North American Journal of Fisheries Management
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ujfm20

Otolith Microstructure during the Early Life-History Stages of Brown Trout: Validation and Interpretation

Julian J. Dodson [a] , Pascal Sirois [b] , Gaétan Daigle [c] , Philippe Gaudin [d] & Agnès Bardonnet [e]

[a] Département de Biologie, Université Laval, 1045 Avenue de la Médecine, Quebec, Quebec, G1V 0A6, Canada

[b] Laboratoire des Sciences Aquatiques, Département des Sciences Fondamentales, Université du Québec à Chicoutimi, 555 Boulevard de l'Université, Chicoutimi, Quebec, G7H 2B1, Canada

[c] Département de Mathématique et de Statistique, Université Laval, 1045 Avenue de la Médecine, Quebec, Quebec, G1V 0A6, Canada

[d] Unité Mixte de Recherche Ecobiop, Université de Pau et des Pays de l'Adour, Unité de Formation et de Recherche des Sciences de la Côte Basque, Anglet, F-64600, France

[e] Institut National de Recherche Agronomique, Unité Mixte de Recherche Ecobiop, Pôle Hydrobiologie Institut National de Recherche Agronomique, St Pée-sur-Nivelle, F-64310, France

Version of record first published: 26 Jan 2013.

To cite this article: Julian J. Dodson , Pascal Sirois , Gaétan Daigle , Philippe Gaudin & Agnès Bardonnet (2013): Otolith Microstructure during the Early Life-History Stages of Brown Trout: Validation and Interpretation, North American Journal of Fisheries Management, 33:1, 108-116

To link to this article: http://dx.doi.org/10.1080/02755947.2012.746245

North American Journal of Fisheries Management 33:108–116, 2013

ISSN: 0275-5947 print / 1548-8675 online
DOI: 10.1080/02755947.2012.746245

MANAGEMENT BRIEF

Otolith Microstructure during the Early Life-History Stages of Brown Trout: Validation and Interpretation

Julian J. Dodson
Département de Biologie, Université Laval, 1045 Avenue de la Médecine, Quebec, Quebec G1V 0A6, Canada

Pascal Sirois*
Laboratoire des Sciences Aquatiques, Département des Sciences Fondamentales, Université du Québec à Chicoutimi, 555 Boulevard de l'Université, Chicoutimi, Quebec G7H 2B1, Canada

Gaétan Daigle
Département de Mathématique et de Statistique, Université Laval, 1045 Avenue de la Médecine, Quebec, Quebec G1V 0A6, Canada

Philippe Gaudin
Unité Mixte de Recherche Ecobiop, Université de Pau et des Pays de l'Adour, Unité de Formation et de Recherche des Sciences de la Côte Basque, Anglet F-64600, France

Agnès Bardonnet
Institut National de Recherche Agronomique, Unité Mixte de Recherche Ecobiop, Pôle Hydrobiologie Institut National de Recherche Agronomique, St Pée-sur-Nivelle F-64310, France

Abstract

We examined the extent to which otolith microstructure provides an accurate estimate of age, growth, and early life history transitions during the period between hatching and 1 week after emergence in Brown Trout *Salmo trutta* exposed to natural variations in ambient water temperature. All fry analyzed possessed a prominent check on the observed date of hatching. After hatching, daily growth increments were visible on sagittal otoliths. There was no evidence for the formation of an emergence check mark and no statistically significant evidence that emergence and daily temperature fluctuations interacted to form check marks. However, daily temperature fluctuations may influence the formation of check marks, largely based on an observed increase in the proportion of fish possessing checks on the days following the two largest temperature fluctuations observed during the experiment. There was no evidence that feeding or stressing emergent fish contributed to the formation of an emergence check mark. The observed proportionality of somatic and otolith growth in conjunction with daily growth increments and the formation of a prominent hatch mark provides the opportunity to back-calculate somatic length distributions and to document the hatching, dispersal, growth, and survival of the early life history stages of Brown Trout in nature.

The analysis of age and growth information contained in the otoliths of fishes has become a highly useful tool for fisheries scientists and managers that provides information about age, growth rate, life history, and recruitment in exploited fish stocks (Stevenson and Campana 1992; Panfili et al. 2002). When applied to the otoliths of the early life history stages of fishes, one can estimate the age of individuals as growth increments are typically deposited on a daily basis (Pannella 1971). In many species, the timing of major life history transitions such as hatching, first feeding, or metamorphosis can be determined, because such transitions are often accompanied by the deposition of distinctive increments, or check marks, within otoliths. Furthermore, individual growth trajectories may be reconstructed from the relative spacing between growth increments as there is generally a proportional relationship between the growth

*Corresponding author: pascal_sirois@uqac.ca
Received May 3, 2012; accepted October 29, 2012

of the otolith and the somatic growth of the fish (Campana 1990).

The interpretation of otolith microstructure relies on two assumptions. Firstly, deposition rates of increments must be of regular and known periodicity, and secondly, the back-calculation of growth rates assumes that there is a proportional relationship between growth of the otolith and somatic growth of the fish (Campana and Jones 1992). Previous studies on salmonids have demonstrated that otolith microstructure provides a reliable record of age between hatching and shortly after emergence in Arctic Char *Salvelinus alpinus* and Brown Trout *Salmo trutta* (Mosegaard and Titus 1987), Atlantic Salmon *S. salar* (Meekan et al. 1998), and Chinook Salmon *Oncorhynchus tshawytscha* (Neilson and Geen 1982). On the other hand, several authors have attempted to demonstrate that the relationship between otolith and fish size at the time of emergence is weak or absent (Arctic Char, Mosegaard et al. 1988; Brown Trout, Titus and Mosegaard 1991; Atlantic Salmon, Wright et al. 1990; Metcalfe et al. 1992). The lack of correlation between otolith and fish size at emergence is quite different from that observed only a few weeks later, when there is a significant concordance between otolith and fish size (Titus and Mosegaard 1991; Wright et al. 1991; Metcalfe et al. 1992).

To account for this apparent contradiction, some workers have hypothesized that variation in otolith size reflects differences in metabolic rate among individuals, so that fish with high metabolic rates tend to have larger otoliths, but are not necessarily larger in somatic size (Mosegaard 1990; Titus and Mosegaard 1991; Metcalfe et al. 1992). As individuals with higher metabolic rates grow faster, otolith size and fish size become correlated once relatively large size differences develop after a few weeks of life in the stream habitat. The hypothesized uncoupling of otolith and fish growth early in life proposed by these studies has done little to encourage the use of otolith microstructure in reconstructing early life history events in salmonids.

Alternatively, the weak relationship between otolith and fish size at emergence recorded in previous studies may simply be an artifact of measurement error and the truncation of size ranges in regression analyses associated with taking brief "snapshots" at some specific point in time along the developmental trajectory (Meekan et al. 1998). These latter authors examined the development of the relationship between otolith size and body size in Atlantic Salmon between hatching and emergence by repeatedly measuring individual fish. Weak but significant linear regressions were found between the back-calculated radius of the sagittal otoliths and standard length (SL) of alevins measured on five separate occasions. However, when pooled among sampling times, these variables were highly correlated, with variation in otolith size explaining almost all of the variation in SL of alevins (Meekan et al. 1998). Thus, the examination of otolith microstructure provides the means to reconstruct the growth history of individual fish since hatching and to back-calculate somatic length distributions at otolith check marks associated with known life history transitions or environmental effects.

Although the Brown Trout is chiefly a European species, it has been widely spread by humans. Self-sustaining populations have been established in 26 countries beyond the species' native range. In North America, introductions began in the late 19th century, and naturalized populations now occur in 34 of the 50 states of the USA and in 9 of the 10 provinces of Canada (Jonsson and Jonsson 2011). Because of its role in reducing native fish populations (particularly those of other salmonids), the Brown Trout is considered as one of the "100 worst invasive alien species" by the Invasive Species Specialist Group (Lowe et al. 2000). In North America, the ecology of the early life history stages of Brown Trout is poorly understood, and such basic information is necessary for the management of this important alien species. More specifically, the ability to back-calculate size at age, based on validated otolith microstructure, during the weeks and months after emergence provides estimates of growth rate and a measure of competitive success in the presence of sympatric native salmonids. Back-calculated growth trajectories also provide estimates of size-selective mortality and the development of alternative life history tactics (Aubin-Horth et al. 2005). Size at age early in life is an important determinant of whether salmonids remain resident in freshwater streams or adopt the migratory life style (Aubin-Horth et al. 2005), a life history decision of critical importance in predicting the long-distance dispersal capabilities of invasive salmonids (Thibault et al. 2010).

To promote the utility of exploiting otolith microstructure in understanding the ecology of the early life history stages of Brown Trout, we examined the extent to which otoliths provide an accurate estimate of age, growth, and early life history transitions during the period between hatching and 1 week after emergence in fish exposed to natural variations in ambient temperature. A previous attempt at validating the microstructure of the otoliths of Brown Trout alevins under controlled temperature conditions reported daily growth increments, but provided no supporting data, and concluded that otolith growth rate was not coupled to somatic growth rate, when expressed as wet mass (Mosegaard and Titus 1987). In the present experiment, we aimed to validate (1) the formation of a hatch mark by associating known dates of hatching with the formation of distinctive increments (check marks), (2) daily deposition by comparing counts of increments within otoliths with the known age of individuals, (3) the formation of an emergence mark by associating known dates of emergence with the formation of check marks, (4) that exogenous feeding and experimental manipulation contribute to the formation of the emergence check mark, (5) that fluctuations in ambient water temperature influence the formation of check marks, because strong daily temperature fluctuations are known to generate check marks on otoliths (Volk et al. 1999), and (6) the proportionality of somatic and otolith growth in Brown Trout.

METHODS

Laboratory Protocol

Experimental group 1.—To identify the hatch mark (objective 1), validate daily increment deposition between hatching and emergence (objective 2), and validate the proportionality of somatic and otolith growth between hatching and emergence (objective 6), eyed embryos were obtained from the controlled crosses of two female and five male Brown Trout obtained from Bertiz River, a tributary of the Bidassoa River in northwestern Spain (1°37′W, 43°10′N); date of fertilization was December 13, 2007. Several hours after fertilization, eggs were transported to the Lapitxuri field station (Station d'hydrobiologie INRA, Saint-Pée-sur-Nivelle) located on a tributary of the Nivelle River in southwestern France (1°29′W, 43°17′N). The eggs were incubated together in a vertical flow incubator until the eyed stage and then in stream channels. Both structures were supplied with water diverted from the Lapitxuri Brook. Temperature thus mirrored the natural temperature regime. Photoperiod was adjusted to follow the natural photoperiod (10 h light :14 h dark until mid-February, and then 11 h light : 13 h dark). On January 18, 2008, we placed 25 eyed embryos in each of four small incubators (10 cm height, 6.3 cm in diameter; filled with gravel) that were buried in the gravel of two parallel stream channels (50 cm wide, 3 m long). Fish could not emerge from these incubators. One incubator was removed every 7 d posthatch (February 5, 12, 19, and 26, 2008), and alevins were sacrificed to identify the hatch mark and to validate daily growth increments and the proportionality of somatic and otolith growth between hatching and emergence. A sample of 25 eyed embryos was held in a control incubator located in a third channel, free of gravel, and monitored daily to record the date of hatching, and hence, the beginning of the sampling schedule of the four additional incubators. These embryos all hatched on January 30, 2008, following 12 d of incubation in the stream tank.

Experimental group 2.—To validate daily increment deposition after emergence (objective 2), validate the formation of an emergence mark (objective 3), validate that fluctuations in ambient water temperature influence the formation of check marks (objective 5), and extend the relationship between otolith and fish size to include postemergent fry (objective 6), a second experimental protocol was established. On January 18, 2008, 345 Brown Trout eyed embryos were placed in two incubators (20 cm in height, 8 cm in diameter) buried in gravel in two tanks supplied continuously with the same water as the stream channels. Incubators were equipped with emergence traps such that newly emerged fry could leave the gravel but were retained for daily sampling. The emergence traps were monitored daily until no emergence was observed for seven consecutive days.

Emergence of fry started on February 28, 2008. The last fish emerged on March 5. All fry sampled at emergence were held for a period of 7 d in incubators without food before being sacrificed to ensure that any mark laid down at emergence was succeeded by seven daily growth increments. The last fry was sacrificed on March 11. These fish thus provided a temporal sequence of emergence over a period of 8 d.

To validate that exogenous feeding and experimental manipulation contribute to the formation of the emergence check mark (objective 4), two experiments were conducted. On February 29, 60 emergent fry from experimental group 2 were divided into two groups of 30 and held in basins located within the stream channels. One group was fed ad libitum for a period of 7 d with live *Daphnia* sp. The second group was not fed over the 7-d period. We predicted that the stress of the transition from endogenous to exogenous feeding would accentuate the emergence check mark. On March 1, 100 emergent fry were divided into two groups of 50 and held in separate basins for 7 d. Group 1 was subjected to stress by subjecting them to a 15-min period of anesthetization (with phenoxyethanol), individual manipulation with forceps, and transfer to a petri dish for recovery before being moved to the holding basin. The second group was transferred directly to the holding basin with no additional manipulation. We predicted that the stress of handling would accentuate the emergence check mark.

Before being sacrificed, all fish were anesthetized with phenoxyethanol in a small petri dish, photographed with a Canon S70 camera connected to a Leica M76 binocular microscope along with a millimeter scale, and then placed in 95% ethanol in microcentrifuge tubes for subsequent otolith extraction. Fish were measured to the nearest 0.01 mm using the Image J (version 1.42) software prior to fixation. Preserved alevins were measured once again before otolith extraction.

Otolith Microstructure Analysis

Sagittal otoliths were removed from the pre- and postemergent fry under a dissecting microscope using fine forceps. Otoliths were mounted on a microscope slide with thermoplastic glue and those of larger individuals (postemergent) were polished with a 3- or 5-μm lapping film. Otoliths were measured using an image-analysis system (SigmaScanPro 5.0) connected to a light microscope at 400 × to 1,000 × magnification. Because of the presence of several accessory primordia, measurements were taken along a postrostral axis and included the hatch mark radius (μm), otolith postrostral radius (μm), and increment counts (Figure 1). All otoliths were analyzed twice by the same reader at an interval of >1 month, and each count estimate was ranked according to the confidence of the reading. If the CV (SD/mean × 100) in the number of increments counted between the first and the second reading did not exceed 10%, the single best increment estimate was used as the age estimation (Campana and Jones 1992).

Statistical Analysis

The formation of daily increments was validated by regressing the number of days after the date of hatching (calendar age) with the number of increments distal to the hatch mark (estimated age) and then comparing the intercept and the slope of the relationship to a value of 0 and 1, respectively, using the

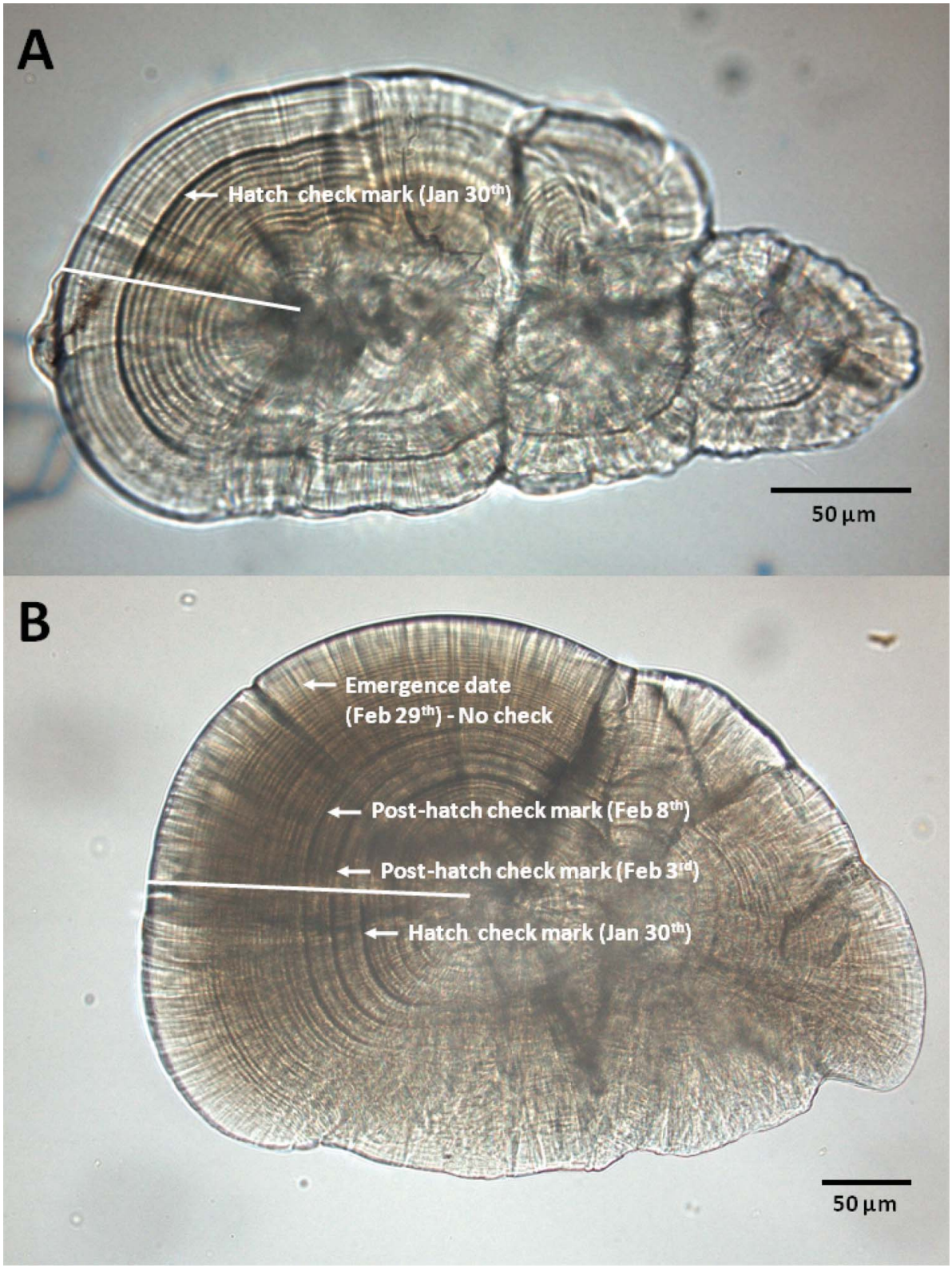

FIGURE 1. The sagittal otoliths of a Brown Trout fry aged (**A**) 7 d posthatch (400 × magnification) and (**B**) 37 d posthatch (200 × magnification). The hatch mark (January 30, 2008) is indicated on both otoliths. The horizontal line on both otoliths represents the postrostral radius. [Figure available in color online.]

usual *t*-test statistics on regression parameters. Each of these two statistical tests followed a Student distribution with $n - 2$ degrees of freedom, where n is equal to the sample size.

From the same regression model, confidence intervals (CIs) for the mean value and prediction intervals for a new observation were calculated. This was done first to estimate the calendar age from a specific number of growth increments on the otolith. In this case, the confidence and prediction intervals are related to variation on the *y*-axis. This was also done to estimate the age read on otoliths from a specific calendar age, and in that case, the confidence and prediction intervals are related to variation on the *x*-axis. The latter estimates were calculated using the inverse regression technique (Neter et al. 1985).

Embryos in the control incubator hatched on January 30 and all analyzed fry had a prominent otolith check mark on this date. Thus, we assumed that the hatch mark of all analyzed fish corresponded to January 30 $\pm$ the calculated margin of error (see below). As numerous other check marks were observed, we assessed the contribution of emergence and daily temperature fluctuations to the formation of these check marks by calculating the proportion of fish possessing a check mark between the day after hatching and the end of the experiment 42 d later (January 31 to March 12). To do so, we employed a logistic regression model. The response was a binary variable corresponding to the presence (1) or absence (0) of a check mark on each fish and for each of the 42 d of the experiment. However, because check formation on any given day was not independent of check formation on previous days, the generalized estimating equation (GEE) estimation approach was used with an autoregressive correlation structure. Two independent variables were considered in this model: the date of emergence was entered as a binary variable (date of emergence scored as 1, all other dates scored as 0) and daily temperature fluctuations were entered as a continuous variable (maximum daily temperature minus minimum daily temperature). As the estimated age was not without error (see Results), we used another GEE logistic regression model with a new binary response variable corresponding to the presence (1) or absence (0) of at least one check mark on each fish for each day of the experiment $\pm$ 2 d. The first and the last 2 d of the experiment were removed for this analysis to take into account the 2-d margin of error. Thus, the time series for this model was 38 d long, beginning on February 2 and ending on March 10.

We also used χ^2 tests to test the hypothesis that a greater proportion of fed (or stressed) fish possessed a check mark on the date of emergence (or the date of emergence $\pm$ 2 d) than the proportion of unfed (or unstressed) fish possessing a check mark on the date of emergence (or the date of emergence $\pm$ 2 d). To ensure that fish provided with food in fact did feed, we compared the wet mass of unfed fish and fed fish after 7 d of exposure to food using a *t*-test with unequal variances. Finally, we tested the proportionality of somatic and otolith growth by regressing log SL on log postrostral radius of the otolith. All of the foregoing statistical analyses were conducted with SAS (SAS version 9.2; SAS Institute, Cary, North Carolina).

RESULTS

Brown trout eyed embryos were transferred to the stream channels on January 18, 2008, at a temperature of 10.5°C (Figure 2). Temperatures subsequently fluctuated around a mean of 9.4°C (SD = 1.2), with several daily fluctuations surpassing 2°C.

Validation of Hatch Marks and Check Formation

All embryos held in the control incubator hatched on January 30, 2008. A total of 249 otoliths were examined but 57 (23%) were rejected due to their unreadability or to the 10% criterion. The remaining 192 fry all possessed a prominent check on January 30 that we assumed to correspond to the hatch mark (Figures 1, 3). The hatch mark occurred at a postrostral radius of between 60 and 75 μm in 80% of the fish examined (Figure 4). A variable proportion of fry possessed check marks throughout the duration of the experiment, and a large proportion possessed check marks shortly after the transfer of eyed embryos to the stream tanks 12 d before hatching (Figure 3).

Validation of Daily Increment Deposition

After hatching, daily growth increments were detected on sagittal otoliths (Figure 5). The relationship between the number of growth increments and known age was significant ($r^2 = 0.99$). The intercept was not significantly different from 0 ($t = -0.47$, df = 190, $P = 0.638$). The slope, however, was significantly different from a slope of 1 ($t = 2.57$, df = 190, $P = 0.011$). Age based on the otolith growth increments slightly underestimated the true age after fry were 6 d old. However, the 95% CI for the estimation of the mean of calendar age varied from ± 0.164 to ± 0.357 d and in all cases was less than 1 d for the duration of the experiment.

Emergence, Temperature Fluctuations, and Check Formation

Emergence occurred between February 28 and March 5, but there was no evidence for the formation of an emergence check mark (Table 1). Furthermore, there was little evidence that daily temperature fluctuations influenced the formation of check marks, or that emergence and daily temperature fluctuations interacted to form check marks (Table 2). Considering the margin of error of ± 2 d in the estimation of a new observation for calendar age (Table 1), our analyses revealed that 37.6% (SE = 3.2%) of fry possessed check marks during emergence compared with 42.9% (SE = 1.2%) of fish with check marks formed on all other days between hatching and the end of the experiment, but excluding the dates of emergence (± 2 d). If we exclude the margin of error, 7.6% (SE = 2.8%) of fry possessed check marks formed during emergence compared with 12.8% (SE = 0.4%) of fish with check marks formed on days excluding the dates of emergence (Table 1).

When including the margin of error, daily temperature fluctuations appeared to have influenced the formation of check marks, although the *P*-value was marginally nonsignificant ($P = 0.087$; Table 2). This relationship appears to be largely related

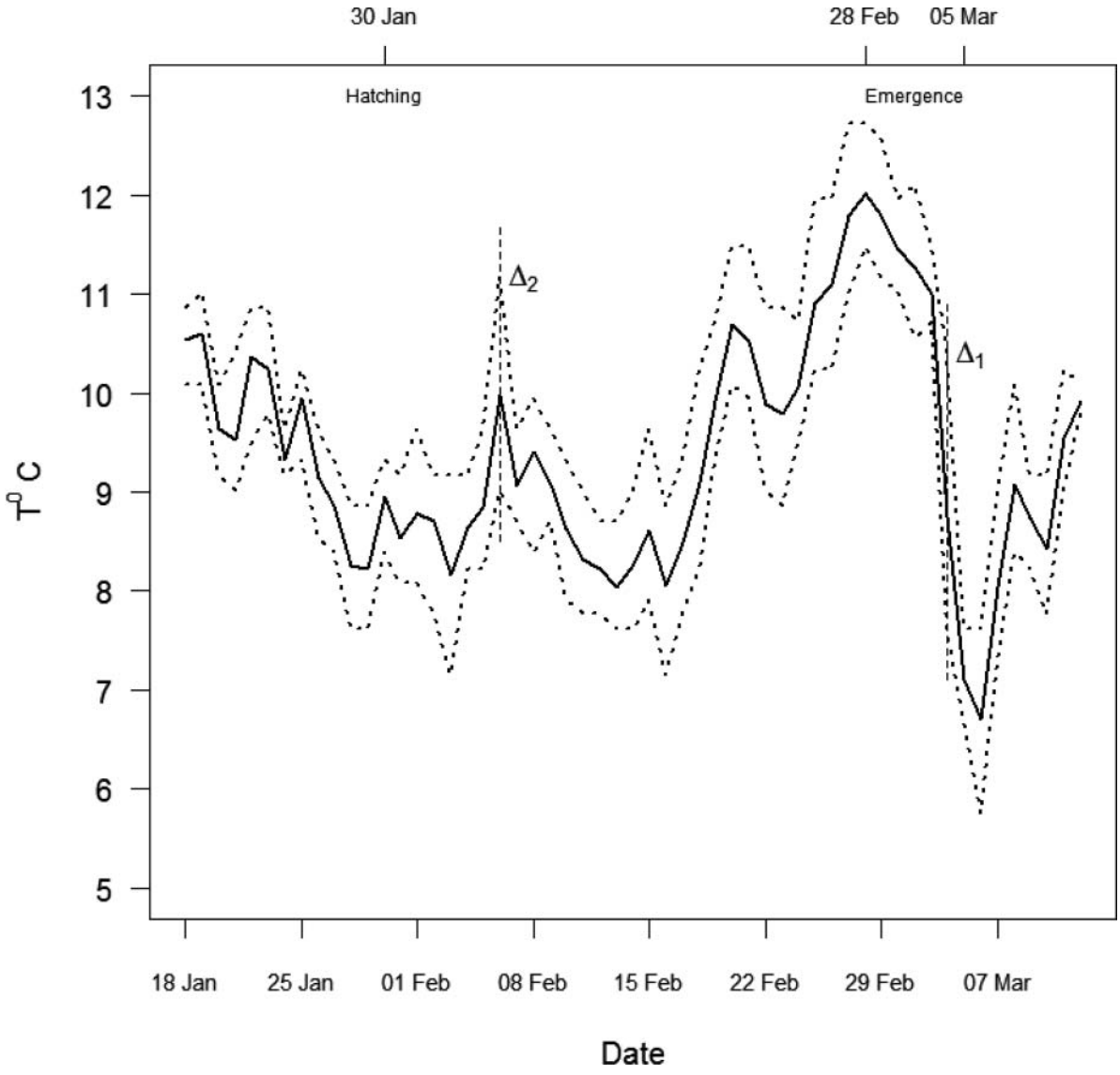

FIGURE 2. Mean (middle solid line), maximum (upper dotted line), and minimum (lower dotted line) daily water temperatures measured during the experiment. The date of hatching (January 30) and the dates of emergence (February 28–March 5) are indicated. Δ_1 = the largest daily temperature fluctuation observed (maximum − minimum = 2.8°C) recorded on March 4, 2008. Δ_2 = the second largest daily temperature fluctuation observed (maximum − minimum = 2.2°C) recorded on February 6, 2008.

to an increase in the proportion of fish possessing checks on the days following the two largest temperature fluctuations observed during the experiment (Δ_1 and Δ_2, Figure 2; Figure 3). However, the paucity of large daily temperature fluctuations during the course of the experiment weakens the statistical power of the regression analysis to adequately estimate the significance of this relationship.

There was no evidence that feeding or stressing emergent fish contributed to the formation of an emergence check mark. Although a significant increase in wet mass occurred among fry fed for 7 d (unfed fry [$n = 30$] mean wet mass = 0.119 g [SE = 0.001 g]; fed fry [$n = 30$] mean wet mass = 0.139 g [SE = 0.003 g]; $t = -6.19$, df = 41.45, $P < 0.0001$), no significant difference was observed in the proportion of fed and unfed fry possessing check marks on the date of emergence (excluding the margin of error: $\chi^2 = 1.00$, df = 1, $P = 0.316$; including the margin of error: $\chi^2 = 0.048$, df = 1, $P = 0.826$). Similarly, no significant difference was observed in the proportion of stressed and unstressed fry possessing check marks on the date of emergence (excluding the margin of error: $\chi^2 = 0.0$, df = 1, $P = 1.0$; including the margin of error: $\chi^2 = 0.186$, df = 1, $P = 0.666$).

Proportionality of Somatic and Otolith Growth

There was a significant relationship between postrostral length and SL of the fish during the course of the experiment (Figure 6). Variation in natural log otolith size explained 89%

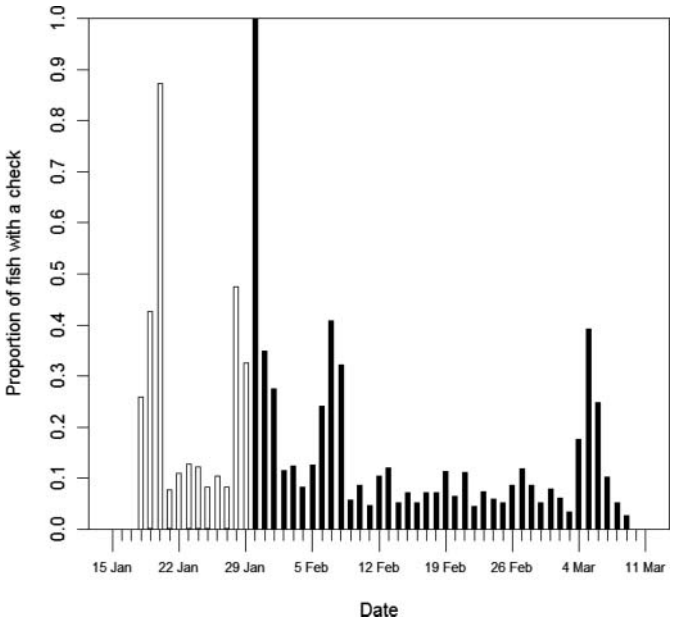

FIGURE 3. The proportion of Brown Trout fry possessing daily otolith check marks throughout the duration of the study. Hatching occurred on January 30, 2008. Open bars identify the prehatching period (embryos were transferred to the laboratory on January 18, 2008). Filled bars identify the posthatching period.

of the variation in the natural log SL of Brown Trout between 1 and 42 d of age.

DISCUSSION

The otoliths of pre- and postemergent Brown Trout provide the means to reconstruct the growth history of individual fish and to back-calculate somatic length distributions. Hatching was marked by a strong check mark on the otoliths of all fish and growth increments were formed on a daily basis thereafter, permitting the age determination of Brown Trout fry and the determination of hatching date of fry captured in nature. There was also evidence that daily growth increments were formed prior to hatching, with prominent check marks forming in the days after the transfer of eyed embryos to the stream tanks (Figure 3). The proportionality of somatic and otolith growth provides the opportunity to back-calculate somatic length distributions and reconstruct individual growth trajectories early in the life of Brown Trout using such back-calculation models as the biological intercept method (Campana 1990) or the time-varying growth method (Sirois et al. 1998).

Surprisingly, we failed to validate the formation of an emergence check mark. The proportion of fish with check marks formed on the day of emergence was no different from the proportion of fish exhibiting check marks outside the period of emergence. The manipulations designed to accentuate the emergence mark (feeding and stressing fish at emergence) failed to generate consistent check marks on the otolith. However, a precipitous drop in temperature that occurred at the end of the period of emergence (starting on March 4, Figure 2) coincided with an increase in the percentage of fish with emergent check marks to between 50% and 60% (Table 1). Therefore, a strong temperature fluctuation may have coincided with emergence on these dates to create the appearance of an emergence check mark. A similar increase in the proportion of fish with a check

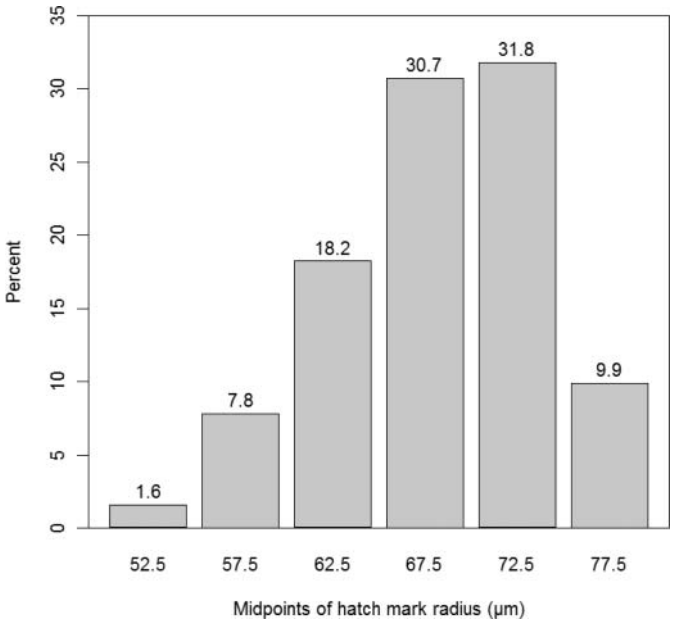

FIGURE 4. The distribution of hatch marks along the postrostral radius of Brown Trout otoliths. Numbers above bars represent actual percentage values.

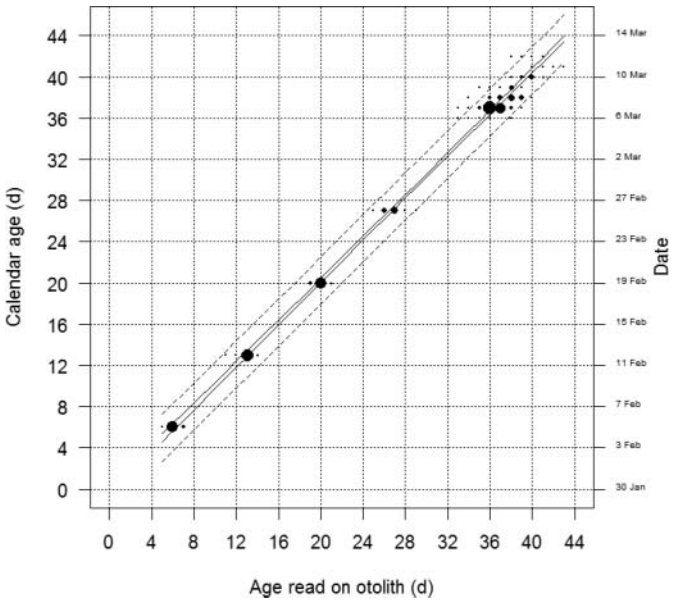

FIGURE 5. Relationship between the number of days since hatching (calendar age and date) and the number of otolith growth increments (age read on otolith) for Brown Trout fry ($n = 192$). Solid lines define the 95% confidence interval for the estimation of the mean of calendar age and the dashed lines define the 95% prediction interval. Diameter of data points is proportional to the number of observations.

TABLE 1. The mean proportion of Brown Trout fry possessing check marks on the date of their emergence and on the date of their emergence ± 2 d. The mean proportion for all emergence dates combined is a weighted mean, accounting for variation in sample sizes among dates of emergence.

Emergence date (day/month/year)	*N*	Mean proportion	SD	SE
On date of emergence				
28/02/08	6	0.167	0.373	0.152
29/02/08[a]	39	0.103	0.303	0.049
01/03/08[b]	30	0.067	0.249	0.046
02/03/08	12	0.000	0	0
03/03/08	16	0.063	0.242	0.061
04/03/08	6	0.000	0	0
05/03/08	5	0.600	0.490	0.219
All emergence dates	114	0.096	0.295	0.028
On date of emergence ± 2 d				
28/02/08	6	0.333	0.471	0.152
29/02/08[a]	39	0.359	0.480	0.049
01/03/08[b]	30	0.233	0.423	0.046
02/03/08	12	0.333	0.471	0
03/03/08	16	0.500	0.5	0.061
04/03/08	6	0.500	0.5	0
05/03/08	5	0.600	0.490	0.219
All emergence dates	114	0.360	0.480	0.045

[a]Includes fed and unfed fry.
[b]Includes stressed and unstressed fry.

TABLE 2. Results of the GEE logistic regression model to relate check formation with emergence date of Brown Trout and daily temperature fluctuations. Two regression models are presented: one with the margin of error of the prediction of a new observation of calendar age included (check marks on day *i* ± 2 d), and one without (check marks on day *i*). *Z* = results of *Z*-test, max–min = difference between the maximum and minimum recorded temperature, emerge = date of emergence, max–min × emerge = interaction between daily temperature fluctuation and emergence.

Parameter	Estimate	SE	*Z*	*P*
Margin of error not included				
Intercept	−1.887	0.178	−10.63	<0.0001
Max–min	−0.022	0.115	−0.19	0.846
Emerge	0.975	0.858	1.14	0.256
Max–min × emerge	−1.052	0.707	−1.49	0.137
Margin of error included				
Intercept	−0.384	0.071	−5.41	<0.0001
Max–min	0.065	0.038	1.71	0.087
Emerge	0.282	0.342	0.83	0.409
Max–min × emerge	−0.340	0.263	−1.29	0.197

mark occurred a day after the second most important daily temperature fluctuation, leading to the marginally nonsignificant relationship revealed by the regression analysis (Table 2).

The coincidence of emergence and strong daily temperature fluctuations may explain the apparent contradiction between the present results and those reported by Titus and Mosegaard (1991). Those authors reported the formation of an emergence check mark during an experiment with Brown Trout fry. During their experiment, however, fish were held at 4°C as preemergence-stage fry and then acclimated to a temperature of 14°C at the time of emergence. A temperature increase of 10°C at the time of emergence most probably contributed to the formation of the check mark. Such high temperature changes induce more distinguishable check marks than the smaller temperature changes (on the order of 2°C) recorded here for Brown Trout and for other salmonids (Volk et al. 1999).

The susceptibility of Brown Trout otoliths to form reliable thermal otolith check marks needs validation, but this is a promising avenue of pursuit as the use of thermal marking is a simple and relatively unobtrusive way of marking large numbers of fish. Patterns of light and dark bands akin to bar codes can be created on the otolith by exposing fish to relatively short periods of cold or warm water relative to ambient temperature (Volk et al. 1999). The technique has been used successfully in Atlantic Salmon (e.g., Letcher and Terrick 1998; Aubin-Horth and Dodson 2002), but not in Brown Trout to the best of our knowledge. Brown Trout have been the subject of several recent studies using tetracycline antibiotics (e.g., Meerbeek and Bettoli 2005) and Alizarin fluorochrome compounds (e.g., Baer and Rösch 2008) for marking large numbers of young fish, but the use of such compounds are either restricted or banned in many European and North American jurisdictions. Thermal marking is free of such concerns and, in combination with microstructure analysis, has the potential to be a useful tool for studying the hatching, dispersal, growth, and survival of the early life history stages of Brown Trout.

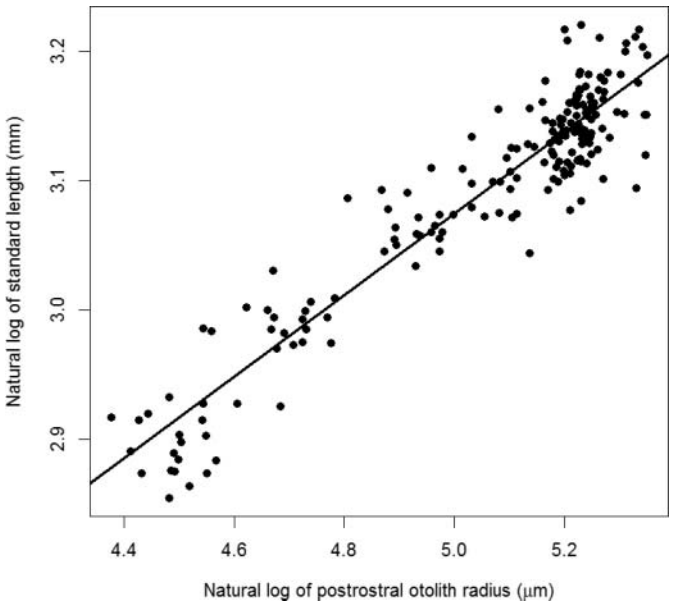

FIGURE 6. The proportionality of somatic and otolith growth in Brown Trout fry. A simple regression relates the natural log of postrostral otolith radius to the natural log of standard length: $y = 0.316x + 1.495$, $n = 192$, $r^2 = 0.89$.

ACKNOWLEDGMENTS

This experiment was conducted during the senior author's sabbatical leave at the Station d'hydrobiologie, Pole Hydrobiologie INRA, UMR Ecobiop, St Pée-sur-Nivelle, France. We thank Anne-Lise Fortin and Isabelle Poirier, Université du Québec à Chicoutimi, for otolith microstructure analysis and Stéphane Glise and François Guéraud, Station d'hydrobiologie, INRA, for the experimental design and egg production.

REFERENCES

Aubin-Horth, N., and J. J. Dodson. 2002. Impact of differential energy allocation in Atlantic Salmon (*Salmo salar*) precocious males on otolith-somatic size proportionality: a longitudinal approach. Canadian Journal of Fisheries and Aquatic Sciences 59:1575–1583.

Aubin-Horth, N., D. A. J. Ryan, S. P. Good, and J. J. Dodson. 2005. Balancing selection on size: effects on the incidence of an alternative reproductive tactic. Evolutionary Ecology Research 7:1171–1182.

Baer, J., and R. Rösch. 2008. Mass-marking of Brown Trout (*Salmo trutta* L.) larvae by alizarin: method and evaluation of stocking. Journal of Applied Ichthyology 24:44–49.

Campana, S. E. 1990. How reliable are growth back-calculations based on otoliths? Canadian Journal of Fisheries and Aquatic Sciences 47:2219–2227.

Campana, S. E., and C. M. Jones. 1992. Analysis of otolith microstructure data. Pages 73–100 *in* D. K. Stevenson and S. E. Campana, editors. Otolith microstructure examination and analysis. Canadian Special Publication of Fisheries and Aquatic Sciences 117.

Jonsson, B., and N. Jonsson. 2011. Ecology of Atlantic Salmon and Brown Trout: habitat as a template for life histories. Springer, New York.

Letcher, B. H., and T. D. Terrick. 1998. Thermal marking of Atlantic Salmon otoliths. North American Journal of Fisheries Management 18:406–410.

Lowe, S., M. Browne, S. Boudjelas, and M. De Poorter. 2000. 100 of the world's worst invasive alien species: a selection from the global invasive species database. Invasive Species Specialist Group, Auckland, New Zealand.

Meekan, M. G., J. J. Dodson, S. P. Good, and D. A. J. Ryan. 1998. Otolith and fish size relationships, measurement error, and size-selective mortality during the early life of Atlantic Salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 55:1663–1673.

Meerbeek, J. R., and P. W. Bettoli. 2005. Mass-marking Rainbow Trout and Brown Trout fingerlings with oxytetracycline. Journal of Freshwater Ecology 20:555–559.

Metcalfe, N. B., P. J. Wright, and J. E. Thorpe. 1992. Relationships between social status, otolith size at first feeding and subsequent growth in Atlantic Salmon (*Salmo salar*). Journal of Animal Ecology 61:585–589.

Mosegaard, H. 1990. What is reflected by otolith size at emergence: a reevaluation of the results. Canadian Journal of Fisheries and Aquatic Sciences 47:225–228.

Mosegaard, H., H. Svedäng, and K. Taberman. 1988. Uncoupling of somatic and otolith growth rates in Arctic Char (*Salvelinus alpinus*) as an effect of differences in temperature response. Canadian Journal of Fisheries and Aquatic Sciences 45:1514–1524.

Mosegaard, H., and R. Titus. 1987. Daily growth rates of otoliths in yolk sac fry of two salmonid species at five different temperatures. Pages 221–227 *in* S. O. Kullander and B. Farnholm, editors. Proceedings of the V congress of European ichthyologists. Swedish Museum of Natural History, Stockholm.

Neilson, J. D., and G. H. Geen. 1982. Otoliths of Chinook Salmon (*Oncorhynchus tshawytscha*): daily growth increments and factors influencing their production. Canadian Journal of Fisheries and Aquatic Sciences 39:1340–1347.

Neter, J., W. Wasserman, and M. H. Kutner. 1985. Applied linear statistical models, 2nd edition. Irwin, Homewood, Illinois.

Panfili, J., H. de Pontual, H. Troadec, and P. J. Wright, editors. 2002. Manuel de sclérochronologie des poissons. Ifremer-IRD, Brest, France.

Pannella, G. 1971. Fish otoliths: daily growth layers and periodical patterns. Science 173:1124–1127.

Sirois, P., F. Lecomte, and J. J. Dodson. 1998. An otolith-based back-calculation method to account for time-varying growth rate in Rainbow Smelt (*Osmerus mordax*) larvae. Canadian Journal of Fisheries and Aquatic Sciences 55:2662–2671.

Stevenson, D. K., and S. E. Campana, editors. 1992. Otolith microstructure examination and analysis. Canadian Special Publication of Fisheries and Aquatic Sciences 117.

Thibault, I., R. D. Hedger, J. J. Dodson, J. C. Shiao, Y. Iizuka, and W. N. Tzeng. 2010. Anadromy and the dispersal of an invasive fish species (Oncorhynchus mykiss) in eastern Quebec, as revealed by otolith microchemistry. Ecology of Freshwater Fish 19:348–360.

Titus, R. G., and H. Mosegaard. 1991. Selection for growth potential among migratory Brown Trout (*Salmo trutta*) fry competing for territories: evidence from otoliths. Canadian Journal of Fisheries and Aquatic Sciences 48:19–27.

Volk, E. C., S. L. Schroder, and J. J. Grimm. 1999. Otolith thermal marking. Fisheries Research 43:205–219.

Wright, P. J., N. B. Metcalfe, and J. E. Thorpe. 1990. Otolith and somatic growth rates in Atlantic Salmon parr, *Salmo salar* L.: evidence against coupling. Journal of Fish Biology 36:241–249.

Wright, P. J., D. Rowe, and J. E. Thorpe. 1991. Daily growth increments in the otoliths of Atlantic Salmon parr, *Salmo salar* L., and the influence of environmental factors on their periodicity. Journal of Fish Biology 39: 103–113.