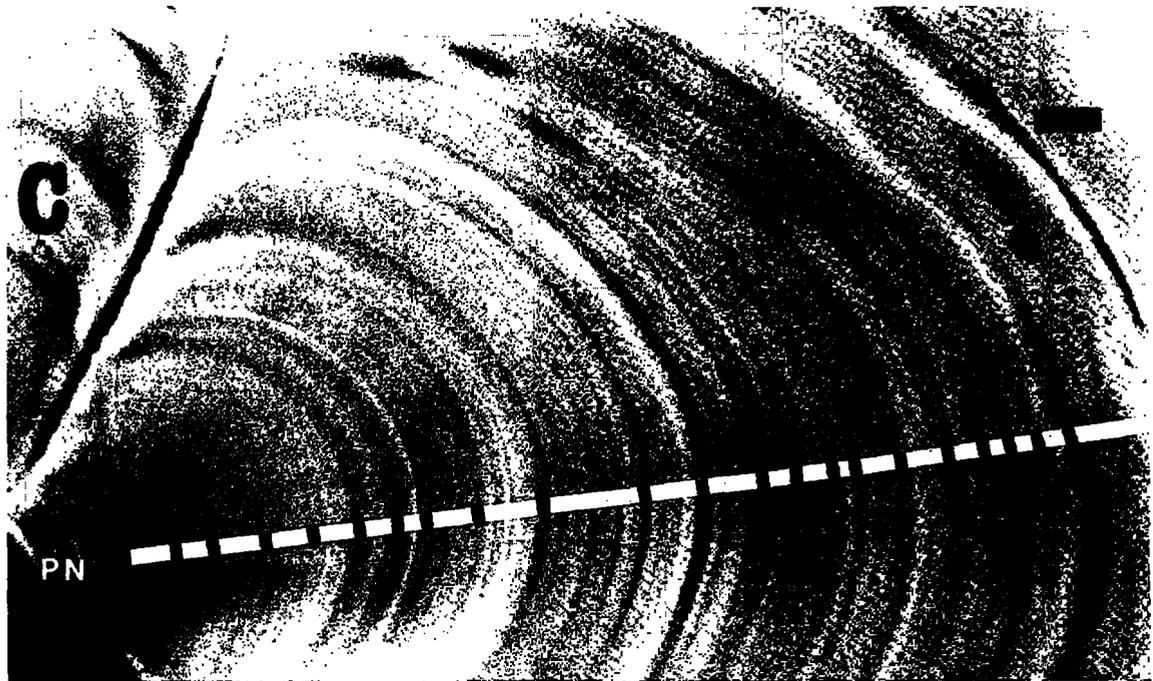
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FIGURE 4.—Growth increments on the polished sagittae of larval plainfin midshipman. Subdaily increments are visible between some of the indicated daily increments. Daily increments became more clear with age, but were most prominent/consistent in width in (A). Bar = 30 μm.

PN = peripheral nucleus. (A) Hatched under a diel light cycle; (B) hatched under a diel temperature cycle; (C) hatched under a constant environment.

irregular in width (Fig. 5C). In contrast, increments of fish transferred to the constant environment as juveniles were visually faint, becoming more prominent after 2-3 wk. Juveniles transferred from a constant environment to a cyclic regime deposited similar-appearing increments before and after transfer. However, posttransfer increments tended to be more regular in width than in constant environment fish; the change generally became apparent 2-4 d after transfer. Visual contrast of daily increments may have increased in the fluctuating temperature regime, but the change was not consistent among all otoliths. No such change was evident among the posttransfer increments of fish shifted from 24L/CT to 14L:10D/CT, although the incidence of subdaily increments appeared to decrease. Fish transferred from the constant environment to either of the cyclic regimes produced daily increments after transfer; high increment counts (Table 1) were derived from the irregular, pretransfer region of the otolith.

DISCUSSION

Daily growth increments were deposited on the otoliths of plainfin midshipman under a variety of environmental conditions. My results indicated that light, temperature, age, and an endogenous circadian rhythm may all influence the production and/or appearance of daily and subdaily increments. However, some of the variables tested interacted to a large degree, and their influence on increment production was subject to alteration through time.

A cyclic light regime influenced increment production in larval fish more than any other variable tested. Under a natural photoperiod, daily increments were produced from the time of hatch. In contrast, constant light conditions disrupted the production of posthatch increments, resulting in a high incidence of prominent nondaily increments (>1 increment/24 h) and irregular increment widths. My observations are consistent with those of Taubert and Coble (1977), who observed numerous, nondaily increments in larval *Tilapia* hatched under constant light conditions. Those authors concluded that light acted as a zeitgeber for an endogenous rhythm and that without a cyclic photoperiod, daily increment production was not possible. My results only partially support their conclusion. Photoperiod entrained daily increment production in newly hatched midshipman. However, in the absence of cyclic light or temperature stimuli, an endogenous circadian rhythm of increment deposition became apparent after an acclimation
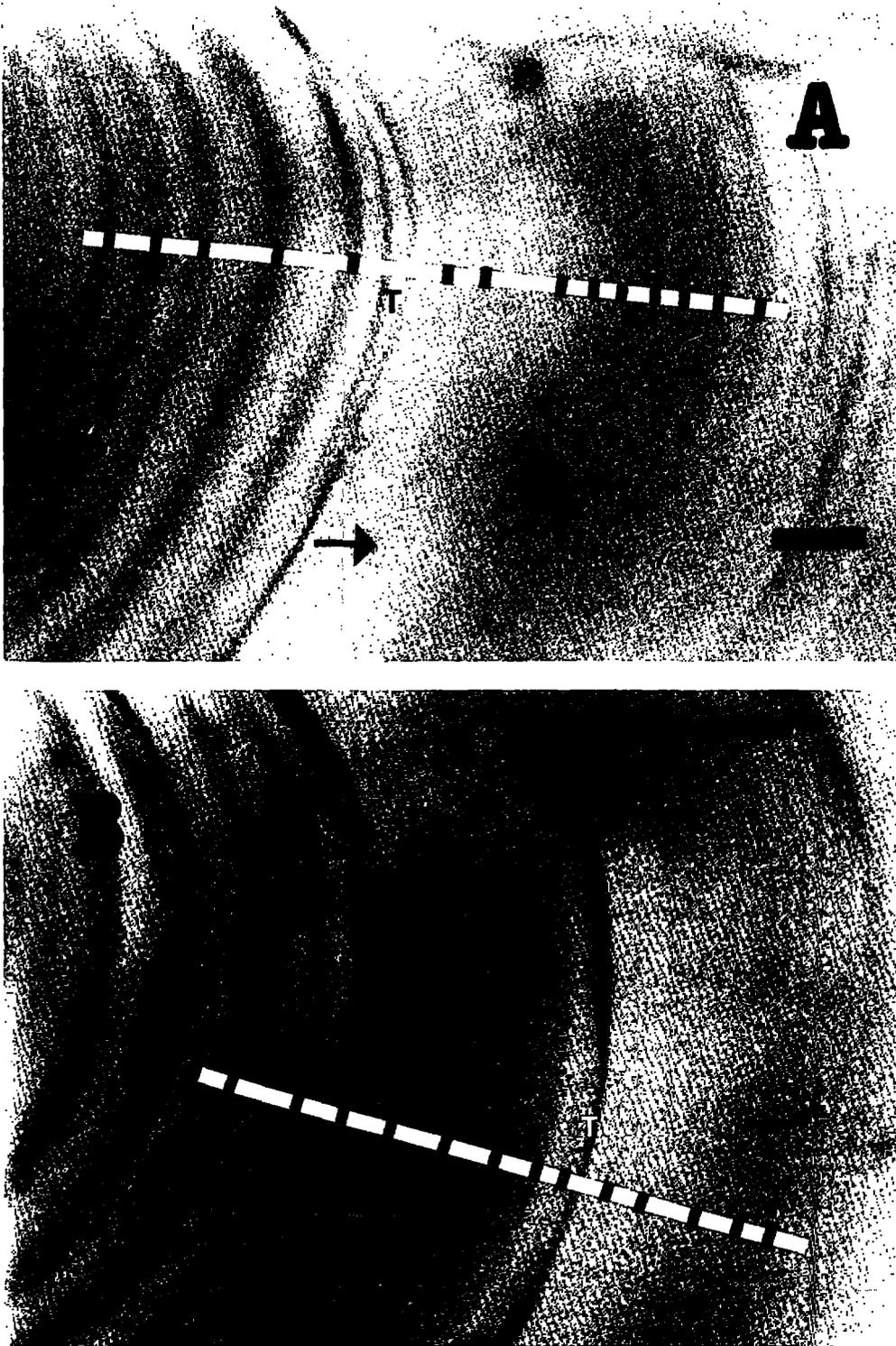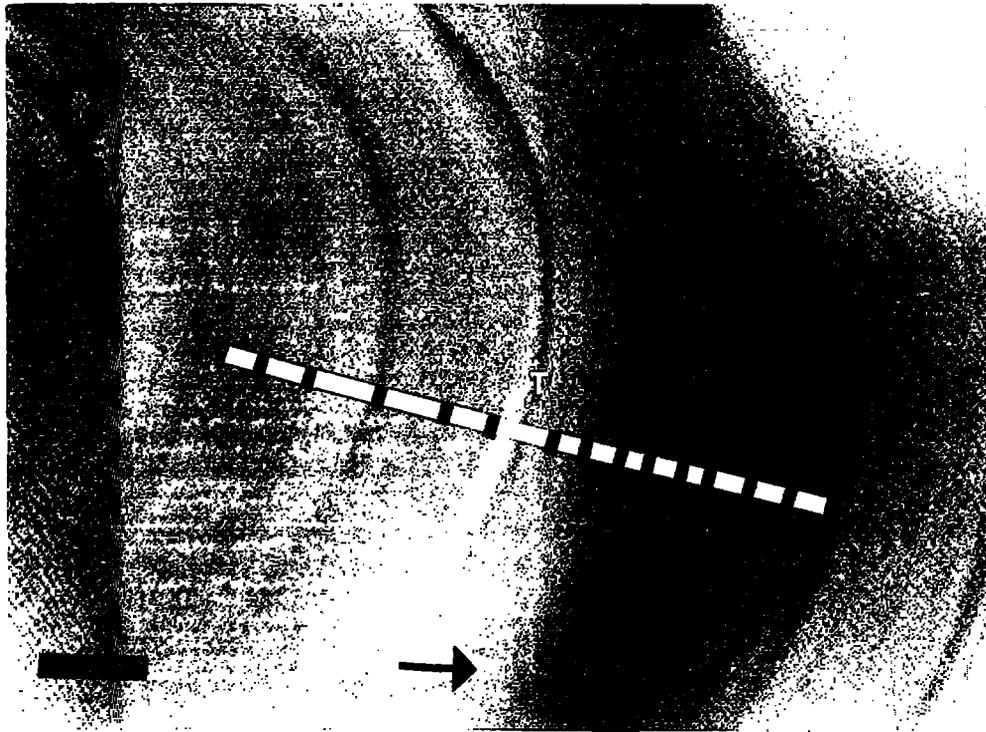
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FIGURE 5.— Growth increments in sagittae of plainfin midshipman produced before and after transfer to a constant environment. Fish hatched under 24L/CT produced clearer daily increments than those transferred from a different

environment. Daily increments are indicated, as is direction of sagittal growth (arrow). T = transfer check. Bar = 30 μm.
(A) 14L:10D/CT to 24L/CT; (B) 24L/14T₁:10T₂ to 24L/CT; (C) 24L/CT to 24L/CT.

period of 2-4 wk. Therefore, photoperiod acted as a zeitgeber for an endogenous rhythm during the early larval stages, but became unnecessary with increasing age. The nondaily increments produced after hatch in this study (and that of Taubert and Coble 1977), probably comprised both daily and subdaily increments. The combination resulted in the deposition of more than 1 increment/24 h.

If a constant photoperiod was present at hatch, an endogenous rhythm of increment deposition became apparent after an acclimation period. Acclimation also occurred when older fish were transferred from a natural light cycle to constant light conditions. However, the pattern of increment production during acclimation differed at the two ages (Table 2). The larval fish acclimation period may be analogous to that of newborn rats transferred from a diel photoperiod to constant conditions. An arhythmic activity pattern continues for almost 2 wk in rats before an endogenous circadian rhythm becomes apparent (Davis 1981).

The length of the acclimation period could not be determined with accuracy. A shift in increment appearance after transfer from a constant to a cyclic environment generally occurred in 2-5 d. The reverse transfer resulted in almost nonexistent increments

TABLE 2.—Age effects on growth increment production in otoliths of plainfin midshipman, *Porichthys notatus*, reared under three artificial environments.

Larvae	Juveniles
Light important as zeitgeber	Light not important as zeitgeber.
Daily & subdaily increments similar during acclimation to 24L	Faint daily increments, but subdaily increments dissimilar during acclimation to 24L
Long acclimation to 24L	Short acclimation to 24L
Immature circadian rhythm	Mature circadian rhythm

for a period of 5 d, but the visual contrast of the growth patterns improved over the subsequent 10-15 d. Therefore, the critical stage of the adaptation process appears to have been completed in 2-5 d. This result is consistent with that of Tanaka et al. (1981), who observed a 6-d transitory period of increment formation when a 24-h light-dark cycle was suddenly reversed.

Age-related changes in endogenous circadian rhythms have not been examined in fishes. Mammalian studies indicate that endogenous rhythms often appear after birth; once present, cycle amplitude tends to increase with time until the rhythm is "mature" (Davis 1981). *Porichthys* larvae hatched under constant light appear to fit this pattern. Daily and subdaily increments were not easily
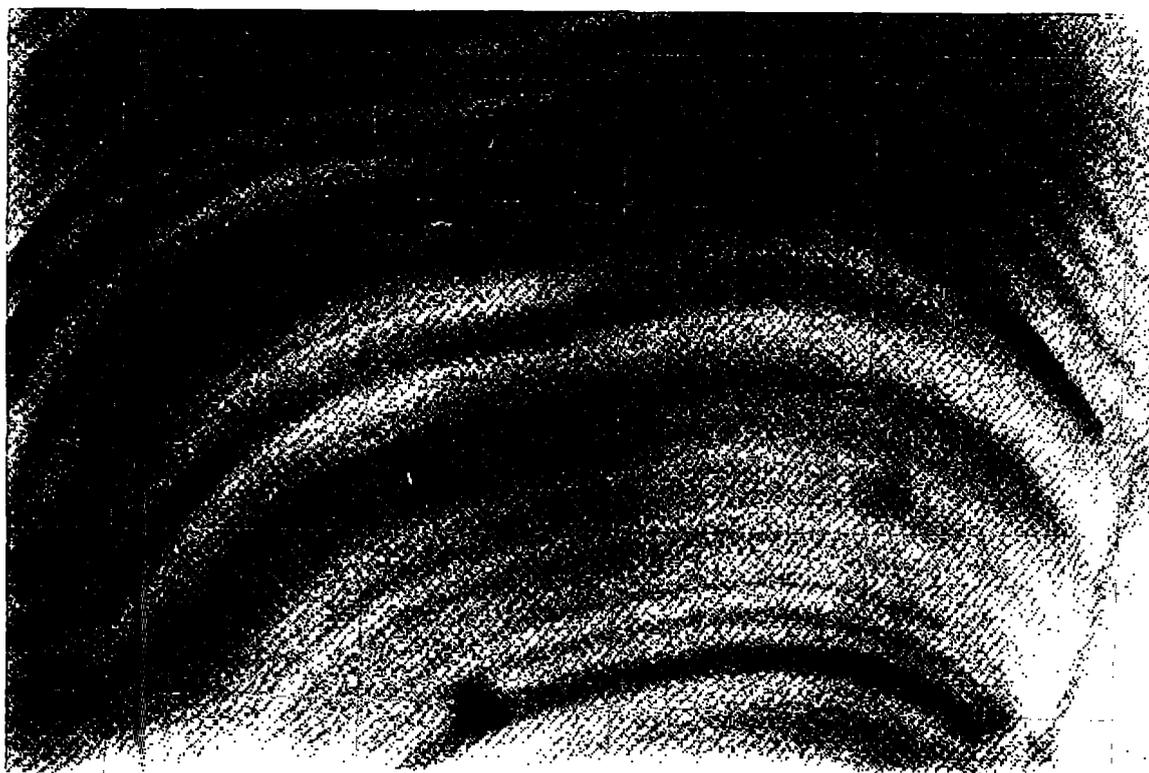
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FIGURE 6.—Daily growth increments produced on the sagittae of plainfin midshipman after 15-25 d of rearing under a diel temperature cycle. The increments were visually prominent and sharply delineated relative to those produced under other environmental regimes. Bar = 20 μm.
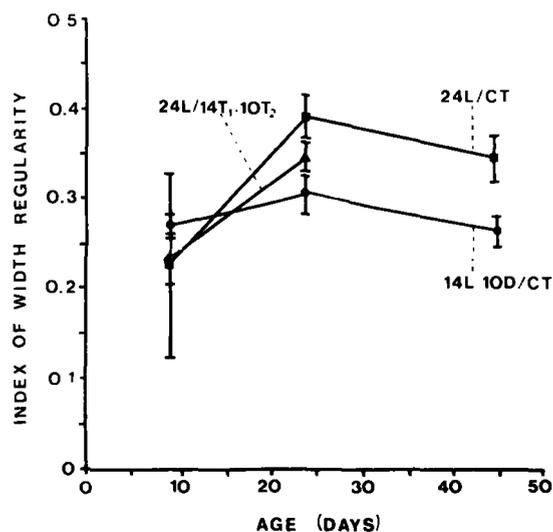

FIGURE 7.—Index of daily increment width regularity as a function of age for otolith samples of plainfin midshipman from each of the three experimental environments. Bars represent ±1 SE.

differentiated at first, suggesting that the circadian deposition rhythm was not yet mature. Maturation apparently occurred by days 10-20. Early larval increments were only indistinct temporarily in the 14L:10D/CT fish, suggesting that the cyclic photoperiod entrained the maturing rhythm fairly quickly. In addition, very young animals may be more responsive to a diel light cycle, due to age-related characters of the rhythm cycle (Sacher and Duffy 1978). For instance, the metabolic rate of newly hatched rats is very sensitive to changes in light level, while older rats are less affected. In this study, larval fish exposed to a constant environment took longer to produce daily increments than did juvenile fish, suggesting an analogy with the rat study. Similar age-related results were reported by Gibson et al. (1978) in an ontogenetic study of flatfish activity cycles. A constant photoperiod eliminated a diel activity cycle in larval plaice (*Pleuronectes platessa*), but had no such effect on juveniles of the same species.

Increasing age of midshipman was correlated with decreasing increment width and fewer subdaily

increments in all environments. However, foremost among the age-associated effects (Table 2) was the prominence of daily increments in juveniles relative to larvae. Distinction between daily and subdaily increments was seldom difficult in juveniles (outside of the acclimation period) unlike the situation in larval otoliths. If this age-related difference in daily increment formation is universal, daily increment counts in larvae may be unreliable relative to slightly older fish. This suggestion has serious implications for the application of growth increments in aging larval fish. Similarly, the absence of definitive criteria for differentiating daily and subdaily increments could cause problems in aging field-collected fish. Subdaily increments can be numerous and confusing in some species (Campana, unpubl. data).

The demonstration of and age-related rhythm and the existence of an acclimation period may have resolved some of the conflicting results in the literature concerning the zeitgeber effect of light. In a previous study, a constant light regime did not influence the production of daily increments in juvenile starry flounders (Campana and Neilson 1982). The flounders were about 8 mo-old, suggesting that the necessary acclimation period would be short. In addition, the fish were exposed to the experimental environment for 2 wk prior to tetracycline injection (marking the start of the experiment); it is probable that acclimation occurred during this period, resulting in clear daily increment production by the time the experiment began. An analogous explanation may explain the results of another study, where chinook salmon eggs, reared in darkness, produced daily increments after hatch (Neilson and Geen 1982). The embryos were held in total darkness for 50 d before hatch, suggesting that their endogenous circadian rhythm had time to acclimate before hatch.

A fluctuating temperature regime did not entrain increment production under constant light conditions. Fish reared in this environment produced more increments than would be expected of daily production, similar to those of 24L/CT fish. The variance of larval increment counts was similar to that produced under a constant environment, both of which were significantly larger than the 14L:10D/CT variance (Bartlett's test, $P < 0.01$). Once acclimation occurred, daily increments were produced through an apparently endogenous periodicity, and not through temperature entrainment of an internal clock. However, the formation of a broad, optically dense, sharply delineated opaque zone in postacclimation daily increments indicates that temperature fluctuation did affect increment production. The opaque portion of a daily increment consists of calcium carbonate and a proteinaceous matrix, with the latter component predominating (Brothers 1981; Mugiya et al. 1981). Falling temperatures, such as would occur at night, may have increased the proportion of protein deposited in the opaque region, resulting in an increment that had increased visual contrast. Accentuation of contrast renders increments visually prominent, and could easily be interpreted as an entraining mechanism. Diel temperature fluctuations noticeably accentuated increment contrast in young chinook salmon otoliths (J. D. Neilson[2]). A correlation of increasing protein deposition with decreasing temperature suggests that the broad opaque zone formed during the low temperature, 10-h, experimental "night", overlaid the opaque zone formed under circadian control through a 3-h period (Mugiya et al. 1981). If temperature does exert a "masking" effect (Enright 1981), a low temperature-induced opaque zone would appear independently of any endogenous circadian rhythm of deposition. Therefore, multiple daily oscillations in temperature could conceivably produce a distinct increment after each cycle, in addition to the daily increment formed under endogenous control. In some situations, the masking effect of temperature fluctuations may be substantial, obscuring most of the increments formed through the action of an endogenous rhythm of deposition (E. B. Brothers[3]). This hypothesis is consistent with studies that demonstrated that temperature cycles do not entrain daily increment production (Campana and Neilson 1982; Neilson and Geen 1982), but can influence increment formation (Brothers 1981).

My results suggest that a diel light cycle entrains an endogenous circadian rhythm of increment deposition. Increasing age mitigated the zeitgeber effect of photoperiod, while temperature fluctuation influenced increment appearance, rather than periodicity. In other studies, the incidence of subdaily increments was correlated with feeding periodicity (Neilson and Geen 1982; Campana 1983). The fact that so many variables may affect increment deposition suggests that the environment does not influence the rhythm of otolith deposition directly, but acts through some penultimate process. Metabolic rate is susceptible to environmental influence, as well as being subject to an endogenous circadian rhythm (Matty 1978) that changes with age (Davis 1981). However, metabolic rate is in turn

regulated by endocrine levels, and it may be the environmental modulation of endocrine rhythms that ultimately controls increment periodicity on the otolith (Menaker and Binkley 1981). Endocrine secretion often follows a circadian pattern (Simpson 1978) and, in mammals at least, is closely linked to the circadian pacemaker itself (Menaker and Binkley 1981). Hormones regulate many aspects of metabolism and growth, including skeletal calcification (Simpson 1978). Therefore, it seems reasonable to postulate that those factors that entrain and/or moderate the circadian rhythm of endocrine secretion will have a subsequent effect on increment deposition in the otolith.

ACKNOWLEDGMENTS

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