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Daily and sub-daily otolith increments of larval and juvenile walleye pollock, *Theragra chalcogramma* (Pallas), as validated by alizarin complexone experiments

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Abstract

Walleye pollock (*Theragra chalcogramma*) were reared from eggs to the juvenile life stage to study daily increment formation in the sagittae otoliths, which are routinely used for age and growth analyses. The apparent deposition of sub-daily growth increments becomes problematic for determining fish age from the late larval stage throughout the juvenile (young-of-the-year) development stage. Otolith marking experiments were conducted to determine interpretation criteria to differentiate between daily and sub-daily increments. Immersion of larval and transforming walleye pollock in 25 mg/l of alizarin complexone (ALC) for 6 h once a week produced a fluorescent mark on the day of staining. Evidence of six well defined and equally spaced increments counted between the weekly ALC marks validated the deposition of daily increments. The daily increments gradually increased in width as the fish/otolith grew. The criteria for determining the presence of sub-daily increments between the daily increments were (1) weak optical definition and (2) a sudden change in incremental distance that lasted for one or two increments and were approximately <0.5 μm in width. Growth problems that occurred during the experiments were identified on otoliths as reductions in daily incremental widths and optical definition, which continued for several days. Otoliths from field-collected fish have also shown similar changes in daily increment properties during the juvenile stage, which may be an indicator of an environmental influence. The criteria for defining different increment types help to resolve our current age determination issues for late larval and early juvenile stage walleye pollock from the Gulf of Alaska. Published by Elsevier B.V.

Keywords: Walleye pollock; Otolith microstructure; Increment validation

1. Introduction

Gulf of Alaska juvenile (young-of-the-year) walleye pollock (*Theragra chalcogramma*) otoliths are, in general, easily interpreted for daily increments but occasionally the daily increments are not well defined and a potential problem with sub-daily increments makes confident age assessment difficult. Sub-daily increments have been observed in the otoliths of walleye pollock as early as the beginning of juvenile transformation (12 mm standard length (SL)). Daily otolith increment formation has been validated for laboratory-reared walleye pollock during the larval and early juvenile stages (Nishimura and Yamada, 1984; Bailey and Stehr, 1988) from hatching up to 100 days, but not to the completed juvenile stage (40 mm SL from Brown et al., 2001). From these early experiments, it was determined that the first daily increment is deposited on the lapilli and sagittae at hatching. Deposition continues on a daily basis with the increments about 1 μm apart until the yolk is mostly absorbed and exogenous feeding begins (about 5–6 days at 6 °C). Once the fish are exogenously feeding, gradual increases in the distances between the increments begin, depending on the quality of the rearing conditions (temperature, prey quality, etc.). The clarity and definition of daily increments of laboratory-reared walleye pollock otoliths are often not as distinct as those found on otoliths from wild fish. Increment definition is a common problem in laboratory rearing experiments when it is not possible to grow the fish in a true natural environment (Campana, 2001).

In the past, and without the proper validation of increment types of late larval and juvenile walleye pollock otoliths from the Gulf of Alaska, daily increments have been interpreted as having well defined and consistently spaced increments with gradual increases in incremental distances as the otolith/fish grows. The

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presence of sub-daily increments in-between daily increments was suspected when weakly defined increments were accompanied by sudden changes in increment widths. If the increment type were to be misinterpreted, then the potential for over-ageing fish at a young age was possible. This type of reading error would result in a false reporting of a slow growth rate for a cohort of fish that may have actually been growing at an average rate or higher. For this reason, a validated criterion is needed to define the differences in increment types for young-of-the-year walleye pollock so that there is consistent interpretation of the increment types during the reading process.

Daily otolith increment validation studies of other species, which either involved rearing fish from the egg stage or marking the otoliths at a known age with a fluorescent stain such as oxytetracycline (OTC) or alizarin complexone (ALC) (Tsukamoto, 1988; van der Walt and Faragher, 2003; Welsford, 2003) have proven successful for many different fish species in fresh and saltwater. These types of validation experiments have also resulted in the interpretation of sub-daily increments in otoliths of larval and juvenile fish as less defined increments with inconsistent spacing (Campana, 1984; Powell et al., 2000). Although some species have otolith increments that are not easily interpreted as daily or sub-daily, as reported by Volk et al. (1995) for pink salmon (*Oncorhynchus gorbuscha*), most daily incremental structures may be enhanced by subjecting the fish to a fluorescent stain, which is incorporated into the otolith on the day of staining. In this study, larval and juvenile walleye pollock were immersed in the fluorescent stain ALC to determine the criteria necessary to define and validate the daily versus sub-daily increments in laboratory-reared otoliths to assist in our interpretation of otolith increments from fish caught in the Gulf of Alaska.

2. Materials and methods

2.1. Specimen collection and laboratory rearing conditions

Walleye pollock adults (three females, two males) were collected from the Gulf of Alaska in March of 1992 and stripped of their gametes aboard the NOAA ship *Miller Freeman* to provide fertilized eggs for shipment to the Alaska Fisheries Science Center for rearing. The eggs were maintained at $3 \pm 0.1\,^{\circ}C$ aboard ship, and then reared at $6 \pm 0.3\,^{\circ}C$ in the laboratory in 120 L containers with a 14-h light and 10-h dark cycle to simulate conditions in the wild. The hatch date range for the eggs used for these experiments was April 14–April 16 (day of year 105–107) with 50% hatch on April 15. After first feeding, a random selection of 100 larvae was made and split into two groups and placed into 20 L rearing containers. One group was untreated and maintained under the same conditions as the group that was used for ALC experiments. Water temperature was maintained at $6\,^{\circ}C$ during the early larval stage and then slowly increased to $8 \pm 0.3\,^{\circ}C$ through the late larval and juvenile stages. Larvae were fed daily with rotifers (*Brachionus plicatilis*), and then graduated to copepod nauplii and copepodites (*Acartia* sp.) collected from a local lagoon. Freshly hatched *Artemia* sp. were fed to the growing larvae as they became capable of eating larger prey items.

2.2. ALC marking experiments and staining procedures

To determine the minimum amount of ALC needed to mark daily increments with minimal fish mortality, preliminary exposure experiments were conducted. Several concentrations of ALC were tried until a low concentration with short exposure duration produced strong marks on the otoliths on the day of marking. Fish were immersed once every 7 days in the morning hours so that the ALC would be incorporated into the increment formed on the day of marking. Fish were removed from the 20 L holding tank and immersed in the ALC in 4 L containers and monitored for mortality during the staining procedure. After each immersion period, larvae were removed and dipped several times into clean seawater to thoroughly rinse off any remaining ALC before being returned to their holding tank. The untreated fish were reared at the same temperature and food concentrations as the experimental fish, but they were not removed (except for natural mortalities) or handled in any way to allow the fish to potentially grow at an uninterrupted rate in an attempt to demonstrate "normal" growth. Fish were sampled randomly from each rearing tank once a week and preserved in 95% ethanol. All tanks were carefully monitored several times a day for dead larvae which were also preserved in 95% ethanol.

2.3. Otolith processing and photography

Otoliths were removed and mounted to microscope slides with clear acrylic nail polish. Otoliths from fish larger than 12 mm SL were processed for reading following the procedures of Brown and Bailey (1992). Briefly, otoliths were mounted in resin and ground in the sagittal plane until the nucleus and all daily increments were visible. The slides were kept in dark storage boxes in a cool, dry environment to avoid possible fading of ALC marked increments. All slides were viewed and photographed at 100×, 400×, and 1000× with transmitted light and ultraviolet (UV) light through a compound microscope to detect ALC marks on the otoliths. Otolith photographs were taken by mounting a Nikon SLR 35 mm camera loaded with tungsten light balanced film onto a Zeiss Axioscope compound microscope equipped with UV lighting. Photographs were also taken of field-caught Gulf of Alaska walleye pollock otoliths preserved at similar times (June–July) to the laboratory fish to compare increment types.

2.4. Otolith increment measurements

Only the sagittae otoliths with optimum increment definition and clarity were chosen for increment width measurements. One sagitta otolith from each fish was selected for increment measurements. Larval otoliths were read and measured along the maximum diameter axis routinely used for ageing. Due to the shape change that developing walleye pollock otoliths undergo during growth, the larval and juvenile daily increment widths could not be measured on a consistent axis from the center of the nucleus to the edge of the otolith for all otolith samples. Increment widths were measured along the ageing axis for the larval growth and then the axis shifted towards the ventral edge of the

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Table 1
Exposure experiments to determine minimum alizarin complexone (ALC) dosage for maximum survival of larval walleye pollock

Experiment	Number of fish	ALC dosage	Exposure time	Mortality percentage (%)	Rearing temperature	Marked increment intensity
1	10	Untreated	NA	0	6 °C	NA
2	10	100 mg/l	24 h	100	6 °C	No mark, died in ALC
3	10	50 mg/l	24 h	50	6 °C	Strong mark, mortality high
4	10	25 mg/l	24 h	20	6–7 °C	Strong mark
5	10	25 mg/l	6 h	0	6–7 °C	Strong mark

otolith during the juvenile growth period. The combination of these two ageing axes is the ageing path used for juvenile walleye pollock otoliths from the field, but the increment widths for the juvenile growth period would not necessarily be the maximum widths available. The maximum increment widths occur along the axis from the nucleus to the anterior tip of the otolith. Since the occurrence of sub-daily increments is a concern along the reading axis of the otoliths, the selected method for increment width measurements was the most applicable. Daily and sub-daily increments were measured using a compound microscope at 1000× with an ocular micrometer, which is divided into 1 μm intervals. Larval and juvenile otoliths have been routinely read and measured using a compound microscope instead of an image analysis system in this laboratory even though an image analysis system would have allowed for more precise measurement of the increment widths. Increments identified and measured between 1 μm intervals were recorded as <0.5 μm since the resolution limit of a compound microscope at 1000× is often questionable for measurements below 1 μm. A mean increment width was calculated for the daily increments between each ALC staining event. The increments suspected as being sub-daily were recorded as <0.5 μm and the number noted to determine if sub-daily increments were deposited on a regular basis as the otolith continued to grow.

2.5. Otolith age versus known age

Both sagittae otoliths were aged for each fish unless one was damaged or had poor increment definition. The final otolith age for each fish was recorded as the mean of five reads from each otolith by a single reader. For a fish where the otolith ages were drastically different between otoliths, two ages were recorded for the fish. To determine "normal" growth in the untreated fish, as well as in the fish that underwent ALC treatments, the following procedure was used to determine if the number of otolith increments read was a reliable estimate of age. Because the hatch range of the fish was known, it was possible to use the date of preservation to calculate a known age range. If the age of the fish was within this range, then only daily increments had been read. If the fish age was above the known age range, then sub-daily increments were included in the increment count. Otolith ages that were below the known age range were interpreted as lacking daily increment formation or the increment compression was severe enough to be below the resolution limit of the microscope.

3. Results

Preliminary staining experiments determined the concentration and duration necessary to obtain ALC marked otolith increments (Table 1). From these preliminary experiments, the optimal ALC exposure duration to immerse the fish once every 7 days was 25 mg/l for 6 h (Table 2). Weekly ALC immersions produced sagittae otoliths with six daily increments between the ALC marks (Fig. 1). Although the lapilli were marked with ALC just as strongly as the sagittae, they were not used due to the sagittae being the larger otoliths that are routinely used for age determination. From examination of the ALC otoliths, it was observed that the daily increments in-between the ALC marks were well defined and easy to interpret. The mean increment widths between the ALC marks were variable due to the differences in fish/otolith size at the dates of marking (Table 3). Fish that grew well demonstrated a gradual change in daily increment widths with progression in length-at-age and occasionally had

Table 2
Treatment and preservation dates for walleye pollock exposed to alizarin complexone (ALC) to define daily increments

Mark #	Marking date	ALC dosage (mg/l)	Start time/duration	# ALC fish	# Untreated fish	Date of preservation	Preserved SL (mm)
1	27-May-92	25	1030/6 h	48	5	27 May–2 June	4.16–10.08
2	3-June-92	25	0830/6 h	28	5	3 June–9 June	4.80–7.72
3	10-June-92	25	1040/6 h	20	5	10 June–16 June	6.56–8.32
4	17-June-92	25	1030/6 h	17	4	17 June–23 June	5.60–12.00
5	24-June-92	25	0930/6 h	10	3	24 June–30 June	8.20–20.32
6	1-July-92	25	1000/6 h	9	3	3 July	8.96
7	8-July-92	25	0900/6 h	6	3	8 July–14 July	9.76–12.48
8	15-July-92	25	0800/6 h	6	3	*	*
9	22-July-92	25	0800/6 h	6	3	28 July	31.80
10	29-July-92	25	0900/6 h	5	3	*	*

Decreasing numbers of fish available for continued weekly ALC exposure was due to periodic sampling of fish and mortality during handling. Fish were preserved in 95% ethanol. No fish sampled in time intervals noted with an asterisk (*) to allow fish to grow.

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Table 3
Mean widths of daily and sub-daily increments measured between ALC marks 1 and 2 (1–2), 2 and 3 (2–3), etc. at 1000× for larval and juvenile walleye pollock

SL (mm)	Daily 1–2	Sub-daily 1–2	Daily 2–3	Sub-daily 2–3	Daily 3–4	Sub-daily 3–4	Daily 4–5	Sub-daily 4–5	Daily 5–6	Sub-daily 5–6	Daily 6–7	Sub-daily 6–7	Daily 7–8	Sub-daily 7–8	Daily 8–9	Sub-daily 8–9	Daily 9–10	Sub-daily 9–10
6.48	0.8	None	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
6.16	0.9	None	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
4.80	1.0	None	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
8.24	1.7	None	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
8.32	1.0	<0.5 (1)	2.0	None	*	*	*	*	*	*	*	*	*	*	*	*	*	*
7.24	1.3	None	1.6	None	*	*	*	*	*	*	*	*	*	*	*	*	*	*
7.12	1.7	None	2.0	None	0.5	None	*	*	*	*	*	*	*	*	*	*	*	*
8.20	1.7	None	2.0	None	2.7	<0.5 (2)	*	*	*	*	*	*	*	*	*	*	*	*
20.32	1.7	None	2.0	None	3.0	<0.5 (1)	3.0	<0.5 (1)	*	*	*	*	*	*	*	*	*	*
11.04	1.7	<0.5 (1)	2.8	<0.5 (3)	3.7	<0.5 (2)	3.2	None	*	*	*	*	*	*	*	*	*	*
8.96	1.7	None	2.3	None	3.0	None	2.0	None	3.7	<0.5 (3)	*	*	*	*	*	*	*	*
9.76	1.8	None	2.0	None	1.9	None	1.0	None	0.5	None	*	*	*	*	*	*	*	*
14.90	1.0	None	2.0	None	2.0	None	1.0	None	1.0	None	1.0	None	1.0	None	1.0	None	1.0	None
25.70	1.0	None	2.0	None	2.0	None	3.0	None	3.0	<0.5 (3)	3.0	None	3.0	None	3.0	None	2.0	None
35.50	1.7	None	2.5	None	3.0	None	3.0	None	3.0	None	3.0	None	3.0	None	3.0	None	4.0	None

Untreated fish

SL (mm)	Comments
7.52	Daily increments 1 μm in width until first feeding mark then 2 μm, no sub-daily increments
12.00	Daily increments same as above with progression to 3 μm, 2 sub-daily increments <0.5 μm each
51.00	Daily increments same as above with progression to 4 μm, 4 sub-daily increments <0.5 μm each

Otoliths with optimum increment clarity were chosen for measurements to illustrate progression in increment widths. Units for all increment widths are in μm. The numbers in parentheses were the number of sub-daily increments observed. Otolith measurements from untreated fish are noted below.
* unavailable measurement data.

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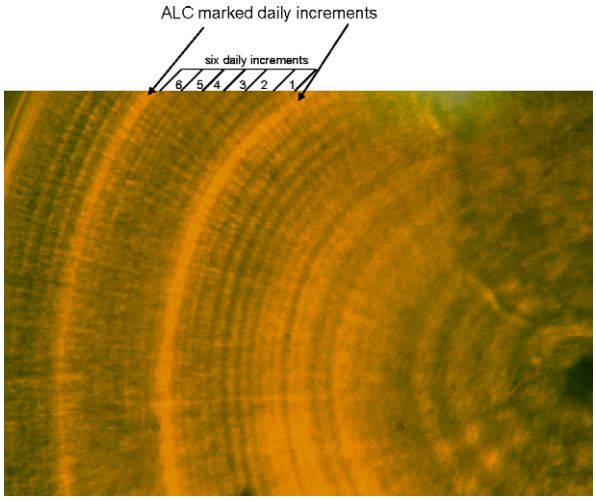

Fig. 1. ALC marked larval walleye pollock (11.04 mm SL) otolith at 1000×. Light used for photography is half transmitted and half UV. Note consistent daily increment widths between ALC increments with sub-daily increments between the daily increments.

smaller increments between the well-defined daily increments that were <0.5 μm and hence interpreted as sub-daily increments (Fig. 2). Fish that did not grow well (did not continue to increase in length) throughout the duration of the experiments had daily increments that did not increase in width with age. These fish also had daily increments widths of approximately 0.5 μm just before they died with no sub-daily increments interpreted at any place on the otoliths. ALC and untreated fish demonstrated the change in increment widths with age as a progression of daily increment widths from 1 to 4 μm to the otolith edge with the occasional sub-daily increment faintly visible at <0.5 μm between the daily increments.

The difference in optical definition of the daily and sub-daily increments was made apparent when the depth of field

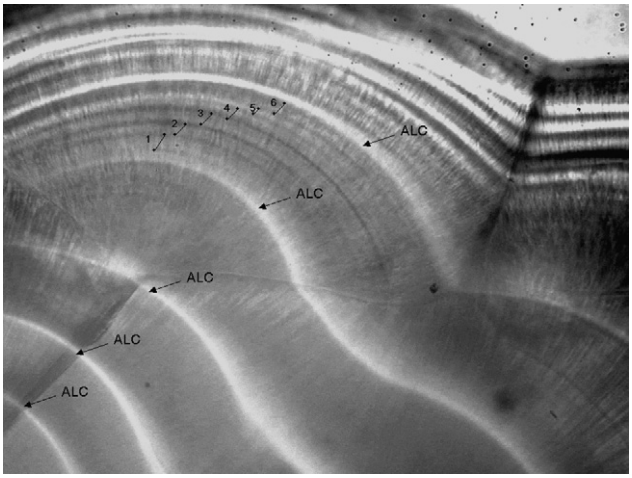

Fig. 2. ALC marked juvenile walleye pollock (35.5 mm SL) otolith at 400×. Six labeled bands highlight the daily increments with several sub-daily increments visible between the daily increments. Note changes in increment widths but optical definition is consistent between ALC marks. Focal depth and otolith shape may affect the increment widths, but not the over-all optical definition of the daily increments.

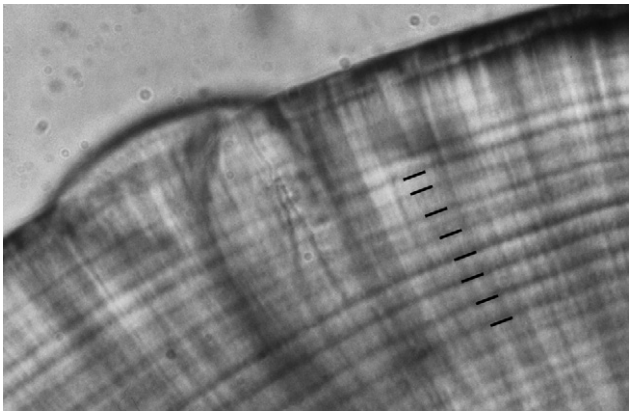

Fig. 3. Daily increments in an otolith from a transforming juvenile walleye pollock (23.5 mm SL) from the Gulf of Alaska at 1000×. Note the sub-daily increments that are barely visible between some of the well-defined and evenly spaced daily increments that are highlighted by the thick black lines.

was changed while viewing through the microscope. By focusing the objective up and down in small steps, it was possible to optically "move" the daily increments slightly due to their three-dimensional structure, but they were still visible. A slight change in the intensity of the transmitted light also helped to determine the optical definition of the increments. This was not the case for sub-daily increments. Changing the depth of field while observing these less defined increments resulted in a temporary elimination of the sub-daily increments from view. These observations were also applicable to otolith increments from fish caught in the field, which have been observed to have sub-daily increment structures that occur randomly throughout the late larval and juvenile life stages (Fig. 3).

The results from the ALC treated and untreated fish (Table 4) were used to define the types of increments that were observed for walleye pollock in laboratory conditions. Otoliths with daily increments were easily interpreted as well defined structures accompanied by reasonable fish length (mm SL), otolith lengths, and ages that were within the known age range of the fish. Sub-daily increments were not only identified by their faint definition and small increment widths, but the total age of the fish was older than expected when the sub-daily increments were included in the total increment count. Otoliths from fish that were under-aged were identified as having non-daily increments with smaller standard length and otolith length measurements as well as having incremental distances that were often tightly spaced in comparison to other otoliths. The fish that were identified as having non-daily otolith growth were considered to be from fish that were stunted. Stunted fish had otoliths with very closely spaced incremental structures just before the otolith edge. The otoliths from these fish did not have six increments visible between all ALC marks. When combinations of non-daily and sub-daily increments were observed within the same otolith, the estimated age was close to the expected age but the size of the fish and otoliths were small, which helped to confirm that there was a growth problem. This was best illustrated by two untreated fish of the same age, but their final otolith and SL sizes were drastically different (Fig. 4).

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Table 4
Walleye pollock otolith specimens from weekly alizarin complexone (ALC) experiments used to determine increment types which may interfere with accurate estimates of fish age

# ALC marks	SL (mm)	Otolith length (μm)	Known hatch range	Date preserved	Known age range	Otolith age	Increment type	Comments
1	8.80	109	105–107	149	42–44	41	Daily	Died after ALC treatment
1	10.08	135	105–107	149	42–44	43	Daily	1 increment after ALC
1	5.12	47	105–107	155	48–50	18, 25	Non-daily	Stunted
2	6.72	78	105–107	155	48–50	49	Daily	ALC on edge
2	5.52	52	105–107	156	49–51	45	Non-daily	Stunted
2	6.16	94	105–107	156	49–51	50	Daily	1 increment after ALC
Untreated	7.52	97	105–107	160	53–55	54	Daily	
2	4.8	62	105–107	161	54–56	30, 33	Non-daily	Stunted
3	8.24	150	105–107	162	55–57	57	Daily	ALC on edge
3	8.32	182	105–107	167	60–62	63	Sub-daily (1)	
3	6.56	99	105–107	167	60–62	51	Non-daily	Stunted
Untreated	12.00	275	105–107	170	63–65	66	Sub-daily (1)	
4	7.24	120	105–107	171	64–66	53	Non-daily	Stunted
4	8.20	180	105–107	176	69–71	67	Non and sub-daily (2)	Stunted
5	20.32	460	105–107	176	69–71	73	Sub-daily (2)	ALC on edge
5	11.04	275	105–107	182	75–77	83	Sub-daily (6)	
6	8.96	303	105–107	185	78–80	82	Sub-daily (3)	
6	9.76	190	105–107	190	83–85	79	Non-daily	Stunted
10	34.80	1365	105–107	216	109–111	109	Daily	
Untreated	28.20	1007	105–107	222	115–117	115	Daily	
Untreated	51.00	2500	105–107	222	115–117	120	Sub-daily (4)	
10	35.50	1400	105–107	223	116–118	116	Daily	
10	14.90	390	105–107	223	116–118	112	Non-daily	Stunted
10	25.70	930	105–107	223	116–118	121	Sub-daily (3)	

The preservation dates are listed in Julian days for 1992. Fish lengths are reported as 95% ethanol preserved standard lengths (SL). The numbers in parentheses were the number of sub-daily increments observed.

4. Discussion

Weekly exposure to 25 mg/l of ALC for 6 h produced well-marked daily increments on the day of staining in larval and juvenile walleye pollock otoliths. By marking fish every 7 days with ALC, the well-defined and uniformly spaced six increments in between the ALC marks consistently demonstrated which increments to interpret as daily. The difference between daily and sub-daily increments was made obvious by the difference in increment width and a combination of changing the depth of field and light intensity while viewing the otolith through the microscope. If an increment was <0.5 μm, less defined and temporarily disappeared while changing the depth of field, then it was identified as a sub-daily increment. When all sub-daily and daily increments were purposely counted towards the total age of the fish, this demonstrated the effect of over-ageing that sub-daily increments produce. Due to sample numbers being lower throughout the experiment than would have been ideal, statistical analyses and the reporting of such results beyond the calculation of mean increment widths would have only been useful to demonstrate the variance in increment widths between a few fish instead of a representative cohort of fish. Such a variance estimate would not have been useful to determine the difference between the natural variance in increment widths and what was potentially introduced by the individual who was reading and measuring the increments. Other potential sources of vari-

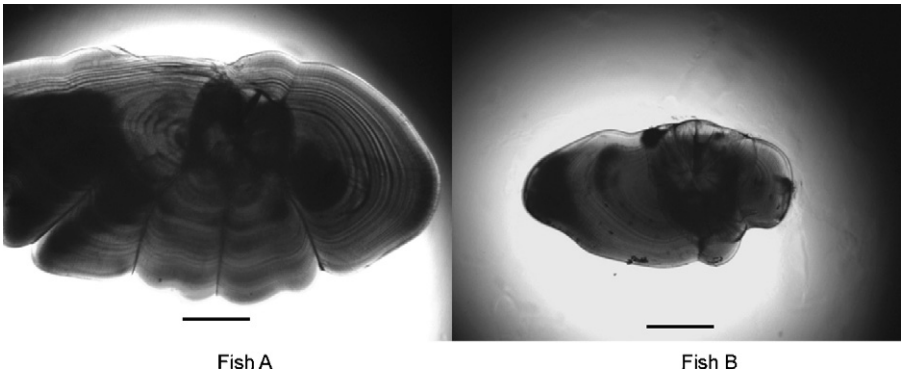

Fig. 4. Otoliths from untreated fish removed from the same rearing tank and photographed at 100× (scale bar = 0.25 mm). The fish are the same age, but development of Fish B and its otoliths were severely stunted. Fish A was 51 mm SL while Fish B was 28.2 mm SL at time of preservation.

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ability between specimens could also have been generated by the increment widths not being measured on a consistent axis for all specimens and that growth problems adversely affected the increment widths in several specimens. The low number of available specimens made it difficult to define the increment widths in terms of repeatable measurements that would be applicable to all specimens from the laboratory or the field. For these reasons, the use of increment widths to determine the difference between daily and sub-daily otolith increments should be used only as a general guide towards the final increment interpretation.

During the course of the marking experiments, several fish did not produce readable daily otolith increments or the expected length-at-age. This helped to immediately identify growth problems. The lack of daily increment formation, and hence stunting of otolith growth, was most likely a result of a poor growth environment as suggested by Taubert and Coble (1977) and Laroche et al. (1982). Geffen (1982) suggested several possible causes that would result in poor growth in the laboratory with the most applicable to this study being the need for increased food diversity that would be more similar to what would be experienced in the wild. Poor nutrition is most likely the explanation for the observed stunted growth in these experiments since growing fish in the wild would be progressing to larger prey items, such as adult copepods and euphausiids, which have higher nutritional values than freshly hatched Artemia. Another indicator of growth problems was the lack of accessory growth centers, which are the first signs of juvenile transformation for walleye pollock (Brown et al., 2001). Accessory growth centers only formed on otoliths of fish that were 20 mm SL or greater by the end of the experiments. The deposition of sub-daily increments seemed to be related to the continued health and growth potential of the fish. When a fish did not continue to grow well and the daily increment widths began to decrease, sub-daily increment deposition did not occur or was not within the resolution limit of the microscope.

A difference in increment definition between field and laboratory otolith increments is often a problem when comparing wild larvae to those reared in the laboratory (Laroche et al., 1982) and is often attributed to a lack of temperature changes that would be experienced in the wild. On occasion, larval otoliths from wild fish have been observed that have groups of daily increments that are closely spaced and less defined increments followed by incremental growth that had returned to expected distances and optical definition. These changes may indicate that the fish had entered a poor and then a more favorable environment for survival resulting in the growth rate of the fish potentially resuming or surpassing its previous rate. This suggests that compensatory growth, as reported by Sogard and Olla (2002) for juvenile walleye pollock, may be occurring at a young age. Depending on the duration of a change in food or environmental conditions, an increase in the potential for mortality and vulnerability to predation would be expected. This may be why we do not see examples of prolonged stunted growth in juvenile fish collected from the field and hence a potential problem with the occurrence of non-daily increments is not a serious concern compared to sub-daily increments when ageing juvenile pollock.

In situations where difficult to interpret otoliths are encountered from wild walleye pollock due to changes in incremental distances or optical definition, it will now be possible to classify the increments as either daily or sub-daily with more confidence. When less optically defined areas exist in the daily increment pattern (which identifies a potential growth problem), more time will have to be spent analyzing these areas or a procedure developed, such as staining or etching, to enhance the daily incremental structures. Future experiments on the effect of constant and fluctuating temperatures on increment definition would be helpful to determine if the occurrence of less defined daily increments from field otoliths are being caused by the temperature being too constant (as in laboratory conditions) within the water layer that the fish vertically migrate through to feed and avoid predators.

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