REVIEWS/SYNTHÈSES

Microstructure of Fish Otoliths

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Otolith microstructure examination has found an increasing number of applications in recent years. However, few workers have critically assessed the assumptions upon which the age and growth inferences are based or considered the potential for environmental modification of microstructural features. This paper reviews present applications and their assumptions and suggests future directions. Particular attention is given to the premises that the frequency of increment formation is constant and that the width of increments is proportional to fish growth. A hypothesis of increment formation is presented which appears consistent with the numerous and often conflicting studies reported to date. The presence of an endogenous circadian rhythm of increment formation is invoked, entrained by photoperiod, but susceptible to modification by other cyclic environmental variables. Increments formed as a result of the circadian rhythm (once per 24 h) may be induced by different processes than those induced through the action of environmental cues (often > 1 per 24 h), thus explaining apparent morphological differences in increment structure noted by some workers. Temperature fluctuations appear to be a primary source of subdaily increments and are a potential source of error in otolith interpretation.

Au cours des dernières années, on a trouvé un plus grand nombre d'applications à l'examen de la microstructure des otolithes. Toutefois, peu de chercheurs ont fait une évaluation critique des hypothèses sur lesquelles sont bassées les conclusions sur l'âge et la croissance ou ont tenu compte des modifications environnementales potentielles des caractéristiques microstructurales. La présente étude fait le compte rendu des applications actuelles et des hypothèses connexes et propose des orientations futures. On porte une attention particulière aux principes selon lesquels la fréquence de formation de zones de croissance est constante et la largeur des zones est proportionnelle à la croissance du poisson. On présente une hypothèse sur la formation de zones qui semble conforme aux nombreuses études souvent contradictoires réalisées jusqu'à maintenant. On a recours à la présence, pour la formation des zones, d'un rythme circadien endogène qui est régi par la photopériode mais sujet à des modifications sous l'effet d'autres variables environnementales cycliques. Les zones formées selon le rythme circadien (une par 24 h) peuvent être induites par des processus autres que ceux qui sont produits sous l'effet de facteurs environnementaux (souvent, plus d'une par 24 h), ce qui expliquerait les différences morphologiques apparentes dans la structure des zones notée par certains chercheurs. Les variations thermiques semblent être une cause importante de la formation de plusieurs zones de croissance en une journée et représentent une source potentielle d'erreurs dans l'interprétation des otolithes.

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Scales, otoliths, opercular bones, fin rays and vertebrae are examples of structures that are commonly used for age and growth inferences. However, interpretation of age and growth from any bony structure of fish is based on the assumptions that periodic features are formed at a constant frequency, and that the distance between consecutive features such as scale circuli or annuli is proportional to fish growth. These assumptions have been questioned, notably in the case of scale annuli, structures previously assumed to be formed yearly. Blacker (1974) has indicated that both age and genetic factors may influence the rate of production of structures commonly referred to as annuli. In addition, environmental variables such as feeding frequency and photoperiod may affect both the rate of formation and distance between successive scale annuli (Bilton 1974), thus complicating or invalidating age and growth inferences. Moreover, conventional age determination methods have little utility for sub-yearling fishes and tropical species, where seasonal marks such as annuli are not generally present.

The relatively new finding by Pannella (1971) and subsequent workers that many teleost fish deposit otolith growth increments with a 24-h periodicity appeared to offer a method of assessing age and growth with greater accuracy and precision than was previously possible. It also offered a means of age determination for species or life history stages where such data were previously unavailable. Daily growth increments in otoliths of teleost fish are now known to be a widespread phenomenon, present in taxa in both freshwater and marine habitats, and in species distributed from the polar regions to the tropics. In addition, the assumptions of constant frequency of formation and proportionality between otolith and fish growth appear to be well met, perhaps more so than is the case with annuli. In this paper, we critically review the evidence supporting these assumptions.

The terminology of otolith microstructure, in common with that associated with other aspects of age and growth studies, is often rather confused with an abundance of synonymous terms whose definitions are based on subjective criteria. For example, growth increments (the terminology used here) have been called rings, lamellae and growth units to name just a few. Primordia, structures found in the nucleus and thought to be centres of accretion, have been referred to as nuclei, foci and kernels. To avoid such ambiguities, we have adopted a standard set of terms referring to the parts of the otolith and its microstructure (Fig. 1). A growth increment is a bipartite structure, usually formed over 24 h, consisting of incremental and discontinuous zones (terminology of Mugiya et al. 1981). When viewed with a light microscope, the incremental zone appears as a broad, translucent band, while the discontinuous zone is relatively narrow and opaque. When a series of daily growth increments is etched with a weak acid and viewed with a scanning electron microscope (SEM), incremental zones appear as relatively wide, lightly-etched areas while the discontinuous zones are narrow and deeply-etched (Fig. 1).

Incremental otolith growth occurs through differential deposition of calcium carbonate and protein over a 24-h period. Under high magnification (> 1000×), the crystal structure is apparent with the long axis of the crystals oriented perpendicularly to the growth increments. Individual crystals are enmeshed in a proteinaceous matrix, a band of which apparently terminates crystal growth at each end (the discontinuous zone) (Dunkelberger et al. 1980). However, use of the term "discontinuous" is somewhat misleading in that some continuity of the crystalline network is often maintained. The crystalline structure of the lightly etched zone is thought to be largely of calcium carbonate in the aragonite configuration (Mugiya et al. 1981) although vaterite, another mineral form of calcium carbonate, is occasionally present (Carlstrom 1963; Campana 1983a; Brothers 1984). A matrix of the protein otolin (M.W. 150,000), characterized by a high abundance of acidic amino acids, makes up much of the narrow, deeply etched zone (Degens et al. 1969; Dunkelberger et al. 1980; Ross and Pote 1984). The matrix structure is sheet-like, with tightly packed fibers 8 nm in diameter. Ultrastructural details of both the crystalline network and the organic matrix are presented elsewhere (Irie 1960; Degens et al. 1969; Dunkelberger et al. 1980; Watabe et al. 1982; Mann et al. 1983).

While nothing is known of the matrix formation cycle in otoliths, the cyclic nature of calcium deposition has been elucidated by workers using the radioisotope ^{45}Ca. Mugiya (1974) showed that macular cells secreted ^{45}Ca into the endolymphatic fluid surrounding the otoliths. Mugiya et al. (1981) noted that the rate of accretion on various parts of the otolith was proportional to the concentration of adjacent macular cells. They also demonstrated that the rate of calcium deposition on goldfish (*Carassius auratus*) otoliths slowed around sunrise, possibly as a result of reduced macular cell secretion. This circadian rhythm of calcium deposition is almost certainly under endocrinological control (Simkiss 1974; Dacke 1979), yet parallels between scale and otolith growth are difficult to identify. For example, scale growth ceases under conditions of food deprivation, and in cases of severe stress, resorption may occur. Similar phenomena have not yet been documented for otoliths. Otoliths continue to grow under conditions of food deprivation (Marshall and Parker 1982; Campana 1983a; Neilson and Geen 1984), while fish stressed by exertion (Campana 1983b) or by exposure to low pH (Geen et al. 1985) do not show evidence of otolith resorption. Thus, under many circumstances, otoliths provide a more representative growth record than do scales. A further advantage of using otolith microstructure examination for age determination is that otoliths are often the first calcified structures that appear during the early development of teleosts. Otoliths may then have utility for age determination when scales are absent or for species where scale growth is irregular or unpredictable.

That otolith size reflects fish size so closely serves to underscore its utility for growth studies. The conservative nature of otolith growth relative to scale growth may reflect the functions of each: the otoliths as organs of equilibrium and hearing presumably must maintain precise configurations with respect to other parts of the fish's otic apparatus, whereas the scale's function as part of the integument may permit greater latitude in functioning as part of the calcium reserves.

In this paper, we review the existing and potential applications of otolith microstructure examination in the fields of fisheries biology and management. In addition, we critically assess how well otolith growth reflects fish age and growth at the daily level of precision.

Methodology

Microstructural examination of otoliths is a three-stage process consisting of otolith mounting, preparation and observation. Specific techniques vary with the age and size of otolith, increment width and clarity, degree of resolution required, available equipment and application. In this section, we briefly review and contrast the techniques now available.

Of the three pairs of otoliths that occur in teleost fish, the sagittae are usually the largest and most commonly used for microstructure studies. Of the other two pairs of otoliths (the lapilli and asterisci), the former are useful in circumstances where the sagittae are small and comparatively delicate (i.e. in cyprinids), and indeed may be preferred to sagittae in many other species of fish.

All fish otoliths are acellular, mineralized structures. As such, decomposition under relatively dry conditions does not occur over the short term (< 3 yr). Dry storage and/or mounting on standard microscope slides prior to microstructural examination are therefore common practices. Mounting media include instant glue, thermoplastic cement, and liquid media such as Euparal. The use of Permount is not recommended, since it is not rigid enough to allow subsequent grinding. Liquid storage in 95% ethanol is also possible. However, acidic solutions are incompatible with calcareous structures such as otoliths, even at mildly acidic pHs of 6.0–6.5. Therefore, partial otolith dissolu-

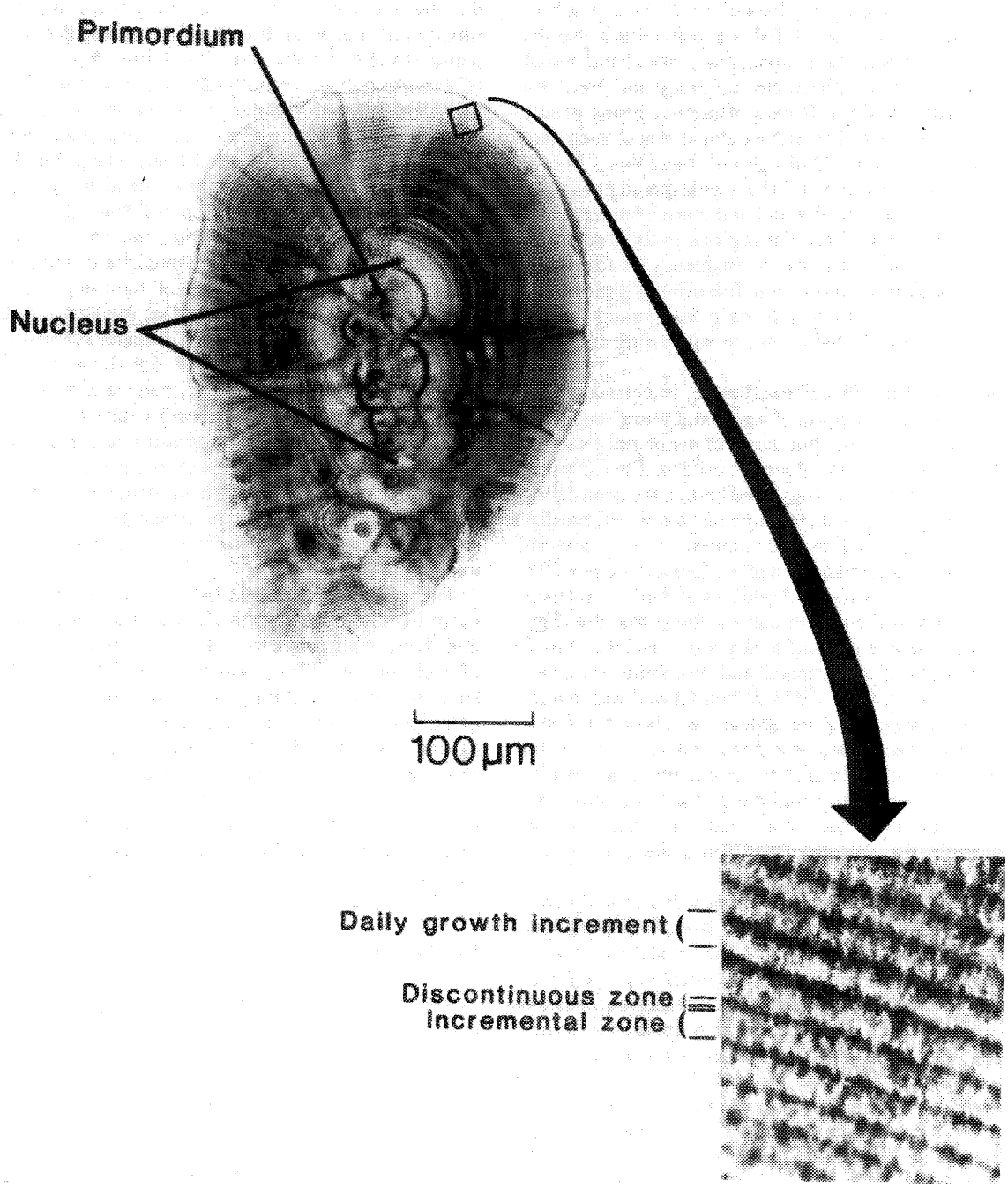

FIG. 1. Incremental growth sequence in a juvenile chinook salmon otolith (sagitta) as viewed with transmitted light. As indicated, the periphery of the nucleus is delimited by the first contiguous increment encircling all central primordia.

tion may be expected of fish/otoliths stored in buffered formalin or solutions containing <70% ethanol (Radtke and Waiwood 1980). Due to surface area to volume considerations, dissolution rates and otolith size are inversely related, rendering larval otoliths most susceptible to acidic degradation.

Where otolith preparation is necessary, its purpose is to enhance the distinction between the incremental and discontinuous zones that comprise each bipartite growth increment. Visual differentiation of adjacent growth increments is only possible because of the difference in light transmission characteristics between each component of a given increment. Therefore, daily increments can be discerned only when viewed as adjacent transverse sections; this necessitates focusing along the midplane of one of the three primary otolith orientations.

Several methods are available for enhancing light microscopic images; all act to reduce refractive effects and increase light transmission through the otolith. Increased transparency of overlying material can be obtained through use of clearing media such as Euparal or immersion oil (Radtke and Dean 1982). However, overclearing cannot always be reversed and will reduce visibility of microstructural features. Sectioning, grinding, polishing, and acid-etching physically remove the calcareous overburden and are frequently used techniques for otolith preparation (Wild and Foreman 1980; Wilson and Larkin

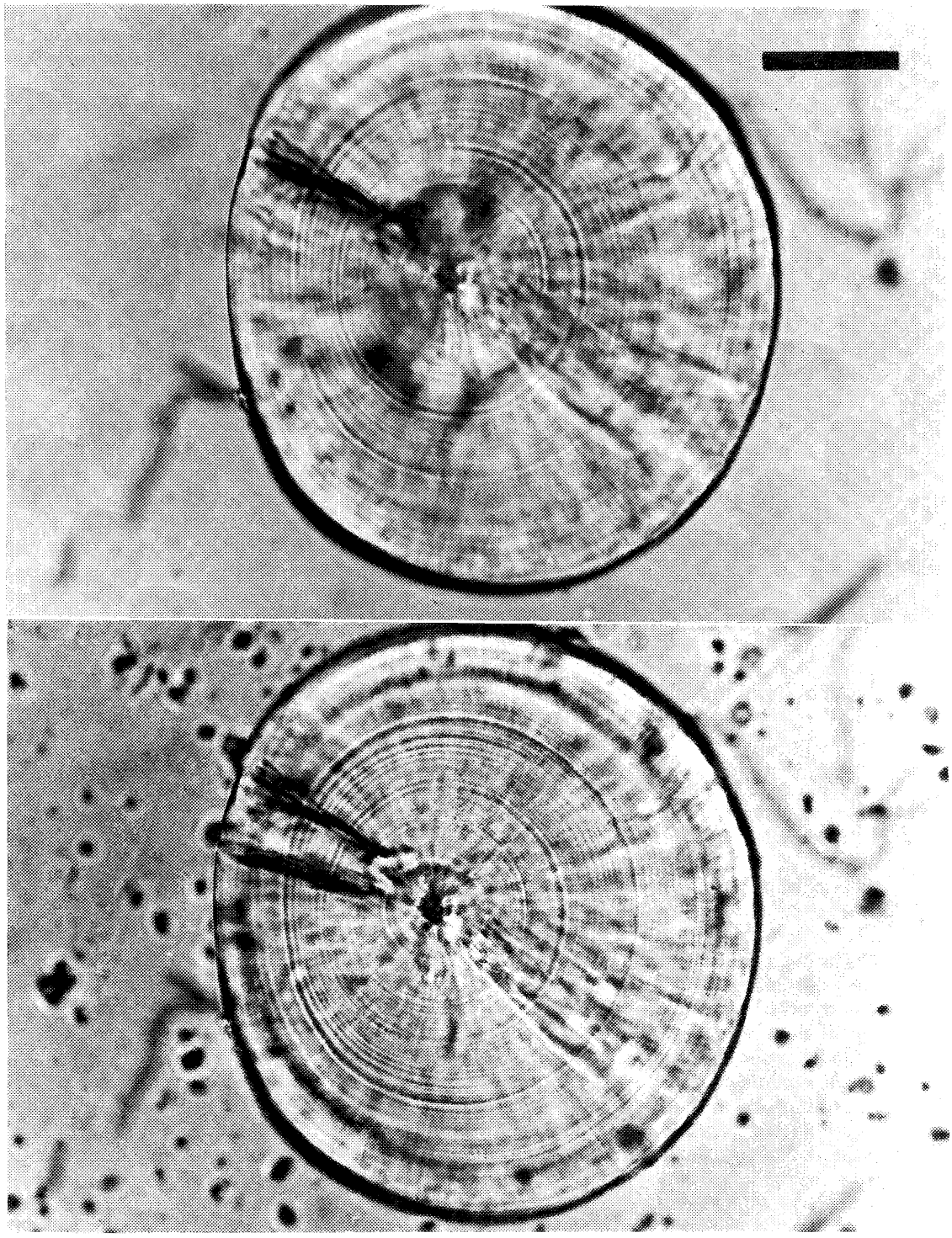

FIG. 2. Comparative photomicrographs of a single wild Atlantic herring otolith before and after preparation for light microscopy. Differences are most apparent in the perinuclear region where the most material was removed during grinding. Top, unground; bottom, ground and polished. Bar = 30 μm.

1980; Campana and Neilson 1982). Overgrinding of otolith edges due to beveling of hand-held preparations can be eliminated through use of grinding jigs that maintain an even grinding plane (Neilson and Geen 1981). Grinding from both sides (to form a thin section) requires more preparation time, but can result in preparations of superior quality (Campana 1984a). Caution must be observed during application of all of these techniques, since they are irreversible and can lead to ruined preparations if carried out past the midplane. Nevertheless, removal of material above the midplane almost invariably improves the resolution of increments observed microscopically, even in the case of translucent larval otoliths (Campana 1984b). Comparative micrographs of ground and unground otoliths from wild herring (*Clupea harengus*) larvae demonstrate the difference that grinding can make, particularly in the perinuclear area (Fig. 2).

Certain techniques in light microscopy can also improve viewer resolution of growth patterns. Use of crossed polarizing filters can be helpful in locating larval fish otoliths both in the initial dissection and on the microscope slide. Microscope–video systems serve to enlarge the image, simplifying subsequent increment counts and measurements. While these systems do not improve resolution, electronic enhancement of contrast is possible.

Whereas otolith preparation for light microscopy is designed to enhance light transmission differences between incremental and discontinuous zones, preparation for SEM and acetate impressions takes advantage of differences in chemical composition between the zones. Both SEM and acetate techniques rely on differential etching to produce contoured surfaces amenable to topographic examination. Contrary to expectation, weak acid solutions such as 1–2% HCl appear to remove material from the proteinaceous zone rather than the calcified area (Pannella 1980a; Mugiya et al. 1981). The initial suggestion of Brothers et al. (1976) that the acid solution removed material from the calcified zone at a greater rate has since been reconsidered (E. B. Brothers, 3 Sunset W., Ithaca, NY 14850, pers. comm.). Although HCl solutions are still widely used for etching (Pannella 1971; Neilson and Geen 1982), calcium chelators such as ethylenedinitrilo tetraacetic acid (EDTA) give better results in some situations (Radtke and Waiwood 1980; Campana 1984b). Regardless of the etching agent used, etching times must be regulated to ensure that specimens are not overetched and microstructural features lost. Etched samples may then be gold coated for SEM (Neilson and Geen 1982) or used as molds in the preparation of acetate impressions (Pannella 1980b). Note that the preparation of acetate impressions is under the same grinding and etching constraints as that of SEM. However, unlike SEM, microstructural detail can be lost during creation of impressions, while visual artifacts and limited resolution can be introduced during the subsequent light microscopic examination.

The advantages and disadvantages of the various otolith examination methodologies are linked to the size, morphology, and features of the otolith to be examined. Light microscopy is a fast and flexible means of observation, where otolith preparation and equipment needs are minimal, and focal lengths can be adjusted to follow the contours of nonplanar increments. Limitations include the need for samples translucent enough to allow light transmission, potential confusion of increments with visual artifacts, and a resolution limit that may not be sufficient to differentiate increments of narrow width. The light transmission constraint requires sufficient grinding to prepare a thin section of the most opaque otolith in the sample. Although some workers have rejected the largest, generally fastest-growing otoliths due to special grinding requirements (Geffen 1983), this practice can introduce a directed bias into later calculations. Light micrographs provide a medium for count and measurement purposes (Wilson and Larkin 1980; Campana and Neilson 1982), but their fixed focal length may introduce more visual artifacts than would have been observed through a light microscope. However, with SEM, resolution exceeds that needed for observation of the most narrow increments known, and visual artifacts do not occur. In addition, counts and measurements can be made from photographs, which generally reduces observer error. Restrictions on SEM use include the need for expensive specialized equipment and more involved sample preparation. Unlike the preparation for light microscopic examination, grinding of otoliths for SEM is a mandatory procedure and must be carried out precisely to the midplane to ensure adequate etching. Therefore, SEM is not appropriate for the observation of otoliths with nonplanar increments. Moreover, etching criteria for otoliths from species such as plaice (*Pleuronectes platessa*) have not yet been fully developed (Blacker 1975), and in others, etching success may vary with location on the otolith. Age-dependent etching times can complicate preparations of extended growth sequences. In addition, the preparation of larval otoliths can require specialized techniques (Campana 1984b).

While a given examination procedure may be preferred in certain situations, light microscopy and SEM will generally produce increment counts of similar accuracy and precision if the increments are of sufficient width (i.e. > 1 μm, see Fig. 4) (Campana 1983a). The benefit of SEM use, with its greater preparation time, comes when narrow increments (< 1 μm) are being viewed or if increment widths are being measured. SEM measurements of increment width require a perpendicular angle between the planar surface of the otolith and the impinging electron beam, either through adjustment of the specimen stage or alteration of the path of the electron beam. In general though, increment width measurements are more precise when digitized from SEM photographs than when measured with a light microscope under conditions of variable focal length. The increased use of image analysis systems (Methot 1981) and drawing tubes (Bolz and Lough 1983) has reduced the magnitude of errors associated with measurements obtained using light microscopy. Use of hand counters and blind-labelled preparations has similarly reduced bias during counting. However, errors derived from misidentification of microstructural features cannot be circumvented through use of one observation technique or another (with the exception of visual artifacts). Structures that can be confused with daily growth increments under a light microscope, such as subdaily increments and some checks, can also be confused when viewed with SEM. Edge effects are another source of error common to both techniques, although causes differ. Under a light microscope, transmitted light is refracted at obtuse angles at the otolith edge due to the curved surface. Increments so affected may appear to be laterally compressed or disappear from view altogether. With an SEM, errors due to edge effects are of similar magnitude, but are due to over- or under-grinding at the point furthest from the grinding focus (nucleus). This problem is most accentuated in larval otoliths (Campana 1984b).

Validation of daily increment formation is a necessary prerequisite to otolith microstructure work of any kind. Substantial errors may be introduced into an analysis if the structures observed are subdaily but not interpreted as such. Validation procedures include the use of known-age fish in the laboratory (Brothers et al. 1976; Neilson and Geen 1982), sequential sampling of a population where age-selective migration/mortality does not occur (Struhsaker and Uchiyama 1976), or the introduction of dated marks onto the otoliths. The latter technique can be applied to fish in either the laboratory or the wild and can use chemical or intrinsic marks. Chemical marking techniques commonly employ oxytetracycline hydrochloride, which is incorporated into growing calcified tissues within a day of application (Wild and Foreman 1980; Campana and Neilson 1982). The incorporated compound is fluorescent under ultraviolet light, accurately marks the date of application, and resists fading during prolonged storage in the dark (up to at least 4 yr, E.B. Brothers, pers. comm.). Administration can be through injection (100 mg/kg fish) (Wild and Foreman 1980; Campana

and Neilson 1982), dietary intake (250 mg/kg fish) (Weber and Ridgway 1967), or branchial uptake (30 min in 0.1% saline/ 0.004% tetracycline) (Neilson and Geen 1984), although incorporation times limit the frequency of sequential marks that can be applied (Kobayashi et al. 1964). Acetazolamide, a carbonic anhydrase, has also been used as a chemical marker, but with questionable success (Mugiya 1977; Ralston and Miyamoto 1983). Chemical markers for SEM use may also be available. Some elements such as strontium act as calcium analogs during crystal formation. Since heavy elements are detectable with the X-ray line scans available on most SEMs, one should be able to associate the daily increment corresponding to the application date with a peak in strontium abundance. Although this technique has apparently been applied to the study of growth increments in squid statoliths (Hurley et al. 1985), we have found that similar marks in fish otoliths could not be resolved with the required definition (J.D. Neilson, unpubl. data).

Dated marks can be placed on otoliths through other than chemical means. By taking advantage of some of the intrinsic growth processes of otoliths, natural or induced temporal markers can be used to validate the frequency of increment formation. For example, hatch checks are prominent features of many otoliths and are often used as benchmarks for increment counts (Marshall and Parker 1982; Neilson and Geen 1982). Since they do not necessarily form on the day of hatching, their age of formation must be independently determined for the species under examination. Artificially induced checks can be formed through imposition of stress on known dates (Pannella 1980a; Campana 1983b; Ralston and Miyamoto 1983), although this is not a recommended procedure in situations where other checks also exist which might confuse later identification. An alternate approach might be that of sudden shifts in growth rate that can result in marked changes in increment width (Mugiya and Muramatsu 1982). The usual example of this type of marking scheme involves the sudden provision of large quantities of food to fish over a period of several days (Struhsaker and Uchiyama 1976; Victor 1982). Again, caution is required of this type of approach, since lag times may exist between the provision of extra food and its subsequent reflection on the otolith (Neilson and Geen 1984). As with the use of chemical markers, preliminary work must include a study of the elapsed time between application and appearance on the otolith.

Incremental Growth of Otoliths

Accurate interpretation of microstructural growth patterns in wild fishes requires knowledge of those factors that may affect the production of one increment per day. Environmental and physiological variables such as photoperiod, temperature, feeding, growth, and an endogenous circadian rhythm may all fluctuate cyclically, and all have the potential to influence otolith deposition. Except in laboratory studies, the history of exposure to such variables will generally be unknown. Since modification of the cycle of daily increment production will introduce an error into the microstructural examination, the accuracy and limits of a given application may be affected by these variables. In the following section, we summarize those details of otolith growth relevant to an understanding of the periodic nature of increment formation. Subsequently, we review the evidence concerning microstructure–environment interactions and present a hypothesis of increment formation that is consistent with much of the published work to date.

If the initiation of otolith growth in fishes parallels that suggested for other vertebrates, it is probable that the otolith begins as one or more partially calcified primordia exocytosed by cells in the inner ear. Nucleation points on the primordia would regulate the axial accretion of calcium carbonate, although some form of control would also stem from the surrounding matrix (Mann et al. 1983). The number of primordia produced is probably reflected in the number visible in the more developed otolith and is presumably under genetic control. Fusion occurs some time before hatch, at least in salmonids (Geffen 1983; Neilson et al. 1984). Once incremental growth has begun, the organic matrix is a likely template for the crystallization of calcium carbonate (Degens et al. 1969; Lowenstam 1981; Ross and Pote 1984). If this is so, either calcium carbonate accretion or otolin matrix formation or both must vary over a diel cycle to account for the bipartite structure of a growth increment (Dunkelberger et al. 1980; Mugiya et al. 1981; Watabe et al. 1982). Diel variations in calcium deposition have been well documented (Mugiya et al. 1981; Mugiya 1984), but matrix formation is not as well understood. Matrix formation is probably a continuous process, although there is evidence for differing amounts between the crystalline and proteinaceous zones (Dunkelberger et al. 1980). However, regardless of which otolith component varies cyclically, the existence of daily increments indicates that one or more of the factors influencing deposition rate must vary on a daily basis. The determination of these factors, and their potential variability, is the focus of concern from an applied point of view.

The influence of photoperiod on increment formation has been documented numerous times, although the results are seemingly contradictory. An influential role for photoperiod was suggested when reversal of the light–dark cycle reversed the order of formation of discontinuous and incremental zones in goldfish (Tanaka et al. 1981). In addition, daily increments were not formed in the otoliths of young *Lepomis* (Taubert and Coble 1977) or *Fundulus* (Radtke and Dean 1982) in the absence of a 24-h light–dark cycle. However, disruption of daily increment production through an abnormal photoperiod has never resulted in a 1:1 correspondence between increment formation and the light cycle (Taubert and Coble 1977; Campana and Neilson 1982; Geffen 1982). Moreover, Brothers (1981) reported that light was of secondary importance to increment formation, while other workers reported no inhibition of daily increment formation under either constant light (Campana and Neilson 1982; Geffen 1982; Neilson and Geen 1982) or dark conditions (Neilson and Geen 1982). Fishes of different ages and species were used in these apparently conflicting studies. Although interspecies differences may also exist, the response of increment formation to photoperiod was age-mediated in at least one species (Campana 1984c).

Temperature is a second environmental variable that may potentially entrain increment formation. There is no evidence that daily increment formation is inhibited under constant temperature conditions high enough to promote growth (Taubert and Coble 1977; Campana and Neilson 1982; Neilson and Geen 1982, 1984; Radtke and Dean 1982; Campana 1984c). Nor does increment number vary between temperature levels (Neilson and Geen 1982; Radtke and Dean 1982), except where low temperature has resulted in cessation of fish growth (Taubert and Coble 1977; Marshall and Parker 1982). However, Geffen (1983) has suggested that both photoperiod and temperature influenced increment number in Atlantic salmon (*Salmo salar*) embryos through proximal effects on metabolic rate. Apparently, increments were not formed on a daily basis under either

normal or abnormal light and temperature regimes, although increment formation rates of both less than and greater than 1 per 24 h were reported. Unfortunately, low sample sizes and possible methodological problems limit the value of Geffen's study. In addition, the results of her first experiment were inconsistent with those of her second (increment deposition rates varied by a factor of two between identical light/temperature treatments). In an analogous study of rainbow trout (*Salmo gairdneri*) embryos, J.D. Neilson (unpubl. data) found only daily increment formation under various temperature regimes.

While fluctuating temperatures do not appear to be necessary for daily increment formation, their presence can disrupt or otherwise influence a circadian cycle of otolith deposition. Although no data were presented, Brothers (1981) was the first to note that nondaily increment formation could be induced by short-term temperature fluctuations. Quantitative substantiation of this hypothesis was provided by Neilson and Geen (1984), who reported that a temperature cycle with a 12-h period resulted in the formation of 2 increments/24 h. Furthermore, Campana (1984c) noted that a daily temperature cycle enhanced the visual contrast between adjacent discontinuous and incremental zones in a growth sequence produced under a 24-h light–dark regime. Clearly, a fluctuating temperature regime has the potential to influence the otolith microstructure of wild fishes.

The effect of feeding cues on otolith deposition is more equivocal than is the response to temperature. Increment number appears to be unaffected by food deprivation, at least when body energy reserves are sufficient to enable limited skeletal growth to occur (Marshall and Parker 1982; Campana 1983a; Neilson and Geen 1984; Volk et al. 1984). Such reserves may not have been available to starved northern anchovy (*Engraulis mordax*) larvae, resulting in the cessation of otolith growth (Methot and Kramer 1979). However, Atlantic cod (*Gadus morhua*) larvae continued to deposit daily growth increments for at least 14 d after hatch, despite the absence of food (S.E. Campana, unpubl. data).

It is unlikely that feeding acts as an entraining factor for increment formation, since changes in feeding time are not correlated with shifts in the time of formation of incremental or discontinuous zones (Tanaka et al. 1981). Yet there is evidence that feeding periodicity is correlated to the frequency of increment production. Young chinook salmon (*Oncorhynchus tshawytscha*) fed more than once per day produced significantly more increments than did those fish fed once daily, although increment number was not directly proportional to feeding frequency (Neilson and Geen 1982, 1984). Although no data were presented, a similar suggestion was made by Pannella (1980a). These results have not been substantiated by other studies where daily increment production was unaffected by multiple daily feedings (Taubert and Coble 1977; Campana 1983a). However, the conflicting results may be more a matter of interpretation than of substance. Using an SEM (with its attendant high degree of resolution), Neilson and Geen (1982, 1984) made no attempt to differentiate between daily and subdaily increments; all increments, both daily and subdaily, were enumerated. Only those increments considered to be daily were included in the results of the other, light-microscopic studies. Since Campana (1983a) noted that the incidence of subdaily increments appeared to increase with feeding frequency, it is probable that multiple feedings do induce formation of new growth increments, although their reduced width and visual prominence may allow their identification as subdaily increments. Note that otolith response to feeding periodicity is not restricted to ultradian cycles. When rainbow trout were fed at 3-d intervals, every third growth structure that resulted was of increased prominence. Similar results were not obtained with starry flounders (*Platichthys stellatus*), suggesting that interspecies differences in otolith response may have been related to metabolic rate (Campana 1983a).

The variables discussed to this point have been considered for their direct influence on cyclical otolith growth. However, these and other factors are also the primary controllers of fish growth. In a 1982 paper, Geffen challenged the current theories of increment formation by suggesting that increment number was a simple function of growth rate in larval turbot (*Scophthalmus maximus*) and herring. Since daily increment formation has not been confirmed in a number of slow-growing larvae reared in the laboratory (Laroche et al. 1982; Lough et al. 1982; Campana 1984b), verification of Geffen's (1982) hypothesis could invalidate many applications of otolith microstructure examination.

There is little doubt that cessation of skeletal growth is associated with reduced or intermittent daily increment production; otolith annulus formation is an example of this phenomenon (Taubert and Coble 1977; Victor and Brothers 1982). Other workers have demonstrated that the "growth rate limitation hypothesis" does not apply above an unknown, low growth rate (Taubert and Coble 1977; Barkman 1978; Radtke and Dean 1982). Validation of daily increment formation in faster growing larval turbot indicates that the growth rate threshold must lie between 0.15 and 0.26 mm/d for that species (Rosenberg and Haugen 1982; Geffen 1982). Since the natural growth rates of a number of temperate, pelagic larvae can be very low (Bolz and Lough 1983; Campana 1984b), Geffen's (1982) hypothesis must be taken seriously. However, an alternate hypothesis for apparent nondaily increment formation is one of limited observer resolution, which can be linked to three factors (Campana 1984b):

(1) Constant temperature — there is no doubt that constant temperatures, such as those found in a laboratory environment, reduce increment contrast and render growth patterns more difficult to see (Campana 1984c; Neilson and Geen 1984). Exposure to diel temperature fluctuations, even if through vertical migration (Fortier and Leggett 1983), would be expected in a natural environment. Growth structures in wild-caught larvae are usually more clear than those observed in laboratory-reared specimens (Laroche et al. 1982; Lough et al. 1982; Campana 1984b).

(2) Otolith preparation — despite their small size and translucency, larval otoliths contain narrow growth increments which can be obscured by overlying material, even when larger increments are clearly visible without otolith grinding (Campana 1984b). In many of those studies not able to verify daily increment formation, otolith preparation was limited or absent (Laroche et al. 1982; Lough et al. 1982; Geffen 1982). In such cases, the absence of grinding may have biased increment counts below the actual number.

(3) Resolving power of light microscopy — due to the diffractive nature of light, objects separated by less than 0.20 μm cannot be resolved (Eastman Kodak Co. 1980). This is the theoretical resolution limit of a light microscope, assuming perfect optics, an objective with a high numerical aperture, ideal sample preparation, and a source of illumination with a relatively short wavelength, i.e. green. Therefore, if otolith growth structures are narrower than this value, they will be underrepresented in an increment count.

Geffen (1982) reported that increment counts in her slow-growth treatments were substantially lower than those expected of daily formation. However, in one of the slow-growth treatments, three of eight larvae were less than 20 mm long after 94 d. Allowing 5 d for yolk-sac absorption (Lough et al. 1982), the otoliths of these larvae would have grown 12–34 μm in 89 d. If daily increments were indeed formed in these otoliths, their mean width should have been 0.13–0.38 μm, a range that drops substantially below the resolving power of any light microscope. Considering the curvilinear nature of the otolith length – fish length relationship (Lough et al. 1982), the last-formed increments would have been wider than this, and the first-formed narrower. Thus, verification of daily increment formation via light microscopy would have been theoretically impossible. Note also that similar calculations, coming to similar conclusions, can be made using the data of other studies reporting nondaily increment formation (Laroche et al. 1982; Lough et al. 1982; Campana 1984b). Although this argument does not reject the "growth rate limitation hypothesis" suggested by Geffen (1982), it does suggest that resolution limitations are a viable explanation of the results reported elsewhere. In all cases, the slowest-growing otoliths would have the greatest number of nonresolvable increments, thus giving the appearance of a correlation between increment number and growth rate. A test of this hypothesis would require SEM examination of larval otoliths, a technique that has not yet been fully developed. However, it is interesting to note that SEM was used to detect narrow daily increments in chinook salmon reared at 5°C (Neilson and Geen 1982), whereas increment formation had apparently stopped at this temperature when assessed by light microscopy (Marshall and Parker 1982).

Despite the number of variables that have been suggested as influencing otolith deposition, we present here a hypothesis of increment formation that is consistent with much of the published work to date. The major concepts in the hypothesis are not novel, yet their synthesis is. Incorporation of interspecific differences was not required. The basic premise of the hypothesis is that daily increment formation is linked to an endocrine-driven, endogenous circadian rhythm. We suggest that this circadian rhythm is entrained at an early age by photoperiod, but that certain environmental variables can "mask" the rhythm. Endocrine secretion displays a circadian periodicity in many animals (Simpson 1978; Jacklet 1981; Menaker and Binkley 1981), and through the intermediary of metabolic rate, ultimately controls most physiological processes, including skeletal deposition (Simpson 1978). Circadian rhythms in general have been documented at several system levels in fishes (Gibson et al. 1978; Eriksson and van Veen 1980; Godin 1981). Since the basic features of endogenous circadian rhythms differ little among taxa, expected features of a piscine rhythm would include free-run capability (continuation of a circadian rhythm in the absence of periodic stimuli) and entrainment to a daily environmental cue (zeitgeber) such as the light–dark cycle or a diel temperature fluctuation (Jacklet 1981; Takahashi and Zatz 1982). During the free-run phase, endogenous rhythms generally drift from a 24-h periodicity; cycles of 21–26 h are common (Jacklet 1981). Entrainment coordinates the drifting rhythm to an appropriate environmental cue, a phenomenon known as phase-shifting the circadian rhythm. However, entrainment to a cue with a periodicity differing by more than 2–4 h from 24 h is generally impossible. An exception would be a cycle with multiple harmonics of 24 h, a phenomenon known as frequency demultiplication (Marler and Hamilton 1966). Only one environmental variable may act as the entraining factor (usually the light–dark cycle), although others may act to mask an endogenous rhythm (Enright 1981). A masking agent does not phase shift the endogenous rhythm or affect the period during free-run. However, it can influence the resultant cycle directly, thus obscuring the effect of the endogenous rhythm and inducing a cycle with a frequency equal to that of the masking agent. This frequency does not have to be daily, or even close to daily. As an example of this phenomenon, consider the entraining effect of a light–dark cycle on the activity pattern of fish in a predator-filled environment. Normally the activity cycle would be circadian. Introduction of particularly high levels of predators at specific hours of the day might curtail activity at these hours, thus inducing noncircadian activity cycles. However, the predators would merely mask the influence of the innate rhythm, and it would once again become apparent upon removal of the predators.

Given the characteristics of an endogenous circadian rhythm, surprisingly few tests of its potential association with cyclic otolith growth have been made. Daily increment formation in the absence of periodic environmental cues is suggestive of free-run, but the possibility of the presence of uncontrolled variables cannot be excluded (Taubert and Coble 1977; Campana and Neilson 1982; Geffen 1982; Marshall and Parker 1982; Neilson and Geen 1982.) Experiments in which abnormally short or long photoperiods were used demonstrate only that photoperiod does not act as a masking agent (Taubert and Coble 1977; Campana and Neilson 1982; Geffen 1982, 1983). However, Tanaka et al. (1981) provide support for the hypothesis that an endogenous circadian rhythm entrained by photoperiod synchronizes daily increment formation. Through SEM examination of goldfish otoliths, they were able to demonstrate that a phase-shifted photoperiod phase-shifted the time of daily increment production. An analogous change in the time of feeding produced no such response. Thus, photoperiod appears to have acted as a zeitgeber for the circadian rhythm.

Evidence is also derived from mammalian studies, where endogenous rhythms often appear after birth. Once present, cycle amplitude tends to increase with time until the rhythm is "mature" (Davis 1981). In experiments with plainfin midshipman (*Porichthys notatus*), Campana (1984c) documented an age-modulated disruption of daily increment formation under constant light conditions. The effect of photoperiod disruption decreased with increasing fish age, as has been noted in both fish (Gibson et al. 1978) and mammalian (Sacher and Duffy 1978; Davis 1981) endogenous rhythms. Due to the developmental maturation of endogenous cycles, acclimation of fish to constant light would be expected to occur more rapidly in juvenile than in larval fish. In fact, such a phenomenon has been observed in midshipman (Campana 1984c). Therefore, this evidence and the study of Tanaka et al. (1981) strongly implicate photoperiod as a zeitgeber for an endogenous circadian rhythm. Moreover, this hypothesis is consistent both with those studies where photoperiod effects were absent (through acclimation and use of older fish) (Campana and Neilson 1982; Neilson and Geen 1982) and those studies where constant light disrupted daily increment formation (since newly hatched, unacclimated fish were used) (Taubert and Coble 1977; Radtke and Dean 1982). Of course, proof of the existence of an endogenous rhythm will probably never be obtained, due to the possible existence of uncontrolled variables in any experimental design.

If photoperiod acts as a zeitgeber, any effects manifested on

otolith deposition by temperature and feeding must be due to masking effects. Temperature masking was indicated by a 1:1 correspondence between temperature fluctuations and growth increments, even in the presence of several cycles per day (Brothers 1981; Neilson and Geen 1984). Reinforcement of an endogenously formed daily increment occurred when temperature reduction occurred at night (Campana 1984c). Similar but weaker effects appear to explain the influence of feeding periodicity. In the case of both variables, masking probably occurs at the level of metabolic rate, since the latter is known to be susceptible to environmental influence (Matty 1978). Probable interactions between metabolic rate and activity may also explain the results reported by Neilson and Geen (1984), where artificially induced activity cycles resulted in the formation of otolith increments of a similar frequency. Metabolic rate in turn controls the differential deposition rates of calcium and protein on the otolith, resulting in the bipartite structure of a daily growth increment. Physiologically, this is consistent with the study of Mugiya et al. (1981) where evidence of a circadian rhythm of calcium uptake in goldfish was presented. Uptake of labelled calcium ceased near dawn each day and was associated with reduced plasma levels of calcium and a concurrent reduction in deposition on the otolith. Since plasma calcium levels are internally regulated (Norris et al. 1963; Simkiss 1974; Nordlie and Whittier 1983), it is likely that masking agents similarly modify calcium uptake and/or deposition. If this is verified, resolution of the controversy concerning the morphological differences between daily and subdaily growth increments may be possible. Our hypothesis predicts that one increment per day should be produced at relatively regular intervals due to the circadian rhythm. Asynchronous temperature or feeding fluctuations would then form increments in addition to the one already expected. Since the latter growth structures may be formed at irregular intervals, and since the degree of calcium uptake inhibition will be a function of the strength of the masking agent, the increments so formed may be irregular in width and contrast. In fact, these are the criteria that are often assessed subjectively to characterize increments as subdaily (Taubert and Coble 1977; Campana and Neilson 1982; Marshall and Parker 1982). Quantification of these criteria has been attempted (Campana 1984c). However, on the basis of our experience, such classifications can sometimes be difficult and imprecise (Campana 1984c). The difference between increments formed as a result of an endogenous rhythm (daily) and those induced through other means (subdaily) appears to be a quantitative and not a qualitative one, despite the difference in the processes ultimately responsible for their formation. Therefore, development of fully reliable distinguishing characteristics may prove to be difficult. However, while it is essential to determine the rate of increment formation for an accurate estimate of age from otolith microstructure, a categorization of increments as daily or subdaily is not an absolute requirement. Of course, an age estimate derived from any microstructural feature is always based on the assumption that the frequency of increment formation does not vary during the period of study. Unfortunately, it is often difficult to assess the validity of this assumption when studies of natural populations are being made and subdaily increments are present. For this reason, differentiation of daily and subdaily increments may be warranted, even if subjective criteria must be used.

There are several experiments that can be done to test key parts of the increment formation hypothesis. One of the best would test for the period drift predicted of free-run. However, observational error would probably be of the same magnitude as the expected drift from a 24-h cycle of increment production. Another experiment could test for phase-shifting of increment formation through phase-shifted circadian temperature fluctuations; if temperature acts as a masking agent, phase-shifted increment production should not occur. Further, masking by a 12-h temperature cycle should result in the formation of two increments every 24 h, with reversion to a daily pattern upon elimination of the cycle. Finally, observation of an endogenous circadian rhythm of otolith protein deposition would substantiate our hypothesis. Further substantiation occurs naturally, since structures similar in appearance to daily increments have been observed in Arctic (Townsend and Shaw 1982), Antarctic (Townsend 1980), and deepsea fishes (Rannou and Thiriot-Quievreux 1975). The absence of diel light and/or temperature cues in these environments is consistent with the presence of an endogenous circadian rhythm in the resident fishes.

Since this hypothesis of increment formation allows for the interaction of environmental variables with an endogenous rhythm, it allows the prediction of the degree to which environmental variation may disrupt the formation of a reliable daily growth sequence in wild fishes. With photoperiod as a zeitgeber, masking effects of light are not possible and nondaily increments cannot be formed under variable light regimes. In lightless environments, a free-running circadian period could occur, but a 2-h drift per day would only introduce an 8% error. The only other potential source of error would be the longer acclimation period of newly hatched fish in the absence of a zeitgeber, resulting in a more confused growth pattern during the first week(s) of life.

As a masking agent, feeding periodicity can introduce subdaily increments into the otolith. Multiple daily feedings occur in many fishes (Keast and Welsh 1968; Grove et al. 1978; and others). However, since feeding-induced increments are often fainter than those resulting from the circadian rhythm (Campana 1983a), their presence should be recognizable.

Temperature fluctuations have the greatest potential for the introduction of subdaily increments into the daily increment sequence. Temperature appears to be a strong masking agent, and increments induced through its action can be as prominent (Neilson and Geen 1984) or more so (Brothers 1981; pers. comm.) than those produced as a result of the circadian rhythm. Midday migrations to colder water can result in the formation of two increments per day, as may occur in intertidal starry flounders migrating seawards on a midday low tide (Campana 1984a). In this case, the midday increment was easily differentiated from the nightly one, but migrations between less similar temperature regimes might have resulted in more confusing growth structures. However, in most environments, temperatures fall at night, thus reinforcing the formation of the daily increment. In such instances, cyclic environmental cues act in an additive fashion to the endogenous rhythm and result in increased increment definition in a natural environment relative to a temperature-controlled laboratory setting. Thus, it would appear that daily increment formation is to be expected in most natural environments. The influence of environments with anomalous temperature regimes, coupled with knowledge of horizontal and vertical movements of fish on a diel basis, is an area for further research.

Increment Width

Given a sequence of growth increments of known periodicity

and a relationship between otolith and fish size, estimation of the fish length corresponding to otolith size at each of the marks should be possible. Indeed, this is the basis for the back-calculation of size at age from both scale and otolith annuli (Bagenal and Tesch 1978; Carlander 1981). Back-calculation could be carried a step further by relating the width of an annual growth zone to the otolith–fish size relationship, thereby determining growth rate at age. This approach is seldom justified, since the date of annulus formation is not well defined and may vary with the size, age, and ambient environment of the fish. Since the date of formation of a daily increment can be well defined, these constraints do not apply to the same degree at the microstructural level. Consequently, one should be able to examine an incremental series to estimate both size at age and the instantaneous growth rate over short time periods — at least on average. Preliminary evidence in support of this hypothesis has been presented both experimentally (Wilson and Larkin 1982; Volk et al. 1984) and through observation (Struhsaker and Uchiyama 1976; Pannella 1980a; Victor 1982). Under an environment promoting regular growth, the influence of various temperature (Neilson and Geen 1982, 1984; Campana 1984a) and ration (Struhsaker and Uchiyama 1976; Volk et al. 1984) regimes was proportional to mean increment width. However, a close correspondence between fish and otolith growth at the daily level has not yet been demonstrated. Since a measure of instantaneous growth rate would be of undisputed value, we review here those factors that are known to influence increment width and its relationship to fish growth and otolith morphology.

Daily increment width must reflect fish growth (either allometrically or isometrically) if the two growth patterns are linked by a smoothly monotonic curve. Therefore, the potential value of increment width measurement as a growth indicator rests solely upon the characteristics of their mutual relationship. Under circumstances where the otolith–fish size relationship is temporarily disrupted, increment width loses its proportionality to fish growth.

Several studies have reported an uncoupling or disruption of the otolith–fish size relationship under suboptimal conditions (Brothers 1981; Neilson and Geen 1982; Marshall and Parker 1982; Campana 1983a, 1984c). In one study, an abnormal photoperiod resulted in noncharacteristic otolith growth relative to fish growth (Campana 1984c). Manipulated photoperiods may not occur naturally, but their influence demonstrates that some environmental variables can induce uncoupling. Continued otolith growth through periods of starvation is well documented, even after cessation of linear fish growth (Brothers 1981; Campana 1983a). Although increment width decreased through the period of food deprivation (Campana 1983a; Neilson and Geen 1984), this phenomenon was probably linked to metabolic rate and may not be evident in fishes of low metabolic rate (Campana 1983a).

The processes responsible for continued otolith growth during starvation are presently unknown. The conservative nature is emphasized by the apparent absence of otolith resorption under stressful conditions (Simkiss 1974; Campana 1983b), despite its occurrence in other parts of the skeletal system (Bilton 1974; Simkiss 1974; Ichii and Mugiya 1983b). Uptake of calcium for subsequent deposition on the otolith occurs via a closely regulated branchial pathway (Simkiss 1974; Mugiya et al. 1981; Campana 1983b); little is taken up from dietary sources (Ichii and Mugiya 1983a). Therefore, there is no direct link between calcium uptake and ration level. After uptake, plasma levels passively regulate calcium deposition rate on the otolith (Mugiya et al. 1981). Nothing is known of the relationship between calcium uptake and growth rate, leaving no a priori reason why there should be a direct correspondence between short-term fish and otolith growth. Considering the gradual shifts in metabolic activity that occur upon food deprivation (Phillips 1969), a gradual response of increment formation is not surprising. Indeed, a number of studies to date suggest that otolith growth represents a running average of fish growth rates, with the length of the "run" inversely proportional to metabolic activity (Struhsaker and Uchiyama 1976; Neilson and Geen 1982, 1984; Campana 1983a, 1984a). In an indirect comparison of increment width as a measure of growth, Wilson and Larkin (1982) noted an inexplicable but significant nonlinearity in their regression. In addition, a lagged response of otolith growth to fish growth may be common (Neilson and Geen 1984; M. J. Bradford, Department of Biological Sciences, Simon Fraser University, pers. comm.)

Several other factors may introduce significant intraspecific variability into the otolith–fish relationship. One such factor is the size of the otolith nucleus, which is in turn regulated by the number and spacing of otolith primordia (Neilson et al. 1984). Primordial numbers and placement vary substantially among species, but are typified by at least two types of patterns: a scattered pattern such as that observed in salmonid otoliths (Fig. 1), and a clustered one, such as that of flounders (Fig. 3). Among the species we have examined, the latter pattern occurs more frequently. Within a given species, the size of the nucleus is environmentally determined, at least in part (Neilson et al. 1984). Thus, nucleus length can vary sufficiently among individuals that the otolith–fish relationship is significantly affected (West 1983; Neilson et al. 1984). This in turn has the potential to affect the accuracy of back-calculated growth rates.

Formation of accessory primordia (referred to as "peripheral nuclei" in Campana 1984b) in the otoliths of juvenile fish may also confuse the interpretation of daily increment width. Through an unknown mechanism, these secondary growth centres develop after metamorphosis in many fish species as foci for further otolith deposition (Brothers 1984; Campana 1984b). While we refer to these foci as accessory primordia, we do so acknowledging the possibility that these structures are not analogous to the primordia found in the nucleus. The primordia occur asymmetrically around the central nucleus. Since the size of the otolith will vary with the location of the primordia, substantial variance can be introduced into the otolith–fish size relationship. More importantly, individual growth increments may encircle both primordial types, but with substantially different widths around each (Campana 1984b) (Fig. 3). Therefore, increment widths measured in two different fields on the same otolith will not necessarily be comparable.

Check Formation

Checks, or discontinuities, are characteristic of most otolith growth sequences, where they may record periods of perturbation or stress to the fish. Checks may occur at apparently random locations in the growth record, or delimit incremental patterns of weekly or fortnightly periodicity (Pannella 1971, 1980a; Campana 1984a). In both cases, check location can be associated with a date of formation by enumerating the daily increments between the check and the otolith periphery (date of sampling). Use of this technique has demonstrated that nonperiodic check formation is generally associated with periods of stress or sexual

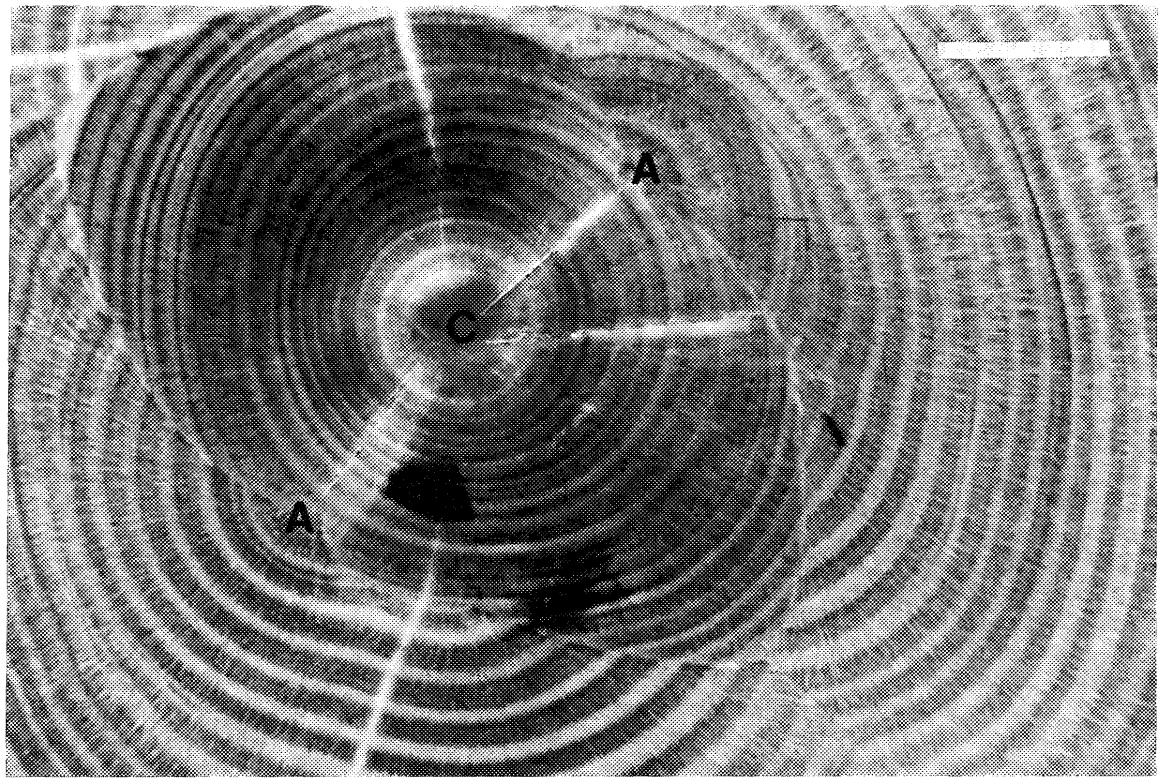

FIG. 3. Central (C) and accessory (A) primordia in the sagitta of a wild, juvenile starry flounder. Note the change in width of a given daily increment as it passes from one field to another. Bar = 50 μm.

maturity (Pannella 1980a; Campana 1983b), while periodic series may be linked to the lunar cycle (Pannella 1980a; Campana 1984a). Since a check may represent a growth interruption of unknown duration, their ubiquity may prove to be a significant problem in the accurate interpretation of a growth sequence. However, the association of some checks with stressful events also suggests their potential utility in defining the size and age at which life history transitions occur. Moreover, dated marks, useful in validation studies, may be formed on otoliths through population-wide stress phenomena such as storms. However, little is known of the physiological processes responsible for check formation despite substantial variance in their visual prominence.

Transmission of light through checks differs relative to other microstructural features, resulting in structures that are immediately distinguishable from surrounding increments. Under SEM, checks appear more deeply etched than surrounding discontinuous zones (Fig. 4). The enhanced visibility suggests that some aspect of otolith deposition has been disrupted. Since otolith growth occurs through cyclic deposition of calcium carbonate and protein, anomalous incorporation of one or both of these components may occur during check formation. Experiments in which young coho salmon (*Oncorhynchus kisutch*) were stressed while immersed in ^{45}Ca water demonstrated that check formation was associated with reduced calcium deposition on the otolith, although concurrent protein deposition was not monitored (Campana 1983b). These results may explain why check prominence can vary with the level of stress experienced. If the reduction in otolith calcium deposition is proportional to stress level, then the calcium:protein ratio will be reduced accordingly in the corresponding growth increment. It is the difference in the ratio between the two components of a bipartite growth increment that accounts for its visual identity. Specifically, the discontinuous zone consists primarily of a proteinaceous matrix, while the incremental zone has a higher calcium content (Dunkelberger et al. 1980; Mugiya et al. 1981). Enhancement of these bipartite composition differences should have a parallel effect on visual prominence. This process describes that suggested to occur during formation of a stress-induced check. However, stress experiments through which calcium and protein deposition were concurrently monitored during check formation have not yet been conducted. Therefore, it is not known if protein incorporation is also reduced through periods of stress. Moreover, quantification of the relationship between check prominence and level of stress has not yet been attempted.

Although consistent with the experimental results, the above hypothesis does not account for some of the observed check characteristics. Otolith growth interruptions may occur regionally: either they may not encircle the nucleus (Campana 1984a), or they may occur in only one of the otolith pair (E.B. Brothers, pers. comm.). Either situation suggests an interruption of otolith growth at the local level. More puzzling are observations of discontinuities that intersect other growth increments (Pannella 1980a; Campana 1984a). Although only present in some taxa, the resemblence of these structures to fault zones (in a geological sense) may be more than coincidental. Refraction of light through the interface between two regions of different refractive indices might result in an unusually prominent structure that we interpret as a check (E.B. Brothers, pers. comm.). However, there is no evidence that such is the case, nor mechanisms to suggest why the interface should be present. An alternate hypothesis was presented by Pannella (1980a), who suggested that checks may demarcate regions of otolith resorption. Resorp-

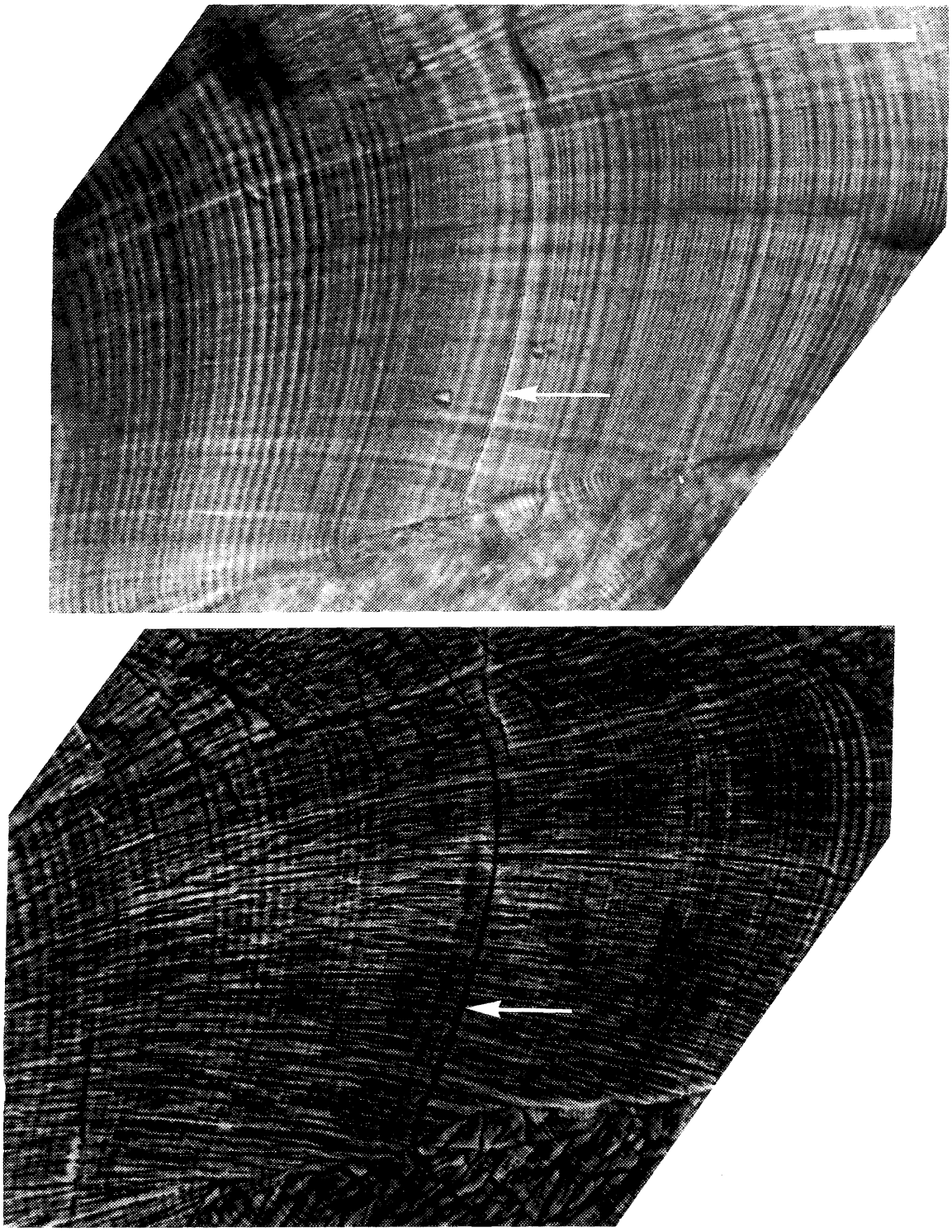

FIG. 4. A check, or growth interruption (indicated by arrow), in a lapillus of the cyprinid *Couesius plumbeus*. Top, viewed with a light microscope; bottom, viewed with a SEM. Bar = 30 μm.

tion could account for some of the characteristics of checks, but the limited experiments performed to date indicate that otolith deposition cannot be reversed (Simkiss 1974; Campana 1983b; Ichii and Mugiya 1983b). We conclude that stress-induced checks represent regions of reduced or interrupted otolith growth and that interregional differences in check prominence may occur.

Stress checks may represent isolated periods of perturbation to individual fish, or a population-wide process recorded on the otoliths of all of the fish. Large-scale stress factors such as storms may disrupt incremental growth in an entire population of bivalves (Kennish 1980), although they are less likely to do so in the case of mobile fish. However, physiologically derived stress can be a nearly universal phenomenon, even though the resultant check is a function of age, not of date. For example, hatch checks are formed in many species of fish and are often

used as benchmarks from which increment counts are made (Marshall and Parker 1982; Neilson and Geen 1982). Presumably, hatching or an associated event is a physiologically stressful period in a fish's life. Hatch checks formed on other than the day of hatching may reflect interspecies differences in the timing of physiologically stressful events (Balon 1984). The presence (Wilson and Larkin 1980) or absence (Brothers and McFarland 1981; Campana 1984b) of checks at other life history transitions may be a function of the physiological stress experienced during the transition, although no studies have been conducted in this area.

Discontinuities need not be induced by stress. Perhaps as a result of localized otolith growth interruptions, check frequency increases as a function of age, particularly in sexually mature fish. Spawning is one potential source of these checks (Pannella 1971, 1974, 1980a). In addition, check formation in marine fish otoliths has been linked to the lunar cycle (Pannella 1971, 1974, 1980a; Campana 1984a). Through an unknown process, starry flounder otolith growth was interrupted at 1- or 2-week intervals in synchrony with the phases of the moon (Campana 1984a). Although the lunar cycle induces a 2-wk period in the tidal cycle, weekly periods are generally not evident. Therefore, the weekly frequency of check formation could not be explained. Checks formed at 2-wk intervals have been used in age determination studies (Rosenberg 1982), but are generally considered to be too variable for this purpose (Campana 1984a; E.B. Brothers pers. comm.).

Present Applications of Microstructure Examination

Age Determination

As we noted earlier, the rate of formation of otolith growth increments theoretically permits age determination with high precision, often to the daily level. Such studies are perhaps the most obvious application of microstructure examination. Accordingly, most workers have reported results related to this aspect of microstructure research. However, as was shown in the previous section, the apparent rate of increment formation may vary, thus causing difficulty in the interpretation of age from otolith microstructure. Few studies have provided validation of the frequency of formation of increments. Indeed, the results of many studies carry the explicit assumption that the otolith increments were formed daily. Studies that have presented data allowing the precise determination of the rate of formation have usually relied on the use of fish of known age (Neilson and Geen 1982; Wilson and Larkin 1982) or on the incorporation of time markers (Wild and Foreman 1980; Campana and Neilson 1982; Neilson and Geen 1984) which allow the interpretation of periodicity of otolith growth from a dated reference, as discussed earlier. A compilation of workers who have conducted studies of the frequency of increment formation is given in Table 1. The number of studies where no confirmation of the frequency of increment formation was provided or where they were assumed to be daily greatly exceeds the number of studies shown in Table 1.

In addition to providing a means for determining ages to the daily level of precision, growth increments have utility for age determination at coarser levels of precision. For example, some workers have used growth increments to corroborate the occurrence of annuli (Pannella 1971; Taubert and Tranquilli 1982; Victor and Brothers 1982). Pannella (1971) noted that daily growth increments were present throughout the first 3–4 yr of life in the red hake (*Urophycis chuss*) and were compressed in the region corresponding to winter growth. Victor and Brothers (1982) used a similar approach to determine the location of the first annulus in a temperate cyprinid. However, the utility of otolith microstructure in such instances may be limited, as discrete growth increments often become unclear after about 200 d (Brothers 1979). Nevertheless, Brothers noted that in some tropical species, recognizable growth increments may persist over the first 2 or 3 years of life. Ralston and Miyamoto (1983) used a rigorous approach to describe the growth of the Hawaiian snapper (*Pristipomoides filamentosus*) entailing the use of otolith microstructure, tetracycline validation techniques, and modal analyses and concluded that otolith growth adequately represented fish growth when time was measured on a scale of years. In general, however, we feel that the utility of otolith microstructure examination for age determination to the annual level of precision is questionable beyond the first year of life, particularly in species found in temperate zones. Growth increments are often so compressed during the winter slow-

TABLE 1. List of studies reporting the frequency of otolith increment formation, including those where a non-daily frequency of formation was observed.

Reference[a]	Species
Pannella 1971	*Urophycis chuss*
Struhsaker and Uchiyama 1976	*Stolephorus purpureus*
Brothers et al. 1976	*Engraulis mordax*
	Leuresthes tenuis
Taubert and Coble 1977	*Tilapia mossambica*
	Lepomis gibbosus
	L. cyanellus
	L. macrochirus
Barkman 1978	*Menidia menidia*
Radtke and Waiwood 1980	*Gadus morhua*
Wilson and Larkin 1980	*Oncorhynchus nerka*
Wild and Foreman 1980	*Thunnus albacares*
	Katsuwonus pelamis
Mugiya et al. 1981	*Carassius auratus*
Tanaka et al. 1981	*Tilapia nilotica*
Bailey 1982	*Merluccius productus*
Campana and Neilson 1982	*Platichthys stellatus*
Geffen 1982, 1983	*Clupea harengus*
	Salmo salar
Neilson and Geen 1982	*O. tshawytscha*
Laroche et al. 1982	*Parophrys vetulus*
Radtke and Dean 1982	*Fundulus heteroclitus*
Rosenberg and Haugen 1982	*Scophthalmus maximus*
Tsuji and Aoyama 1982, 1984	*Pagrus major*
	Engraulis japonica
Victor 1982	*Thalassoma bifasciatum*
	Halichoeres bivittatus
Victor and Brothers 1982	*Semotilus corporalis*
Ralston and Miyamoto 1983	*Pristopomoides filamentosus*
Campana (1983a, 1983b, 1984c	*Salmo gairdneri*
	Oncorhynchus kisutch
	Porichthys notatus
Crecco et al. 1983	*Alosa sapidissima*
Williams and Clarke, 1983	*Herklotsichthys quadrimaculatus*
Radtke 1983, 1984	*Euthynnus pelamis*
	Mugil cephalus
Volk et al. 1984	*Oncorhynchus keta*

[a]If more than one worker has studied the frequency of increment formation in a given species, the first published work is listed

growth period that the identification of discrete increments is difficult.

At an intermediate level of age precision, Pannella (1971) has indicated that patterns of fortnightly frequency were apparent in the red hake otoliths he examined. The fortnightly patterns were attributed to lunar influences, expressed through tidal influences (Pannella 1980a). Such patterns usually consisted of a cluster of six or seven thin daily increments followed by a group of six to eight thick increments. Conceivably, where such sequences recur on a regular basis, they may have utility for age determination. Rosenberg (1982) used counts of fortnightly growth increments to estimate the size at age of young English sole (*Parophrys vetulus*) in Oregon estuaries. However, Brothers (1979) noted that the appearance of lunar patterns is often irregular and difficult to demonstrate critically. Campana (1984a) noted that lunar cycles of otolith growth (expressed through increment width and contrast when viewed with a light microscope) were correlated with tidally modulated variables, particularly temperature.

Among the problems associated with using growth increment counts for age determination, the occurrence of checks, or growth interruptions, is one of the most serious. Thus, examination of otolith microstructure for age determination is less useful in older fish, as intermittent otolith growth leading to check formation may cause difficulties (Brothers et al. 1976; Ralston and Miyamoto 1983).

Just as otolith growth interruptions such as spawning checks can introduce errors into age determinations based on annuli counts, subdaily increments may be incorrectly interpreted as daily growth increments. In addition, narrow increments may be overlooked, particularly in studies using light microscopy. Distinction of daily growth increments from subdaily increments is usually on the basis of subjective criteria such as increment width or appearance (Taubert and Coble 1977; Marshall and Parker 1982; Campana 1984c). However, as was mentioned earlier, differentiation is often difficult and probably represents a significant source of error in some species (Campana and Neilson 1982). Since the variance of counts due to misinterpretation is cumulative, heterogeneity of variance may result when regressions of increment counts on fish age are calculated. The extent of the problem is probably species-specific and related to fish age. However, use of one of the several methods currently available for validation of age estimates removes some of the uncertainty regarding the presence of subdaily increments.

A final source of error is that associated with the date of formation of the first increment. The date of first increment formation may vary from before hatching as with chinook salmon (Neilson and Geen 1982) to the time of first feeding as in anchovies (Brothers et al. 1976). In the absence of data on the age of first increment production, some workers have assumed that it commenced at the onset of an event of biological significance in the fish's life history. For example, Wilson and Larkin (1980) assumed that increment formation in sockeye salmon (*Oncorhynchus nerka*) commenced at the time of emergence of the alevins. However, subsequent studies (Neilson and Geen 1982; Marshall and Parker 1982) have shown that increment formation commenced considerably before hatching. The seemingly erroneous assumption by Wilson and Larkin (1980) was probably responsible for the poor correspondence between observed and expected increment counts, particularly in young fish.

Growth Rate Estimation

Increment widths can provide important information on the day to day growth of fishes (Brothers 1981). In fact, some of the first workers in this field have recognized the potential of otolith microstructure for detailed studies of growth. As noted by Volk et al. (1984), more conventional means of estimating fish growth, such as mark–recapture experiments, suffer from drawbacks which include possible tagging effects on growth, the need to mark large numbers to ensure significant returns, possible size-related mortality between marking and recapture of fish and the requirement for relatively long periods between marking and recapture. However, only recently have researchers taken advantage of the potential of otolith microstructure in that regard. The difficulty in obtaining precise measurements of features that are often <2 μm wide may account for the relatively few detailed studies of growth to date.

To circumvent the problem of measuring individual increments, many authors have chosen to measure the change in otolith radius or diameter as a proxy variable for fish growth (Struhsaker and Uchiyama 1976; Taubert and Coble 1977; Marshall and Parker 1982; Wilson and Larkin 1982, and others). Ralston and Miyamoto (1983) noted that authors using such otolith measurements have not discussed the limitations of the method. With these studies, average daily growth rates were reported. While measurements of otolith radius or diameter are readily obtained, the potential value of discrete increment width data as precise indicators of daily growth is reduced in proportion to the number of increments that comprise the measurement of otolith growth. An alternate approach for studying fish growth entails the plotting of fish size versus increment counts. Many workers have employed this technique for studying relative growth rates of fish obtained from different habitats or environmental conditions (Kendall and Gordon 1981; Volk et al. 1984). Methot (1981) measured the outer three daily increments of larval fishes as indicators of recent growth. However, as shown earlier, such an approach is sensitive to the effects of a lagged response in the relationship between fish growth and otolith growth.

Studies where increment width data are related to the growth of individual fish represent a more powerful application of otolith microstructure examination. Geen et al. (1985) used otoliths from individually branded chinook salmon fry to study short-term growth responses to various pH regimes. However, even in this instance, average increment widths were used and the data smoothed by use of running means. Potentially, the most revealing studies of fish growth from otolith microstructure are those where daily growth of individual fish is compared with the widths of corresponding growth increments. However, we are not aware of any such work. The paucity of such studies may be related in part to the difficulty in obtaining short-term growth data for individual fish as well as precise increment width data, where use of an SEM is often desirable or even necessary.

Due to the lack of detailed studies of the relationship between increment width and fish growth on a daily basis, various workers hold somewhat equivocal views of how closely increment width reflects fish growth at the daily level of precision. Among the more obvious factors that could complicate the interpretation of growth from otolith microstructure are the occurrence of checks or growth increments that are formed at an unknown rate. As noted by Marshall and Parker (1982), Cam-

pana (1983a) and Neilson and Geen (1984), otolith growth may continue under conditions of food deprivation. M.J. Bradford (pers. comm.) has noted that the response of otolith growth to changing ration in chinook salmon fry is not instantaneous. Thus, there is evidence that the widths of growth increments are not necessarily proportional to instantaneous growth rates on the dates in question.

The choice of radius along which increment widths are measured is significant. Because otoliths are not perfectly circular objects, it is not possible to select any one radius that intersects all increments at 90°. To circumvent the problem, relatively short sequences of increments should be selected for width measurements. Alternatively, a perpendicular line may be drawn from each point of intersection of the radius and the increments to allow width measurements in a consistent fashion. Asymmetry of otolith growth along the depth axis, the third dimension, may also cause difficulty in increment width measurements. Correction for curvature in that dimension is difficult and we are unaware of any studies that have quantified or corrected for such error.

Other sources of intraspecific variation in the otolith–fish length relationships are age-related. A curvilinear (or allometric) otolith length–fish length relationship is characteristic of many species during the larval phase (Methot 1981; Laroche et al. 1982; Lough et al. 1982; Campana 1984b), while a linear (isometric) relationship is usual for juveniles (Messieh 1975; Rosenberg 1982; Campana 1984b). Unless the size at metamorphosis is known, back-calculations of growth from juvenile to larva generally cannot be done. Campana (1984b) has documented that in starry flounder larvae, increasing increment widths were better correlated with increasing age rather than increased growth rate, at least before the larvae reached metamorphosis. Crecco et al. (1983) found that growth of larval American shad (*Alosa sapidissima*) as inferred from increment counts and length data, decreased as they approached metamorphosis. However, increment width data were not available in this instance to assess whether otolith microstructure continued to reflect fish growth.

We conclude that the use of increment widths as indicators of instantaneous fish growth rate must be done with caution, unless conducted under rigorous laboratory control or when the calculations are averaged over several days or weeks. Further research on factors influencing otolith deposition and its relation to fish growth would be valuable to further assess the utility of this application of otolith microstructure examination.

Detection of Life History Transition

Several authors have used changes in increment width to indicate transitions in the life history of fish, usually the movement of fish from one habitat to another. Movements from freshwater to an estuarine environment, for example, might be associated with a different water temperature or feeding regime. Such changes might be reflected in the otolith microstructure and would be expected in some species on the basis of laboratory experiments where temperature and feeding regimes were varied (Neilson and Geen 1982, 1984). Neilson et al. (1984) used changes in otolith increment width and appearance to identify the time at which juvenile chinook salmon entered an estuary. Campana (1984b) has used changes in increment width to detect the point of metamorphosis in a series of growth increments from otoliths of starry flounders.

Growth interruptions or changes in increment appearance such as contrast (when viewed with a light microscope) may also have utility for detection of life history transitions. For example, Brothers et al. (1983) estimated the duration of larval life in 12 families of Australian reef fishes by identifying, through subjective examination of various species-specific criteria, the point on the otolith corresponding to when the larvae settled. However, the authors noted that the transitions were often difficult to detect and could only be resolved when they were at least three or four increments from the otolith periphery. In contrast, Victor (1982) apparently had little difficulty in detecting a settling mark on the otoliths of bluehead wrasse (*Thalassoma bifasciatum*). Brothers and McFarland (1981) interpreted stepwise transitions in increment width and the presence or absence of subdaily increments as representing five different life history stages in juvenile French grunts (*Haemulon flavolineatum*). In papers by Brothers and Thresher (1985) and Thresher and Brothers (1985) the hypothesis that prolonged larval life is correlated with broad geographic distribution was tested using examination of growth increments formed during the pelagic life of Indo-Pacific coral-reef fish. In such studies, the timing of initiation of growth increment formation is a possible source of error. If increment production does not commence with the onset of pelagic life then the estimates of the duration of pelagic phase will be in error.

A preliminary study (J.D. Neilson, unpubl. data) of the transition between pelagic and demersal life history stages of Scotian Shelf gadoids did not detect the presence of a transition or settling mark as noted in tropical forms. The absence of a check may reflect the fact that the transition to the demersal life-style apparently does not occur abruptly (P. A. Koeller and J. D. Neilson, unpubl. data).

Checks associated with hatching have been used as reference points from which counts or measurements of increments have been made (Neilson and Geen 1982). While this is an appropriate procedure for species where there is reason to believe that significant stress or other factors associated with hatching might somehow be reflected in the otolith microstructure, there is some indication that for certain species, hatching may be a physiologically insignificant event (Balon 1984). In such cases, a distinct check may not be apparent such as was found in chinook salmon otoliths (Neilson and Geen 1982).

It is worthwhile to note that life history transition studies based on otolith microstructure are subject to the same constraints as are other applications of microstructure examination. Indeed, it may be necessary to conduct separate experiments to determine the rate of increment formation and relationship with fish growth at each stage of a fish's life history.

Estimates of Recruitment and Mortality

Healey (1982) was able to demonstrate size-selective mortality through examination of distributions of scale circulus spacing. As scale circulus spacing is proportional to fish growth, examination of the successive distributions of circuli spacing as the cohort ages may be used to demonstrate the occurrence of size-selective mortality. Scale circuli are analogous structures to otolith increments, but are not formed at as great or as constant a rate. Therefore, there is potential advantage in using otolith increment examination to provide the age-structured data necessary for such studies. Neilson (1984) applied a similar methodology based on an examination of increment widths to demonstrate the possibility of size-selective mortality during the early ocean life of chinook salmon. Crecco et al. (1983) used ages determined from the otolith microstruc-

ture of American shad to determine survivorship curves and mortality estimates. Rosenberg and Haugen (1982) examined individual growth trajectories of larval turbot obtained from back-calculation using otolith growth increments and were able to show that slower growing individuals died at a greater rate. West (1983) located emergence checks on otoliths of sockeye salmon and developed a relationship between otolith nucleus dimension (defined by the extent of the emergence check) and fish length. He then examined frequency distributions of the nucleus measurements in successive samples of the cohort and was able to identify when the selective mortality occurred. However, due to the nature of such analyses, it is not possible to assess the extent of total mortality, only whether size-selective mortality exists. Methot (1982) used otolith microstructure examination to determine the birthdate distribution (defined as the date of yolk absorption) of larval northern anchovy. These data allowed both a comparison of survival between two subsequent year-classes and the determination of intraseasonal patterns of survival.

Use in Taxonomic Studies

Brothers (1984) provided a review of the utility of otolith microstructure examination for systematic ichthyology which was based largely on internal and external morphological characteristics, including the relative sizes and time of formation of the different otoliths. He also speculated on the systematic applications of descriptions of primordia number and type, as well as the value of increment width data and increment appearance in taxonomic studies. Given the variation in microstructural features which are often found in fish of similar age within a species, we feel that the utility of such data for interspecies comparisons might be limited. However, detailed analysis of growth from otolith measurements appears to have promise for stock differentiation. West (1983) found significant stock differences among linear regressions of (ln) fork length versus (ln) total otolith length of emergent sockeye salmon fry from various populations. Conceivably, the measurement of individual increment widths would have revealed the same stock differences. In addition, Uchiyama and Struhsaker (1981) found that on the basis of otolith microstructure, skipjack tuna (*Katsuwonus pelamis*) from the eastern Pacific grew at a slower rate than those from the central Pacific.

Other microstructural features of otoliths appear to have less utility for racial identification. Neilson et al. (1984) reexamined the hypothesis of Rybock et al. (1975) who suggested that otolith nucleus lengths had utility for separating sympatric races of rainbow and steelhead trout. The former authors used microstructural examination to better define the extent of the otolith nucleus. However, in contrast with the findings of the earlier authors, Neilson et al. (1984) were unable to demonstrate significant differences in otolith nucleus length between the two races. In this instance, the exact delineation of the otolith nucleus using microstructural preparation techniques allowed more precise measurements of the nucleus length.

Future Applications of Otolith Microstructure Examination

Many of the applications described in the previous section have not yet reached their full potential. The detailed study of fish growth, for example, has not developed as quickly as anticipated, perhaps due to methodological difficulties. This, in turn, has slowed research on problems such as the relationship between larval fish mortality and subsequent recruitment. However, progress has been made and further developments are expected. There also remain applications of otolith microstructure examination which have received little or no attention from researchers to date. Some of these areas of research are described below.

Despite its pivotal position as a regulator of fish abundance, there are few reliable predictors of year-class success prior to recruitment to the fishery. Both environmental variables and biological processes may influence recruitment, although it is unlikely that either acts in isolation (Lasker 1981). Assessment of the causal processes has been difficult; numerous parameters have been correlated with cohort survival, yet few provide any predictive power (Sissenwine 1984). However, fish growth results from the cumulative influence of both biotic and abiotic factors, and may well be a causal mechanism of year-class strength. Most of the commonly noted correlates of recruitment (temperature, food abundance and predator abundance) have direct influences upon fish growth, which in turn are reflected in the incremental growth sequence in the otolith. Of equal importance is the fact that the growth sequence spans both the larval and juvenile stages of the life history. Thus, no assumptions concerning the "important" ages are necessary. With the determination of size at age and growth rate at age from the otolith microstructure, information can be collected concerning the age at which year class strength is determined. This can be accomplished through collection of independent estimates of cohort size over a range of years, which in turn can be correlated with the growth at age data in the development of a predictive model of recruitment. If such a model was validated, otoliths from prerecruit fish could be examined to estimate the size of the year-class. Although currently feasible, this approach has not yet been pursued. However, less direct applications of otolith microstructure have yielded encouraging results in this field, both through analysis of birth date distributions (Methot 1982) and through determination of mortality rates at age (Crecco et al. 1983).

The identification of spawning locales is another research area in which otolith microstructure examination can be applied. Larvae of a given species, collected over a broad geographic area, can be assigned ages derived from daily increment counts. This information can be integrated with data on regional water circulation patterns to map patterns of larval drift. Given the duration of the egg stage, and approximate current speeds, back-calculated hatch dates can then be used to infer the general area of spawning. Of course, this technique would be most useful for species that spent little time in the egg stage and spawned in discrete locations.

One of the most promising fields to be developed in the future is that of otolith isotope analysis on a spatial scale equivalent to that of a daily growth increment. Previous workers have demonstrated that the ratio of stable oxygen (^{18}O:^{16}O) and carbon (^{13}C:^{12}C) isotopes in whole otoliths of wild fish reflect the ambient temperature in which the fish lived (Devereux 1967; Degens et al. 1969; Mulcahy et al. 1979). Recent laboratory experiments have suggested that physiological processes may alter the predicted isotope ratios, but that a relationship with water temperature does exist (Radtke 1984). Furthermore, studies with fish and other aquatic organisms have indicated that increasing nitrogen isotope (^{15}N:^{14}N) ratios in bone are correlated with increasing trophic levels (Schoeninger and DeNiro 1984). Given the fact that otolith material is apparently not

recycled after deposition and that stable isotopes are incorporated in a manner influenced by the ambient environment, it appears likely that isotope analyses of given daily increments would reflect both the ambient temperature and feeding habits of a fish on the dates in question. In other words, this type of analysis could allow the reconstruction of an individual fish's past movements and general feeding habits at a daily level of resolution. Until now, technological constraints have restricted isotope analyses to that of whole otoliths. However, the ion microprobe can be used to determine isotope ratios on a spatial scale of 10–25 μm (Karsten et al. 1982; Exley 1983), which approaches that of short sequences of growth increments. Calibration of the technique with temperature profile data would allow inferences to be made with respect to migration routes and even diel vertical migrations. In conjunction with more standard increment measurements, individual otoliths could be analyzed to provide a daily record of size at age, growth rate at age, life history transitions, ambient temperature, and trophic status. Although such data would be useful in the study of most fishes, otoliths could prove to be the only data source for rare or infrequently sampled species. For example, resolution of the North American eel (*Anguilla rostrata*) – European eel (*A. anguilla*) taxonomic controversy (Williams et al. 1984) may be possible through such an examination.

Stock identification is another field in which otolith microstructure analysis may be valuable. Multivariate analyses of elemental composition are based on the assumptions that ambient elemental concentrations vary by region and are incorporated into tissues with growth. The elemental load at hatch is considered to be one of the few natural tags of wild fish (Lapi and Mulligan 1981). Through elemental analysis of whole fish (Calaprice 1971) and scales (Lapi and Mulligan 1981), a limited form of stock discrimination has been demonstrated. However, in both cases, the structure/fish analyzed was exposed to waterborne elements in areas and at times other than that of hatch. Analysis of the perinuclear region of the otolith would remove this potential source of variance and may increase discriminatory powers. Use of more sophisticated devices such as the ion microprobe would further enhance resolution. A related application would take advantage of the chronological sequence of growth increments and the elements incorporated into each: line scans with an ion microprobe might allow the detection of both specific pollutants and the date on which the fish were first exposed to them.

In summary, the potential of otolith microstructure research illustrated in previous sections remains largely unrealized. Major hindrances to further progress include the question of interpreting the presence of subdaily increments in otoliths from fish populations in natural environments, the poorly understood relationship between increment width and somatic growth, and inadequate methods for preparing otoliths that have a degree of concavity along the sagittal plane (often the case in older fish). In our view, such problems represent the most appropriate subjects for future microstructure research. Once resolved, many of the far-ranging applications proposed thus far may then be feasible.

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