Otolith and fish size relationships, measurement error, and size-selective mortality during the early life of Atlantic salmon (*Salmo salar*)[1]

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Abstract: The development of the relationship between otolith and body size in Atlantic salmon (*Salmo salar*) between hatching and emergence was examined by repeatedly measuring individually identified fish. Otolith growth increments were deposited daily in the period between hatching and emergence. Comparison of back-calculated otolith size and standard length using least squares regression analyses revealed a weak relationship between these variables at each of the 5-day sampling intervals. However, when data sets were pooled among intervals, variation in otolith size accounted for 98% of the variation in alevin length. A computer simulation demonstrated that levels of measurement error similar to those documented in our study resulted in the failure of regression analyses to detect strong relationships between otolith and fish size. Mortality that occurred during the experiment was strongly size selective. This truncated the size ranges of fish in cross-sectional data sets and thus reduced the ability of regression analysis to detect relationships between otolith and fish size. We propose that the weak relationship between otolith and fish size at emergence recorded in previous studies was an artifact of measurement error and the truncation of size ranges in regression analyses. Differences in alevin size at emergence were present at hatching and had been propagated by growth.

Résumé : Nous avons examiné le développement du rapport entre la taille des otolithes et la taille corporelle chez le saumon atlantique (*Salmo salar*), entre l'éclosion et l'émergence, en mesurant à plusieurs reprises chacun des poissons marqués. Au cours de cette période, les cercles de croissance se sont déposés tous les jours sur les otolithes. La comparaison de la taille des otolithes et de la longueur standard obtenues par rétrocalcul à l'aide des analyses de régression des moindres carrés a révélé un faible rapport entre ces variables pour chacun des intervalles d'échantillonnage de 5 jours. Toutefois, lorsque les ensembles de données ont été regroupées entre intervalles, la variation de la taille des otolithes représentait 98% de la variation de la longueur des alevins. Une simulation sur ordinateur a montré que des taux d'erreur de mesure semblables à ceux de notre étude étaient attribuables au fait que les analyses de régression ne permettaient pas de déceler de relations fortes entre la taille des otolithes et la taille du poisson. La mortalité enregistrée pendant l'expérience était fortement sélective selon la taille, ce qui a tronqué les plages de taille des poissons dans les ensembles de données transversales, et a donc réduit la capacité de l'analyse de régression à déceler les rapports entre la taille des otolithes et la taille du poisson. Selon nous, le faible rapport entre la taille des otolithes et la taille du poisson au moment de l'émergence enregistré dans des études précédentes était un artefact de l'erreur de mesure et de la troncature des plages de taille dans les analyses de régression. Les différences de taille chez les alevins à l'émergence étaient présentes à l'éclosion et elles se sont maintenues tout au long de la croissance.

[Traduit par la Rédaction]

Introduction

The analysis of age and growth information contained in the otoliths of young fish has become a standard technique in fisheries science. Typically, growth increments are deposited within otoliths on a daily basis and thus provide an estimate of an individual's age (Jones 1992). In many species, the timing of major life history events such as hatching or the initiation of first feeding can also be determined, since these may coincide with the deposition of distinctive increments within the otolith (Victor 1982). Furthermore, past histories of growth may be reconstructed from the relative spacing between growth increments (Campana 1990).

The reconstruction of growth patterns from otoliths relies on two assumptions. Firstly, deposition rates of increments must be regular and invulnerable to systematic errors when the otolith is analyzed. Secondly, the back-calculation of growth rates assumes that there is an allometric relationship between

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growth of the otolith and somatic growth of the fish. In recent years, a large number of studies have drawn attention to the problems of error and bias associated with these assumptions (Campana 1992; Neilson 1992). In a few cases, workers have reported that increments within the otolith were not formed on a daily basis (Geffen 1982; Campana 1984), while other studies have shown that the relationship between otolith and somatic growth may be lagged (Molony and Choat 1990) or very weak (Mosegaard et al. 1988; Secor and Dean 1989; Wright et al. 1990). When such problems occur, otoliths will be of limited use in the back-calculation of growth rates.

In the weeks after emergence, strong selection occurs in Atlantic salmon (*Salmo salar*) and other salmonids, with the larger, faster growing members of the cohort occupying and defending the most profitable territories in the streambed (Elliot 1990). The smaller, slower growing fish are forced downstream and may eventually die. Among those that survive, relative dominance status and competitive ability determine subsequent life history patterns (Metcalfe et al. 1990; Metcalfe 1991). Individuals that grow quickly may migrate to the sea after only 1 year in streams, while slow-growing fish delay migration, remaining in the stream for at least a second year (Thorpe 1977, 1989; Wright et al. 1990). The probability of survivorship and the establishment of a territory is correlated with otolith size, so that fast-growing, dominant individuals generally have larger otoliths at emergence than the slow-growing members of the same cohort (Metcalfe et al. 1992). This implies that the process of selection must begin at the initiation of first feeding.

As salmon otoliths are formed in the developing embryos prior to hatching (Geffen 1983), the records of growth retained in these structures may provide a means of examining the growth rates and size of fish during the earliest stages of the life history. However, Geffen (1983) found that during the period between hatching and exogenous feeding when the alevins reside in the redd, otolith growth increments may not be deposited at daily intervals. Furthermore, there appears to be little relationship between otolith and fish size at the time of emergence (Metcalfe et al. 1992). These problems seriously impair efforts to reconstruct growth histories from the otoliths of young salmon.

The lack of correlation between otolith and fish size at emergence and first feeding stands in marked contrast with patterns found only a few weeks later in the life history, when deposition rates within the otolith follow a regular daily pattern and there is a good 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 attempted to explain the convergence of otolith and fish growth rates as a function of the physiological process of otolith formation in salmon. This argument proposes that during the period prior to and immediately following emergence, 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 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.

There are, however, alternative explanations of changes in the strength of the relationship between otolith and fish size in young salmon. At emergence, variability in alevin size is small. This restriction of size ranges has important implications, since comparisons of otolith and fish size are typically made using least squares regression methods that are vulnerable to sample truncation effects (Campana 1990; Campana and Jones 1992; Murdoch and Doherty 1997). Furthermore, it will be difficult to detect real differences among individuals if errors in measuring small otoliths and alevins are relatively large (Meekan 1997). Since variation in growth rates among alevins will have cumulative effects on relative size, fry collected some weeks or months after emergence will display a much greater range of sizes at any given age. In contrast, measurement errors should remain constant and will be proportionally smaller, relative to size differences among individuals. Consequently, any relationship between otolith and fish size will be easier to detect using regression analysis. Thus, variation in size at age and the relative importance of measurement error may account for the lack of correlation between otolith and fish size early in the life of Atlantic salmon.

Here, we examine the extent to which otoliths provide an accurate estimate of age and growth of Atlantic salmon during the period between hatching and emergence. The accuracy of age estimates was determined by comparing counts of increments within otoliths with the known age of individuals. The development of otolith and fish size relationships was examined by repeatedly measuring individually identified fish in the period between hatching and emergence. Relationships between otolith and fish size in these fish were compared with those of alevins collected at hatching and emergence.

Methods and materials

Experimental design and sampling

Embryos were obtained from sea-run salmon caught in the Sainte-Marguerite River, Québec, Canada, in the fall of 1994. These were placed in a flow-through incubator that drew water directly from a tributary branch of the river. Within the incubator, eggs were spread in thin layers on perforated racks. At hatching, which occurs in the early spring, alevins migrate into gravel at the bottom of the incubator. After about 20–25 days, yolk reserves are exhausted, and alevins emerge from the gravel to be carried by the current to the outflow pipe. Alevins are then netted and released into the river.

On May 18, 1995, a few days before hatching, 100 embryos were placed in a fine-mesh cage within the incubator and examined daily for hatching. Alevins were removed on the day of hatching, filmed using a video camera, and then frozen. A sample of 100 emergent alevins that were collected on the first day of their appearance at the outflow pipe of the incubator was also filmed and preserved in liquid nitrogen. In addition to these regular samples of the population held within the incubator, the individual growth rates of 200 alevins were recorded. These individuals were haphazardly selected just before hatching, placed in individually numbered containers in the incubator, and monitored daily for hatching. At hatching, containers were removed and the alevins filmed. Containers were then replaced in the incubator. This process was repeated at 5-day intervals for 20 days, at which time the surviving alevins were removed, filmed, and preserved in alcohol. This gave a total of five repeated measures of these individuals. The final days of this experiment coincided with the emergence of the majority of alevins from the gravel in the incubator. During the period of the experiment, alevins in the incubator were exposed to natural cycles of light and water temperature. Average water temperature in the incubator and in the stream that supplied the

flow-through system was 10°C (SD = 3.6) and ranged from 5.0°C at the beginning of the experiment to 15.5°C at the end.

Otolith and size analysis

Sagittae and lapilli were dissected from the alevins using fine forceps and a dissecting microscope with a polarized light source. They were cleaned and placed in thermoplastic glue on a microscope slide. Otoliths from the larger individuals (those near to or at emergence) were ground on lapping film to the level of the core of the otolith. Preparations were viewed using a compound microscope at magnifications of 250 and 400× that was linked by a high-resolution video camera to a video monitor and computer. Increments were counted and measured along a constant axis in one of the pair of sagittae (the largest of the pairs of otoliths) with the aid of an image analysis program (OPTIMAS). Total area of the otolith was also measured. Similarly, standard lengths and area of the yolk sac of alevins were obtained from the videotape records.

Data analysis

Otolith – somatic growth relationships

Standard lengths and otolith dimensions were compared using regression analysis. However, for individuals that were repeatedly measured, this analysis could not be used on the entire data set of length and otolith measurements (the five sampling intervals), since data points collected at different times were not independent. The complete data set was analyzed by fitting the following random coefficient model to the data using restricted maximum likelihood techniques (PROC MIXED, SAS V6):

$$y_{ij} = \rho + \delta_{\rho_i} + (\beta_1 + \delta_{\beta_{1i}})x_{ij} + (\beta_2 + \delta_{\beta_{2i}})x_{ij}^2 + \varepsilon_{ij}$$

where y_{ij} is the fork length and x_{ij} is the otolith radius for the ith individual at the jth time period, ρ, β_1, and β_2 represent the intercept, slope, and quadratic terms, respectively, and δ_{ρ_i}, $\delta_{\beta_{1i}}$, and $\delta_{\beta_{2i}}$ are independent random deviations from the intercept, slope, and quadratic terms and are assumed to be distributed as $NI(0, \sigma_\rho^2)$, $NI(0, \sigma_{\beta_1}^2)$, and $NI(0, \sigma_{\beta_2}^2)$, respectively. The error term, ε_{ij} was assumed to be distributed as $N(0, \sigma_{jj}^2)$ and $Cov = (\varepsilon_{ij}, \varepsilon_{i*j}) = 0$ for $i \neq i*$ and $Cov = (\varepsilon_{ij}, \varepsilon_{i*j}) = \sigma_{jj*}$ for $i = i*$. This covariance structure was used to account for the correlations between measurements made on the same individual through time (Laird and Ware 1982; Chambers and Miller 1995).

Measurement error

To quantify the error involved in measurements of otolith dimensions, the radius and area of the otoliths of 10 haphazardly selected alevins were remeasured five times. The error generated from measurements of standard length was estimated by reanalyzing all videotapes taken during the course of the experiment, 2 months after they were initially analyzed.

The influence of measurement errors on the ability of regression analyses to detect relationships between otolith and fish size at emergence was examined in a computer simulation. Otolith radius was used to generate predicted standard length using the regression relationship calculated for fish at the end of the 20-day experiment. It was assumed that a perfect correlation existed between these variables (i.e., $r^2 = 1$). The effect of measurement error was evaluated by adding a random error component to observed otolith radii and predicted standard lengths using the RANUNI function of SAS (v.6). This error component had a mean of 0 and a standard deviation that was chosen to correspond to the size of the errors estimated from the repeated measurements of the otolith radii and standard lengths of the same individuals. A total of 500 data sets were generated, each containing 97 paired observations of otolith radius and standard length. Regression analyses were then used to estimate the mean r^2 and its standard deviation from the 500 data sets. The standard deviation of the error

component added to the observed otolith radius and predicted standard lengths was then reduced and the entire simulation repeated. The influence of differing measures of otolith size on regression analyses was examined by repeating the process outlined above using otolith area (with appropriate regression model and error components) rather than radius in the simulation.

Size-selective survivorship

We examined the influence of selective mortality on survivorship during our experiment by comparing frequency distributions of standard length at hatching of individuals that survived the 20-day period of the experiment with those of individuals that had died 5, 10, and 15 days after the experiment began. As the death of an alevin did not always coincide with a day during the experiment when standard lengths were measured, dead individuals were discarded after they were removed from containers held in the incubator. Consequently, frequency distributions of otolith size of individuals that survived could not be compared with those of alevins that died. However, we were able to compare the growth of survivors with that of dead alevins by reconstructing growth trajectories from videotape records taken in the time periods prior to their death. As samples sizes at each time interval (i.e., 5, 10, 15, and 20 days) were unequal due to mortality, these repeated measures data sets were analyzed as a mixed linear model (Laird and Ware 1982; Chambers and Miller 1995) using PROC MIXED, SAS (v.6).

Results

Age

The sagittae and lapillae of alevins that had been placed in individual containers at hatching and then killed after 20 days had an average of 23.5 (SD = 1.3, range 17–27) and 22.68 (SD = 1.5, range 17–25) growth increments, respectively. In all individuals, there was a distinctive mark near the centre of the otolith that was formed about 20 increments before the end of the experiment (Fig. 1). As this mark was also present at the margin of otoliths of newly hatched alevins, it was probably formed just prior to or on the day of hatching. In the region of the otolith within the hatchmark, two or three faint increments were visible (Fig. 2), which must have been deposited prior to hatching. Thus, the rate of increment deposition in otoliths of alevins was daily between hatching and emergence.

The density of growth increments, as visually assessed, was markedly reduced during the last 2 or 3 days of daily deposition in otoliths of individuals that had completely absorbed yolk reserves before the end of the experiment (Fig. 1). This transition was also found in the otoliths of all alevins that were collected from the incubator at emergence. In most individuals, this transition usually occurred two or three increments prior to the edge of the otolith (Fig. 3). These additional increments on the edge of the otolith most probably represent the number of days between yolk sac absorption and emergence from the gravel and arrival at the outflow pipe for collection.

Variation in size and growth rate

Of the 200 embryos that were placed in individual containers, 111 survived to 20 days after hatching. The standard lengths of these alevins (15.75 mm, SD = 0.52) were not significantly different from those of the population within the incubator at the start of the experiment (15.77 mm, SD = 0.44) (one-way ANOVA, $p > 0.05$). However, at 20 days after hatching, the mean size of alevins held in individual containers

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Fig. 1. Sagittal otolith of an alevin sacrificed 20 days after hatching and grown in an individual container. N, nucleus; H, hatch mark; YA, yolk sac absorption mark. The straight white line indicates the radius of the otolith, and the white flags indicate 20 daily growth increments. Yolk sac absorption occurred 17 days after hatching. Also note the presence of growth increments prior to the hatch mark.
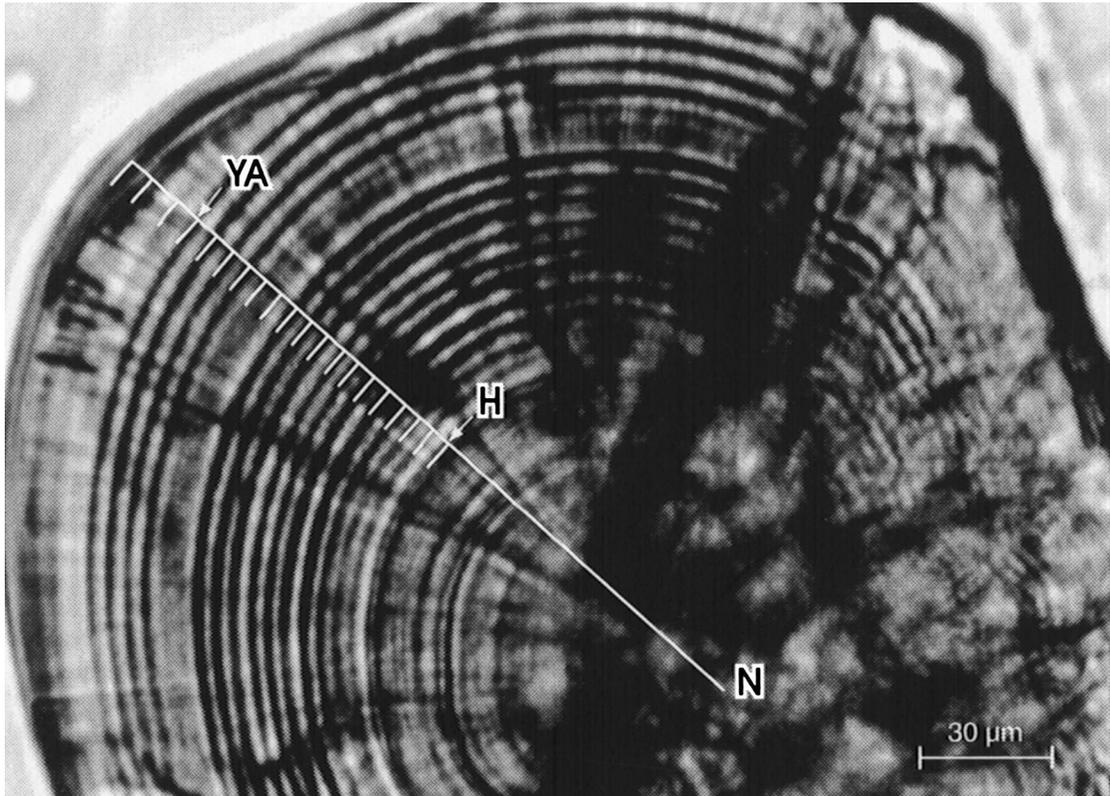
Fig. 2. Sagittal otolith of an alevin taken on the day of hatching. N, nucleus; H, hatch mark. The straight black line indicates the radius of the otolith, and the black flags identify five increments deposited prior to the hatch mark.
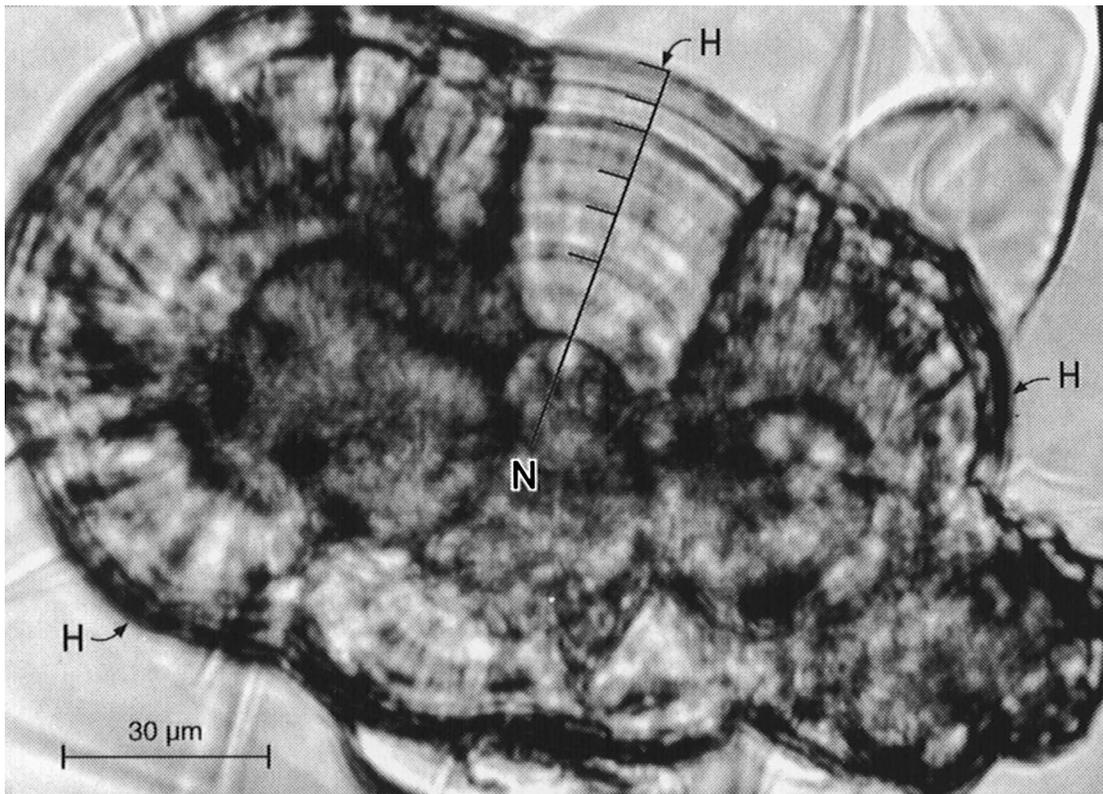
Fig. 3. Sagittal otolith of an alevin taken on emergence in the first compartment of the incubator. N, nucleus; H, hatch mark; YA, yolk sac absorption mark. The straight white line indicates the radius of the otolith, and the white flags indicate daily increments. This alevin absorbed its yolk sac 16 days after hatching and emerged from the gravel 6 days later.
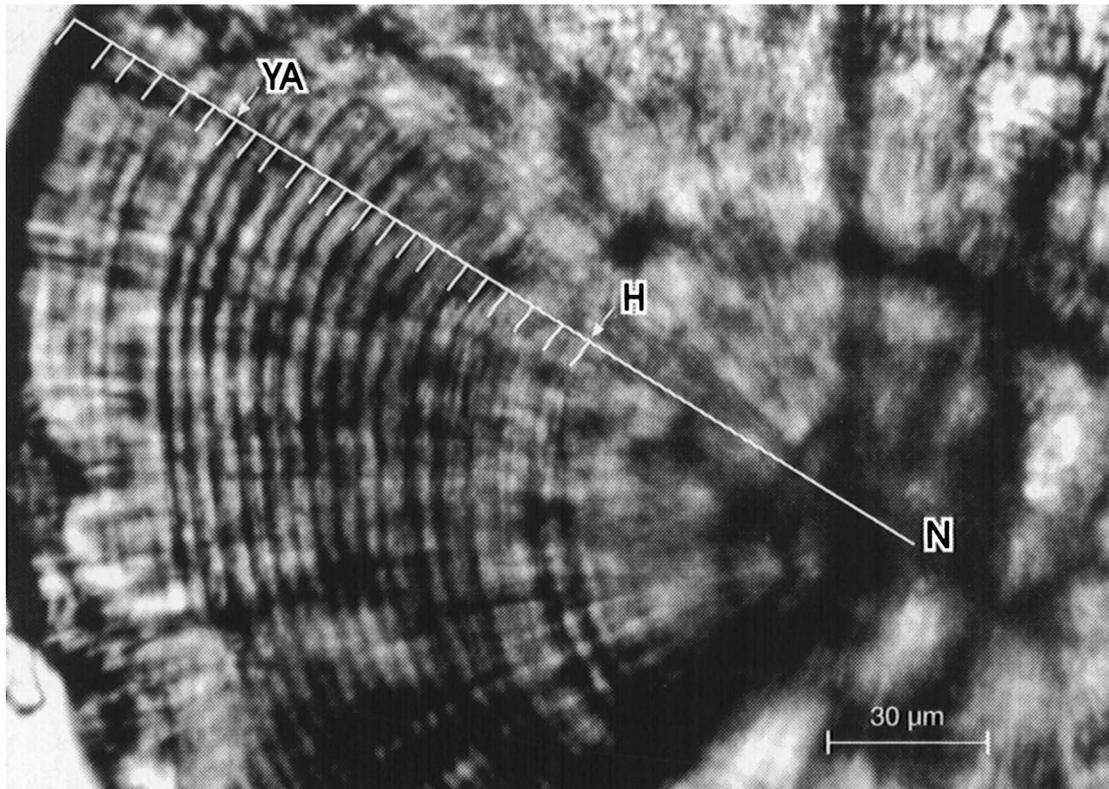
(23.50 mm, SD = 0.71) was smaller than that of alevins that emerged from the incubator (24.95 mm, SD = 1.10) (one-way ANOVA, $p < 0.001$). Alevins held in individual containers grew at rates of 0.57 mm·day^{-1}.

Otolith and fish size

As increments found within the otoliths of alevins were deposited at daily intervals, the relationship between otolith size and standard length could be examined for alevins that were maintained in individual containers by back-calculating the radius of the otolith on the days when measurements of size were made. Of the 111 alevins that survived until the end of the experiment, 97 were available for otolith analysis. Fourteen alevins were excluded, as clear images could not be obtained from videotapes or otoliths were lost or broken during dissection and polishing.

Significant but weak linear relationships were found between the back-calculated radius of the sagitta and standard length of the alevins (obtained from videotape images) on each of the five sampling occasions (Figs. 4A–4E). At best, variation in the radius of the sagitta could account for only 17% of the variance in fish size. There was some improvement in the strength of relationships between otolith and fish size when otolith area was used as a measure of otolith size. When total areas of sagittae were compared with the standard length of alevins collected at hatching and at the end of the experiment, there was a marked increase in the r^2 values of the relationship between otolith and fish size, with variation in the total area

of the sagittae accounting for 39 and 44% of the variation in standard length at hatching and after 20 days, respectively.

When a linear model (PROC MIXED) was used to describe the relationship between the entire data set of standard length and otolith radii measurements (the five sampling intervals), these variables were highly correlated, with variation in otolith size explaining 95% of the variance in fish size. The addition of a quadratic term explained a small, but significant, amount of variability ($p < 0.0001$), with variation in otolith size explaining 98% of the variation in standard length. Thus, in contrast with the regression analyses conducted on cross-sectional data sets from each time interval, the analysis showed that when pooled among sampling times, these variables were highly correlated, with variation in otolith size explaining almost all of the variation in standard length of alevins (Fig. 4F). The covariance parameter estimates for the intercept ($\sigma_{\beta_0}^2$), slope ($\sigma_{\beta_1}^2$), and quadratic ($\sigma_{\beta_2}^2$) components were 0.4266, 0, and 0, respectively. This suggested that while fish hatched at different sizes, trajectories of fish and otolith growth were parallel among individuals. To confirm this result, the relative sizes of otolith radii of alevins at hatching were compared with their radii at emergence. Alevins were equally divided among three categories (small, medium, and large) on the basis of otolith radius firstly at hatching and then again at the end of the experiment. The composition of these categories was compared using a chi-square test (Table 1). This analysis was then repeated using standard lengths of alevins at hatching and emergence (Table 1). As in the results of the modeling of otolith and fish size, these comparisons showed that the

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Fig. 4. Relationships between otolith radius and standard length of salmon larvae sampled at (A) hatching and (B) 5 days, (C) 10 days, (D) 15 days, and (E) 20 days after hatching; (F) data sets pooled among sampling dates. Data points from each sampling date are identified.
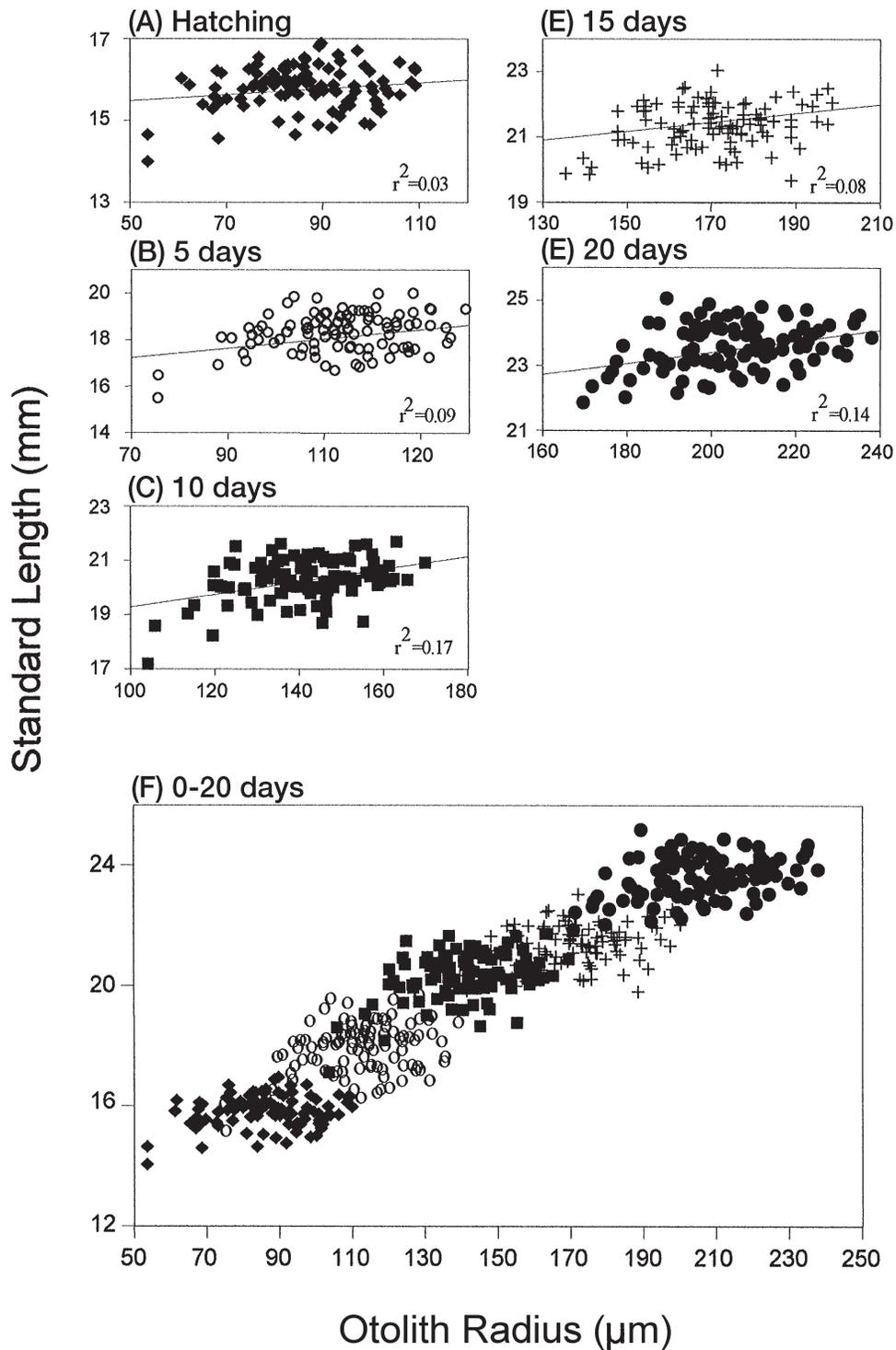
relative differences in otolith size and standard length among alevins at hatching were retained during the course of the experiment. Almost 70% of individuals that had small otoliths at hatching also had relatively small otoliths at the end of the experiment. No individuals that were classified as having small otoliths at hatching were reclassified as having relatively

large otoliths at the end of the experiment. Similarly, 74% of alevins that were classified as having relatively large otoliths at hatching were also classified as having large otoliths after 20 days, while no individuals that had relatively large otoliths at hatching were reclassified as having small otoliths after 20 days. A similar result was found in the comparison of

standard lengths of alevins at hatching and at the end of the experiment, although these results were less clear-cut. Between 44 and 52% of alevins that were classified as large, medium, or small on the basis of length remained in those categories at the end of the experiment.

Measurement error

To determine the contributions of measurement error to differing estimates of otolith size, data sets of remeasurements of the same individuals were analyzed as one-factor ANOVAs. The relative amount of variability contributed to the entire data set by differences in size among individuals and by replicate measurements of the same individual (measurement error) was then calculated using the method given in Snedecor and Cochran (1973, p. 280). In the analysis of otolith area, remeasurements of the same otoliths showed that measurement errors were small relative to differences among individuals and contributed only 2.44% of the total variability in the data set. In contrast, analysis of repeated measures of otolith radii showed that measurement error contributed 38% of the total variability in the data set. The greater levels of error associated with measurements of otolith radius, when compared with those of otolith area, were probably due to locating a consistent axis of measurement and aging errors. In these small individuals, the characteristic pattern of otolith growth found in older fish had not yet occurred, making it difficult to reliably measure the same axis within the otolith. In contrast, measurement of otolith area did not require age analysis and could be easily calculated.

Reanalysis of the videotapes showed that measurement error in estimates of standard length averaged 0.48 mm. These errors did not differ significantly among time periods and ranged from 0.56 to 0.31 mm (Table 2). As there was no major increase in the variability of standard lengths among alevins between hatching and 20 days, there was no consistent decline in the size of errors relative to the size range of alevins during the course of the experiment. Data sets for each time period were analyzed separately using one-factor ANOVAs and the relative contributions of measurement error at each time period calculated using the method given in Snedecor and Cochran (1973). These showed that the contribution of measurement error ranged between 10 and 65% of the total variability in the data sets.

The results of the simulation study (Table 3) demonstrated that levels of measurement error comparable with those that occurred in our study had a major influence on the strength of regression relationships calculated between otolith and fish size at emergence. Inclusion of levels of error in regression analyses associated with our measures of otolith radius and standard length resulted in a weak relationship (mean $r^2 = 0.202$) between otolith and fish size. Strong relationships between otolith and fish size ($r^2 > 0.75$) were not evident until levels of measurement error associated with otolith radius and standard length were reduced by 30 and 75%, respectively. When otolith area was used as a measure of otolith size the correlation between otolith and fish size substantially improved. At levels of error estimated to have occurred in our study, otolith area and fish length were moderately correlated (mean $r^2 = 0.475$). Reduction of measurement errors by 50% resulted in an increase in the mean value of r^2 to 0.786.

Table 1. Composition of three relative size categories (small, medium, and large) of otolith radius and standard length of individually identified salmon larvae at hatching and 20 days after hatching.

20 days after hatching	Hatching		
	Small	Medium	Large
Otolith radius			
Small	23	10	0
	69.7%	27.0%	
Medium	10	16	8
	30.3%	43.2%	25.8%
Large	0	11	23
		29.7%	74.2%
Standard length			
Small	14	16	6
	51.8%	31.8%	19.4%
Medium	7	17	9
	25.9%	43.6%	29.0%
Large	6	10	16
	22.2%	25.6%	51.6%

Note: For each size category at hatching, the table shows the number and percentage of fish that remained in the same category or were reclassified in other size categories 20 days later. Chi-square analyses for both size variables were significant (chi-square = 52.428, df = 4, p = 0.001 for otolith radius; chi-square = 11.36, df = 4, p = 0.02 for standard length).

Size selection

Comparison of frequency distributions of standard length at hatching of surviving alevins with those of fish that died during the 20-day experiment showed that mortality progressively removed the smallest members of the cohort, with alevins that were smallest at hatching being the first to die (Fig. 5). Size selection was not linked to growth rates. The mixed linear model comparison of growth trajectories revealed a significant interaction between alevin growth rate and time period of measurement (F = 7.47, df = 6, 186, p < 0.0001). Some dying fish were growing slower than the survivors of the 20-day experiment; others grew at similar rates. For example, those individuals that died between 5 and 10 days were not growing slower than surviving fish from 1 to 5 days after hatching. In contrast, fish that died 10–15 days after hatching were growing more slowly than surviving fish in the period from 0 to 10 days after hatching. Fish that died between 15 and 20 days after the start of the experiment displayed variable growth rates. These individuals grew more slowly than survivors up to 5 days after hatching, but then grew at the same rate as survivors until 15 days after hatching.

Discussion

Age

The otoliths of Atlantic salmon alevins provided a reliable record of age between hatching and emergence. Daily deposition of increments during this period has also been recorded in other salmonids including Arctic char (*Salvelinus alpinus*) and brown trout (*Salmo trutta*) (Mosegaard and Titus 1987) and chinook salmon (*Oncorhynchus tshawytscha*) (Neilson and Geen 1982). However, Geffen (1983) suggested that in Atlantic salmon, entrainment to daily rates occurs only after emergence. In Geffen's study, alevins were raised under conditions

Table 2. Errors associated with estimates of standard length measured from videotape records.

	SD (mm)		s^2	% variation
Hatching	0.537	Between	0.0918	
		Within	0.1703	65
5 days	0.477	Between	0.4849	
		Within	0.1174	24
10 days	0.563	Between	0.4300	
		Within	0.1680	39
15 days	0.505	Between	0.3074	
		Within	0.1493	48
20 days	0.316	Between	0.5068	
		Within	0.0538	10

Note: SD is the standard deviation of measurement error for each sampling date. These were derived from two independent measurements of standard length of each of 97 individuals at each sampling date. Data sets for each sampling date were analyzed using one-factor ANOVAs. Estimates of the variance components (s^2) associated with differences among individuals (between) and between replicate measures of the same individual (within) are shown. The percentage of variation in the data sets attributable to differences between replicate measurements of the same individual (measurement error) was calculated according to the formula given in Snedecor and Cochran (1973).

of constant temperature and regular cycles of light and dark, while in the present study, alevins were exposed to natural cycles of light and temperature. Such differences in experimental conditions might account for these apparently contradictory results, as Neilson and Geen (1982) have shown that daily cycles of temperature may be important for the entrainment of daily patterns of deposition in otoliths of young salmonids.

While it is possible that the daily pattern of increment deposition between hatching and emergence may have been influenced by the regular removal of alevins from the incubator for filming, this appears unlikely. The process of removal, filming, and replacement of alevins in the incubator was rapid and was usually completed in less than 10 min. No distinctive marks could be found on the otoliths that might have corresponded to these 5-day events.

Measurement error

Otolith and fish size of Atlantic salmon alevins were strongly correlated in the period between hatching and emergence. Modeling of the relationship between these variables suggested that variability in otolith size during this time could account for up to 98% of the variation in fish size. This strong relationship was not detected when linear regression was used to compare otolith and fish size for each of the 5-day sampling periods. Our simulation suggested that this was largely due to the measurement errors associated with size estimates. The use of otolith radius in the simulation resulted in a mean correlation coefficient between otolith and fish size of 0.2. About 21% of the r^2 values calculated by the simulation were lower than the actual coefficient calculated for alevins sampled 20 days after hatching (0.14). Substitution of otolith area as a measure of otolith size in the simulation resulted in a mean correlation of 0.475, a value similar to that calculated for data sets obtained from emergent alevins (0.44). These results imply that the changes in the strength of correlation coefficients that

occurred when otolith area, rather than radius, was used as a size estimate in regression analyses were largely due to a reduction in measurement error.

Difficulties involved in aging of fish and measuring a consistent radius on the otolith may have contributed to the larger errors associated with measures of radii. In contrast, otolith area could be easily measured from whole otoliths and did not require age analysis. Secor and Dean (1992) and Neilson et al. (1985) observed that otolith area provides a more reliable estimate of size than otolith radius. The latter authors demonstrated that because of variation in the number and arrangement of primordia that fuse to form sagittae prior to hatching, area is a better estimate of otolith size than any unidimensional measure. They also suggested that variation in otolith size caused by variation in the number of fused primordia may weaken the otolith and fish size relationship at hatching, although no analysis was presented to support this proposition. West and Larkin (1987) found a change in the strength of the correlation coefficient from about 0.12 to 0.52 (which is comparable with the results of the present study) when radii measurements were replaced by estimates of sagittae area in regression analyses of fish and otolith size of juvenile sockeye salmon (*Oncorhynchus nerka*). We propose that the differing amounts of error associated with these measures of otolith size may account for this result.

The simulation demonstrated that reduction of errors in measurements of standard length had a greater effect on the strength of correlation coefficients than reductions in measurement errors of otolith size. Larger errors associated with standard lengths might account for the greater degree of reassortment of individuals among standard length categories than among otolith size categories in chi-square comparisons of fish at hatching and at the end of the experimental period. However, it is difficult to judge if the measures of fish and otolith size in our experiment were less reliable than those of previous studies, since few explicitly stated any estimate of error. As fish size was measured from videotapes, errors may have been greater than those associated with the direct measure of preserved individuals. Obviously, this latter technique is not possible in a longitudinal study of development.

Size selection

The source of mortality among individually contained alevins is unknown, although it was clearly size selective. Analysis of growth trajectories of fish that died during the experiment (reconstructed from videotape records) displayed no clear association between growth rate and mortality. Fish that died were smaller at hatching than those that survived, but in many cases were growing at the same rate as surviving fish during much of the experimental period. The removal of fish that were relatively small at hatching would have weakened the correlation between otolith and body size. Since individual otolith and fish size trajectories were parallel, size-selective mortality after hatching effectively truncated the length distributions of fish at emergence that could be used in regression analyses. This is also likely to have strongly influenced the importance of measurement error, by reducing the range of length differences among individuals. While the propagation by growth of size differences among survivors during the course of the experiment could have compensated for such an effect, this does not appear to be the case in our study. At

hatching, the largest and smallest fish in the sample of 200 fry differed in standard length by 5.52 mm, while after 20 days, the 111 surviving individuals differed in standard length by a maximum of only 3.85 mm.

The effect of this sample truncation on the strength of regression relationships between fish and otolith size can be easily demonstrated. An expansion of the range of standard lengths of older fish can be achieved by randomly sampling 97 measurements (the total number of surviving individuals for which we had measurements of both standard length and otolith size) from the data sets collected 15 and 20 days after hatching. These subsamples were made without replacement (i.e., an individual selected at one time interval was not included in the subsample from the next time interval) to avoid any autocorrelation between measurements made on the same fish at different times. This resulted in a data set where the range in standard lengths was 4.4 mm. In the regression analysis of fish that survived until 20 days after hatching, the range in standard lengths was 3.85 mm and otolith and fish size were weakly correlated ($r^2 = 0.14$). When this subsampling was used to artificially expand the size range of fish used in the analysis (to 4.4 mm), the correlation between otolith and fish size increased to 0.66.

The effect of sample truncation on the ability of linear regression analyses to detect significant relationships is well recognized in growth and otolith studies of larval fish (Smale and Taylor 1987; Campana 1990). When body size and age are compared, sample truncation occurs when unbiased samples of all size groups in the population cannot be obtained as a result of gear selectivity (Murdoch and Doherty 1997) or due to size-selective mortality (Smale and Taylor 1987) or to growth effects on otolith size (Campana 1990). In such cases, sample truncation will result in the calculation of spurious regression lines where the slope and intercept are poor estimates of the actual values for the sample. Campana (1990) and Murdoch and Doherty (1997) recommended that the intercepts of lines should be constrained to biologically realistic values to overcome this problem. In our study, this effect of sample truncation can be illustrated by extrapolating the relationship between otolith and fish size to the average size of the alevins at hatching. For the data set collected at 20 days after hatching, the regression line gave a predicted length at hatching of 21.4 mm, a value that is obviously incorrect, since the average size of alevins at the beginning of our experiment was 15.7 mm. However, sample truncation, in this case due to size-selective mortality, will not only influence the slope and intercept of a regression line, but can also affect the strength of a correlation between the variables that are being compared. This will occur due to a reduction in the size range of individuals in the sample, which will increase the relative importance of measurement error.

In previous studies of Atlantic salmon, sample truncation has occurred as an artifact of the selection of experimental subjects. Metcalfe et al. (1992) compared otolith – fish size relationships in young salmon from two samples of individuals that were collected at different times. There was no significant correlation between otolith size and fork length at emergence in a small sample ($n = 19$) of fish collected immediately after emergence ($r^2 = 0.155$, fig. 1 in Metcalfe et al. 1992). A moderate correlation between these variables ($r^2 = 0.665$) was found in a larger sample of fish collected 3 months after

Table 3. Summary of results of a computer simulation of differing amounts of measurement error on the strength of correlations between otolith and fish size of salmon larvae at emergence.

	Length (mm)	r^2	SD	Min.
Radius (μm)				
2	0.12	0.828	0.024	0.727
	0.25	0.540	0.059	0.382
	0.50	0.232	0.069	0.046
5	0.12	0.762	0.033	0.669
	0.25	0.499	0.059	0.318
	0.50	0.221	0.065	0.043
7	0.12	0.699	0.039	0.586
	0.25	0.459	0.064	0.259
	0.50	0.202	0.065	0.017
Area (μm²)				
292	0.12	0.941	0.007	0.910
	0.25	0.789	0.027	0.703
	0.50	0.489	0.057	0.301
584	0.12	0.937	0.008	0.905
	0.25	0.786	0.027	0.683
	0.50	0.488	0.061	0.307
1170	0.12	0.921	0.011	0.891
	0.25	0.772	0.031	0.669
	0.50	0.475	0.060	0.278

Note: A detailed description of the simulation protocol is given in the Methods and materials section. A total of 500 data sets each containing 97 data pairs was used in each run of the simulation. Initially, the simulation used otolith radius as a measure of otolith size. The entire simulation was then repeated using total area as a measure of otolith size. The values shown for radius, area, and length are the standard deviations of the error component (mean = 0) that were added to the data sets calculated by the regression. The largest of each of these is equivalent to the error associated with size measurements in our experimental study. These were estimated from repeated measurements of the same individuals (see Methods and materials). The r^2 is the mean value calculated from the 500 data sets and SD is the standard deviation of this mean in each run of the simulation. The minimum r^2 calculated for the 500 data sets are shown.

emergence (fig. 2 in Metcalfe et al. 1992). Otolith radius ranged from 210 to 245 μm in the sample of fish collected at emergence, while in the sample collected at the later date, otolith radius at emergence ranged from 120 to 200 μm. Given that the ranges of otolith radii at emergence in these two samples do not overlap and that the range of radii in the fish collected at emergence was less than half that of fish collected 3 months after emergence, it is clear that the emergent fish used in their analysis were not representative of the population sampled at the later date. A second example of this problem is seen in Titus and Mosegaard (1991), where the young salmon used in their experiment were the progeny of a single pair of adults. While there is considerable variability in size of offspring among different parents, the progeny of one pair tend to be of a similar size (Titus and Mosegaard 1991). This artificial restriction of size ranges of emerging fish may have influenced the ability of regression analysis to detect significant relationships between otolith and fish size at emergence.

By taking a longitudinal approach to the comparison of otolith and fish size, we have shown that a strong relationship exists between these variables after hatching in young Atlantic salmon. This has important implications for studies that have proposed that selective mortality occurring in populations of

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Fig. 5. Numbers of survivors and fish that were dead at days 5, 10, 15, and 20 as a function of size-class (standard length) at hatching.

Atlantique), situated at the Ste-Marguerite River, Sacré-cœur sur le fjord du Saguenay, Québec.
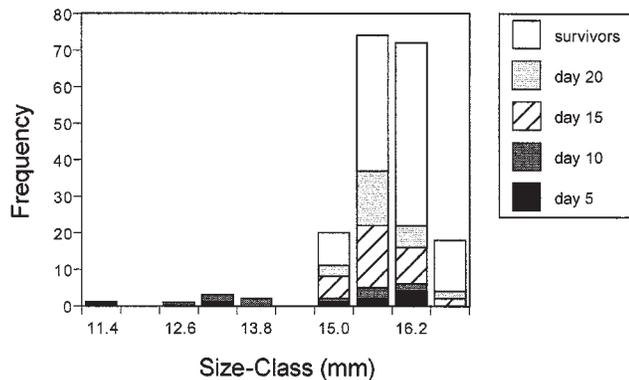
young salmon in the period immediately following emergence operates on differences in metabolic rate that occur independently of variation in size among individuals (e.g., Mosegaard 1990; Titus and Mosegaard 1991; Metcalfe et al. 1992). These assume that the ability of regression analyses to detect correlations between otolith and fish size does not vary, irrespective of the ranges of size and age of fish examined in the study. Our work shows that this assumption is false. Small fish and their otoliths cannot be measured without error. The interaction of this error and sample truncation, due to size-selective mortality and (or) the selection of experimental subjects by investigators, may be able to explain the results of these previous studies, without the need to invoke processes of selection based on variables other than the size of young fish. However, traits such as size, growth, and metabolic rate are often highly correlated during the early life history of fish (Chambers et al. 1988; Hare and Cowen 1997). For this reason, our study does not exclude the possibility that selection for metabolic rate in young salmon does indeed occur, although determining the relative importance of any early life history trait during selection is likely to be difficult (Hare and Cowen 1997). However, changes in the apparent strength of the relationship between otolith and fish size in young salmon are not sufficient evidence to conclude metabolic rate selection without considering the importance of measurement error and sample truncation. If selection is size based (see Meeken et al. 1998), then the parallel nature of the otolith – fish size trajectories observed here indicates that it operates on differences in the relative sizes of alevins that are present at hatching.

Acknowledgments

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