

Does water calcium content influence the distinctness of daily growth increments in the otoliths of larval whitefish (*Coregonus lavaretus* L.)?

R. Eckmann

Limnological Institute, University of Konstanz, Germany

Summary

In larval and juvenile whitefish (*Coregonus lavaretus* L.) from Lake Constance, Germany, the otolith increments are deposited daily, whereas daily deposition could not be confirmed in larval whitefish from Lake Pyhäselkä, Finland. The calcium concentration in Lake Constance is high (around 1.3 mM), while calcium deficiency is typical for Finnish lakes (around 0.15 mM). Therefore, the hypothesis that the distinctness of daily otolith increments in whitefish is related to water calcium content was tested by rearing three groups of Lake Constance whitefish in water of 0.2, 1.3 and 4.7 mM Ca. The eggs were incubated in lake water (1.3 mM Ca), and the larvae were acclimated to the experimental calcium concentrations on the day of hatching. After 39 days of *ad libitum*-feeding with *Artemia* nauplii, the three groups did not differ significantly in total length, wet and dry weight, and otolith length and width. The daily increments were easily recognizable, and contrast between dark (D)- and light (L)-zones was the same in the fish of all test groups. For the experimental set-up of this study, and particularly the range of calcium concentrations tested, the hypothesis that water calcium content influences the distinctness of daily otolith increments was rejected.

Introduction

The analysis of daily otolith increments is a powerful tool for studying the early life history of fish. The microstructure of fish otoliths and hence the visual appearance of daily increments, however, is influenced by a variety of factors such as temperature, feeding regime and photoperiod (Neilson and Geen 1982; Campana and Neilson 1985). Only when the visual contrast between the mineral-rich L-zone and the matrix-rich D-zone (*sensu* Secor et al. 1995) is strong enough, and when subdaily increments are not confounded with daily increments, can the latter be reliably evaluated with regard to number and increment width.

In larval and juvenile coregonids, daily deposition of otolith increments has been validated (Rice et al. 1985; Eckmann and Rey 1987) and increment analysis has been subsequently used to study early life history aspects in bloaters, *Coregonus hoyi* Gill. (Rice et al. 1987) and lake whitefish, *Coregonus lavaretus* L. (Eckmann and Pusch 1989; Rey and Eckmann 1989). With vendace, *Coregonus albula* L., and lake whitefish from Finnish lakes, however, the daily deposition of otolith increments could not be validated (Huuskonen and Karjalainen 1993, 1995), mainly due to the presence of subdaily increments. Therefore, the growth rates could not be estimated from the increment widths.

Four hypotheses have been proposed that might account for

this apparent discrepancy in otolith microstructure between coregonids from different locations. (1) In the Finnish lakes, light intensity was lower than 100 lux for only about 2 h each night, and complete darkness was never observed (Huuskonen and Karjalainen 1995). Hence, prolonged feeding time per day and lack of a clear zeitgeber may account for the high number of subdaily increments. (2) Since the number of subdaily increments tended to increase with growth rate (Huuskonen and Karjalainen 1995), the lack of well-defined daily increments may be due to high growth rates in Finnish coregonids. (3) Multiple feedings per day may induce the formation of subdaily increments (Neilson and Geen 1982), possibly via peaks in the specific dynamic action that are associated with distinct meals during the day (Huuskonen and Karjalainen 1998). (4) Calcium content in Finnish lakes is almost an order of magnitude lower than in lake Constance, so that the low optical contrast between L- and D-zones might be caused by the calcium deficiency in Finnish waters (Huuskonen et al. 1998).

Huuskonen et al. (1998) tested for population-specific differences in otolith microstructure by rearing Finnish and Lake Constance whitefish under similar conditions of water quality and photoperiod (14 h light per day). Otolith increments were formed daily and were clearly recognizable in all of the populations tested. Thus, there were no population-specific effects. The authors therefore suggested that environmental effects, i.e. lower calcium content in Finnish lakes together with the effect of growth rate on subdaily increment formation, may account for the confounded microstructure in vendace and whitefish otoliths from Finnish lakes.

To test for the effect of water calcium concentration on the distinctness of daily otolith increments, three groups of Lake Constance whitefish larvae that originated from the same egg batch were reared under similar conditions of temperature, photoperiod and food regime, but in waters of different calcium content.

Materials and methods

Eggs and milt were obtained from spawners caught with gill nets in Lake Constance in December 1996. Fertilized eggs were incubated in glass jars at $4 \pm 0.3^\circ\text{C}$ and larvae hatched by early March. Three rearing units were stocked with 500 larvae each. A rearing unit consisted of an 80 L glass aquarium which contained the larvae and a 60 L reservoir tank for temperature control. Water was recirculated between the reservoir and the aquarium via a standard aquarium filter at a rate of 2–3 L/min. The temperature was adjusted to about 12°C .

Calcium concentrations were maintained at about 0.2, 1.3, and 4.7 mM, respectively, by either adding deionized water to

Table 1

Physical and chemical parameters in three units of 140 L volume each, during a 41-day rearing experiment with coregonid larvae. Parameters were determined prior to the daily water exchange which amounted to 14–21 % of the total volume. Mean values \pm 1 SD or ranges are given, n is the number of measurements

	Unit 1	Unit 2	Unit 3
Temperature ($^{\circ}$ C) (n = 36)	12.4 \pm 1.0	12.3 \pm 0.9	12.4 \pm 0.9
Conductivity (μ S/cm) (n = 33)	70–390	370–610	1040–1400
pH (n = 31)	6.8–7.2	7.5–8.2	7.5–8.5
Ca (mM) (n = 14)	0.18 \pm 0.02	1.32 \pm 0.09	4.72 \pm 0.31
NH ₄ (μ M) (n = 14)	2.5–61	2.0–64	1.9–52
Total no. of dead larvae	16	19	23

tap water, using tap water alone (filtered and ozonized water from Lake Constance), or by adding calcium chloride to tap water. In the treatments with medium and high calcium content the pH was controlled by the addition of hydrochloric acid. To avoid accumulation of ammonia, the aquaria were cleaned daily and between 20 and 30 L of water (14–21 % of the total volume) were exchanged. Temperature, conductivity and pH were controlled daily, while calcium and ammonia concentrations were determined three times per week prior to the daily water exchange. The larvae were fed with live *Artemia* nauplii to satiation several times per day between 0800 h and 1700 h. Illumination from overhead fluorescent tubes was controlled to mimic the local photoperiod (12.5 h light in March, 14.5 h light in April). Complete darkness was provided during the night.

After 11, 25 and 39 days of rearing, the length and weight were determined for about 20 larvae from each treatment. Samples for otolith analyses were taken on days 17 and 31. Sagittae were dissected out and embedded in epoxy resin. The length and width were measured on one sagitta from each of 10 fish per sample on day 17, while both sagittae from each of 10 fish per sample were analysed on day 31. Photographs were taken from ground and polished otoliths. The experiment ended on day 41.

Results

Growth in terms of length and wet weight did not differ between treatments. After 39 days of rearing at 12.3–12.4 $^{\circ}$ C (Table 1), mean total length ranged from 27.5 to 27.8 mm and average wet weight per fish was between 0.138 and 0.141 g (Table 2). This corresponds to specific daily growth rates of 2.3–2.4 % in terms of length and of 7.8–7.9 % in terms of weight across the 39-day rearing period.

The otolith lengths did not differ significantly between treatments on either sampling date, while the widths differed between two treatments on the second sampling date (Table 3). Visual contrast between L- and D-zones was always high and was virtually the same in all three treatments (Fig. 1). Increment counts were, therefore, performed on samples from all three treatment groups combined. Otolith increments averaged 16.4 in 17-day-old larvae (range: 15–18, n = 23), while in 31-day-old larvae 29.5 increments could be counted on average (range: 28–31, n = 28). In both cases the observed values differed significantly from the expected values (two-tailed t-test; 17 days:

t = 3.44, degrees of freedom (d.f.) = 22, P = 0.0023; 31 days: t = 11.67, d.f. = 27, P = 0.0001). These results, however, should not be mistaken as evidence for nondaily increment formation. The differences of 0.6 and 1.5, respectively, between the increment counts and age of larvae were mainly due to one or two weakly defined increments at the otolith edge and to problems with proper identification of the hatch check. Subdaily increments were not detected in most cases or they were so weakly defined that they caused no problem in the recognition of daily increments.

Discussion

As in previous studies (Eckmann and Rey 1987; Klink and Eckmann 1992), where similar conditions of photoperiod, temperature and feeding had been provided, daily increments were deposited on the sagittae of Lake Constance whitefish from the day of hatching onwards and subdaily increments were negligible. This result is not compatible with the hypothesis of Huuskonen et al. (1998) who suggested that the recognition of daily increments in whitefish otoliths becomes difficult when specific growth rate in terms of length exceeds 2 % per day during the first weeks of life. In the present study the specific daily growth rates were 2.3–2.4 % during 39 days. Huuskonen and Karjalainen (1995) observed 2.3 % in the fastest growing whitefish from Lake Phyäselkä during 37 days, yet could not identify daily increments unequivocally. Therefore, high growth rate does not necessarily lead to the formation of subdaily increments.

The feeding frequency has been reported to affect subdaily increment formation (Neilson and Geen 1982). An explanation has been proposed recently of how various peaks of specific dynamic action that are associated with feeding peaks may lead to the formation of subdaily increments (Huuskonen and Karjalainen 1998). The results of the present study, in which the larvae received several feedings per day at discrete times, do not support this concept. After each feeding, *Artemia* nauplii remained in the aquaria, but soon settled to the bottom from where they were only occasionally picked up by the whitefish larvae. Consequently, there were several discrete peaks of food intake per day in the present study. In the enclosure study of Huuskonen and Karjalainen (1995), the fish were not given additional food but preyed on zooplankton that was expected to be freely available in the enclosures. Thus, since food intake was much more pulsed in the present study, feeding frequency during a 12.5 or 14.5 h light period does not seem to be an important determinant for subdaily increment formation in whitefish. Under conditions of no complete darkness during the night, however, food intake may occur throughout 24 h. In this case, specific dynamic action might fluctuate in an unpredictable fashion and will probably never decrease to a level that is attained by larvae which experience several hours of complete darkness. Under these conditions, daily increments might be poorly defined due to the lack of a major decrease of specific dynamic action.

Water calcium concentration can affect otolith calcification (Wright et al. 1992), although it has yet to be shown that lower concentrations of environmental calcium result in lower visual contrast between L- and D-zones and thus in confounded otolith microstructure. The present study was specifically designed to test for a possible effect of environmental calcium concentration on daily increment formation. The results show that water calcium concentration has no influence on visual contrast of daily otolith increments or on subdaily increment formation.

Table 2
Growth of lake whitefish (*Coregonus lavaretus* L.) during 39 days in three rearing units with water of different calcium concentrations (cf. Table 1). Total length and wet weight at hatching were 11 mm and 6.5 mg. Total length (mm) values are means \pm 1 SD measured in samples of size n, while wet weight (mg) is an average of n individuals. Specific growth rate (SGR) was calculated as $100 (\ln \text{value}_2 - \ln \text{value}_1) / (t_2 - t_1)$

Age (days)	Unit 1			Unit 2			Unit 3		
	n	Total length	Wet weight	n	Total length	Wet weight	n	Total length	Wet weight
11	20	15.5 \pm 0.6	nd	20	15.8 \pm 0.6	nd	20	16.1 \pm 0.9	nd
25	20	21.6 \pm 0.7	44.1	21	22.0 \pm 0.7	44.8	20	22.3 \pm 0.8	46.7
39	22	27.8 \pm 1.5	140.3	21	27.5 \pm 1.4	137.5	21	27.8 \pm 1.3	140.9
SGR over 39 days		2.4	7.9		2.3	7.8		2.4	7.9

nd, not determined

Table 3
Lengths and widths of saccular otoliths of lake whitefish (*Coregonus lavaretus* L.) larvae, reared in waters of different calcium concentrations (cf. Table 1). At age 17 days, one sagitta from each fish was measured, while at age 31 days both sagittae were measured. Sample size was 10 fish in all cases. Values (μm) are means \pm 1 SD. Means with the same superscript do not differ significantly (χ^2 test, P = 0.05)

Age (days)	Unit 1		Unit 2		Unit 3	
	Length	Width	Length	Width	Length	Width
17	188 ^a \pm 20	158 ^b \pm 20	196 ^a \pm 15	169 ^b \pm 10	193 ^a \pm 13	163 ^b \pm 11
31	338 ^c \pm 16	254 ^{d,c} \pm 10	333 ^c \pm 30	245 ^d \pm 21	347 ^c \pm 20	258 ^c \pm 10

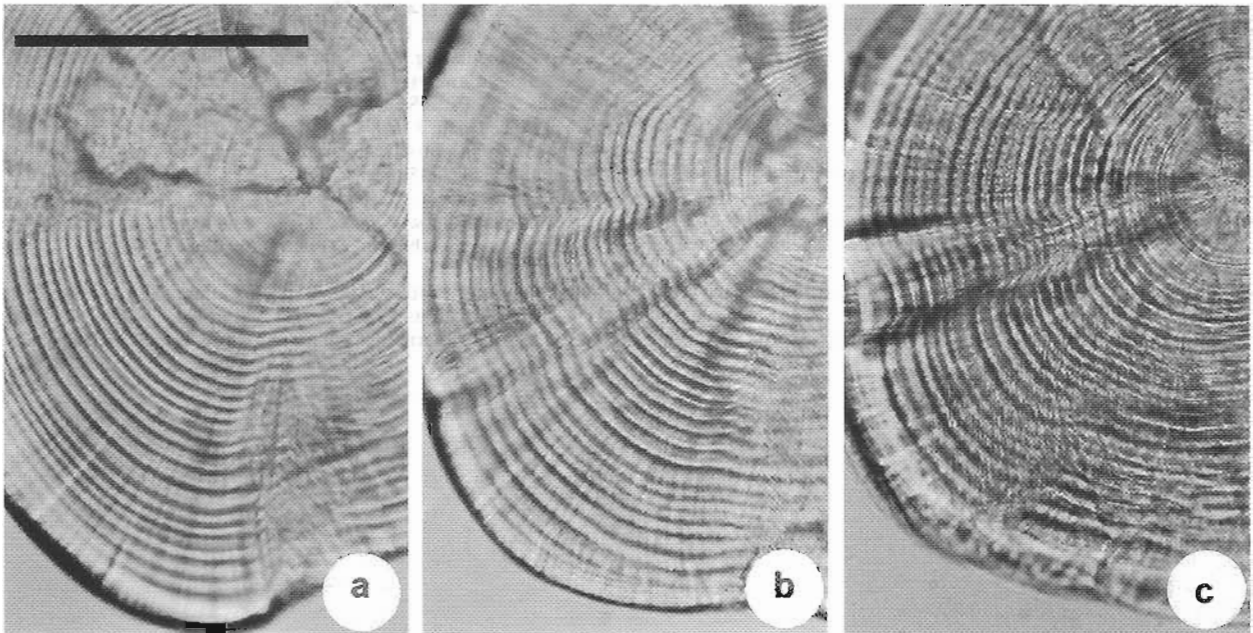


Fig. 1. Sagittae from 31-day-old lake whitefish (*Coregonus lavaretus* L.) larvae, reared in waters of different calcium concentrations (a, 0.2 mM, b, 1.3 mM, c, 4.7 mM). Sagittae were ground and polished, and photographs were taken and processed under identical conditions. Daily increments are clearly recognizable, subdaily increments are largely absent. Contrast between L- and D-zones does not depend on water calcium concentration. Bar: 100 μm

This is true at least for whitefish larvae from Lake Constance during their first weeks of life and for the range of calcium concentrations tested.

The foregoing analyses demonstrate that neither growth rate, nor feeding frequency during 12.5 of 14.5 h of daylight or water calcium content seem to affect increment formation on the

sagittae of coregonid larvae to such an extent that the recognition of daily increments becomes particularly difficult or even impossible. The only parameter whose effect on otolith increment deposition in larval coregonids has not been experimentally evaluated to date is photoperiod. Increment formation seems to be linked to an endocrine-driven, endogenous

circadian rhythm that is entrained by photoperiod (Campana and Neilson 1985). The strong influence of photoperiod on calcium deposition on the otoliths has repeatedly been demonstrated (Mugiya et al. 1981; Mugiya 1984; Wright et al. 1992). When a well-defined zeitgeber is lacking during the first days or weeks of life, a circadian rhythm of increment formation may not be entrained. Photoperiod conditions at Lake Puruvesi may have been deficient in this sense, so that a circadian rhythm of increment deposition was not entrained in the larvae studied by Huuskonen and Karjalainen (1995). The lack of a rhythmic activity pattern of whitefish around midsummer in the sub-Arctic region (Müller 1978) supports the concept that under these particular photoperiod conditions a clear zeitgeber may be lacking.

In summary, Huuskonen and Karjalainen (1995) and Huuskonen et al. (1998) have suggested four parameters that might account for the lack of clearly defined daily otolith increments in Finnish coregonids: growth rate, feeding frequency, water calcium content and photoperiod. The first three parameters appear not to be decisive and so photoperiod seems to be the single most important factor. It may influence increment deposition directly (lack of a clear zeitgeber) or indirectly via food uptake and, hence, elevated metabolic rate throughout 24 h.

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Author's address: Professor Dr. Reiner Eckmann, Limnological Institute, University of Konstanz, D-78457 Konstanz, Germany. Email: reiner.eckmann@uni-konstanz.de