

Impact of differential energy allocation in Atlantic salmon (*Salmo salar*) precocious males on otolith-somatic size proportionality: a longitudinal approach¹

Nadia Aubin-Horth and Julian J. Dodson

Abstract: We studied juvenile Atlantic salmon (*Salmo salar*) males that become precociously mature or not at age-1+ to test the hypothesis that differential energy allocation affects the relationship between otolith size and fish size and to validate the use of a back-calculation method to estimate size over 30 weeks. We used a longitudinal approach by repeatedly measuring marked fish and obtaining corresponding otolith radius measurements. Differential energy allocation of mature males did not affect the proportionality ratio between otolith and somatic size. Short-term otolith growth varied with short-term somatic growth, but only weakly with temperature. Some correlation coefficients of the covariation of otolith growth estimated over a longer time interval with somatic growth were significantly greater than the short-term estimate. For mature and immature males, back-calculated lengths accurately estimated the observed individual length on practically all occasions. These results indicate that back-calculation can be used to estimate size for Atlantic salmon with different energy allocation patterns. Variable strength of coupling of otolith and somatic growth depending on time interval suggests that these processes are completed on different time scales.

Résumé : Nous avons étudié des juvéniles mâles de saumon atlantique (*Salmo salar*) qui deviennent sexuellement matures de façon précoce ou non à l'âge 1+ pour tester l'hypothèse selon laquelle une allocation différentielle de l'énergie affecte la relation entre la taille de l'otolithe et celle du poisson et pour valider l'utilisation d'une méthode de rétro-calcul permettant d'estimer la taille antérieure sur trente semaines. Nous avons utilisé une approche longitudinale en mesurant de façon répétée des poissons marqués et en obtenant les mesures correspondantes du rayon de l'otolithe. La présence d'une allocation différentielle de l'énergie chez les mâles précoces n'a pas affecté le rapport de proportionnalité entre la croissance de l'otolithe et du poisson. La croissance de l'otolithe à court terme variait avec la croissance somatique à court terme mais marginalement avec la température. Les coefficients de corrélation calculés pour la covariation de la croissance de l'otolithe et somatique estimées sur de plus longues périodes étaient parfois significativement plus élevés que l'estimé à court terme. Les tailles rétro-calculées estimaient adéquatement la taille individuelle observée à presque toutes les occasions, et ce pour les mâles matures et immatures. Ces résultats indiquent que le rétro-calcul peut être utilisé pour estimer la taille de saumon atlantique présentant des patrons différents d'allocation d'énergie et que la croissance otolithique et somatique sont des processus complétés sur des échelles temporelles différentes.

Introduction

Individual size is a significant variable in determining many important life-history traits in a great number of species (Stearns 1976). Survival (Good et al. 2001) and age at maturity (Hutchings and Jones 1998) are examples of such size-related traits. In the wild, individual size and growth can be documented using otolith readings and back-calculation methods. Otoliths carry an everlasting record of an individ-

ual's life events and environmental variation, reflected in the incremental growth that characterizes otolith development (Campana and Jones 1992). Otolith-based size estimation methods assume that increments are deposited on a regular basis and that proportionality exists between fish growth and otolith growth (Campana 1990). These two assumptions have been verified, directly or indirectly, in a number of species (e.g., Volk et al. 1984; Secor et al. 1989; Sirois et al. 1998). The somatic-otolith size relationship is usually taken as a

Received 28 November 2001. Accepted 8 May 2002. Published on the NRC Research Press Web site at <http://cjfas.nrc.ca> on 30 September 2002.
J16627

N. Aubin-Horth. Département de biologie, Université Laval, Cité universitaire, Québec, QC G1K 7P4, Canada.

J.J. Dodson. Département de biologie, Université Laval, Cité universitaire, Québec, QC G1K 7P4, Canada.

¹Contribution to the program of CIRSA (Centre interuniversitaire de recherche sur le saumon Atlantique) and GIROQ (Groupe interuniversitaire de recherche en océanographie du Québec).

²Corresponding author (e-mail: julian.dodson@bio.ulaval.ca).

proxy to verify the assumption of proportionality of somatic and otolith growth before using a back-calculation model (Campana 1990). Otolith and somatic growth proportions are not necessarily uniform, either among individuals or among periods. Several studies suggest that proportionality ratios are variable among individuals that present dissimilar developmental rates even though they are from the same life stage (e.g., "the growth effect"; Secor et al. 1989; Campana 1990). In addition, temporal changes in the proportionality of fish growth and otolith growth, linked to changes in life-stages or to growth-rate variations during the lifetime of an individual, have been documented (Sirois et al. 1998; Neuman et al. 2001). For example, the proportionality ratio is affected by the onset of different growth patterns in juvenile Atlantic salmon (*Salmo salar*) entering the smolting phase (upper modal-length group (UMG)) or not (lower modal-length group (LMG)). Otolith growth continues while somatic growth virtually ceases for the nonmigrating part of the population. This results in a curvilinear relationship between otolith and somatic size for these individuals (Wright et al. 1990). These authors and others coined the term "uncoupling" to describe these divergences of proportionality ratios among types of individuals. It was suggested that this resulted from otolith growth being primarily influenced by factors that have an effect on metabolism, like temperature. This would result in uncoupling when, for example, a given factor would inhibit somatic growth while exerting a positive effect on otolith growth at the same values (Wright 1991; Barber and Jenkins 2001).

Studies have shown that there is no proportionality between mean somatic growth and corresponding mean otolith radius growth of groups of fish on a short time scale (e.g., 6 to 15 days growth interval; Bradford and Geen 1987; Secor et al. 1989). This absence of relationship is observed even when a strong relationship is found between otolith size and fish size, and between otolith growth and somatic growth on a longer time scale for the same individuals (e.g., 50 days; Bradford and Geen 1987). The main argument that emerges from these studies is that the covariation of somatic size and otolith size observed in many empirical studies does not automatically demonstrate a causal relationship between daily or short-term somatic growth and otolith growth, as is often assumed (Secor et al. 1989; Wright et al. 1990). Considering this, short-term estimation of length using otolith size could turn out to be inadequate even in the presence of a correlation between otolith size and somatic size (Bradford and Geen 1987). Alternatively, Meekan et al. (1998) argued that this lack of correlation between daily or short-term somatic and otolith growth is not a biological phenomenon in certain cases, but rather an artefact of not respecting the underlying assumptions of correlation and regression analyses when truncated size distributions are employed.

Atlantic salmon is an ideal model to study the variability of otolith and fish size proportionality. In this typically anadromous fish, a sizeable fraction of the juvenile male population becomes sexually mature after 1–3 years of life, without leaving fresh water, at a size 5–10 times smaller than their anadromous counterparts (Hutchings and Myers 1994). The resource allocation of these mature males diverges from immature males of the same cohort, based on

their lower somatic growth rate during the period of gonad formation in laboratory studies (Rowe and Thorpe 1990). It is, however, not known if the slope of the relationship between otolith size and fish size of precociously mature males diverges at some point from the slope of immature individuals owing to differential energy allocation. In Atlantic salmon, the presence of a relationship between otolith size and fish size and the regular deposition of increments has been verified from hatching to exogenous feeding by Meekan et al. (1998) and from this stage until they are aged about 300 days by Wright et al. (1991).

The objectives of the present study, using male juvenile and precociously mature Atlantic salmon as a case study, were to first compare the slope and intercept of the relationship between otolith size and somatic size to test the null hypothesis that this relationship does not differ between the two types of male. The second objective was to test the assumption of proportionality in individual fish and otolith growth over a short time scale. The third objective was to directly validate the use of a back-calculation model to estimate somatic length from otoliths at a series of dates over a 30-week time scale.

Materials and methods

As proposed by Secor et al. (1989), laboratory-reared fish should be exposed to similar variations and ranges of temperature observed in nature to address these objectives. Fish should also be measured frequently for comparison of short-term (1–15 days) otolith and somatic growth. We used a longitudinal approach by repeatedly measuring, at intervals of 15 days, otolith and somatic sizes of individually marked male fish held in the laboratory in semi-natural conditions.

Laboratory conditions

One hundred age-1+ Atlantic salmon parr were transferred to an environment-controlled chamber in January 1999. These individuals came from crosses between wild breeders, originating from the Ste-Marguerite River (Québec). They were raised in a fish hatchery environment during their first year of life. They were individually identified with coloured elastomer marks, measured (fork length, 0.5 mm) and weighed (wet weight, 0.01 g) on 11 March 1999. They were fed a commercial fish diet 2–4 times a day during daylight, the total daily amount varying according to mean weight and the number of individuals. Natural photoperiod, by signalling the arrival of spring and the potential for growth, plays an important role in the onset of sexual maturity in juvenile Atlantic salmon males (Duston and Saunders 1992). These fish were therefore exposed to conditions of temperature and photoperiod varying in a similar fashion to those encountered in the Ste-Marguerite River in 1998 (CIRSA database, Université Laval, Cité Universitaire, Québec, QC G1K 7P4, Canada, unpublished data). Temperature varied between 1.4 and 18.1°C during the course of the experiment and the simulated day varied between 10 and 15 h. Accidental mortality occurred in May 1999. Considering the number of remaining individuals, and assuming a 1:1 sex ratio, it appeared necessary to add new individuals to assure statistically valid comparisons, particularly as we could not predict the number of mature males present at

the end of the experiment. We therefore transferred, on 26 May 1999, 54 additional individuals originating from the same parents and the same basin as the original individuals. These new fish thus experienced different temperatures (water temperature was warmer and more stable) and photoperiod during the period from March to May in the hatchery basin. They were acclimated during the next 15 days and individually marked, measured, and weighed on 9 June 1999. Mean size of the added fish (78.1 mm, standard deviation (SD) = 1.1) did not differ from mean size of individuals (78.7 mm, SD 1.1, *t* test on natural logarithm (ln)-transformed data, *p* = 0.7) already in the basin on this date.

Otolith thermal marking and somatic size measurements

To relate otolith size to somatic size, otoliths were marked every 15 days from March to October 1999 using a thermal marking technique based on the procedures of Akinicheva and Rogatnykh (1996) and Letcher and Terrick (1998). Marking was performed to ensure that otolith measurements could be reliably linked to somatic size measurements, as Wright et al. (1991) suggested that individual otolith increments are difficult to identify at low constant temperatures. The mark was created by varying water temperature by 7°C for a 24-h period. The first marking period involved an increase in temperature, whereas all other periods involved a decrease in temperature. Water temperature was modified in the main basin while the individuals were being maintained at normal temperatures in an aerated confinement basin. All individuals were measured and weighed during this temperature adjustment period. Fish were individually anesthetized in a smaller container using MS-222 (tricaine methanesulfonate) to obtain reliable length and mass measurements along with an accurate elastomer-code reading with a minimum of handling stress. Fish were then transferred to the main basin at the marking temperature. This temperature was maintained for 24 h, and thereafter re-adjusted to the normal thermal regime mimicking natural conditions over a period of 5–10 h without removing the fish. This thermal marking – measurement step was repeated at an interval of 15 days. At the end of the experiment, no trace of the first marking trial could be found on any otolith, indicating that thermal marking carried out by increasing the temperature for 24 h when fish were held at low winter temperatures (1.5°C) was not successful for fish of this size. However, lowering temperature, which was used when the ambient temperature was greater than 8°C, was clearly effective in creating a distinctive mark on the otolith. All fish were sacrificed by an overdose of MS-222 on 22 October 1999 after 225 days of experimentation. This date corresponded to the appearance of precocious sexual maturity in the wild. Experimental subjects were measured with an image analyser and weighed. Sex and maturity status were determined by dissection. Sagittal otoliths were extracted, mounted on a glass slide with thermoplastic cement with the sulcus face up, and lightly ground on fine abrasive paper to improve visibility of marks (Meekan et al. 1998).

Image analysis

Otolith radius length was measured using a microscope with 200× magnification, coupled to a frame grabber, using

a calibrated image analysis system (Scion Image for Windows 2.0, Frederick, Md.). Using a digitized and calibrated image of the otolith, the length (in micrometres) of a line starting in the central nucleus and traversing each detectable mark induced by thermal variations was measured on a constant axis, determined by forming a 45-degree angle with the posterior axis in the ventral region. Measurements were made on individuals in a random order and on a “blind” basis, with the reader not knowing to which individual the otolith image belonged.

Calculations and statistical analysis

Observed somatic size range and growth-rate pattern

Mean sizes attained (mm) and corresponding 95% confidence intervals at each marking event were calculated. They were compared between mature and immature males, with observation dates taken as a factor using a repeated-measures analysis of variance (ANOVA), as measurements made at each date were not independent samples. An Akaike information criterion method was used to determine which covariance structure was the most appropriate for our dataset. We compared a simple model with compound symmetric, autoregressive, and heterogeneous autoregressive models. The covariance structure that best fitted the dataset was heterogeneous autoregressive and it was incorporated in the ANOVA. The analyses were made on ln-transformed data.

Thermal regime

Mean daily temperatures were computed from readings taken automatically every 60 min. These daily values were used to compute mean temperature experienced by individuals during the growth interval (between two observation periods). They varied between 2 and 17°C.

Somatic–otolith size relationship

The characteristics of the relationship (slope and intercept) between otolith size and fish size for immature and precocious males were analysed using a repeated measures ANOVA model, as measurements made at each date were not independent samples. First, an Akaike information criterion method was again used to determine which covariance structure was the most appropriate for our dataset. We compared a simple model (no correlation) with compound symmetric, autoregressive, and heterogeneous autoregressive models. The covariance structure that fitted best the dataset was heterogeneous autoregressive. We applied a random-coefficient model, which takes into account the detected temporal correlation of the length measurements in the analysis of the characteristics of the relationship between otolith and fish size. It permitted us to explore the potential effect of sexual maturity on the otolith size – fish size relationship by estimating the slope and intercept of this relationship for immature and mature males separately and by comparing them while taking advantage of our repeated measures design. Data were ln transformed prior to analysis. The linear structure of the relationship was verified by examining the standardized residuals from the regression line. Failure to reject the null hypothesis does not necessarily mean that there is no effect of the treatment tested (maturity) on the relationship between otolith and fish size (Peterman 1990). Indeed,

the failure to reject the null hypothesis when it is false may be related to a low power of detection leading to a type II error (Sokal and Rohlf 1995). We therefore used a power analysis to test, a posteriori, our capacity to detect differences in slopes and intercepts of the otolith–somatic length relationship for immature and mature males as a function of our sample sizes, keeping in mind that we could not control the number of males in each category present at the end of the experiment.

Coupling of short-term otolith growth with somatic growth and temperature

The coupling of somatic and otolith growth at measurement time t was verified using absolute growth during the 15-day interval between two successive measurement – thermal marking occasions, obtained by subtracting individual length (mm) observed at time $t - 1$ from length at time t . The same calculation was applied to otolith radius–length data. The presence of a relationship between the two variables was assessed using a repeated-measures ANOVA to take into account that several measures were used from the same fish. The same analysis was repeated for somatic and otolith growth calculated over longer time intervals (30, 45, 60, ... to 135 days). We then compared correlation coefficients obtained for growth of the otolith and of the fish over different time intervals. The fact that repeated measures on the same individual were used was accounted for by using a sample size for the calculation of the critical value based on the number of fish used to calculate the correlation instead of the total number of observations. This is a very conservative analysis, as only large differences between correlation coefficients can be detected. A Bonferroni correction method was used to set the α level considering the number of comparisons made between coefficients (36 comparisons).

A correlation analysis was performed for otolith growth and mean temperature over 15 days. The significance of the correlation coefficient obtained was again determined with a sample size equal to the number of fish used in the experiment instead of the total number of observations, as numerous observations of otolith growth were obtained from each fish in the correlation analysis.

Direct validation of back-calculation of individual somatic length

Back-calculation of somatic length at each thermal marking date was achieved with the biological intercept method (Campana 1990). Direct validation of the back-calculation method was achieved by comparison of the estimated individual length with the actual measured length at each thermal marking date for each individual using a linear regression analysis. The proportion of variation in observed length explained by back-calculated length was used as a quantitative measure of accuracy. The slope value obtained was also compared with a theoretical slope of 1 using a t test. This analysis was performed separately for immature and mature males on ln-transformed data. The absolute percentage of divergence of back-calculated individual lengths from observed values (nontransformed data, $100 \times (\text{observed} - \text{back-calculated}/\text{observed})$) was calculated for each fish and for each measurement date separately before being averaged among individuals for a given date.

Mean values and 95% confidence intervals of back-calculated length at each measuring occasion were calculated on original data for the immature and precocious groups separately. They were compared with the mean somatic growth trajectories observed for their respective groups.

Results

Fifty-three females, 51 immature males, and 13 precocious males were identified at the end of the experiment. Twenty-four individuals had “vateritic” otoliths, where the carbon crystal formation is different from the more common “aragonit” type (Campana and Jones 1992). Vateritic otoliths cannot be used to determine the age of old individuals. Forty-five males in total were available for otolith analysis, 34 immature and 11 mature. Twenty-six percent of males were therefore precociously mature at sacrifice. Although thermal marks were readily found on most otoliths, not all of them could be retraced adequately on some individuals owing to the presence of fissures or chipped parts created during otolith preparation. This resulted in variable sample sizes for a given sampling date and an unequal number of repeated measures among individuals. Individuals from the original group established in January had between four and 14 otolith radius–somatic size paired measurements available for analysis (median of 10), whereas the individuals added in May had between six and nine measurements (median of 8).

Somatic size range and growth-rate pattern

Individual size varied between 58 and 136 mm, and 60 and 109 mm, respectively, for immature and mature males for the duration of the experiment. Mean length varied among marking dates ($p < 0.0001$), but not between immature and mature males ($p = 0.07$). The interaction term was not significant ($p = 0.08$). However, these results suggest that the growth patterns of the two groups were not parallel (Fig. 1).

An a posteriori analysis showed that length differed between mature and immature males on marking dates 13 to 16 ($0.010 > p > 0.003$). However, when a Bonferroni correction of the α level of acceptance was used to account for the number of comparisons made (corrected $\alpha = 0.003$), a significant difference was found between mean size of mature and immature males only on occasion 16, even though the trend was apparent in the 45 days preceding sacrifice.

Somatic–otolith size relationship

A significant relationship between otolith radius size and fish length measured at regular intervals was observed for immature and mature males, with otolith size explaining a significant proportion of the variation of fish size in a repeated-measures ANOVA ($p < 0.0001$, Fig. 2). We could not reject the null hypothesis of no significant difference in the slope ($p = 0.06$) and the intercept ($p = 0.63$) of the otolith and somatic size relationships of immature and mature males. We verified the power of our analysis to detect differences in the slope and the intercept given the sample sizes of mature and immature male parr. A power of 80% for an analysis is a desirable value to minimize type II error (Peterman 1990). Considering our sample size and the standard error of the slopes, our power to detect a significant dif-

Fig. 1. Mean somatic length observed on each marking–measuring occasion (15-day interval) for age-1+ immature (open circles) and mature (solid circles) Atlantic salmon (*Salmo salar*) males. The 95% confidence intervals associated with these values are represented by the horizontal lines.

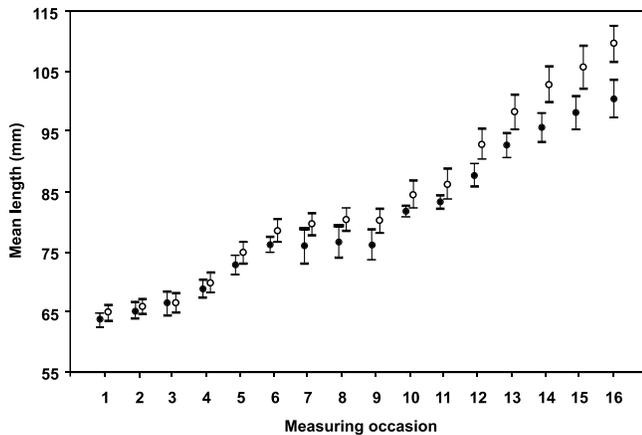
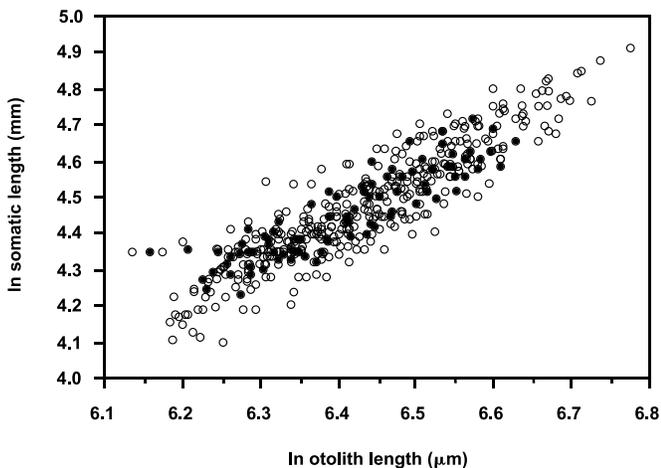


Fig. 2. Otolith radius size and corresponding fish length obtained for immature (open circles) and mature (solid circles) Atlantic salmon (*Salmo salar*) males for all marking–measuring occasions combined.



ference between the slopes of the otolith–fish size relationships of the two groups (5.6% difference) was 48%. Simulations also showed that we had a power of 82% to detect a difference of 8.5% in the two slopes with our experimental design. For the intercept, our analysis showed that we had a power of only 8% to detect the 0.15% difference observed between the two intercepts, considering our sample size and the variability of the two estimates. However, simulations also showed that our analysis had a power of 80% to detect a significant difference of only 0.9% between the two intercepts.

Coupling of short-term otolith growth with somatic growth and temperature

Coefficients of correlation between otolith growth and somatic growth for different growth intervals varied between 0.29 (15-day interval) and 0.81 (75-day interval) (Table 1). These correlations were all statistically significant ($p < 0.001$). The

correlation coefficient calculated for a growth interval of 15 days was significantly different ($p < 0.05$) from coefficients calculated for intervals of 75, 90, and 105 days. The coefficient calculated for a growth interval of 75 days was also different from the coefficients calculated over 30 and 45 days (Table 1). There was no significant covariation of mean temperature and short-term (15 days) otolith growth, although it was nearly statistically significant, as the correlation coefficient observed was 0.29, whereas a coefficient of at least 0.30 was needed to be considered significantly different from zero at the corrected α level of significance.

Direct validation of the back-calculation method

There was a linear relationship between individually back-calculated lengths estimated with the biological intercept model for a given fish and date and the corresponding observed lengths for immature ($r^2 = 0.90$, $p < 0.0001$) and mature ($r^2 = 0.87$, $p < 0.0001$; Fig. 3) males. The slopes of these relationships did not differ significantly from a theoretical slope of 1 ($p > 0.05$). Individually back-calculated lengths with the biological intercept model departed from observed lengths by 1.3–11.2% on average for immature males, depending on the sampling date, with a median difference of 5.0% for the whole experiment. Back-calculated individual lengths of mature males differed from corresponding observed lengths by 1.5–4.3% with a median difference of 3.3%.

When group mean length values were calculated for each measurement date, back-calculated lengths were within the 95% confidence interval (CI) of actual mean lengths for eight measurement dates (out of 13) in immature fish and seven out of 11 measurements for mature males (Fig. 4a, 4b). When the 95% CI of the back-calculated values were also taken into account, 85% of the mean size estimates fell within the 95% CI of observed mean size for immature fish, whereas 100% of estimates did for mature males.

Discussion

Somatic–otolith size relationship

Our longitudinal approach in a controlled environment mimicking natural conditions of temperature and photoperiod led to the development of sexual maturity in one male out of five. This is comparable to the incidence of precocious maturity observed in many rivers at this latitude (Myers et al. 1986). The observation that individual growth patterns of immature males diverged from growth trajectories of maturing males at a particular point in the growing season was also comparable to earlier laboratory studies (Rowe and Thorpe 1990). This divergence in size of individuals of the same age living in the same conditions is related to a different energy allocation strategy for sexual maturation. Our study further demonstrated that the proportionality ratio of somatic and otolith growth was not affected by this onset of differential energy allocation to maturation in Atlantic salmon males.

The absence of differences in the slopes and intercepts of the otolith–somatic size relationship supports the assumption of linearity of this relation for the two groups. In addition, the absence of a difference in the intercept justifies the use of a common biological intercept for immature and mature

Fig. 3. Individual back-calculated lengths and corresponding observed lengths for immature (open circles) and mature (solid circles) Atlantic salmon (*Salmo salar*) males. Regression equation lines are represented for immature (dotted line) and mature (solid line) males separately.

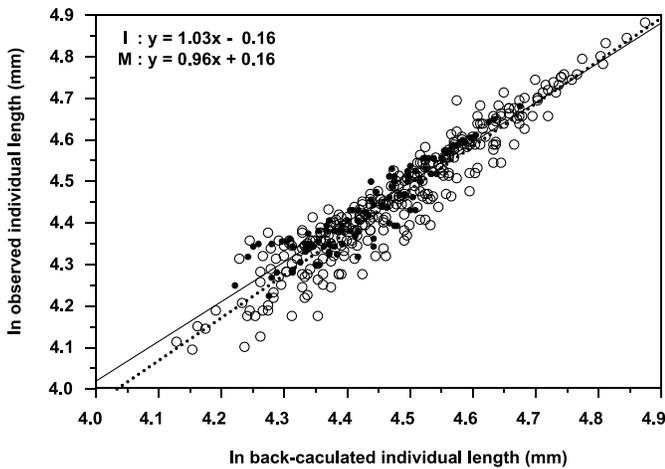


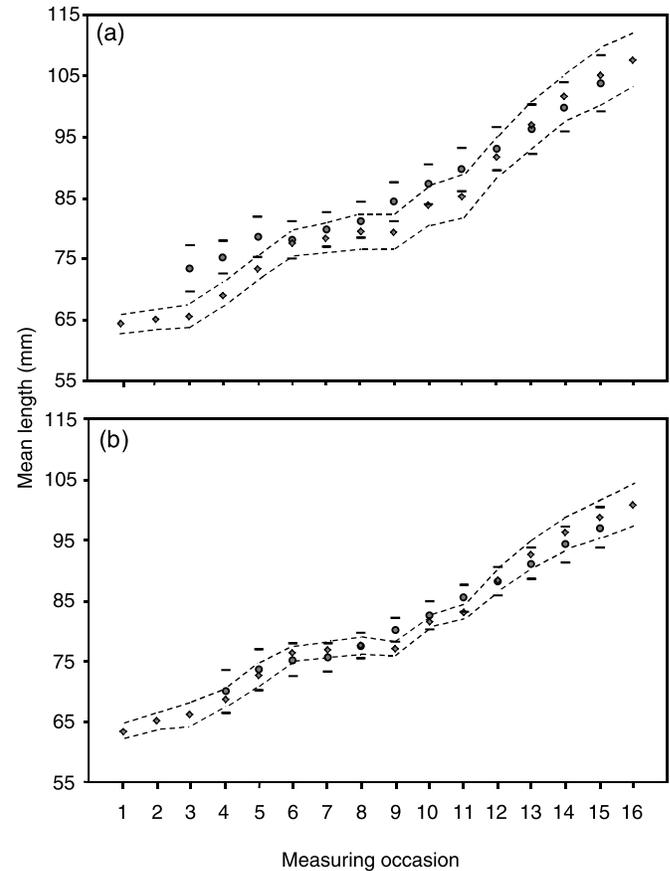
Table 1. Correlation coefficient (*r*) of otolith and somatic growth variations of age-1+ Atlantic salmon males calculated over different time scales (growth interval).

Growth interval (days)	<i>r</i>	<i>N</i>	Significantly different coefficients
15	0.29	45	
30	0.41	43	
45	0.40	44	
60	0.66	44	
75	0.81	42	15, 30, 45
90	0.78	39	15
105	0.76	41	15
120	0.70	40	
135	0.67	32	

Note: Number of fish available depending on length of interval is indicated (sample size; *N*). Coefficients presenting significant differences (at the $\alpha = 0.05$ level) from the value in a given row are represented in the last column by their growth-interval length.

males. However, the failure to reject null hypotheses when they are false has important implications for the use of the biological intercept method to back-calculate body lengths based on otolith microstructure. A curvilinear relationship impedes the use of the biological intercept method, as it biases back-calculation estimates (Campana 1990). Significant differences in the biological intercepts of the two groups would obviously bias back-calculated somatic lengths of both groups. The a posteriori power analyses permit the evaluation of the relative importance of such biases. Power analysis revealed that if a difference of 5.6% in the two otolith–fish size slopes was significantly different from zero in the true population, there would be a 52% probability of not rejecting the null hypothesis (even though it was false) because of sample size and variance. On the other hand, there was an 82% probability of finding statistically significant effects of maturation on the slope of this relationship if the true difference between the slopes of the two groups in the

Fig. 4. Mean length and 95% confidence interval (CI) estimated using back-calculated lengths values (mean: solid circles; 95% CI: horizontal lines) and observed lengths (mean: solid diamonds; 95% CI: broken line) for (a) immature and (b) mature Atlantic salmon (*Salmo salar*) males. Mean values of back-calculated lengths for measurements 1 and 2 of immature individuals are not presented as *N* < 2. Mean values of back-calculated lengths for measurements 1 to 3 of mature males are not presented as *N* < 2.



population would have been 8.5%. Such differences in slopes are relatively minor in the context of back-calculation. Secor and Dean (1992) conducted an experiment where different groups of larval striped bass (*Morone saxatilis*) were differentially fed in the laboratory to generate different growth rates. When excluding a starved treatment, differences varying between 5 and 20% in the slopes of the otolith–somatic size relationship were observed. Thus, the 5.6% difference in slopes documented here is at the low end of variation caused by differing feeding regimes and is related to lower growth of mature males rather than the uncoupling of somatic and otolith growth. This growth effect is integrated in the biological intercept method and does not affect back-calculations (Campana 1990). Finally, the very small variation observed in the intercept between the two groups (0.15%) appears of little consequence. Our experimental design was sufficient to identify a difference of only 0.9% between intercepts as being statistically significant. Differences of this magnitude are less than the precision error in measuring otoliths. Secor and Dean (1992) reported differences of 1.3–2.3% on repeated measures of the same otoliths. Because the difference observed between intercepts in

the present study was six times smaller than this value, its impact on the back-calculation of somatic length is deemed insignificant.

The observation that the slope and the intercept of the relationship between otolith size and fish size are similar between individuals that do not have the same developmental pathways suggests that the slowing down of somatic growth in precocious males is reflected in otolith growth. This result contrasts with work of Wright et al. (1990), who found that the divergent growth patterns observed between UMG and LMG fish were associated with a differentiation in the otolith–somatic size proportionality ratio. Considering that they propose that LMG fish have a lower metabolic resting rate, results from Wright et al. (1990) and the present study suggest that metabolic rates are influential in the age of onset of smoltification, but do not play such an important role in early maturation. Various physiological changes appear at the onset of smoltification, for migrating individuals to face saltwater challenges (Leonard and McCormick 2001). These modifications may be associated with particular metabolic requirements, which only a certain category of individuals (the UMG individuals) would be able to meet. Conversely, Tucker and Rasmussen (1999) did not find differences in metabolic rates between immature and mature male Atlantic salmon juveniles in natural conditions. These findings are consistent with the absence of different otolith–somatic proportionality ratios at the onset of sexual maturity observed in the present study and account for the apparently conflicting results with Wright et al. (1990).

Coupling of short-term otolith growth with somatic growth and temperature

The present study indicates that otolith and somatic growth have significant covariation over short-term periods of 15 days when all growth intervals are considered. Our longitudinal approach permitted us to integrate growth estimates over a time interval that spanned more than one growth period, e.g., >15 days, as proposed by Campana and Jones (1992) as a method to identify the effect of time interval length on the otolith–somatic growth relationship. Covariations of otolith and somatic growth calculated over a longer time period were also significant. Furthermore, the temporal scale of the comparison had a significant effect on the observed covariation of otolith and somatic growth. Correlation coefficients calculated on a longer time interval (75 days) differed significantly from coefficients estimated for the 15- to 45-day intervals. Such differences are probably related to the basal rhythm of these two growth processes and the temporal scale of the measurements. Otolith growth has a period of about 24 h in many species of salmonids (Wright et al. 1991). Direct repeated measurements demonstrate that salmonids also exhibit a cyclic increase of somatic size at different life stages (Aboul-Hosn et al. 2000). However, this cycle does not have a 24-h period. For example, brook trout held in the laboratory were shown to have a growth periodicity of 7–12 days (Harper et al. 1999; Aboul-Hosn et al. 2000). Based on these findings, measuring the somatic growth of fish on a 15-day basis may not be a proper time frame to perceive that growth has effectively occurred. It has been suggested that otolith growth variations can not be related to somatic growth on a very short time

scale, as otolith growth is affected by a wide variety of factors that do not instantly affect somatic growth (Volk et al. 1984; Campana and Jones 1992; Secor and Dean 1992). This may explain the observation in our study of significantly greater correlation coefficients over long-term periods than over the short term. This result is therefore consistent with our significant otolith–fish size relationship and with previous findings by Bradford and Geen (1987). These authors demonstrated that proportionality was nonexistent in juvenile chinook salmon (*Oncorhynchus tshawytscha*) when mean growth rates were observed over a short time interval, whereas the relationship was strong when mean otolith and somatic growth of a group of fish were calculated over a longer period of 50 days. On the other hand, our results contrast with those of Wright (1991), who observed that over a 50-day period, otolith increment width was not correlated to growth rate in mass estimated for 10 juvenile Atlantic salmon. However, this anomalous result may be linked to the inclusion of five UMG and five LMG individuals in Wright's (1991) study. Curvilinearity of the somatic–otolith relationship in the LMG fish (Wright et al. 1990) is present and therefore affects the overall relationship calculated with pooled individuals (Campana and Jones 1992). In the present study, sexual maturity does not affect the linearity of the relationship. Our short-term (15 days) results and Wright's (1991) study may also be influenced in part by a statistical factor rather than a biological one. Meekan et al. (1998) showed that the absence of correlation between short-term somatic and otolith growth in Atlantic salmon fry could be explained by the effect of truncated otolith and somatic size range used in correlation analysis.

The fact that short-term otolith growth was only weakly linked to variations in temperature, an important variable acting upon metabolic rate, suggests that the processes affecting otolith growth are complex. Indeed, Wright (1991) also observed that otolith growth was not linked simply to temperature, as otolith accretion was present without any change in this variable. Furthermore, recent work by Wright et al. (2001) suggested that otolith growth was related to resting metabolism, but that the latter was linked to temperature more directly than the variables regulating otolith growth. They suggested that factors other than temperature play a role in otolith growth, explaining that the link between these two variables is neither linear nor simple. Barber and Jenkins (2001) found that uncoupling between otolith and somatic growth was more intense in high temperature – fasting treatments with otolith growth being apparent and positively correlated to temperature in nongrowing starved fish. In this latter case, the relationship between otolith growth and temperature was simplified by the absence of feeding. However, this type of manipulation leads to death in some instances when rations are too low (Volk et al. 1984) and could not be sustained for a long period in the wild (Schirripa and Goodyear 1997). Therefore, intense uncoupling of otolith and somatic growth, as observed in the Barber and Jenkins (2001) laboratory study, is less likely to be observed in situ, as the exogenous processes that govern otolith growth also regulate somatic growth, and ultimately, life and death in natural conditions. Overall, the present study, coupled with others' results, suggests that otolith growth is governed by many factors and that the relationship be-

tween this variable and somatic growth must not be totally rejected, but rather be taken as a real, albeit complex, relationship (Wright et al. 2001).

Direct validation of back-calculation of individual somatic length

The present study is, to our knowledge, the only study that takes advantage of an individual-based longitudinal approach to evaluate the precision of back-calculations. We directly validated the adequacy of the biological intercept (BI) method to repeatedly back-calculate individual lengths of age-1+ juvenile Atlantic salmon differing in their growth rates among individuals and through time. The BI model permitted the accurate determination of individual length at an earlier moment in life (15–195 days back in time). Back-calculated lengths explained 90 and 87% of variation in observed lengths of immature and mature males, respectively. This result and the 1:1 relationship between individual back-calculated and observed lengths suggest that the BI back-calculation method accurately reconstructed individual growth history. Furthermore, median differences between individual observed length and the corresponding estimated lengths were under 5% for both immature and mature individuals.

On an average basis, mean “group” growth patterns estimated with back-calculated lengths separately for immature and mature males were fairly accurate, as 85% of the mean size estimates fell within the 95% CI of observed mean size for immature fish, whereas 100% of the estimates did for mature males. Specific occasions showed lack of fit of the mean back-calculated lengths to observed data and accounted for the imperfect estimates. This type of result has been related by earlier studies to two different factors: the conservative nature of otolith growth (Mugiya 1987; Secor et al. 1989) and the linearizing effect of the biological intercept method (Secor and Dean 1992; Sirois et al. 1998). Drastic variations of mean growth rates between two successive 15-day periods (e.g., between measurements 8 and 10) were not reflected in corresponding back-calculated values in which growth was more evenly distributed. This suggests that the mean proportionality ratio was larger during this period, with otoliths continuing to grow (being “conservative”) while somatic size changed little. Presence of variations in growth rate over a short-term period was not reflected in back-calculated lengths, though long-term size variations were accurately predicted. This result is in accordance with work by Secor and Dean (1992), who evaluated the precision of different back-calculation methods for larval striped bass. Using a mean length approach, they showed that when somatic growth rates were null or negative at the population level, the biological intercept method would not reflect these variations, since this model linearized the growth trajectory. Sirois et al. (1998) also found this at the population level with larval rainbow smelt (*Osmerus mordax*). This implies that this model gives an averaged version of the growth-rate trajectory at the population level. Clearly, this analysis suggests caution in using back-calculation methods that do not take into account large variations in the proportionality ratio among individuals and over time for fish experiencing significant alterations in growth rates, as proposed by Campana (1990), Secor and Dean (1992), and Sirois et al. (1998). Altogether, this study verified the assumptions behind the

back-calculation method, demonstrated that they are met for juvenile Atlantic salmon, and that it can be used to accurately estimate individual size for age-1+ immature fish, as well as for sexually mature conspecific males, despite their different energy allocation patterns.

Acknowledgments

The authors thank Francis Bérubé, Dany Bussièrès, Dany Garant, Nico Jomphe, Pierre-Alexandre Paradis, and Véronique Thériault, who assisted with laboratory manipulations and maintenance, Christian R. Landry and Pascal Sirois, as well as David Secor and an anonymous referee, who gave important comments on earlier versions of this manuscript. This experimental protocol was revised and approved by the Laval University Animal Protection Committee. Nadia Aubin-Horth was supported by a postgraduate scholarship from NSERC (Natural Sciences and Engineering Research Council of Canada), from GIROQ (Groupe Interuniversitaire de Recherche en Océanographie du Québec), and the partners of CIRSA Inc. (Corporation de soutien aux initiatives de recherche sur le saumon Atlantique). Funding of this project was provided to Julian J. Dodson by NSERC (Collaborative Special Projects), the Fondation de la Faune du Québec, the Government of Québec (Ministère de l'Environnement et de la faune), the Government of Canada (Economic Development), and other financial partners of CIRSA Inc.

References

- Aboul-Hosn, W., Dutilleul, P., and Boisclair, D. 2000. Handling and stocking-density effects on growth rhythms of brook trout, *Salvelinus fontinalis*. *Can. J. Zool.* **78**: 1026–1031.
- Akinicheva, E.G., and Rogatnykh, A.Y.U. 1996. The salmon marking experiment at fish hatcheries: thermal marking. *J. Ichthyol.* **36**: 659–664.
- Barber, M., and Jenkins, G. 2001. Differential effects of food and temperature lead to decoupling of short-term otolith and somatic growth rates in juvenile King George whiting. *J. Fish Biol.* **58**: 1320–1330.
- Bradford, M.J., and Geen, G.H. 1987. Size and growth of juvenile chinook salmon back-calculated from otolith growth increments. *In Age and growth of fish. Edited by R.C. Summerfelt and G.E. Hall.* Iowa State University Press, Ames, Iowa. pp. 453–461.
- Campana, S.E. 1990. How reliable are growth back-calculations based on otoliths? *Can. J. Fish. Aquat. Sci.* **47**: 2219–2227.
- Campana, S.E., and Jones, C.M. 1992. Analysis of otolith microstructure data. *In Otolith microstructure examination and analysis. Edited by D.K. Stevenson and S.E. Campana.* *Can. Spec. Publ. Fish. Aquat. Sci.* No. 117. pp. 73–100.
- Duston, J., and Saunders, R.L. 1992. Effect of 6-, 12- and 18-month photoperiod cycles on smolting and sexual maturation in juvenile Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* **49**: 2273–2280.
- Good, S.P., Dodson, J.J., Meekan, M.G., and Ryan, D.A.J. 2001. Annual variation in size-selective mortality of Atlantic salmon (*Salmo salar*) fry. *Can. J. Fish. Aquat. Sci.* **58**: 1187–1195.
- Harper, D., Martins, H., Boisclair, D., and Dutilleul, P. 1999. Effects of body size and temperature on periodicities in feeding and growth of brook trout (*Salvelinus fontinalis*) and in ammonia concentration of water. *Vie Milieu.* **49**: 261–273.

- Hutchings, J.A., and Jones, M.E.B. 1998. Life history variation and growth rate thresholds for maturity in Atlantic salmon, *Salmo salar*. Can. J. Fish. Aquat. Sci. **55**(Suppl. 1): 22–47.
- Hutchings, J.A., and Myers, R.A. 1994. The evolution of alternative mating strategies in variable environments. *Evol. Ecol.* **8**: 256–268.
- Leonard, J., and McCormick, S. 2001. Metabolic enzyme activity during smolting in stream- and hatchery-reared Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **58**: 1585–1593.
- Letcher, B.H., and Terrick, T.D. 1998. Thermal marking of Atlantic salmon otoliths. N. Am. J. Fish. Manag. **18**: 406–410.
- Meekan, M.G., Dodson, J.J., Good, S.P., and Ryan, D.A.J. 1998. Otolith and fish size relationships, measurement error, and size-selective mortality during the early life of Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **55**: 1663–1673.
- Mugiya, Y. 1987. Phase difference between calcification and organic matrix formation in the diurnal growth of otoliths in the rainbow trout, *Salmo gairdneri*. Fish. Bull. **85**: 395–401.
- Myers, R.A., Hutchings, J.A., and Gibson, R.J. 1986. Variation in male parr maturation within and among populations of Atlantic salmon, *Salmo salar*. Can. J. Fish. Aquat. Sci. **43**: 1242–1248.
- Neuman, M.J., Witting, D.A., and Able, K.W. 2001. Relationships between otolith microstructure, otolith growth, somatic growth and ontogenetic transitions in two cohorts of windowpane. J. Fish Biol. **58**: 967–984.
- Peterman, R.M. 1990. Statistical power analysis can improve fisheries research and management. Can. J. Fish. Aquat. Sci. **47**: 2–15.
- Rowe, D.K., and Thorpe, J.E. 1990. Differences in growth between maturing and non-maturing male Atlantic salmon, *Salmo salar* L., parr. J. Fish Biol. **36**: 643–658.
- Schirripa, M.J., and Goodyear, C.P. 1997. Simulation of alternative assumptions of fish otolith-somatic growth with a bioenergetics model. *Ecol. Model.* **102**: 209–223.
- Secor, D.H., and Dean, J.M. 1992. Comparison of otolith-based back-calculation methods to determine individual growth histories of larval striped bass, *Morone saxatilis*. Can. J. Fish. Aquat. Sci. **49**: 1439–1454.
- Secor, D.H., Dean, J.M., and Baldevarona, R.B. 1989. Comparison of otolith growth and somatic growth in larval and juvenile fishes based on otolith length/fish length relationships. *Rapp. P.-V. Réun. Cons. Int. Explor. Mer.* **191**: 431–438.
- Sirois, P., Lecomte, F., and Dodson, J.J. 1998. An otolith-based back-calculation method to account for time-varying growth rate in rainbow smelt (*Osmerus mordax*) larvae. Can. J. Fish. Aquat. Sci. **55**: 2662–2671.
- Sokal, R.R., and Rohlf, F.J. 1995. *Biometry: the principles and practice of statistics in biological research*. 3rd ed. W.H. Freeman and Company, New York.
- Stearns, S.C. 1976. Life-history tactics: a review of the ideas. *Q. Rev. Biol.* **51**: 3–47.
- Tucker, S., and Rasmussen, J.B. 1999. Using ^{137}Cs to measure and compare bioenergetic budgets of juvenile Atlantic salmon (*Salmo salar*) and brook trout (*Salvelinus fontinalis*) in the field. Can. J. Fish. Aquat. Sci. **56**: 875–887.
- Volk, E.C., Wissmar, R.C., Simenstad, C.A., and Eggers, D.M. 1984. Relationship between otolith microstructure and the growth of juvenile chum salmon (*Oncorhynchus keta*) under different prey rations. Can. J. Fish. Aquat. Sci. **41**: 126–133.
- Wright, P.J. 1991. The influence of metabolic rate on otolith increment width in Atlantic salmon parr, *Salmo salar* L. J. Fish Biol. **38**: 929–933.
- Wright, P.J., Metcalfe, N.B., and Thorpe, J.E. 1990. Otolith and somatic growth rates in Atlantic salmon parr, *Salmo salar* L.: evidence against coupling. J. Fish Biol. **36**: 241–249.
- Wright, P.J., Rowe, D., and Thorpe, J.E. 1991. Daily growth increments in the otoliths of Atlantic salmon parr, *Salmo salar* L., and the influence of environmental factors on their periodicity. J. Fish Biol. **39**: 103–113.
- Wright, P.J., Fallon-Cousins, P., and Armstrong, J.D. 2001. The relationship between otolith accretion and resting metabolic rate in juvenile Atlantic salmon during a change in temperature. J. Fish Biol. **59**: 657–666.