DETERMINING AGE OF LARVAL FISH WITH THE OTOLITH INCREMENT TECHNIQUE

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ABSTRACT

Aging of larval fish from otoliths rests on the assumption that increments are formed daily. Indeed, proper validation of the relationship between increment deposition and age is fundamental to accurate age determination of field-captured fish. To evaluate the universality of daily deposition of otolith increments, the literature was reviewed and exceptions discussed.

Laboratory studies under optimal conditions generally (17 species out of 20) show that larvae deposit daily increments. However, in studies that examined increment deposition under suboptimal or extreme conditions, deposition was not daily in over half of the species. Nondaily deposition caused by extreme conditions (e.g., total starvation, abnormal photoperiod) may not invalidate the otolith increment technique if those conditions do not occur in the field. Nondaily deposition under suboptimal conditions (e.g., low temperature, intermittent starvation) that larvae may face in nature cause concern about this technique for aging field-captured larvae. Deposition in many species has not been examined under suboptimal conditions, nor has the effect of suboptimal conditions been shown on the age at first increment formation. The literature shows that the technique should be validated under both optimal conditions and those that mimic nature.

Otoliths have been used to age fish since Reibisch (1899) first observed annular ring formation in *Pleuronectes platessa* (as reported in Ricker 1975). Assessing age by counting annular rings works well in adults of temperate species where pronounced seasonal changes in growth result in bands (formed from tightly spaced growth increments deposited in the winter) in the otolith which correspond to each year of life. Discovery of fine increments, analogous to annual rings, but instead formed daily, has permitted the age of larval fish to be determined.

While studying temperate water species, Pannella (1971) observed that about 360 fine increments occurred between annular rings and suggested that these were deposited daily. He used this knowledge when reading the otoliths of adult tropical fish (whose otoliths also had fine increments) to show patterns of growth that were grouped into 14- and 28-d cycles (Pannella 1974).

The initial application of the otolith aging technique to larval fish was done by Brothers et al. (1976). Daily increment deposition was verified for northern anchovy, *Engraulis mordax*, and California grunion, *Leuresthes tenuis*, which were reared from eggs in the laboratory. Since this initial application, the otolith increment technique has been used widely to estimate age in at least 29 species of larval fish. It has been used in freshwater and marine species, and applied to field-captured species, at times without adequate validation.

The ultimate purpose in developing the otolith aging technique for application to young fish is the ability to accurately age field larvae and juveniles. If the technique is to be applied directly to the field, based on conclusions drawn from rearing larvae in the laboratory, then the deposition of increments must be daily under conditions experienced in the field during these early life stages. The applicability of this technique relies on the assumption that 1) either surviving larvae (or sampled larvae) are those that grew under moderately good conditions (few larvae under suboptimal conditions survive) or 2) larvae can encounter suboptimal conditions, a proportion of these larvae will survive, and increment deposition is not affected by these suboptimal conditions. The first assumption is difficult to evaluate without using the hypothesis that increments are daily. The second assumption has been tested and the results can be summarized. The second assumption is based on increment deposition being triggered by a zeitgeber, an external factor that entrains a diel cycle within the larvae.

Validation of daily increment deposition under conditions within the natural range of experience of the larvae is fundamental to accurate estimation of age in field-captured fish. When the estimation technique

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used to age larvae is inaccurate, estimates of growth and mortality, which rely on knowledge of age, will also be inaccurate.

The purpose of this paper is to discuss the use of the otolith increment technique to age larval fish. The published literature is used to evaluate the hypothesis, H_0: Larval age is equal to otolith increment count (plus age at first increment deposition) under conditions that are encountered in the field. An additional idea can be evaluated: That time of initial increment deposition is influenced by incubation time.

The paper will discuss the factors which affect deposition of increments, validation studies that have been performed, and application of the technique in the field. Factors which are likely to affect increment deposition in the field must be assessed by the validation procedure. In addition, the adequacy of validation that has been performed is evaluated, and ramifications in field applications are discussed.

FACTORS AFFECTING DEPOSITION RATES

Mechanisms that have been postulated as initiators of differentiation of otolith microstructure are photoperiod, feeding, and temperature. Increment deposition has been tested in the literature under two conditions: 1) tests within the natural range of experience of the fish which could be optimal (nonstressful) and suboptimal (stressful), and 2) abnormal conditions that are wholly outside of their experience.

Taubert and Coble (1977) stated that photoperiod entrained a diel clock that resulted in daily formation of otolith increments. Tanaka et al. (1981) studied the formation of increments in *Tilapia nilotica* using scanning electron microscopy and found that the fast growth (incremental) zone started a few hours after light stimulus and that the slow growth (discontinuous) zone was formed immediately after light stimulus. Neither change in photoperiod length nor feeding time affected increment initiation. Brothers and McFarland (1981), however, reported that the discontinuous zone began near midnight. These results are contradictory, and without further investigations force the conclusion that the temporal formation of increments is species-specific.

Abnormal photoperiods have been shown to disrupt daily increment formation in *Fundulus heteroclitus* (Radtke and Dean 1982) and in *Tilapia mossambica* (Taubert and Coble 1977). Constant light, however, did not disrupt daily increment formation in *Oncorhynchus tshawytscha* (Neilson and Geen 1982) or in *Scophthalmus maximus* (Geffen 1982).

Unlike photoperiod changes, which are regular and gradual in nature, feeding times can occur at irregular intervals and might cause deviations in daily increment deposition. Two studies have tested the effects of feeding within the normal range experienced by fish larvae. Neilson and Geen (1982) found that subdaily increments could be induced through frequent discrete feedings: feeding four times a day resulted in formation of more than one increment in *Oncorhynchus tshawytscha*. Daily and subdaily increments were not distinguished in counts. Tanaka et al. (1981) found conversely that feeding time had no effect on the initiation of increment formation in *Tilapia nilotica*. Larvae were fed once a day, but the times of feeding were changed. Perhaps multiple feeding during the day results in the subdaily increments that sometimes appear in otoliths. The effect of starvation (an extreme circumstance in the field) on increment deposition has been tested in only three species: *Scophthalmus maximus* (Geffen 1982), *Morone saxatilis* (Jones 1984), and *Oncorhynchus nerka* (Marshall and Parker 1982). Geffen raised the turbot larvae on rotifers and *Artemia* until they were 10 d old. Larvae were then starved for 23 d. Jones did not supply exogenous food from hatch onward. Both Geffen and Jones found that starvation disrupted increment formation. Marshall and Parker fed their sockeye salmon larvae for the first 3 wk of life, and then starved them for 2 wk. Marshall and Parker found that starvation over 2 wk had no effect on increment deposition. It is possible that the difference might reflect different age-specific sensitivity to starvation, rather than species-specific responses.

Brothers (1978) has linked temperature as a prime factor in increment deposition. Working with temperate stream populations, he has found that diel temperature changes result in daily increment formation. Brothers (1978) stated that "six or more increments per day may be formed as the result of short term, . . . relatively minor. . . temperature fluctuations." Other investigators (Radtke and Dean 1982; Geffen 1982) found that small temperature changes had no effect on the rate of increment deposition. Apparently, temperature response is also species-specific.

LABORATORY STUDIES OF INCREMENT DEPOSITION

Initial Ring Deposition

When fish are raised in the laboratory from eggs

through the larval stages, two parameters fundamental to application of the increment technique to field populations can be determined: 1) age at first increment deposition and 2) testing of daily increment deposition under artificial conditions. Age at initial increment deposition for 18 species of fish is listed in Table 1. Radtke (1978) speculated that in species having slowly developing embryos, initial deposition occurs at, or before, hatch; in species having rapidly developing embryos, initial increment deposition does not occur until yolk-sac absorption or first feeding. This hypothesis is not substantiated in the currently published literature. Information for nine species of laboratory-reared fish larvae (Table 2) shows no such trend for data currently reported in the literature. Even for the same suborder, Clupeoidei, opposite development and initial increment deposition patterns exist for herring (*Clupea harengus*) and the northern anchovy.

The Case for Daily Increment Deposition

Seventeen species have shown consistent daily deposition of increments under what are presumed to be good conditions for growth. The species that have shown daily increment deposition come from both freshwater and marine habitats and encompass a wide variety of lifestyles. In addition, six species held in the laboratory and sampled over known periods of time demonstrated daily increment deposition (Table 3). Four investigation groups (Struhsaker and Uchiyama 1976 for *Stolephorus purpureus*, Taubert and Coble 1977 for *Lepomis macrochirus*, Campana and Neilson 1982, Wilson and Larkin 1980 for *Oncorhynchus nerka*) brought larvae and juveniles into the laboratory, reared them for a period of time, then correlated increment counts to days of captivity. Schmidt and Fabrizio (1980) took consecutive samples from a field population of *Micropterus salmoides*, which had a short spawning period and correlated the time between samples to the change in mean increment count.

Lack of Daily Deposition Rates

The most controversial results obtained so far come from studies of increment deposition in larval *Clupea harengus* (Table 1). Agreement for daily increment deposition has not been obtained. Studies that observed daily deposition by Gjøsaeter[2] and and Gjøsaeter and Øiestad (1981) indicate that increments are deposited with roughly daily periodicity and that initial increment deposition begins at first feeding (4-5 d). Gjøsaeter and Øiestad (1981) found that 99 increments were formed in 97-d-old larvae. Gjøsaeter, however, cautioned that these results were based on small sample sizes. Lough et al. (1982) reported on larval herring reared in the laboratory that lived until age 18 d. They did confirm that increment deposition began at yolk-sac absorption, but did not find that the increments were daily. In fact, only three increments were laid down within 18 d. Lack of confirmation of daily deposition is easy to dismiss, since the larvae did not survive past 18 d.

However, Geffen (1982) has demonstrated an interaction between growth rate and increment deposition rate. Only under circumstances of very fast growth, 0.42 mm/d (a rate which is faster than growth rates postulated for field animals) did increment deposition approach daily periodicity (0.92 increments/d). It is noteworthy that the growth rates in her study were related to container size; faster growth occurs in bigger containers. The variance of increment count at age is small and homogeneous only under the fastest growth condition (Norway Pond). The increasing variance with age in the other conditions leads to the speculation that some of these larvae were unknowingly starving. However, since the slope of the regression line for the Norway Pond condition is significantly different than 1 increment/d, this result cannot be dismissed. There would be obvious value in repeating these experiments. Geffen also found that increment formation did not begin before yolk-sac absorption and was in agreement with the other investigators on this point. The literature (Table 1) shows only one case (*Oncorhynchus nerka*) where independent investigators have confirmed daily increment deposition (Wilson and Larkin 1980; Marshall and Parker 1982).

Geffen (1982) found that increment deposition was also a function of growth in *Scophthalmus maximus* (Table 4) under various conditions of temperature and photoperiod. Under two conditions—1) 20°C, constant light, and 2) 24°C, 12L:12D—increments were deposited daily. For all other conditions increments were not daily. Under all conditions, deposition rate was a function of length. Although Geffen did not point this out, comparisons of growth at different temperatures can also be drawn from the data. Larvae were grown under 20°C and 24°C, both under a 12L:12D cycle. Larvae grew faster and deposited more increments at 24°C. Such differences in temperature might be used to explain differences in increment deposition except that the other case

[2]Harold Gjøsaeter, Institute of Marine Research, P.O. Box 1870 - 5011 Bergen, Norway, pers. commun. February 1983.

TABLE 1.—Otolith increment deposition for laboratory reared larval fish of known age. ff = first feeding, ysa = yolk-sac absorption, ns = not stated.

Species	Source	Are increments daily?	Time of increment initiation	Validation	*N*	Life history Stage	Rearing conditions				
							Light	Temp	Feeding	Salinity	Other
Clupea harengus	Gjøsaeter and Øiestad (1981)	yes	after ff	correspondence between age and rings Rings = 99, Age = 97 d	10	eggs-100 d	natural	ns	plankton	ns	
	Gjøsaeter (1981)	yes	4-5 d old or ff	slope = 0.95 rings/d	31	6-135 d	ns	ns	ns	ns	
	Geffen (1982)	no	1st rings at ysa	ring deposition depends on growth rate	227	eggs-100 d	18L/6D	8°-14°C	variety	seawater	various container sizes
	Lough et al. (1982)	no	1st ring 4.5 d	1st 3 rings in 18 d	57	eggs-18 d	ns	10°C	plankton	ns	
Engraulis mordax	Brothers et al. (1976)	yes	after ysa	correspondence between age and rings (interaction growth and rings)	88	6-94 d	14L/10D	ns	ns	ns	
Fundulus heteroclitus	Radtke (1978); Radtke and Dean (1982)	yes	from before hatch	slope = 1 ring/d	280 (temp) 270 (photoperiod)	eggs-30 d	several regimes	24°C 30°C	*Artemia*	30‰	
Gadus morhua	Radtke and Waiwood (1980)	yes	day after hatch	correspondence between age and rings	≅40	eggs-30 d	natural	4°C	plankton	18-25‰	2 tank sizes
Lepomis cyanellus	Taubert and Coble (1977)	yes	after swim up	correspondence between age and rings independent of growth	54	eggs-170 d	15L/9D	24°-27°C	plankton and *Artemia*		
Leurestes tenuis	Brothers et al. (1976)	yes	at hatching	correspondence between age and rings	15	eggs-26 d	natural	17°-20°C	*Artemia*	ns	
Menidia menidia	Barkman (1978)	yes		slope = 0.97 rings/d independent of growth	55	eggs-68 d	12L/12D	19.4°-21.6°C	*Artemia*	31‰	
Morone saxatilis	Jones (1984)	sometimes	at ysa	regression analysis	148	eggs-97 d	14L/10D	18°C	*Artemia*	0-10‰	4 L jars
Mugil cephalus	Radtke (1984)	yes	1 day after hatch	regression and correspondence	50	eggs-52 d	ambient	24.0° ± 0.9°C	Rotifers, fish chow	32.0 ± 0.4‰	500 L tanks (flow through)
Oncorhynchus keta	Volk et al. (1984)	yes	at hatch	regression analysis	32(?)	hatch-190 d	ambient	7.6°-10.2°C	Copepods and pellets	29.8-33.6‰	10 L tanks

TABLE 1.—*Continued.*

Species	Source	Are increments daily?	Time of increment initiation	Validation	*N*	Life history Stage	Rearing conditions				
							Light	Temp	Feeding	Salinity	Other
Oncorhynchus nerka	Marshall and Parker (1982)	yes	at hatch but can be interrupted	correspondence between age and rings	≅440	eggs-93 d	natural	ambient >10°C <10°C	fed 3 wk fed versus starved	ns	200 L tanks
Oncorhynchus tshawytscha	Neilson and Geen (1982)	sometimes	1 or more/d	regression and correspondence between age and rings	34 (feeding) 10 (temp) ≅12 (photoperiod)	fry-90 d	12L/12D 24D 24L	4°-12°C	excess 1×/d 4×/d	ns	28 L tanks
Pagrus major	Tsuji and Aoyama (1982)	yes	from hatching	slope = ring/d independent of growth	not given	eggs-30 d	24L 12L/12D	20°C	2×/d variety of food	ns	1,000 L tanks
Parophrys vetulus	Laroche et al. (1982)	yes	4-5 d old	correspondence between age and rings	136	eggs-26 d	14L/10D	12°-13°C	variety	seawater	4 and 8-9 L tanks
Pseudopleuronectes americanus	Radtke and Scherer (1982)	yes	after ysa	regression and correspondence between age and rings	≅200	eggs-34 d	12L/12D	5°-8°C	variety	ns	10 L tank
Salmo salar	Geffen (1983)	no	depends on physiology	regressions and ANOVA	36 (temp) 56 (light and temp)	embryos	24D 12L/12D 6L/6D	8°, 10°, and 15°C	*Artemia*	not applicable	15 cm dishes
Sebastes spp.	Radtke (1980)	yes	from birth	correspondence between age and rings Lab data not presented	not given	not given eggs - ?	12L/12D	ns	ns		
Scophthalmus maximus	Geffen (1982)	no	depends on growth	regressions	72	eggs-23 d	24L 6L/6D 12L/12D	20°C 24°C	rotifers and *Artemia*	seawater	30 L tank
	Rosenberg and Haugen (1982)	yes	at hatch	back-calculated hatch date	62	2-12 d	ambient	18.5°-22.5°C	fed and starved	ns	2,000 m³ tank
Tilapia mossambica	Taubert and Coble (1977)	yes	when they leave mouth	data not presented	≅300	eggs-60 d	15L/9D	24°27°C	trout food	ns	
Tilapia nilotica	Tanaka et al. (1981)	yes	at hatch	correspondence between age and rings	20 (rings) 80 (feeding)	eggs-28 d	12L/12D 18L/6D 6L/18D	27.5°C	ns	ns	60 L tank

TABLE 2.—Relationship between incubation time, egg size, and initial increment deposition: Determing whether species with long incubation and large eggs initiate increment deposition on or before hatch, while species with short incubation and small eggs initiate increments at first feeding or yolk sac absorption. ysa = yolk sac absorption.

Species	Temperature	Source	Incubation time	Source	Initial increment deposition	Source	Egg size (mm)	Source
Clupea harengus	≅10°C	Blaxter (1969)	≅18 d	Blaxter (1969)	4-5 d ysa	See Table 1	0.9-1.7	Blaxter (1969)
Engraulis mordax	11°-21°C	Lasker (1964)	1-5d	Lasker (1964)	≅5 d	Brothers et al. (1976)	≅2	
Fundulus heteroclitus	24°-30°C	Radtke (1978)	14 d	Radtke (1978)	Before hatch	Radtke (1978)	2	Armstrong and Child (1965)
Gadus morhua	4°C	Radtke and Waiwood (1980)	19 d	Radtke and Waiwood (1980)	1 d	Radtke and Waiwood (1980)	1.1-1.6	Blaxter (1969)
Menidia menidia	19.4°-21.6°C	Barkman (1978)	7-10 d at 23°-25°C	Barkmann and Beck (1976)	Before hatch from regression	Barkman (1978)	1.2	Barkmann and Beck (1978)
Morone saxatilis	18°C	Jones (1984)	2 d	Jones (1984)	6-9 d	Jones (1984)		
Parophrys vetulus	20°C	Laroche et al. (1982)	3-3½ d	Laroche et al. (1982)	4-5 d	Laroche et al. (1982)		
Pseudopleuronectes americanus	5°-8°C	Radtke and Scherer (1982)	14 d at 8°C	McPhee[1]	9-10 d	Radtke and Scherer (1982)	0.8	Smigielski and Arnold (1972)
Tilapia nilotica	27°C	Tanaka et al. (1981)	4 d	Tanaka et al. (1981)	At hatch	Tanaka et al. (1981)		

[1]Grace McPhee, P.O. Box 210972, Auke Bay, AK 99821, per. commun. summer 1983.

TABLE 3.—Otolith increment deposition for larval fish maintained in the laboratory over a known time span.

Species	Source	Known-age span	Are increments daily?	Validation	Number of fish
Lepomis gibbosus	Taubert and Coble (1977)	≅6-176 d	yes after swim up	Correspondence between age and rings	
Lepomis macrochirus	Taubert and Coble (1977)	≅6-125 d	yes after swim up	Correspondence between age and rings	
Micropterus salmoides	Schmidt and Fabrizio (1980)	Between 47 and 81 rings	yes	Correlation between change in ring count and time interval	98
Oncorhynchus nerka	Wilson and Larkin (1980)	Between 14 and 26 rings	yes	Slope = 1 ring/d	100
Platichthys stellatus	Campana and Neilson (1982)	8-10 mo old	yes	Slope = 1 ring/d	13 (in situ) 81 (temp and light)
Stolephorus purpureus	Struhsaker and Uchiyama (1976)		yes	Correspondence between rings and days	174

of daily deposition (24L, 20°C) would be an anomaly under this hypothesis.

Ten studies have investigated deposition rates under suboptimal, extreme or varying conditions (Table 4). These studies are important to the understanding of the underlying mechanisms causing increment deposition. Two studies, one by Radtke and Dean (1982) and one by Taubert and Coble (1977), demonstrated disruption of daily increment formation under extreme or abnormal changes in photoperiod. Taubert and Coble (1977) found that in simulated winter conditions, cold temperature and shorter photoperiod resulted in cessation of increment formation in *Lepomis cyanellus*. At and below temperatures of 10°C, growth and increment deposition ceased. If such changes occurred gradually, as occurs in the normal lifetime of fish, acclimation to these temperature changes might be expected through most of the temperature range. Within normal physiological limits (especially where some growth continued), increment deposition would be assumed to continue regularly. However, Marshall and Parker (1982) also found that temperatures below 10°C resulted in cessation of increment deposition in sockeye salmon. Hence two studies have shown that increment deposition is not maintained

TABLE 4.—Otolith increment deposition for known-age larval fish under experiments where various culture conditions were tested.

Species	Source	Conditions of growth				Effect on increment deposition
		Light	Food	Temp	Other	
Clupea harengus	Geffen (1982)				tank size 120 L, 500 L, 310 m^3 4,440 m^3	Increment deposition rate was related to growth rate. Also, larvae grew faster in bigger container and deposited more rings.
Fundulus heteroclitus	Radtke and Dean (1982)	Multiple L/D conditions		24°C 30°C		Temperature affects growth rate, but not increment deposition. Increment deposition rate disrupted under constant dark or under <24-h photoperiod.
Lepomis cyanellus	Taubert and Coble (1977)	15L/9D 10L/14D		4°-25°C		Fewer hours of light and lower temperature resulted in cessation of ring deposition. At 10°C or less, growth ceased, as did increment formation.
Morone saxatilis	Jones (1984)	14L/10D	Fed, starved, intermittent starved, then fed	18°C		Increment deposition rate was disrupted during periods of starvation. Increments not daily in sagittae during 2-3 mo under optimal conditions.
Oncorhynchus nerka	Marshall and Parker (1982)		Fed Starved	<10°C >10°C		Starvation for 10 d did not affect increment deposition. Temperatures <10°C resulted in cessation of increment formation.
Oncorhynchus tshawytscha	Neilson and Geen (1982)	24D 24L 12L/12D	4×/d 1×/d	11°C 5.2°C		Formation of increments was related to feeding frequency. Temperature affected width of increment, not deposition rate. Photoperiod had no effect.
Salmo salar	Geffen (1983)	24D 6L/6D 12L/12D		8°C 10°C 15°C		Rate of ring deposition increased with increased light and temperature.
Scophthalmus maximus	Geffen (1982)	24L 6L/6D 12L/12D	Fed Starved	20°C 24°C		Daily increments formed under 24L-20°C and 12L/12D-24°C. Starvation and 6L/6D interrupted increment formation. Increment formation related to growth rate.
Tilapia mossambica	Taubert and Coble (1977)	24L 24L/12D 15L/9D	Every 3 h Every 6 h Intermittent			Daily increments formed under 24-h photoperiod, not under 36-h cycle nor constant light. Subdaily increments induced. No effect from feeding cycle.
Tilapia nilotica	Tanaka et al. (1981)	12L/12D 18L/6D 6L/18D	3 h before dark 3 h after light			Formation of increment triggered by light stimulus. Feeding time had no effect under 12L/12D.

below certain temperatures. In two other studies where temperatures ranged from 24°C to 30°C (Radtke and Dean 1982) and from 5.2°C to 11°C (Neilson and Geen 1982), these temperatures affected the growth rate and width of increments, but did not alter the increment deposition rate.

Six studies looked at the relationship between feeding and daily increment deposition. Jones (1984), Geffen (1982), and Marshall and Parker (1982) showed opposite effects of starvation on increment deposition. Jones (1984) found that starvation of young larvae for 2 wk resulted in deposition of only one increment every other day. However, in addition to lengthy starvation, the effect of short-term, intermittent periods of starvation was also studied and resulted in nondaily increment formation. Geffen (1982) found that starvation interrupted deposition in larval turbot, while Marshall and Parker (1982) found that starvation for 2 wk had no effect on daily deposition in sockeye salmon. Long-term starvation experiments test for interruption of increment deposition under extreme conditions. To age larvae in the field, it is important to determine the minimum number of consecutive days of starvation needed to affect increment deposition. Once these values are known, it is important to determine whether field larvae actually experience these levels of deprivation.

Three studies looked at feeding time or frequency on increment deposition. Neilson and Geen (1982) found that feeding frequency could induce forma-

tion of subdaily increments in *Oncorhynchus tshawytscha*. Both Tanaka et al. (1981) and Taubert and Coble (1977) found that feeding time had no effect on increment deposition in larval mouthbrooders (*Tilapia nilotica* and *T. mossambica*).

Little agreement has been reached in these studies concerning the effect of light, temperature, or feeding on increment formation. The effects of variability in temperature, food, salinity, and other factors (extreme photoperiods would not be encountered) relate directly to the problems of accurately aging larvae from the field. At the moment, environmental effects appear to be species-specific. Indeed, specific tests of the effect of suboptimal conditions (which are likely to occur in the field) on increment deposition have rarely appeared in the literature. Such analyses, conducted for more species, might confirm the conventional wisdom that deviation from daily deposition rate is abnormal. However, the questions raised by the studies reviewed here (Table 4) remain to be fully addressed or dispelled.

APPLICATION IN THE FIELD

Current Applications

The ability to age larval fish precisely provides more accurate estimates of growth, mortality, and the ability to discern the effects of environmental variables on the first year of life. Rapid growth in the first months of life has commonly been thought to be critical to survival. Evidence in support of this hypothesis (Brothers et al. 1983) and contrary to it (Methot 1983) exists.

The otolith increment aging technique has been

TABLE 5.—Application of the otolith increment aging technique in field grown larvae.

Species	Source	Based on prior validations (validations in Table 1)	Validation source	Sample size	Application
Ammodytes dubious	Scott (1973)	no		71	Back-calculated growth.
Clupea harengus	Graham and Joule (1981)	controversial Geffen (1982) found deposition depended on growth rate. Gjøsaeter and Øiestad (1981) found deposition was daily. See Table 1 for details.	See Table 1 for details	545	Determine hatching dates and delineate cohorts which are followed through time.
	Townsend and Graham (1981)			300	Determine hatching dates and assess growth rates of larval cohorts. Noted cessation of growth in winter.
	Lough et al. (1982)			311	Use age to delineate growth. Fit Gompertz function of length-at-age data.
	Jones (1985)			481	Determination of within-season growth differences based on uncertainty in otolith aging.
Engraulis mordax	Methot and Kramer (1979)	yes	Brothers et al. (1976)	587	Fit Gompertz function to length-at-age data to obtain growth rates. Also mention that starvation slowed increment deposition.
Fundulus heteroclitus	Radtke and Dean (1982)	yes	Radtke and Dean (1982)	not given	Compare length-frequency histograms with increment-frequency histograms. Show relationship between hatching and lunar cycle.
Gadus morhua	Gjøsaeter and Tilseth (1981)	yes	Radtke and Waiwood (1980)	30	Regression of age estimated from morphologic development versus increment counts.
	Steffenson (1980)	yes	Radtke and Waiwood (1980)	138	Back-calculated hatch date from increments. Compare these to field observations of spawning time.
Haemulon flavolineatum	Brothers and McFarland (1981)	no, but refers to data as otolith age		≅306	Correspondence between otolith microstructure and events in the life history. Derive "otolith" growth rates.
Halichoeres bivittatus	Victor (1982)	yes	marked juveniles	10	Determine daily deposition of increments and use to determine settling pattern.
Lepomis macrochirus	Taubert and Coble (1977)	yes	Taubert and Coble (1977)	≅150	Allometric relationship between otolith length and fish length tested for 2 lakes.

applied to larval field populations of many species of fish (Table 5). Most applications have been based on laboratory validation of daily increment deposition for the individual species studied. Some have not. Methot and Kramer (1979), based on validation of daily increment deposition by Brothers et al. (1976), obtained growth rates for wild populations of *Engraulis mordax* by fitting a Gompertz function to length-at-age data. Various other field applications of the increment aging technique are listed in Table 5. Of special interest is a comparison of growth estimates for *Parophrys vetulus* from modal progression of length frequencies and otolith increments (Laroche et al. 1982). Growth based on the increment count method was 2-3 times faster. If the increment count method proves to be accurate, then mortality estimates could be considerably changed.

For at least four species listed in Table 5, laboratory validation was not conducted. These applications assume a given age at initial deposition and daily increment deposition thereafter. The validity of these assumptions depends on the species and on the sensitivity of the application to inexactness in the age estimation. For example, controversial results have been obtained for larval herring, *Clupea harengus*. Geffen (1982) showed that growth rates could be overestimated by as much as three times the actual rate. However, analysis of Gulf of Maine herring data (Jones 1985) showed that differences in growth between larvae hatched early and late in the season could be drawn. Until sensitivity analyses, laboratory verification, or other evidence exists to assure daily increment formation as a universal phenomenon under suboptimal conditions, there will be some doubt about the accuracy of aging field-captured larvae.

Transition from the Laboratory to the Field

A question that remains to be answered when applying laboratory-derived increment deposition

TABLE 5.—*Continued.*

Species	Source	Based on prior validations (validations in Table 1)	Validation source	Sample size	Application
Menidia menidia	Barkman et al. (1981)	yes	Barkman (1978)	105 (lab)	Compare growth in lab and field. Calculate hatching dates. Compare growth between early and late hatched larvae.
Morone saxatilis	Brothers et al. (1976)	no		5	Correspondence between increment estimated age and spawning season. Growth through lifetime of juvenile.
Oncorhynchus nerka	Wilson and Larkin (1982)	yes	Wilson and Larkin (1980)	64	Relationship between fish weight and otolith size. Use daily increments as time marker.
Parophrys vetulus	Laroche et al. (1982)	yes	Laroche et al. (1982)	331	Determine growth of aged field larvae and fit Gompertz and von Bertalanffy functions. Compare length-frequency and otolith techniques.
	Rosenberg and Laroche (1982)	yes	Laroche et al. (1982)	233	Growth during metamorphosis. Relate to age and transformation in morphology.
Pseudopleuronectes americanus	Radtke and Scherer (1982)	yes	Radtke and Scherer (1982)	120	Comparison of length-frequency and increment-frequency histograms for field larvae. Daily growth rate calculated. Compare growth rates over time.
Stolephorus purpureus	Struhsaker and Uchiyama (1976)	yes	Struhsaker and Uchiyama (1976)	213	Built growth curves based on age. Discussion of relationship to feeding. Preliminary study of growth rate difference between areas.
Thalassoma bifasciatum	Victor (1982) Victor (1983)	yes	Victor (1982) marked juveniles	68 103	Determine daily increment deposition. Calculate pattern of settlement based on age estimate.
28 species of coral reef fish	Brothers et al. (1983)	no		210	Determine length of larval life prior to recruitment. Examine otoliths for marker between postlarvae to juvenile.

rates to field populations is the constancy of deposition rates between these environments. Most laboratory studies have occurred under constant temperature and salinity and under conditions of artificial food types and densities and low light intensities compared with the field. Often, increments from otoliths of laboratory-grown larvae are much fainter than those from otoliths of field-captured larvae. Since field conditions can fluctuate to extents that have been shown to cause increment disruption in laboratory situations, a way to verify daily deposition in the field would be an important contribution. A transitional step between the laboratory and the field has been made by Laurence et al. (1979) and Øiestad (1982). Laurence et al. (1979) raised known-age larvae in a flow through enclosure. This study was designed to measure the growth and survival of fish larvae exposed to varying prey concentrations in the field. Modifications of this system could be used to study increment deposition in known-age larvae exposed to field conditions. Øiestad (1982) presented a review of larval fish studies performed in enclosures. Gjøsaeter and Øiestad (1981) reared known-age larvae in large enclosures and determined increment deposition rates (Table 1). Few investigators have used such enclosures for validation of otolith increment deposition rates for field simulated studies. Enclosures should prove particularly valuable for validation and simulation of suboptimal field conditions on growth and increment deposition.

Statistical Applications

Once the veracity of daily increment deposition is established, a wide variety of statistical methods can be used in otolith studies. Statistical methods that have been employed in larval otolith studies have been linear regressions to establish increment deposition rates and curve fitting techniques to establish growth rates from length-at-age data. Linear regression has also been applied regardless of whether it actually fits the data. It is important to check for lack of fit, selection of the appropriate model, and weighting before applying linear regression blindly. It is recommended that, when possible, confidence intervals and standard deviations be included in the data presentation.

Investigators are beginning to relate increment widths, as indicators of growth, with environmental conditions (Methot and Kramer 1979; Lough et al. 1982). When increment widths are correlated directly with environmental factors, either no correlations are seen (Neilson and Geen 1982) or correlations may be spurious. Problems exist in measuring the physical conditions to which the larvae have been exposed, especially since larvae may move from one area to another. In addition, there are questions concerning food availability and its concentration and patchiness. Another consideration in relating growth to environmental conditions is that, as the fish grows, the width of the outer increments decreases proportionately to decreases in length. Better results might be obtained either with covariance analysis or by fitting a growth function to data then using the residuals in correlation tests. Investigations of residuals with exploratory techniques such as principal component analysis or canonical correlation might prove fertile.

Comparison of Scanning Electron and Light Microscopy

Scanning electron microscopy (SEM) has been used to confirm otolith structure (Dunkelberger et al. 1980; Watabe et al. 1982) and to compare increment counts with those obtained by transmitted light microscopy (Radtke and Waiwood 1980; Campana and Neilson 1982; Neilson and Geen 1982; Radtke and Dean 1982; Tsuji and Aoyama 1982; Ralston and Miyamoto 1983). Under optimal conditions, counts using both methods were equivalent except for larval cod. Radtke and Waiwood (1980), using SEM, determined that cod produced daily increments from hatch onward, while Gjøsaeter (1981), using a light microscope, did not observe increment formation until 4-5 d after hatch.

Most investigators did not verify deposition seen with the light transmission microscope with SEM studies. Confirmation with SEM is highly desirable when increments are nondaily. However, extensive use of the technique for field surveys is prohibited by the additional cost and preparation time when compared with light microscopy. In cases where suboptimal or abnormal field conditions may result in nondaily increment formation (Jones 1984), SEM, used in conjunction with ancillary techniques, may assist identification of the proportion of larvae for which age is underestimated with light microscopy.

CONCLUSIONS

The report of the otolith workshop held in Bergen, Norway (Anonymous 1982) stated that the appearance of increments in otoliths of larval fish living in diverse habitats and representing many families, argues strongly for the universality of this phenomenon. Validation that these increments are, indeed,

deposited daily has been reported in 17 out of 20 species (Table 1) grown under optimal laboratory conditions. However, evidence exists that daily deposition can be interrupted under suboptimal and abnormal conditions, or can be dependent on growth rate (Table 6). When the effect of photoperiod is ignored (changes in photoperiod are very gradual in the field), more than 50% of the tests under suboptimal and extreme conditions have shown nondaily increment deposition rates. For other species, tests under suboptimal conditions were not conducted and the effect of these conditions on increment deposition rate is undetermined. The effect of varying conditions on the age at initial increment deposition has also not been addressed. To apply the otolith aging technique to fish from the natural environment, the scientist must either assume that larvae sampled grew under optimal conditions (those exposed to suboptimal conditions died) or verify that the species almost always deposit daily increments under field encountered conditions, or establish the error bounds for the relationship between age and increment count.

Attempts to clarify the natural phenomena that drive daily increment formation have given conflicting results. Photoperiod, feeding periodicity, and temperature fluctuations have all been cited as causing daily increment formation. When these factors are within normal ranges, it is likely, for most larvae, that deposition is daily. However, for larvae experiencing conditions outside tolerable ranges or abnormal conditions, the period of formation is likely to deviate from daily deposition. It is important to determine whether the minimum exposure to suboptimal conditions which result in nondaily deposition is actually experienced by larvae in the field. These hypotheses are amenable to further testing. More basic research on the causation of increment deposition or more extensive testing under a variety of conditions for a given species will yield more information. In situ testing with known-age larvae in enclosures which closely mimic field conditions could yield valuable results. The Bergen otolith workshop report (Anonymous 1982) has recommended that increment deposition be verified for each new species, under a variety of test conditions.

Two issues, cost effectiveness and accuracy, are important in determining whether the otolith increment technique is preferable to length-frequency analysis. Recommendations made in the report from the Bergen otolith workshop (Anonymous 1982) are that "the precision of an age determination... be tested against other available methods... by a cost benefit analysis (i.e. is enough precision gained by using this method to pay the costs and effort in preparation)". A good example would be the results shown in Laroche et al. (1982) when the otolith method was compared with modal progression of length frequencies, estimated growth rates differed by a factor of 2-3. Benefits should also include nonmonetary considerations, such as decrease in error which will propagate through estimates based on age determinations (i.e., growth and mortality). Sensitivity analyses can be used to show situations where more accurate estimates are necessary.

TABLE 6.—Incidence of nondaily increment deposition for species reared under suboptimal and extreme conditions. Stars (★) indicate nondaily deposition caused by exposure to suboptimal conditions; triangles (△) indicate nondaily deposition caused by exposure to extreme conditions; circles (○) indicates no interruption of daily deposition.

Species	Light	Food	Temp	Tank size
Clupea harengus				★
Fundulus heteroclitus	★,△		○	
Lepomis cyanellus	★		★	
Morone saxatilis		★,△		
Oncorhynchus nerka		○	★	
O. tshawytscha	○	○	○	
Salmo salar	△		★	
Scophthalmus maximus	○,△	△		
Tilapia mossambica	△	○		
T. nilotica	★	○		

Specific recommendations for improving reliability and replicability are discussed in the Bergen otolith workshop report (Anonymous 1982). In addition to these, Brothers[3] has suggested that other otoliths, such as the lapillus, be used in analysis.

Aging by the otolith increment technique is a powerful tool. Not only can population estimates of growth and mortality be refined, but growth of individuals can be obtained. Issues such as the importance of environmental factors to survival, the proportion of fast growing larvae to recruitment, and demonstration of compensation in field larvae may become easier to address with the availability of this technique. However, it is equally important to make sure that the technique is based on good scientific technique.

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[3]Edward Brothers, 3 Sunset West, Ithaca, NY 14850, pers. commun. September 1983.

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