Otolith Microstructure of Larval Herring (*Clupea harengus*): Image or Reality?

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When assessed with light microscopy, daily increment formation did not appear to occur in the otoliths of known-age larval herring (*Clupea harengus*). Increment counts underestimated age in all larvae. The age–increment discrepancy increased curvilinearly with age and appeared to stabilize after 50–60 d. Both the magnitude and rate of increase of the discrepancy were consistent with a hypothesis of resolution-limited increment visibility; models of daily otolith growth indicated that discrete daily growth increments would not be resolvable with a light microscope for the first 15–20 d after hatch. The hypothesis was also consistent with the observed effects of otolith polishing, a systematic difference in increment counts between different-sized sagittae in the same larvae, and other published reports of apparent nondaily increment formation in slow-growing pelagic larvae. Previous reports of growth rate limited increment formation appear to provide an empirical description of the same phenomenon. Otolith-based age, growth, and mortality estimates can be expected to be biased if resolution effects are ignored. However, various procedures are available for the identification of potentially sensitive species and samples.

La lecture au microscope optique des otolithes de larves de hareng (*Clupea harengus*) d'âge connu n'a pas permis de mettre en évidence la formation de stries journalières d'accroissement. L'âge de toutes les larves a été sous-estimé. L'écart entre l'âge réel et l'âge estimé augmentait de façon curviligne avec l'âge et semblait se stabiliser après 50 à 60 j. Tant l'ampleur que le taux d'accroissement de l'écart satisfont à une hypothèse voulant que la visibilité des stries d'accroissement soit limitée par la résolution; des modèles de la croissance journalière des otolithes ont indiqué que les accroissements journaliers ne pourraient être mis en évidence par un microscope optique pour les 15 à 20 premiers jours suivant l'éclosion. Cette hypothèse se défend également si on considère les effets observés du polissage des otolithes, les différences systématiques des lectures des sagittas de taille différente provenant des mêmes larves et les rapports faisant état d'une formation apparemment non journalière des stries d'accroissement chez des larves pélagiques à croissance lente. D'autres rapports indiquant que la formation des stries d'accroissement serait limitée par la vitesse de croissance semblent apporter une description empirique du même phénomène. On peut penser que les estimations de l'âge, de la croissance et de la mortalité reposant sur la lecture des otolithes sont biaisées lorsqu'il n'est pas tenu compte des limites de résolution. Ceci dit, il existe diverses méthodes permettant de reconnaître les espèces et échantillons que ce phénomène pourrait toucher.

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In 1982, Geffen challenged the concept that daily growth increments in the otoliths of young fishes provide an accurate index of age. Data collected from known-age larval herring (*Clupea harengus*) suggested that the frequency of increment formation was less than daily in larvae with a suboptimal growth rate. Further, she reported that the rate of increment formation was a curvilinear function of somatic growth rate below some threshold limit. Nondaily increment formation has since been corroborated in larval herring (Lough et al. 1982; McGurk 1984), as well as a variety of other fish species with slow-growing larval phases (Methot and Kramer 1979; Laroche et al. 1982; Bergstad 1984; Campana 1984). Such reports are of particular concern to those who have assumed a daily rate of increment formation in their age-structured analyses (Townsend and Graham 1981; Laroche et al. 1982; Graham et al. 1984; Penney and Evans 1985). There is no reason to doubt the apparent universality of daily increment formation in young fishes under adequate growth conditions (Campana and Neilson 1985). However, the number of reported exceptions, all of which were from temperate pelagic larvae, suggests the existence of some other mechanism which could conceivably complicate or invalidate age interpretations derived from otolith microstructure examination.

Several hypotheses can be invoked to explain apparent

increment formation rates of less than one per day. The growth rate limitation hypothesis of Geffen (1982) has been cited as consistent with the results of a number of studies (Bergstad 1984; McGurk 1984). An alternative hypothesis, that of limited observer resolution, was first presented when it was noted that narrow daily increments could only be seen after adequate otolith preparation (Campana 1984). An expansion of this hypothesis suggested that it was theoretically possible for daily increments to form at or below the resolution limit of even a "perfect" light microscope (Campana and Neilson 1985). Such a situation would result in an apparent increment formation rate that was lower than the true rate. Of course, the resolution and growth rate hypotheses are not mutually exclusive. It is the objective of this paper to demonstrate that novel mechanisms of growth-limited increment formation need not be invoked to explain instances of apparent nondaily increment formation in slow-growing pelagic larvae. Through use of data collected from laboratory-reared larval herring, we will show that an apparently nondaily increment formation rate is to be expected in the otoliths of many pelagic larvae. Realistic light microscopic resolution limits will then be used to reconcile observed and expected increment counts both in this study and elsewhere. Finally, we will assess the implications of our findings with respect to applied otolith microstructure analyses.

Materials and Methods

Herring larvae were reared in the laboratory from fertilization to at least the time of metamorphosis. Fertilized eggs were obtained from hand-stripped adults collected in the Iles Verte region of the St. Lawrence Estuary in June 1985. Larvae were kept in 50-L recirculating, closed-system tanks and fed a continuous supply of rotifers, *Artemia* and/or wild zooplankton; rearing details are available elsewhere (J. Munro and J. A. Gagné, unpubl.) Lighting was fluorescent on a 16 h : 8 h day–night cycle, while temperature was kept roughly constant at 10–12°C. Diel temperature fluctuations, if present, were less than 0.25°C/d. Survival was on the order of 97.8%/d.

Larvae were sampled on a daily basis for the first 35 d of the experiment and at 2- to 8-d intervals thereafter. After having recorded total length to the nearest 0.1 mm (N = 194), all lapillar and sagittal otoliths were removed from a random subsample of the larvae (N = 65), cleared of adhering tissue, and mounted on standard microscope slides with Krazy Glue. Maximum otolith diameter was measured to the nearest micrometre with an ocular micrometer.

Microscopic Examination

Microstructural examinations were made at 1250× with a research quality microscope fitted with planachromat objectives. Theoretical resolution of the system was calculated with the following equation:

$$R = \frac{\lambda}{2NA}$$

where R is the smallest visible distance between two structures, λ is the wavelength of light used, and NA is the overall numerical aperture of the system. Numerical apertures for both the objective and condenser lenses met or exceeded 1.25; however, since only the objective lens was immersed in oil, the NA for the condenser (and hence the whole system) was limited to 0.90–0.95. Accordingly, the theoretical resolution limit of the system under blue light was approximately 0.24 μm. This value is subject to some uncertainty, since several formulae for resolution limits are available (Eastman Kodak Co. 1980). However, functional limits are invariably higher than those derived theoretically and are subject to influence from the visual acuity and microscopic technique of the observer.

Increment counts were made of all sagittae and replicated at least twice by the same reader. Age was unknown to the reader at the time of otolith examination. Lapillar microstructure was difficult to interpret in larger larvae and was not examined further. Counts were made along the longest clear axis between the otolith periphery and a well-defined medial increment hereafter referred to as a "hatch check." While the nature of the hatch check could not be determined with certainty, its diameter (mean ± 95% C.I. = 23.0 ± 0.38 μm) in relation to the size of newly hatched larval sagittae indicated that it was formed at or within several days of hatch. Therefore, the check was used as a clearly defined temporal benchmark from which counts could be initiated. Subdaily increments apparent in many of the older otoliths were treated as such and not counted. While the distinction between daily and subdaily increments can seldom be made with total objectivity (Campana and Neilson 1985), any misinterpretation of subdaily increments as daily increments would serve to strengthen the arguments which are to follow.

Otolith microstructure was examined in specimens both before and after polishing. Increment counts were made of all otoliths prior to polishing and of all those greater than 30 μm in diameter after polishing. The latter were prepared with a graded series of aluminum oxide lapping films (30–3 μm grit size) to a plane just above the midplane. Hatch check diameters of polished specimens were measured to the nearest micrometre; post-hatch growth radii were calculated as one half the difference between the otolith and hatch check diameters. Twelve sagittae prepared for scanning electron microscopy (SEM) were given a final polish to the midplane with a 0.3-μm grit film, etched for 2–4 min in 0.1 M ethylenedinitrilotetraacetic acid (EDTA), and coated with gold–palladium.

Although etching was satisfactory in the region of the otolith formed after 30 d, it was poor in the area of interest (the perinuclear area). If the region of inadequate etching had been restricted to the area where growth increments were poorly resolved with a light microscope, the argument could be raised that no increments were present to be etched. However, some of the increments that were clearly visible with light microscopy remained unetched after EDTA treatment. It is possible that other etching treatments would have produced superior preparations. However, the SEM results available to us could be used neither to support nor reject hypotheses of daily increment formation, and SEM preparations were not continued.

Data Analysis

Daily otolith growth was modelled with both linear and nonlinear regressions. All regression parameters were estimated by least-squares methods. Data input consisted of one otolith observation per larva (selected at random from the two available), except in the resolution model where both sagittae were used. Residuals from the models were given careful examination, particularly near the origin. Models were accepted only in the absence of patterns in the residuals. The significance level was set at 0.05 for all tests.

Results

Increment Formation Frequency and Age

The two pairs of otoliths present at hatch, the sagittae and lapillae, were initially similar in both size and shape. While Lough et al. (1982) referred to the anterior pair as asteriscii, their position and subsequent development indicate that they were indeed lapillae. Sagittal growth in young larvae was exponential relative to larval growth. As a result, increment width tended to increase with age, at least until age 50 d; however, increments were narrow and difficult to see in larvae less than 20 d old.

In virtually all studies where the frequency of increment formation has been determined, a simple linear regression has been fitted to age and increment count data. As a first step, the same approach was adopted here. The resultant slope of 0.78 was both significant and highly correlated ($r^2 = 0.93$), suggesting that increments did not always form on a daily basis. However, the residuals of the linear model were markedly curvilinear at young ages, indicating that a simple linear model is inappropriate for these data (Fig. 1).

As a working hypothesis, increments were assumed to have formed daily during the experiment. Departure from such a model is presented in Fig. 2. The discrepancy between age and increment count increased rapidly with age; in the most extreme case, increment count underestimated larval age by 72%. There was no significant increase in the discrepancy after 30 d; stabilization occurred at a mean level of 17. This age-structured pattern in the discrepancy suggested a daily frequency of increment formation in older fish, and a lower formation rate in the younger fish. Daily increment formation in older fish was substantiated through an age–increment regression slope not significantly different from 1.0 in fish greater than 30 d old.

Increment Formation Frequency and Growth Rate

Larval growth rate varied nonlinearly with age. An average growth rate of 0.29 mm/d was maintained in each tank through day 80, although there were indications of growth cessations at irregular intervals. Mean growth rate before day 40 was significantly lower (0.24 mm/d) than that between days 40 and 80 (0.36 mm/d), while that in the first 9 d was not (0.30 mm/d). Therefore, age-related trends in growth rate may have been present, but were inconsistent through time.

Otolith growth rate was a better predictor of increment formation frequency than was larval growth rate ($P < 0.05$). Otolith size was significantly correlated with larval size. However, in an intralarval comparison of both sagittae from larvae less than 40 d old, increment counts were significantly higher in the larger of the two otoliths (paired t-test, $P < 0.001$). Further, the difference in increment number between the two otoliths was significantly related to the size difference between the two (Fig. 3). Therefore, previous reports of a correlation between larval growth and increment formation frequency (Geffen 1982; McGurk 1984) are more correctly described as a correlation between otolith growth rate and increment formation frequency.

Resolving Power

The influence of reduced resolving power on counting accuracy was demonstrated in a comparison of otoliths before and after polishing. Increment counts in the former were not significantly greater than those in the latter in sagittae with diameters of less than 35–40 μm. Polishing significantly increased both counts and clarity in larger otoliths, and was clearly mandatory when diameters reached or exceeded 50 μm. The age–increment discrepancy (described above) was documented in polished otoliths; however, its magnitude would have been considerably greater in the absence of polishing. Increments obscured by overlying material in the larger otoliths were those nearest the hatch check and were difficult to see under even ideal conditions. However, three rather broad (approximately 1 μm), clearly visible "increments" were visible around the hatch check in most otoliths from larvae greater than or equal to 15 d old. While these "increments" were most apparent in unpolished preparations, they could also be seen after polishing, particularly under conditions of poor focus. Under closer examination, each broad increment could be decomposed into a larger number of narrow increments; the widths of the latter were consistent with those of adjacent increments.

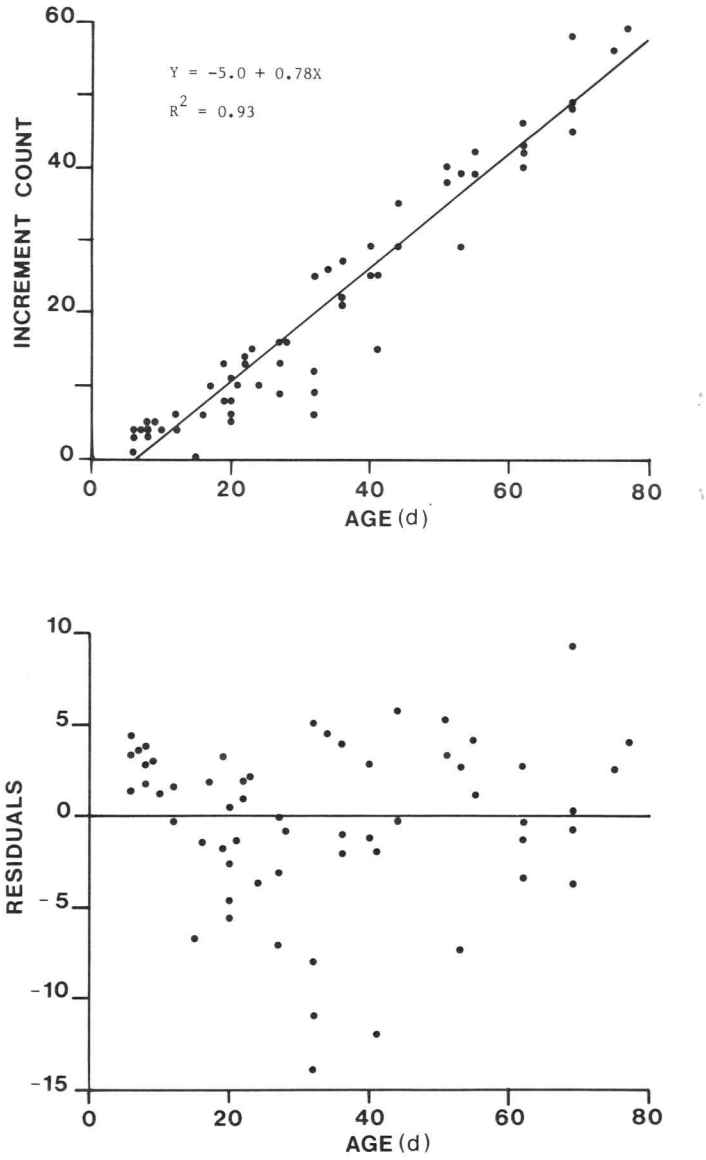

FIG. 1. Increment count as a function of age in laboratory-reared larval herring. The pattern in the residuals indicates that the simple linear regression that has been fitted is inappropriate.

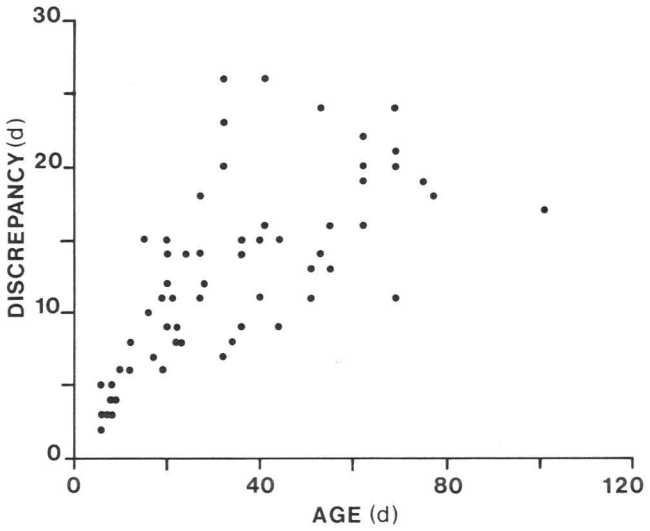

FIG. 2. Discrepancy between age and increment count (age minus count) as a function of age in laboratory-reared larval herring.

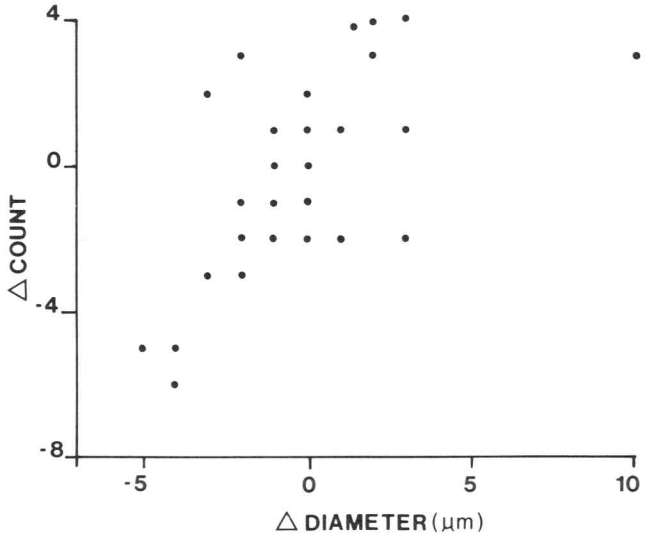

FIG. 3. Intralarval variation in increment count as a function of intralarval variation in otolith diameter. Intralarval variation was assessed as the difference between the two sagittae of a given larva. The relationship is plotted for those larvae ≤40 d old.

Theoretical and Observed Daily Increment Widths

The resolution hypothesis states that larval herring increments formed in the first weeks after hatch will be too narrow to resolve with a light microscope. A direct test of this hypothesis with SEM was inconclusive (see Materials and Methods). Accordingly, the daily growth of the otolith was modelled with the following logistic equation:

$$(1)\quad Y_a = 22.83 + \frac{357.3}{1 + \exp(-0.09647(\text{age} - 60.08))}$$

where Y_a = otolith diameter (micrometres) at age a (Fig. 4). The model was highly significant (Table 1), with the distribution of the residuals indicating that the model fit the data well, including near the origin. Assuming daily increment formation from the date of hatch, theoretical (or expected) increment width at age a (Theor$_a$) was calculated as

$$(2)\quad \text{Theor}_a = (Y_a - Y_{a-1})/2.$$

This assumes symmetric growth on either side of the nucleus along the measurement axis. Visual assessment of otolith shape to day 100 indicated that this was a realistic assumption. Theoretical increment widths increased exponentially from the time of hatch to age 40–50 d; they peaked at age 60 d and declined thereafter (Fig. 5). Given daily increment formation in the herring otoliths, the first 15 increment widths were predicted to be below the limit of resolution of our microscope system. Note that this value is similar to the observed age–increment discrepancy in older (>25 d) larvae (Fig. 2). Even with a "perfect" light microscope (with a resolution limit of 0.16 μm under blue light), it would have been impossible to see the first 11 daily increments.

Apparent increment widths (Appar) were estimated in a manner similar to that of Theor. The logistic equation

$$(3)\quad Y_c = 26.15 + \frac{318.3}{1 + \exp(-0.1341(\text{count} - 41.04))},$$

where Y_c = otolith diameter (micrometres) at increment count c, fit most of the data well (Table 1; Fig. 6). Nonrandomly distributed residuals were noted in otoliths with a diameter of less than 29 μm, but not in larger otoliths. Apparent increment width increased with the first 41 counts and declined thereafter

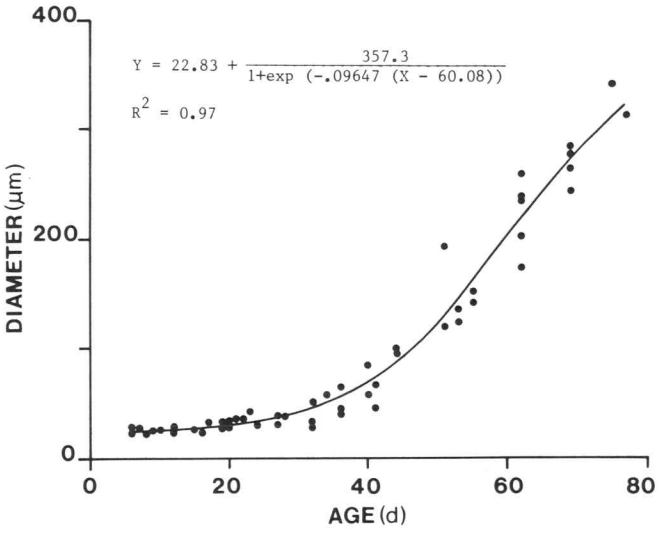

FIG. 4. Relationship between otolith diameter and post-hatch age as fitted with the logistic equation. Model results are presented in Table 1.

(Fig. 7): the general pattern was similar to that observed in the theoretical width model although there was a lag consistent with the age–increment discrepancy. The first seven apparent widths were narrower than the limit of resolution of our microscope (0.24 μm) but considerably larger than those predicted by the theoretical model.

Resolution Model

If inadequate resolution was responsible for the observed discrepancy between age and increment count, it should be possible to reconcile observed and expected counts in a model which assumes that daily increments are visible as distinct structures only if their width exceeds a specified resolution limit. Narrower increments would appear distinct only if pooled with adjacent increments in sufficient number to exceed the resolution limit.

Equation 1 was used to determine the mean pattern of

TABLE 1. Parameter estimates, associated error terms, and ANOVA's for the logistic growth models fitted in Fig. 3 and 5. Theoretical width model: otolith diameter (μm) vs. age (d); apparent width model: otolith diameter (μm) vs. increment count; $Y = a + b(1 + \exp(-c(x - d)))^{-1}$.

Model	Source of error	Sum of squares	df	Mean square	R^2	Variable	Coefficient	SE
Theor	Model	944 105	4	236 026	0.971	a	22.83	3.94
	Error	14 453	60	241		b	357.3	34.8
						c	0.09647	0.01085
						d	60.08	2.18
Appar	Model	940 707	4	235 177	0.964	a	26.15	3.80
	Error	17 851	60	298		b	318.3	21.4
						c	0.1341	0.01519
						d	41.04	1.133

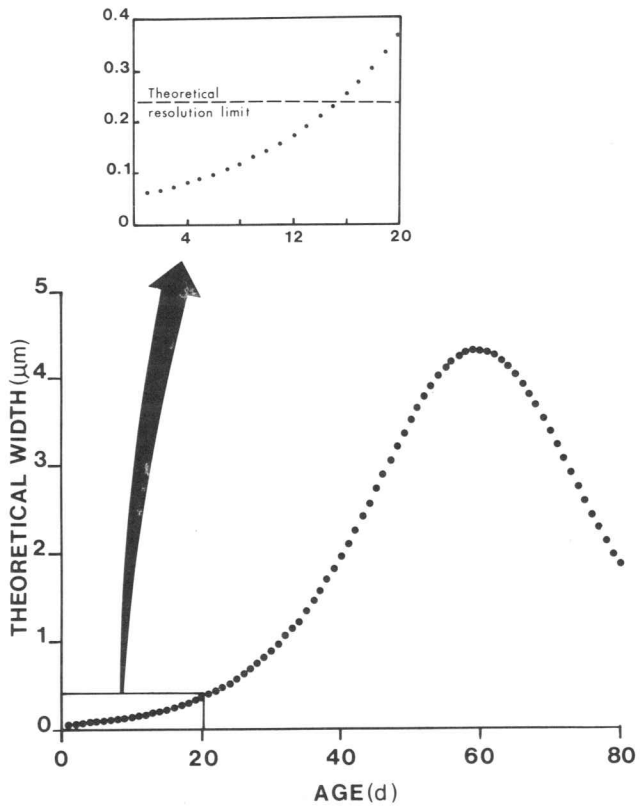

FIG. 5. Theoretical (or expected) daily increment width as a function of age in larval herring. Theoretical values were derived from a fitted otolith growth model (Fig. 4; Table 1).

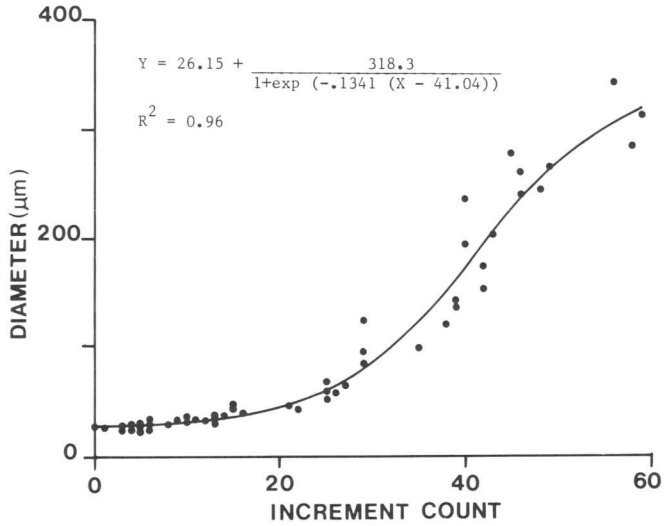

FIG. 6. Relationship between otolith diameter and increment count as fitted with the logistic equation. Model results are presented in Table 1.

expected daily increment widths in our sample of otoliths (Fig. 5). To adjust this pattern to the size of an individual otolith, a scaling factor (SF) was used to adjust the sum of expected increment widths to the observed otolith post-hatch growth radius (PHGR). For otolith i of age a:

$$SF_i = PHGR_i / \sum_{1}^{A} Theor_a$$

where Theor is the theoretical width of the ath daily increment. The model is discrete in that increments narrower than the functional resolution limit (FR) were pooled in integer combinations only.

Mathematically, increment count

$$C = \sum_{1}^{a} C_a$$

for each daily increment in a given otolith, where C_a is calculated according to the following algorithm:

(1) $a = 1$
(2) $n = 0$
(3) If $\left(SF \times \left(\sum_{j=a}^{a+n} Theor_j\right) \geq FR\right)$, go to 6
(4) $n = n + 1$
(5) Go to 3
(6) $C_a = 1$
(7) $a = a + n + 1$
(8) Go to 2.

The fit of the model was assessed through comparison of predicted and observed increment counts. Fit was maximized through iterations where the magnitude and constancy of functional resolution were varied. Maximization criteria consisted of a 1:1 relationship between observed and predicted increment counts, a high correlation coefficient, and a randomized residual pattern. While high correlation coefficients could be achieved when resolution remained invariant with otolith diameter ($R \geq 0.94$), it was not possible to produce a randomized residual pattern and a slope of 1.0 at a constant resolution limit. Models that incorporated linear increases in resolution with otolith diameter produced an improved, but still biased, fit. Fit was maximized and bias minimized when resolution increased with the square root of otolith diameter

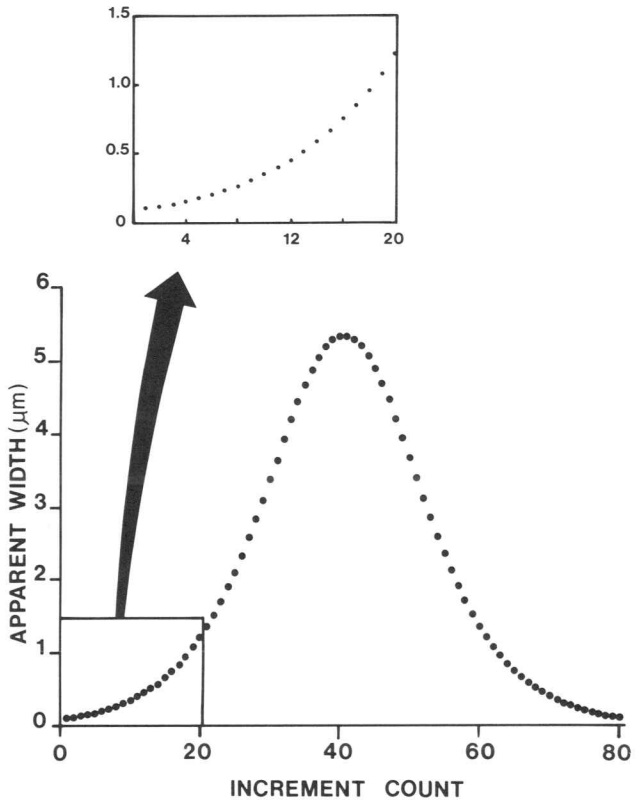

FIG. 7. Apparent increment width as a function of increment count in larval herring. Apparent values were derived from a fitted otolith growth model (Fig. 6; Table 1).

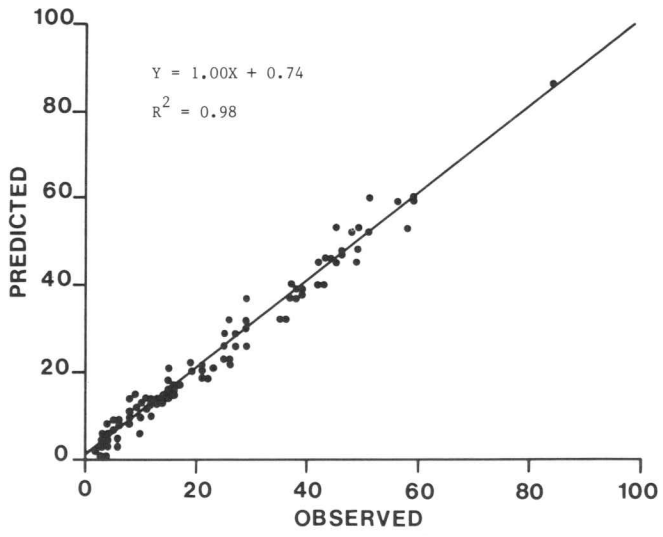

FIG. 8. Relationship between observed and predicted increment counts resulting from a model incorporating resolution-limited visibility of increments. See text for details of model.

(FR = $0.16 + 0.02\sqrt{\text{Diam}}$). The resultant relationship between observed and predicted increment counts had a slope of 1.0, was highly correlated, and was linear (Fig. 8). The model's assumption of a square root relationship between otolith diameter and resolution was justified on the basis of empirical observations of the otolith thickness to diameter ratio and the fact that light transmission is affected by passage through the lower (unpolished) half of the otolith. Functional resolution, as derived from the model, was 0.25 μm for the smallest otolith, increasing to 0.71 μm for the largest specimen. The resolution estimate for the smallest otolith was nearly identical to the theoretical calculation for our microscope system (0.24 μm), thus providing an independent test of the validity of the model and its assumptions.

Discussion

A daily rate of increment formation was not evident in known-age larval herring otoliths when assessed with light microscopy. Simple linear regression estimates of the apparent rate of increment formation (0.78) were consistent with other studies of larval herring with similar growth rates (Geffen 1982: Lough et al. 1982; McGurk 1984). However, the distribution of the residuals indicated that the simple linear model was inappropriate for these data; the apparent increment formation rate was much lower in young larvae than in old. Daily increments were almost certainly formed in larvae older than 30 d. Given the discrepancy between age and increment count in the younger larvae, the question of the most plausible mechanism arises: are daily increments forming with widths below the resolution limit of light microscopy, or are increments forming at irregular, nondaily intervals?

Resolution Hypothesis

The resolution hypothesis provides a causal and empirical explanation of apparent microstructural anomalies through a biologically and physically plausible mechanism. The hypothesis states that daily increments with widths below the functional resolution limit of a light microscope can and do form in some larval otoliths. Since these increments would not be visible as distinct structures, an apparent increment formation rate of less than 1 would result. The strength of the hypothesis lies in its precise mathematical prediction of age-count anomalies and its attendent explanations of apparently unrelated phenomena. It is fully consistent with the results of this and other studies and does not invoke any novel mechanisms of otolith growth.

There is no doubt that resolution plays a role in the interpretation of herring otolith microstructure. The comparison of polished and unpolished otoliths provided the most visible evidence of this: similar results have been reported from larval starry flounder (*Platichthys stellatus*) otoliths (Campana 1984). In situations such as these, where some increments were clearly visible without polishing, increment counts could underestimate increment number as a result of omitting the polishing stage.

Can daily increments form with widths that are unresolvable by light microscopy? The results of the otolith daily growth model indicated that daily otolith growth in the first 2 wk after hatch was insufficient to be resolved as discrete daily growth increments with our light microscope. Given the fit of the model and the magnitude of the predicted values, this conclusion is unambiguous even if increment formation started 4.5–6 d after hatch, as has been inferred elsewhere (Geffen 1982; Lough et al. 1982: McGurk 1984). Support for this conclusion comes from the microstructural examination of chinook salmon (*Oncorhynchus tshawytscha*) otoliths, where SEM was used to detect narrow daily increments formed at 5°C (Neilson and Geen 1982), while increment formation had apparently stopped at this temperature when assessed by light microscopy (Marshall and Parker 1982). Similar conclusions were reached in an examination of striped bass (*Morone saxatilis*) otoliths by both SEM and

light microscopy (Jones and Brothers 1987). In the latter instance, light microscopic increment counts seriously underestimated the number visible under SEM. Therefore, the conclusion that narrow daily increments were formed in the otoliths of the larval herring at or shortly after the time of hatch is consistent with the resolution problems reported by other workers.

Observed increment counts were closely predicted by the resolution model when realistic functional resolution limits were used. The age–increment discrepancy was predicted to increase quickly to 15–20, increasing only slightly thereafter. This prediction was similar to the observed discrepancy, both here and in several other herring studies where growth rates were similar to ours (Geffen 1982; Lough et al. 1982; McGurk 1984). Since compression of a daily increment sequence would be expected to increase as larval growth rate slowed, the resulting decline in apparent increment formation rate would be fully consistent with the results reported elsewhere (Geffen 1982: McGurk 1984). The model also helped explain the observation of three broad perinuclear "increments" in the first 20 d after hatch (Lough et al. 1982; this study) as aggregates of poorly resolved increments. Such an explanation is intuitively appealing in that it avoids the need for novel mechanisms of otolith growth. A third prediction of the model was met in the observed correlation of differential otolith size and differential increment count between the two otoliths of a given larva. Finally, the model correctly predicted a small increase in the accumulated age–increment discrepancy in older larvae in which daily increments were clearly forming. All of the above suggests that the resolution model accounts for all, not just most, of the age–increment anomalies observed in larval herring. Indeed, given the growth-dependent apparent increment formation rate predicted by the resolution hypothesis, we conclude that Geffen's growth rate limitation hypothesis provides an excellent empirical description of the same phenomenon which we describe here. Therefore, we conclude that Geffen's (1982) observations actually support the resolution hypothesis, with the latter providing the theoretical basis for her observations. It should also be noted that an explicit conclusion of the resolution model is that increment formation occurs daily and independently of growth rate and age. Thus, the model is fully consistent with current concepts of increment formation in fish otoliths (Campana and Neilson 1985).

The lower limit of resolution suggested by the resolution model was very similar to that calculated independently for our microscope system. However, the first-formed increment widths predicted by the apparent width model were significantly smaller. Somewhat counterintuitively, this discrepancy is also consistent with the resolution hypothesis. According to the hypothesis, unresolved increments should aggregate in a number sufficient to be resolvable as a unit. Each of these aggregates would be expected to be of similar width. However, the logistic equation used in the Appar model makes no allowance for a series of units with constant widths near the origin, and this is reflected in the residual pattern near the origin. Thus, all three estimates of resolution were consistent with each other.

Implications

The implications of the resolution model extend well beyond interpretive bias in the otolith microstructure of herring larvae. There are a number of larvae for which increment formation rates of less than one have been reported: examples include anchovy (*Engraulis mordax*) (Methot and Kramer 1979), English sole (*Parophrys vetulus*) (Laroche *et al.* 1982), starry flounder (Campana 1984), and sand lance (*Ammodytes americanus*) (S. W. Richards, Little Harbor Laboratory, 69 Andrews Road, Guilford, CT 06437, pers. comm.). All of these species are similar to herring in that they possess a pelagic larval phase with the potential for minimal otolith growth; where increment widths were reported, the lower limit extended well below 1 μm. In other words, these species are expected candidates for resolution-limited increment counts. Presumably there are many others in which resolution limitations may be expected to occur, particularly in colder waters where growth can be slow. Since light microscopy is the most frequently used and convenient of the otolith examination procedures (Campana and Neilson 1985), resolution limitations may already have introduced error and bias into some published estimates of larval age, growth, and mortality. Of course, the magnitude of those errors would be a function of reported age; estimates for young larvae would be more seriously affected than those for older individuals. And given the prediction that age–increment discrepancies would be expected to vary with growth (Geffen 1982; McGurk 1984), empirical corrections for an apparent age–-increment discrepancy (Lough et al. 1982) would be unlikely to be accurate in the absence of independent knowledge of the growth rate through the relevant period.

While not so serious, the age at hatch check formation may also be open to error in resolution-limited otoliths. As first suggested by Lough et al. (1982), an apparently discrete hatch check may actually be composed of an aggregate of unresolved daily increments. This hypothesis cannot be tested with light microscopy alone.

The apparent susceptibility of temperate pelagic larvae to resolution effects is largely a result of their low otolith growth rate relative to that of juveniles. This may account for the few reported examination problems in juveniles. The allometric otolith to body length relationship in larval herring makes the early larval stage particularly sensitive; given such a relationship, daily increment width should increase with age despite constancy in the larval growth rate (i.e Fig, 5), implying that the most serious potential resolution difficulties should occur nearest the nucleus. Similar increases in increment width with age would be expected of other species with an allometric growth relationship. Despite speculation to the contrary (Victor 1986), this form of otolith growth is characteristic of a number of species with pelagic larval phases (i.e. haemulids, Brothers and McFarland 1981; clupeids, Lough et al. 1982; engraulids, Tsuji and Aoyama 1984; gadids, Nishimura and Yamada 1984; pleuronectids, Campana 1984).

Guidelines

Identification of resolution-limited microstructural preparations should be possible in many cases. Direct measurements of increment width, or widths estimated from an observed increment width model such as that described previously (Appar), should provide some indication of the need for further examination. In theory, the calculated resolution limit of the microscope could be used to assess the reliability of the observed increment widths. In practice, however, the former will probably underestimate the true limit due to the influence of sample thickness on resolving power. As a general guideline, we suggest that otoliths where increment widths are less than 1 μm be examined further. Otoliths where increment widths appear to increase in

proximity to the nucleus may also be suspect. Polishing will improve resolution in larger otoliths, but will not necessarily identify resolution-limited increments in small otoliths. A more powerful technique is the comparison of increment counts between left and right otoliths; where counts are greater in the larger of the two otoliths, resolution limitations should be suspected. Of course, such a comparison is only appropriate for the growth zone where increment widths are narrow. Note that this technique is suitable for identifying a resolution problem, but cannot be used to assess the magnitude of the age–increment discrepancy. Indeed, none of the identification measures listed above appears to be suitable for estimating the magnitude of the discrepancy. Where necessary, it may be possible to estimate approximate discrepancies through knowledge of the otolith – larval length relationship, the observed increment width pattern, larval growth rate, and other biological characteristics.

Several procedures are available to maximize resolution during light microscopic examination of otoliths. Polishing and thin sections derived from two-sided polishing can produce superior preparations (Campana and Neilson 1985), thus improving resolution. Resolution can also be improved through careful attention to the effective numerical aperture of the microscope, which can be more important than increased magnification (Eastman Kodak Co. 1980). Substantial increases in both magnification and resolution require application of SEM, a technique that has already been applied successfully to increments unresolvable by light microscopy (Jones and Brothers 1987). However, SEM will not always be appropriate for otolith examination, as was noted in this study. Etching for SEM requires the presence of differences in chemical composition between incremental and discontinuous zones within a daily increment. These differences also render the increment visible with light microscopy as a distinct bipartite structure. Given the low visual contrast characteristic of some larval increments, it may be that SEM will prove no more useful in their examination than will resolution-limited light microscopy.

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