

Back-calculation of previous fish size using individually tagged and marked Atlantic cod (*Gadus morhua*)

L. Li, H. Høie, A.J. Geffen, E. Heegaard, J. Skadal, and A. Folkvord

Abstract: The performance of five back-calculation (B-C) models was tested with individually tagged and multiple alizarin-marked Atlantic cod (*Gadus morhua*) reared at seasonally varying water temperature and food regimes over a 10-month period. The otoliths were transversely sectioned and the otolith growth between marks was measured along the dorsal, distal, and ventral radii and across the otolith width. Observed fish lengths at each marking time were compared with back-calculated lengths using linear and nonlinear scale and body proportional hypotheses (SPH and BPH) and biological intercept (BI) models. Measurements along the dorsal and distal radius with SPH and BI provided more accurate fish length estimates but with lower precision, whereas measurements of the ventral radius consistently produced greater overestimations by any model. Otolith width measurements produced moderately overestimated fish length estimates but with the highest precision and were significantly affected by temperature in all models. Certain combinations of model and otolith dimension were more sensitive to the length of the back-calculation time interval, whereas varying feeding regime induced temporary biases only. The selection of B-C model and otolith dimension thus depends not only on the environmental conditions that the fish experience, but also on the requirements of the application for which the B-C is used.

Résumé : Nous avons testé la performance de cinq modèles de rétro-calcul (B-C) à l'aide de morues franches (*Gadus morhua*) étiquetées individuellement et marquées à répétition à l'alizarine qui ont été élevées à des régimes saisonniers variables de température de l'eau et d'alimentation sur une période de 10 mois. Nous avons sectionné les otolithes transversalement et mesuré la croissance des otolithes entre les marques le long des rayons dorsaux, distaux et ventraux et à travers la largeur de l'otolithe. Les longueurs des poissons observées à chaque période de marquage ont pu être comparées aux longueurs obtenues par rétro-calcul à l'aide des modèles linéaires et non linéaires basés sur les hypothèses de la proportionnalité aux écailles (SPH) et au corps (BPH) et sur l'ordonnée à l'origine biologique (BI). Les mesures le long des rayons dorsal et distal fournissent avec les modèles SPH et BI des estimations plus justes des longueurs des poissons, mais avec une précision réduite, alors que les mesures du rayon ventral produisent une plus forte surestimation avec tous les modèles. Les mesures de la largeur de l'otolithe fournissent des estimations modérément exagérées des longueurs des poissons, mais avec la précision la plus grande; elles sont significativement affectées par la température dans tous les modèles. Certaines combinaisons de modèles et de dimensions des otolithes sont plus sensibles à la longueur de l'intervalle de temps du rétro-calcul, alors que le régime alimentaire variable produit seulement des déformations temporelles. Le choix d'un modèle de rétro-calcul et de la dimension de l'otolithe à mesurer dépend donc non seulement des conditions du milieu subies par les poissons, mais aussi des exigences de l'application visée par le rétro-calcul.

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Introduction

Back-calculation (B-C) is defined as estimating fish size at an earlier time or times on the basis of corresponding measurements of fish size and the dimensions of growth marks in calcified structures (e.g., scales, skeleton, otoliths,

etc.; Francis 1990). B-C is a valuable technique to use to obtain estimates of individual fish growth. Fish size-at-age has been estimated using B-C based on measurements of annual otolith growth marks to fit growth curves (Jones 2000; Colloca et al. 2003) and compare growth rates (Smedstad and Holm 1996; Morita and Matsuishi 2001; Roberson et al.

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2005). Back-calculated length (BCL) can also be obtained at important life history events such as maturity and settlement where these events are marked by distinct otolith features (Vigliola et al. 2000; Brophy and Danilowicz 2003; Colloca et al. 2003). In field studies of fish growth, B-C is the main alternative to mark-recapture studies, and it has the potential advantage that it can be employed on all individuals in the population instead of the limited number of tagged and recaptured fish (Folkvord and Mosegaard 2002).

Various calcified structures are used for B-C, and because sagittal otoliths are the most common structures used for age determination of marine fish species, their use for B-C can provide valuable additional information about individual fish growth. Otoliths are unlike other calcified structures such as spines and scales in that they do not appear to be subject to mineral resorption except under extreme stress (Mugiya and Uchimura 1989). Otoliths can also be marked with fluorescent compounds to mark the actual otolith size at specific dates.

There are several B-C models that differ in their expression of proportionality between fish growth and the growth of the calcified structures, and it is important to validate these models. However, validation of the models has rarely been based on accurate data. The best method for validating B-C performance is to use data from fish that are measured and individually marked with, e.g., a fluorescent chemical and physical tag and then later recaptured, remeasured, and remarked at repeated intervals, producing known increments of growth for comparison (Casselman 1983). Previous studies have used recaptures of tagged fish, but errors are inevitable as there were no precise otolith or scale marks corresponding to the previously measured fish size (Smedstad and Holm 1996; Morita and Matsuishi 2001; Heidarsson et al. 2006). By repeated marking of otoliths or scales Klumb et al. (2001) and Folkvord et al. (2004) could identify precisely previous sizes of these structures but did not have corresponding individual fish measurements and were restricted to comparing mean estimated fish lengths with the average of the population. Individually tagged and marked fish made it possible for studies such as Holmgren (1996), Panfili and Tomas (2001), and Roemer and Oliveira (2007) to validate different B-C applications, but only a few B-C models were used and much more attention was paid to the accuracy than the precision of the models.

Validation of B-C models should include an evaluation of both the accuracy and the precision. Accuracy describes the closeness of BCLs to the observed lengths (L_s). The closer BCLs are to L_s , the more accurate the model is considered to be. Precision describes the repeatability, the degree to which multiple BCLs will be the same or similar relative to the mean L_s both within and between individual fish. A B-C model is called valid for a particular application when it provides both accurate and precise fish length estimates (Francis 1990). It is thus essential to validate and compare widely used B-C models such as linear scale proportional hypothesis (linear SPH), linear body proportional hypothesis (linear BPH), nonlinear SPH, nonlinear BPH (Francis 1990), and biological intercept (BI; Campana 1990), using individually tagged fish that are held over a long time period with repeated measurement and marking.

Another important issue in the validation of B-C models

is the selection of the best otolith dimension for size measurements because the relationship between body size and otolith size depends on the axis, or dimension, chosen for B-C (Panfili and Tomas 2001). Morita (2001) found that measurements along the minor (the shortest) axis of scales of white-spotted charr (*Salvelinus leucomaenis*) provided more accurate estimates of previous lengths with the Fraser-Lee method than those along the major (the longest) axis. Roberson et al. (2005) also compared the accuracy of nonlinear BPH using otolith area and maximum width for B-C in tagged and recaptured Pacific cod (*Gadus macrocephalus*). However, very few studies have validated B-C models together with different dimensions of otolith size. It is of significance to determine which otolith dimension is the best predictor of fish length, especially for Atlantic cod (*Gadus morhua*), an economically important and heavily exploited species.

Few studies have investigated the effect of environmental or growth factors on the deviations of estimated fish length, although the relationship between fish growth and otolith growth has been widely observed to be affected by food-induced variations in growth rate (e.g., Secor and Dean 1992; Holmgren 1996; Folkvord et al. 2000), temperature (e.g., Barber and Jenkins 2001; Otterlei et al. 2002; Fey 2006), and age and ontogeny (e.g., Hare and Cowen 1995; Morita and Matsuishi 2001; Heidarsson et al. 2006). Francis (1990) stressed that both time and time interval had a significant effect on the difference between BCLs using linear SPH and linear BPH after examining eight published data sets. He found a progressive bias, which occurred because the deviation between estimated and observed fish size increased as the length of the back-calculation time interval increased, thus inducing considerable uncertainty in both models. Considering that BCLs are often estimated over many years of growth, especially for size-at-age data, it is important to establish which models and which otolith dimensions are most robust to the time interval that represents the period from the date of capture back to the time corresponding to the earliest length-at-age to be estimated. Additionally, Panfili and Tomas (2001) observed that higher growth rates resulted in increased overestimation of BCL using nonlinear BPH. Therefore, it is necessary to analyze a combination of effects incorporating potentially important factors (temperature, back-calculation time interval, and feeding regime) on the deviation of estimated fish length so that we can better understand the estimation errors and provide useful information for selecting the suitable B-C model and otolith dimension for B-C.

In this study, juvenile Atlantic cod were individually tagged and then measured and their otoliths were marked with Alizarin red S (ArS) at bimonthly intervals. The experimental design provided individual fish measurements on known dates, with corresponding otolith size measurements. The fish experienced natural seasonal temperature variations together with different manipulated feeding treatments to simulate the range of conditions likely in the natural environment. Otolith size measurements were made along four different otolith axes, and the data were used to validate five different B-C models. The purpose of this study was also to examine the effects of temperature, back-calculation time interval, and food treatments on the deviations of BCL.

Materials and methods

Growth experiments

Seventy-six one-year-old, laboratory-reared cod were tagged with internal passive integrated transponder (PIT) tags in March 2002 and were raised in 500 L tanks until 26 June 2002. The fish were then transferred to a 7 m³ tank for the remaining 10 months of the experiment. Bimonthly between 27 June 2002 and 18 February 2003, the fish were removed from their tanks and anaesthetized, measurements of their length and weight were taken, and their otoliths were marked using 24-h immersion in 50 mg·L⁻¹ ArS. The PIT tag IDs of each fish were scanned on every occasion so that the length and weight measurement data could be assigned to individually known fish.

From the beginning of the experiment, the cod were fed with dry pellets at a medium food ration of 0.5% dry weight of mean fish wet weight. This ration was chosen to promote realistic growth rates compared with wild cod of the same size, as estimated by the relationship given by Jobling (1988), and it was maintained until the third alizarin marking on 16 October 2002. The tank was then divided in two by a net and fish were divided equally to each side. The net also prevented food pellets from drifting from one side of the tank to the other side. A low level of food ration (Lo) supporting 20% of maximum growth was offered in one part of the tank and a high food ration (Hi) supporting 80% of maximum growth was offered in the other part of the tank. After 62 days (17 December 2002), half of the fish in each group were exchanged between feeding treatments. This treatment interval ended 18 February 2003 (63 days) (Fig. 1a). In this way, four groups of fish were produced, each experiencing a different feeding history: HiHi (high ration throughout), HiLo (high ration followed by low ration), LoHi (low ration followed by high ration), and LoLo (low ration throughout). All fish were subsequently given food in excess. Forty-three fish remained until the end of the experiment on 22 April 2003. The sagittal otoliths were dissected out after the final measurement. In addition, 33 fish were sampled during the experiment with otoliths collected and lengths measured.

Throughout the experiment, the fish were held in seawater pumped from a depth of 90 m and directed to the tanks after aeration. Water temperature followed that of seasonal ambient natural seawater, increasing from 8 °C at the beginning of the experiment to a maximum of 11.5 °C in late August 2002 and then decreasing to 7 °C in early March 2003 (Fig. 1b). The experiment was carried out within the guidelines of the local animal research board.

Otolith growth measures and calculations

The sagittae of sampled fish were embedded in Epofix resin and sectioned in the transverse plane. Two sections approximately 450 µm thick were cut through the core area with a low-speed diamond wafering saw blade (McCurdy et al. 2002). Digital images of each polished otolith section were taken with transmitted white light and fluorescent epi-illumination. The set of images for 30 fish, using the section with the most clear natural otolith features and ArS marks along the dorsal, distal, and ventral direc-

tions, were selected for further analysis. An additional 24 fish were used for the regression of fish total length (L) and otolith size (O) when we had clear images from the transverse sections of the outer otolith edge. Otolith size was measured using Image Pro[®] software (Media Cybernetics Inc., Bethesda, Maryland). Reference points, such as cracks and corners in the section, were then selected and tagged with coordinates in each image to align the white light and fluorescent images (Fig. 2). The core was identified in the transmitted light image. One point along the growth axis, farthest away from the core visually, was tagged with coordinates for each fluorescent mark and for the otolith margin in the dorsal, distal, and ventral directions on the fluorescent light images. The otolith sizes (at each mark and at termination) were finally calculated as dorsal, distal, and ventral radii and maximum widths (in millimetres) (Fig. 2).

Back-calculation models

The relationship between fish lengths (L) and otolith sizes (O) was described by the following regression models based on the final sampling size of 54 fish from all food treatment groups (Francis 1990):

$$(1) \quad O = a + bL$$

$$(2) \quad L = c + dO$$

$$(3) \quad \ln(O) = \ln(u) + v\ln(L)$$

$$(4) \quad \ln(L) = \ln(w) + k\ln(O)$$

The parameters a , b , c , d , u , v , w , and k were estimated by a linear model applying the fish size and otolith size or prior log-transformations of these.

Five B-C models were used in this study to estimate previous fish size for 30 fish at each marking time. SPH assumes that if a fish at capture has a 10% larger otolith than the average otolith size of the same fish size, then the fish keeps the same proportionality of otolith size throughout its life (Francis 1990). Both linear SPH (eq. 5) and nonlinear SPH (eq. 6) were estimated by linear regression, but nonlinear SPH used log-transformed fish length and otolith size:

$$(5) \quad BCL_i = -alb + (L_c + alb)O_i/O_c$$

$$(6) \quad BCL_i = (O_i/O_c)^{1/v}L_c$$

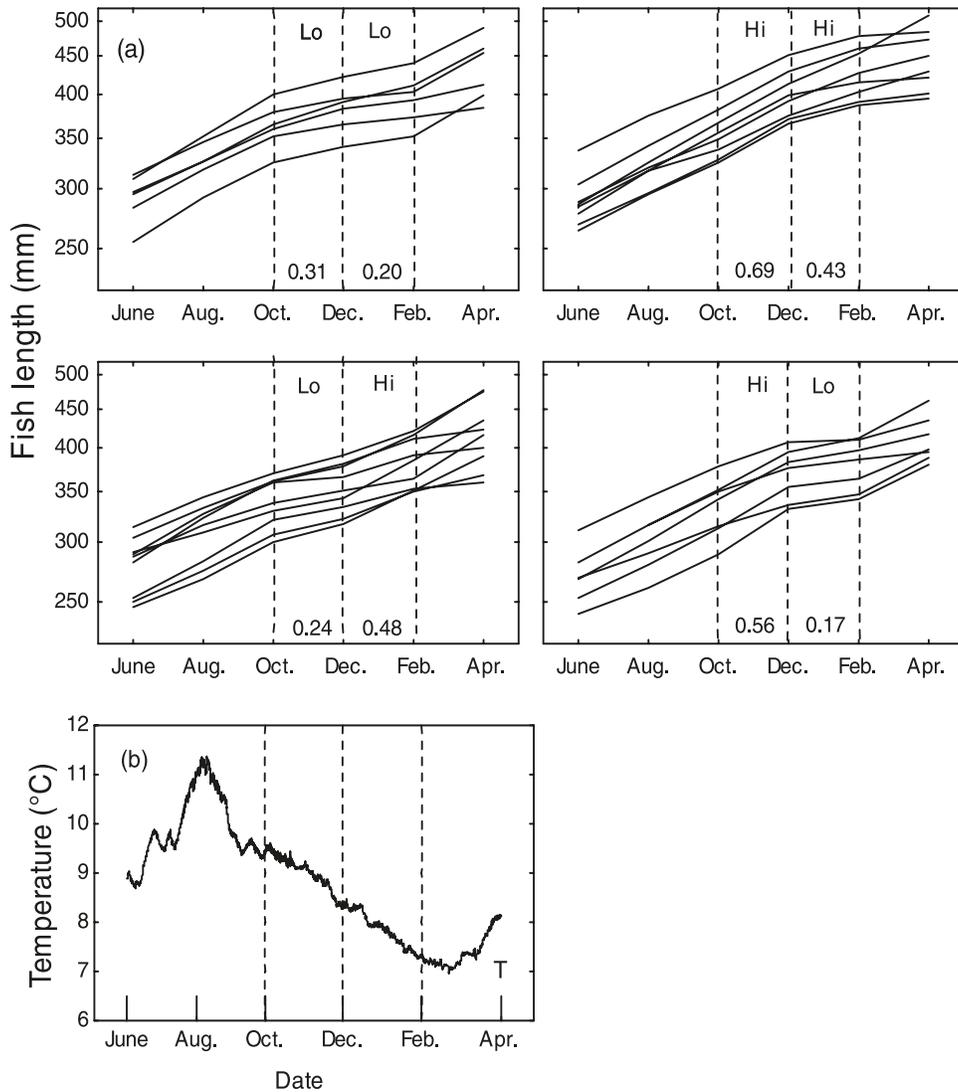
BPH is similar to SPH except that it is fish body size that retains the same proportionality throughout life (Francis 1990). The formulae for linear BPH (eq. 7) and nonlinear BPH (eq. 8) are as follows:

$$(7) \quad BCL_i = L_c(c + dO_i)/(c + dO_c)$$

$$(8) \quad BCL_i = (O_i/O_c)^kL_c$$

Campana (1990) developed BI (eq. 9) with the assumption

Fig. 1. (a) Individual fish lengths (mm, plotted on log-scale) measured at five marking times, with the periods of the different feeding treatments indicated by broken lines. Numbers within the different feeding periods represent average daily length growth rates (mm-day⁻¹) during that time period. (b) Variation in ambient water temperature (°C) over the course of the experiment. Vertical bars show dates of the five Alizarin markings, broken lines show the periods occupied by the feeding treatments, and “T” indicates the termination of the experiment. Feeding treatments: Hi, high ration; Lo, low ration.



that fish length growth and otolith growth become proportional from the point (L_o, O_o) in the early life stage.

$$(9) \quad BCL_i = L_c + (L_c - L_o)(O_i - O_c)/(O_c - O_o)$$

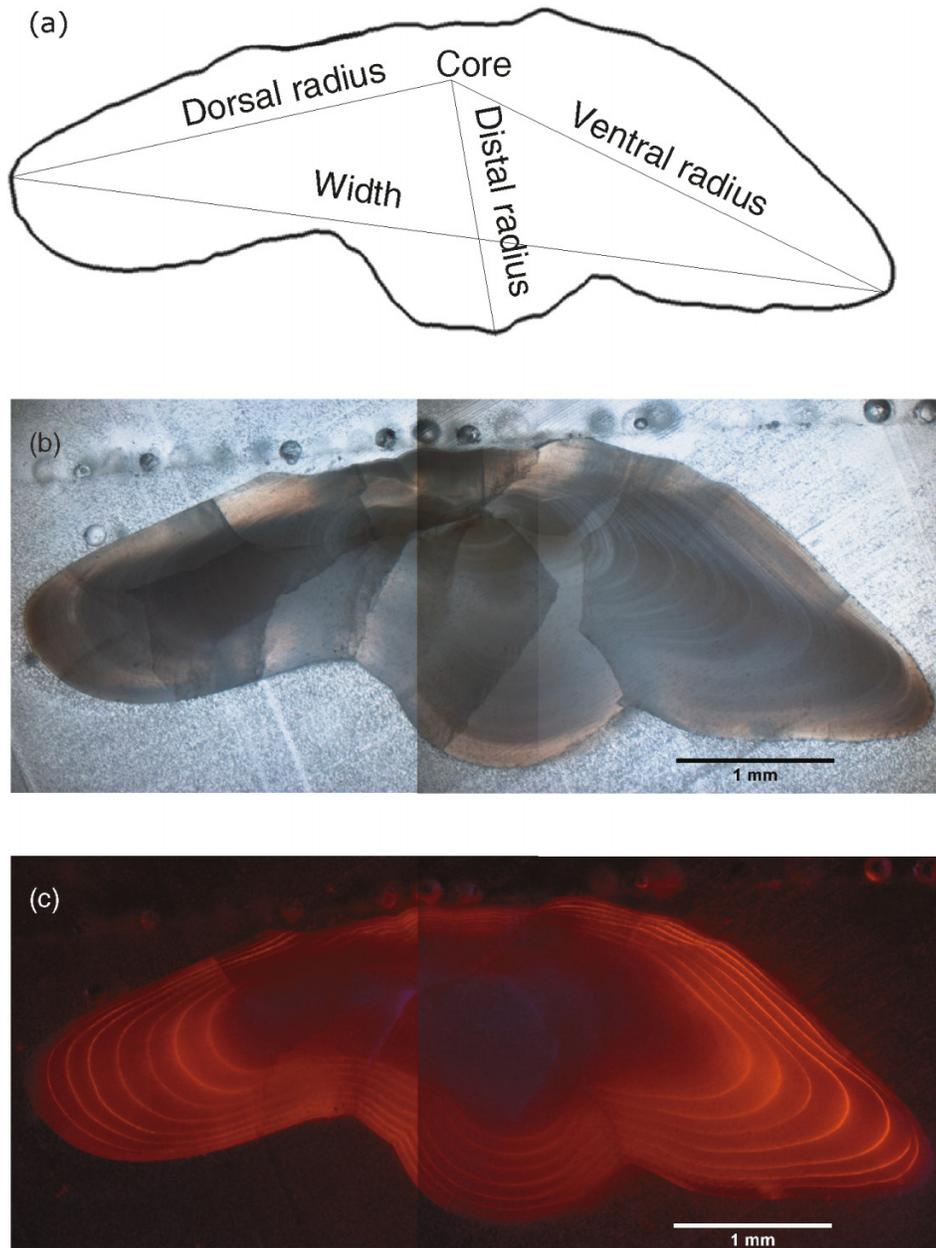
We use the terminology of Francis (1990) except that otolith size (O) is used to represent the size of calcified structure in the model equations. BCL_i is the back-calculated fish body length at marking time i ; L_c is the fish length at the time of final sampling (capture); O_i is otolith size at marking time i ; O_c is otolith size at the time of final sampling, and O_o and L_o are the observed otolith size and fish length when somatic growth and otolith growth become proportional. We used the values in Sinclair et al. (2002): $L_o = 24.4$ mm and $O_o = 0.44$ mm for the dorsal, distal, and ventral radius and 0.88 mm for width assuming that the otolith had not started to become markedly asymmetrical at this size.

Statistical methods

Relative residuals (Res), defined as $Res_{ji} = (BCL_{ji} - L_{ji})/L_{ji}$, were used to show the deviation of BCL_{ji} from L_{ji} for fish j ($j = 1, 2, 3, \dots, 30$) at marking time i ($i = 1, 2, 3, 4, 5$). Fish length increased considerably over the 10-month experiment, and a 50 mm difference between BCL and L had a larger effect in a fish of 250 mm than a fish of 500 mm. However, Res has the effect of standardizing for length and makes it possible to compare precisely the deviation in BCL for fish of different sizes and thus demonstrate the accuracy and precision of each B-C model.

With individual fish as a random factor, mixed-effects models (LME; Pinheiro and Bates 2000) were used to investigate the fixed effects of temperature, back-calculation time interval, and food treatment on Res. The initial LME model included the three factors and their interactions:

Fig. 2. (a) Schematic representation of four dimensions of otolith size in a transverse plane. (b–c) Cod otolith in transverse section viewed with (b) transmitted light and (c) reflected fluorescent light. Note that the otolith has two extra alizarin marks from preliminary marking trials in addition to the five marks imprinted during the experiment.



$$(10) \quad \text{Res}_{ji} = \beta_0 + b_{0j} + \beta_1 \text{treatment}_{ji} + \beta_2 \text{time}_{ji} \\ + \beta_3 \text{temperature}_{ji} + \beta_4 \text{treatment}_{ji} \times \text{time}_{ji} \\ + \beta_5 \text{treatment}_{ji} \times \text{temperature}_{ji} + \beta_6 \text{time}_{ji} \\ \times \text{temperature}_{ji} + \beta_7 \text{treatment}_{ji} \\ \times \text{time}_{ji} \times \text{temperature}_{ji} + \varepsilon_{ji}$$

where Res_{ji} is the i th Res in the j th fish, β_0 is the population mean intercept for all the fish, b_{0j} is a random variable representing the deviation from the population mean intercept for the j th fish, $b_{0j} \sim N(0, \sigma_b^2)$, β_1 to β_7 show the slopes of the corresponding fixed-effect treatments (treatment here means the four food treatment groups), time interval, and temperature and their interactions, ε_{ji} is a random variable represent-

ing the deviation in Res for the observation at marking time i on fish j from the mean Res for fish j , $\varepsilon_{ji} \sim N(0, \sigma^2)$. To refine the model, the factor with the highest p value was removed, starting from the interaction with the highest level, and a new model was checked. This procedure was repeated several times until we obtained the fit model with the lowest Bayesian information criterion (BIC; Pinheiro and Bates 2000).

The response Res is influenced by the experimental factors if the optimal model differs from the model:

$$(11) \quad \text{Res}_{ji} = \beta_0 + b_{0j} + \varepsilon_{ji}$$

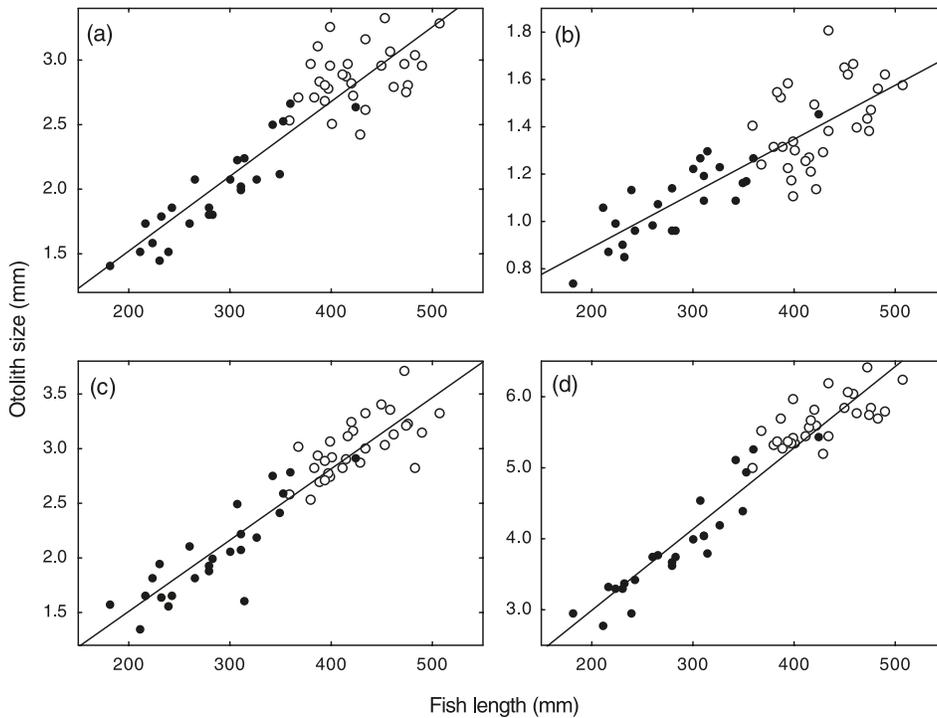
where β_0 is the population mean Res for all the fish over the whole experimental period, representing the accuracy of the

Table 1. Regression summary of final fish length and otolith sizes.

Regression	Otolith dimension	Equations	R ²
Equation 1	Dorsal radius	$O = 0.36 + 0.0058L$	0.84
	Distal radius	$O = 0.43 + 0.0023L$	0.69
	Ventral radius	$O = 0.21 + 0.0065L$	0.88
	Width	$O = 0.70 + 0.0114L$	0.91
Equation 2	Dorsal radius	$L = 6.26 + 144.78O$	0.84
	Distal radius	$L = -17.98 + 301.81O$	0.69
	Ventral radius	$L = 17.82 + 134.13O$	0.88
	Width	$L = -24.33 + 79.75O$	0.91
Equation 3	Dorsal radius	$\ln O = -4.20 + 0.87\ln L$	0.88
	Distal radius	$\ln O = -3.50 + 0.63\ln L$	0.73
	Ventral radius	$\ln O = -4.48 + 0.92\ln L$	0.88
	Width	$\ln O = -3.49 + 0.86\ln L$	0.93
Equation 4	Dorsal radius	$\ln L = 4.98 + 1.01\ln O$	0.88
	Distal radius	$\ln L = 5.62 + 1.14\ln O$	0.73
	Ventral radius	$\ln L = 4.99 + 0.95\ln O$	0.88
	Width	$\ln L = 4.19 + 1.08\ln O$	0.93

Note: $N = 54$.

Fig. 3. Otolith size (mm) in relation to fish length (mm): (a) dorsal, (b) distal, (c) ventral, and (d) width. Open circles represent 30 samples at the termination of the experiment. Solid circles represent 24 fish sampled prior to the end of the experiment. Note different scales on y axes.



B-C model, and b_{0j} and ε_{ji} are random effects specific for fish and residuals, respectively. Combined, the standard deviation of the random effects reflects the precision of the B-C model. Two LME models were also used to detect the long-term food ration effect:

$$(12) \quad \text{Res}_{ji} = \beta_0 + b_{0j} + \beta_1 \text{treatment}_{ji} + \beta_2 \text{time}_{e_{ji}} + \beta_3 \text{treatment}_{ji} \times \text{time}_{e_{ji}} + \varepsilon_{ji}$$

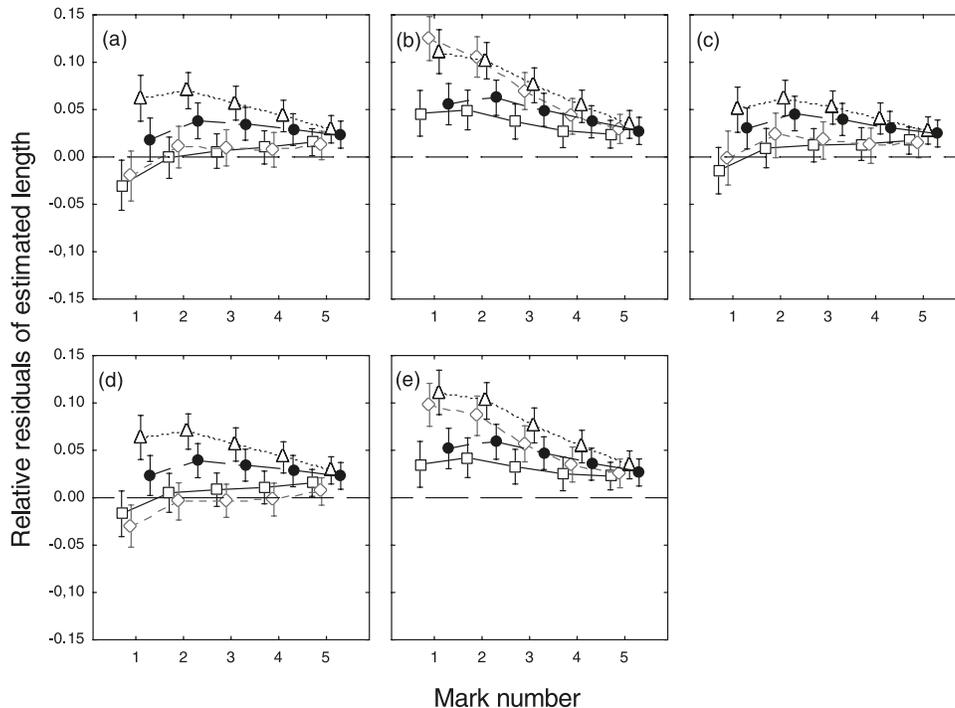
$$(13) \quad \text{Res}_{ji} = \beta_0 + b_{0j} + \beta_1 \text{treatment}_{ji} + \varepsilon_{ji}$$

Equation 12 revealed whether the effect of food treatment

depended on the back-calculation time interval by checking the significance of the interaction between treatments and time. We then used eq. 13 to examine the significance of Res in the four food treatment groups over the whole experimental period to detect any long-term effect.

Because food treatments were carried out only during the period between the third to fifth marking dates, the short-term effects were investigated using an analysis of variance (ANOVA) model for this time interval. Δ_{Res} ($\Delta_{\text{Res}_{ji}} = \text{Res}_{ji} - \text{Res}_{ji-1}$) was calculated, and the time and temperature difference at two adjacent marking times were ignored. Δ_{Res} was analyzed as a function of food ration level during food treat-

Fig. 4. The relative residuals of back-calculation (B-C) estimated fish length (mean \pm 0.95 confidence interval) at each marking time showing the accuracy of the five B-C models: (a) linear scale proportional hypotheses (SPH), (b) linear body proportional hypotheses (BPH), (c) biological intercept (BI), (d) nonlinear SPH, and (e) nonlinear BPH. Horizontal broken lines represent no deviation of estimated lengths from observed fish lengths (Res = 0). Open squares represent dorsal values; open diamonds, distal; open triangles, ventral; and solid circles, width. Means are offset for presentation purposes.



ment period, assuming that food treatment was the only reason for changes of Res.

The level of significance was set at 5% for all the statistical tests used in this study, and all analyses were carried out with S-plus 7.0 Professional software (Insightful Corporation, Seattle, Washington). All linear regression and LME models were diagnosed to test that the assumptions of models were satisfied. If these were not satisfied, the model was removed or altered until it met the requirements and was considered to be a qualified model. All of the results presented were produced by qualified models.

Results

Back-calculation

Both linear and nonlinear models described a close relationship between fish length and otolith size (Table 1; Fig. 3). Multiple R^2 ranged from 0.69 to 0.93, with the lowest value for the distal radius and the highest for otolith width. BCL were estimated by five B-C models for each fish at each marking time and then Res was calculated to evaluate the accuracy of different length estimates (Fig. 4). Using any model, ventral radii consistently overestimated BCL at every marking time and by the largest deviation from true length. Otolith width provided moderately overestimated BCL, and the dorsal otolith axis performed generally well. The distal radii gave accurate BCL estimates with linear and nonlinear SPH and BI but not with linear and nonlinear BPH. Furthermore, linear and nonlinear BPH produced much higher over-

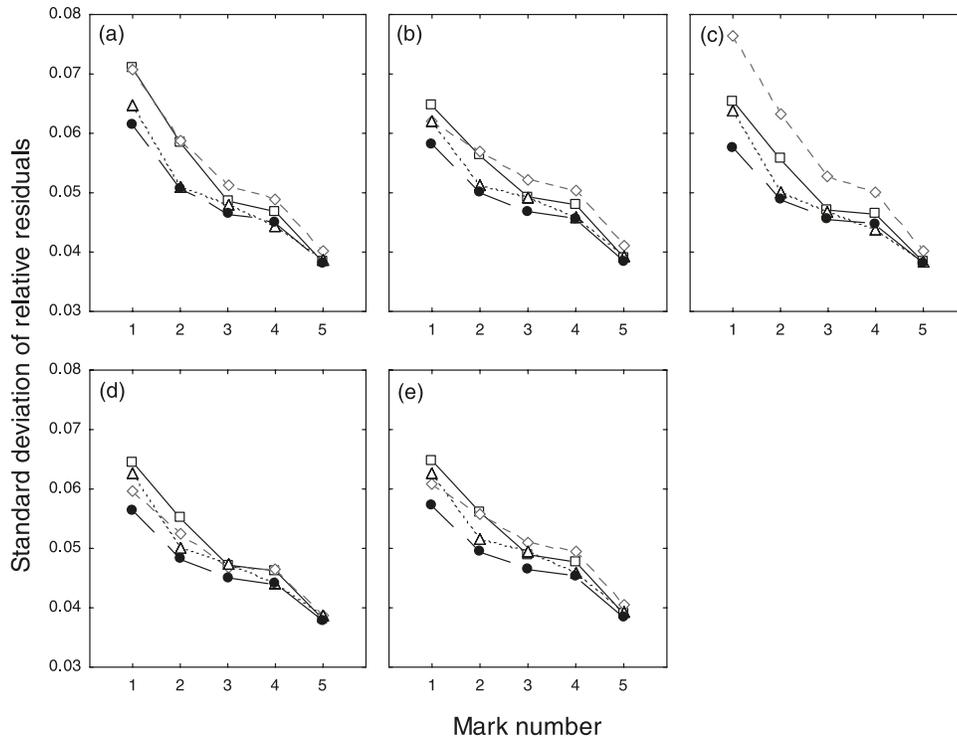
estimates of previous lengths at each marking time with any dimension of otolith size compared with linear and nonlinear SPH and BI. Among the three more accurate models, dorsal and distal radii produced the most accurate estimates of previous fish lengths and had similar Res patterns, with mean Res increasing primarily between the first and second mark and little further change (Fig. 4). Otolith width measurements resulted in Res patterns that appeared to follow the temperature regime, with mean Res values that increased from the first to the second mark and then decreased to the last mark. With BPH models, the mean Res that resulted from dorsal radius and width measurements also seemed to be similar to that of the temperature pattern.

SD of Res increased greatly when going backwards in time from mark 5, suggesting a strong influence of back-calculation time interval on precision (linear model, p value varied from 0.01 to 0.003). The relatively large increase in SD of Res from the fifth mark to the fourth mark may suggest a food treatment effect (Fig. 5). Surprisingly, width consistently demonstrated the best precision with any B-C model, whereas dorsal and distal radii showed the lowest precision depending on the model and marking time. Distal radius with BI yielded the lowest precision of all model and dimension combinations. The nonlinear method also provided more precise estimates than the linear method for SPH.

Time, temperature, and food treatment effects

The Res trajectory of nonlinear SPH-BCL with the four

Fig. 5. Standard deviation of relative residuals at each marking time showing the precision of each of the B-C models at the five different marking times. B-C models: (a) linear scale proportional hypotheses (SPH), (b) linear body proportional hypotheses (BPH), (c) biological intercept (BI), (d) nonlinear SPH, and (e) nonlinear BPH. Open squares represent dorsal values; open diamonds, distal; open triangles, ventral; and solid circles, width.



otolith dimensions indicated differences between and within fish at each marking time (Fig. 6). It is noticeable that the BCLs of most fish were either consistently overestimated or underestimated throughout the experimental period, which indicated relatively good precision within fish. Using LME model eq. 10 for distal radius, the fit model included time as a main fixed effect. When the back-calculated time interval extended backwards to cover two months, the Res decreased about $0.75\% \pm 0.15\%$ (mean \pm standard error, SE). Therefore, underestimation is more likely for the BCLs corresponding to youngest ages. The fit model for the width included temperature as a main fixed effect. When temperature increased 1°C , estimated Res increased $0.66\% \pm 0.25\%$ (mean \pm SE).

The standard deviation within fish (distal radius, 0.029; width, 0.025) and between fish (distal radius, 0.042; width, 0.040) revealed by the LME model (eq. 11) indicated higher precision for width measurements. On the other hand, the mean of Res was closer to zero (-0.006) for distal measurements than for width measurements (0.030), indicating better accuracy.

Significant interaction between time and food treatments (e.g., LME model eq. 12, $P < 0.001$ for Res in nonlinear SPH with both distal and width dimensions) indicated that the food treatment effect varied between marking intervals. Res increased during the period of high food ration and decreased during the period of low food ration, and the Res patterns were generally more similar when using different B-C models than when using different otolith dimensions

(Figs. 7 and 8). However, the different food treatments did not produce significantly different groups over the whole experimental period (e.g., LME model eq. 13, $P = 0.75$ for the distal radius and 0.95 for the width).

One-way ANOVA revealed significant short-term food treatment effects on the Δ_{Res} (e.g., $P < 0.001$ in Res of nonlinear SPH with the four otolith dimensions). In all cases, Δ_{Res} was negative during high food ration periods and positive and mostly significantly different from zero during low food ration periods (Table 2). Thus, BCLs became smaller during high food ration periods and larger during the low food ration periods relative to the observed fish lengths. The largest bias in both time periods was along the ventral direction in the high food ration groups. The absolute change in residuals from high food ration to low food ration periods was similar among the four otolith dimensions, however, averaging 0.042–0.047 and 0.035–0.039 for the mark intervals 3–4 and 4–5, respectively (Table 2).

Discussion

Otolith preparation

Polished transverse sections of otoliths provided high quality images and accurate otolith sizes in this study, but a displaced cut can directly affect the values of otolith sizes and estimated otolith growth (Panfili et al. 2002). Even when cutting through the core at the correct angle, it was difficult to avoid minor lobes at the periphery of the otoliths. A lobe may contribute up to a 0.48 mm (15%) differ-

Fig. 6. Relative residuals (Res) of nonlinear scale proportional hypotheses (SPH) back-calculated length (BCL) for otolith (a) dorsal radius, (b) distal radius, (c) ventral radius, and (d) width for 30 fish from four food treatment groups (LoLo, low ration throughout; HiHi, high ration throughout; LoHi, low ration followed by high ration; HiLo, high ration followed by low ration) at five marking times. Horizontal broken line: Res = 0.

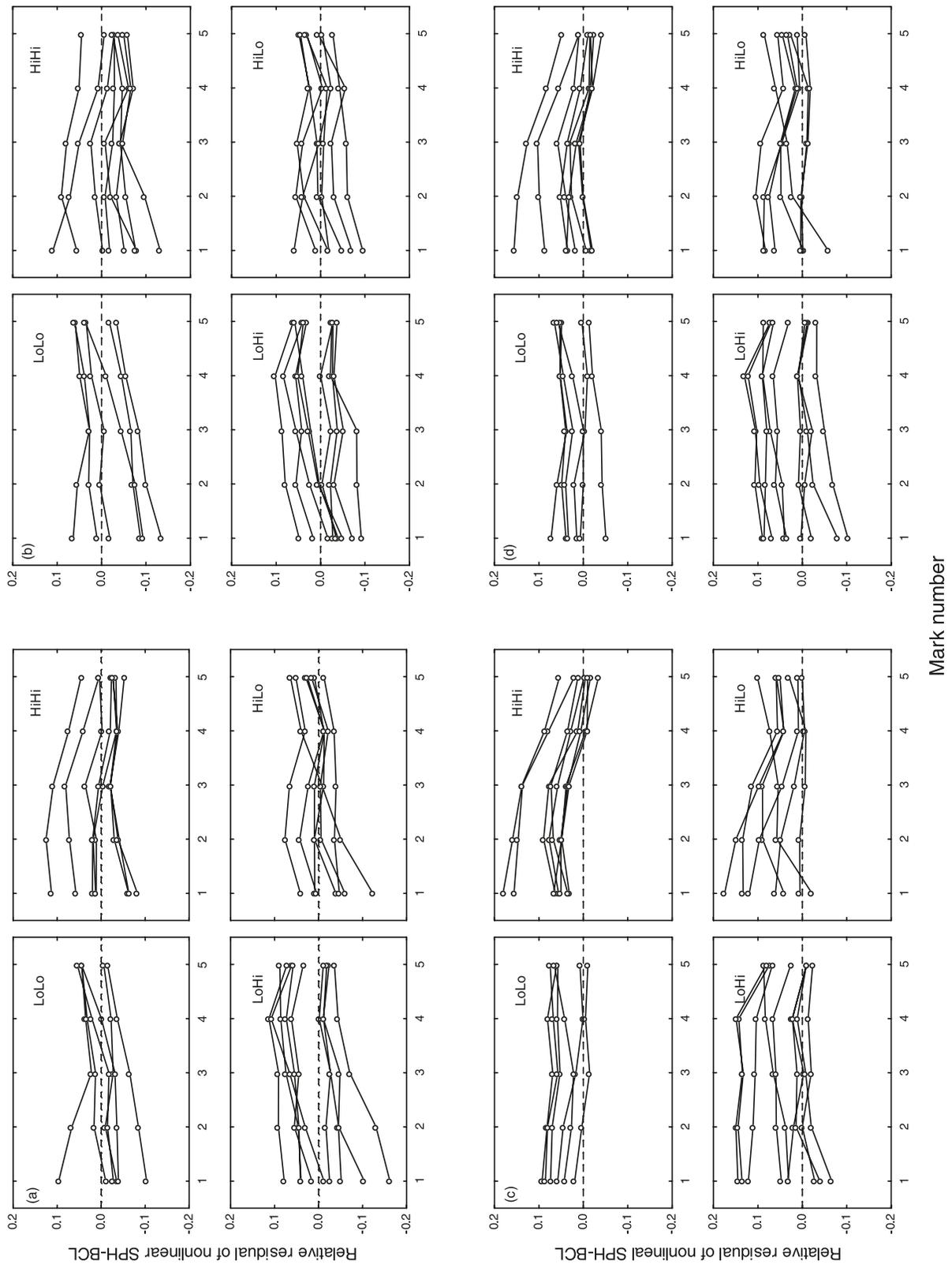


Fig. 7. Relative residuals of back-calculation (B-C) estimated length using nonlinear scale proportional hypotheses (SPH) (mean \pm 0.95 confidence interval) with different otolith measurements. Each panel shows the results for a different food treatment group (*a*, LoLo, low ration throughout; *b*, HiHi, high ration throughout; *c*, LoHi, low ration followed by high ration; *d*, HiLo, high ration followed by low ration) during five marking times. Horizontal broken line: Res = 0. Open squares represent dorsal values; open diamonds, distal; open triangles, ventral; and solid circles, width. Means are offset for presentation purposes.

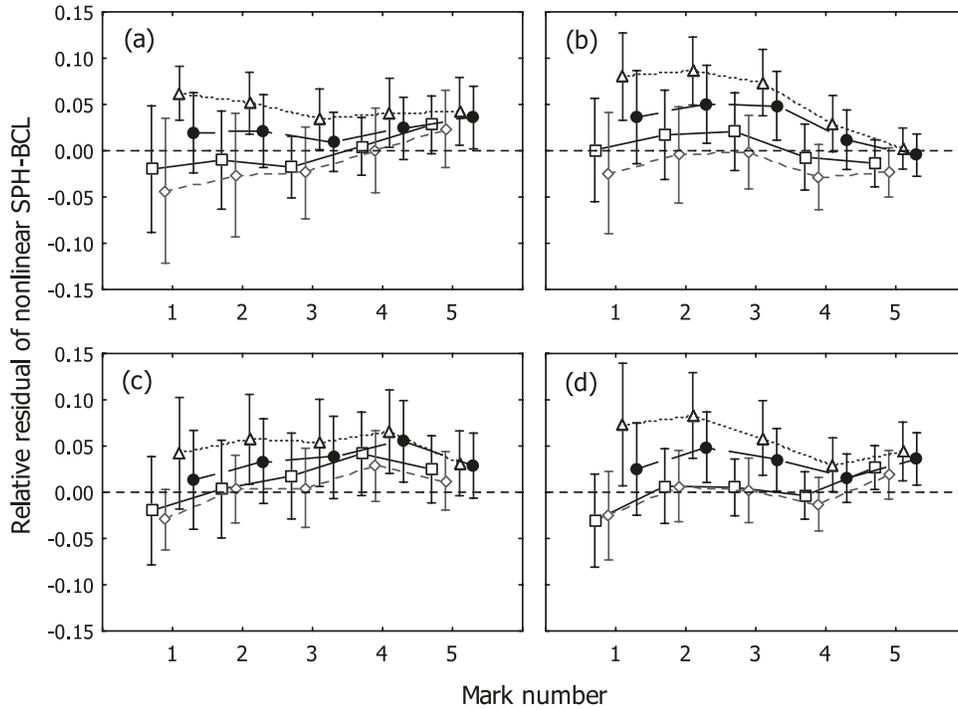


Fig. 8. Relative residuals of back-calculation (B-C) estimated length using five B-C models and otolith width measurements (mean \pm 0.95 confidence interval). Each panel shows the results for a different food treatment group (*a*, LoLo, low ration throughout; *b*, HiHi, high ration throughout; *c*, LoHi, low ration followed by high ration; *d*, HiLo, high ration followed by low ration) during five marking times. Horizontal broken line: Res = 0. Open squares represent BI values; open circles, linear scale proportional hypotheses (SPH); solid circles, linear body proportional hypotheses (BPH); solid triangles, nonlinear SPH; and open triangles, nonlinear BPH. Means are offset for presentation purposes.

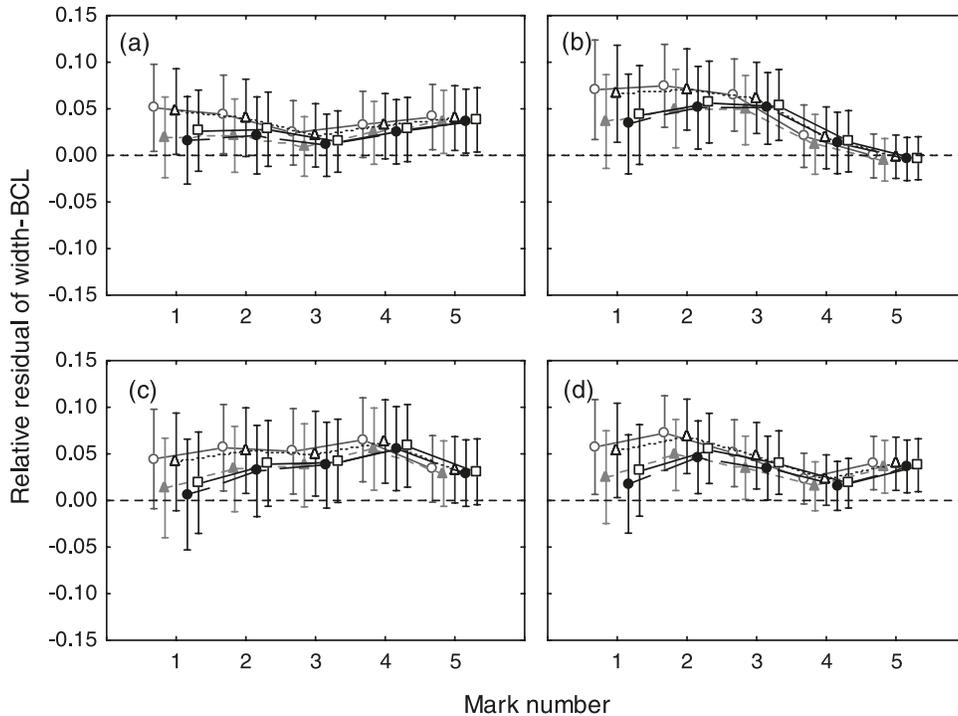


Table 2. Mean ΔRes during the periods of high and low food rations using nonlinear SPH.

Test period	Otolith dimension	ΔRes	
		During high food ration	During low food ration
Marks 3–4	Dorsal radius	-0.0187*	0.0234*
	Distal radius	-0.0213*	0.0239*
	Ventral radius	-0.0372*	0.0101*
	Width	-0.0289*	0.0163*
Marks 4–5	Dorsal radius	-0.0120*	0.0270*
	Distal radius	-0.0061	0.0278*
	Ventral radius	-0.0307*	0.0085
	Width	-0.0217*	0.0167*

Note: An asterisk (*) represents values significantly different from 0.

ence in ventral radius and 0.50 mm (8%) in otolith width. Sharp and clear marks were often visible in images of sections cut exactly along the growth axis of the lobe; otherwise, the marks were wider with blurred edges. Taking into account that the distal radii are approximately one-half the size of the corresponding ventral radii for young cod, a cut through a distal lobe could exaggerate the distal radii, whereas a cut through a ventral lobe would produce a relatively smaller change in ventral radii or otolith width. This could explain the relatively high variation in distal radii relative to fish size. Dorsal radii did not have this problem because there are rarely large lobes on the dorsal side of the cod otolith (Campana 2004).

Statistical methods

Retrospective data are common in fisheries, but the potential for autocorrelation (lack of independence) between such observations is frequently ignored (Jones 2000). Lack of independence is problematic in estimating size-at-age and building growth curves (Seber and Wild 1989). Jones (2000) therefore recommended repeated-measures analysis. The LME model is good at dealing with a combination of fixed and random effects (Pinheiro and Bates 2000). In this study, we used individual fish as a random effect to preclude the correlation between the observations at the five marking times when examining the fixed effects. Additionally, LME is more applicable when random effects are of concern. For example, the LME model (eq. 11) produced mean Res, which indicates accuracy, and at the same time, variance between fish and within fish, which indicates precision. LME models are not yet commonly used in fisheries science but were considered highly suitable for this study.

The choice of otolith dimension and back-calculation model

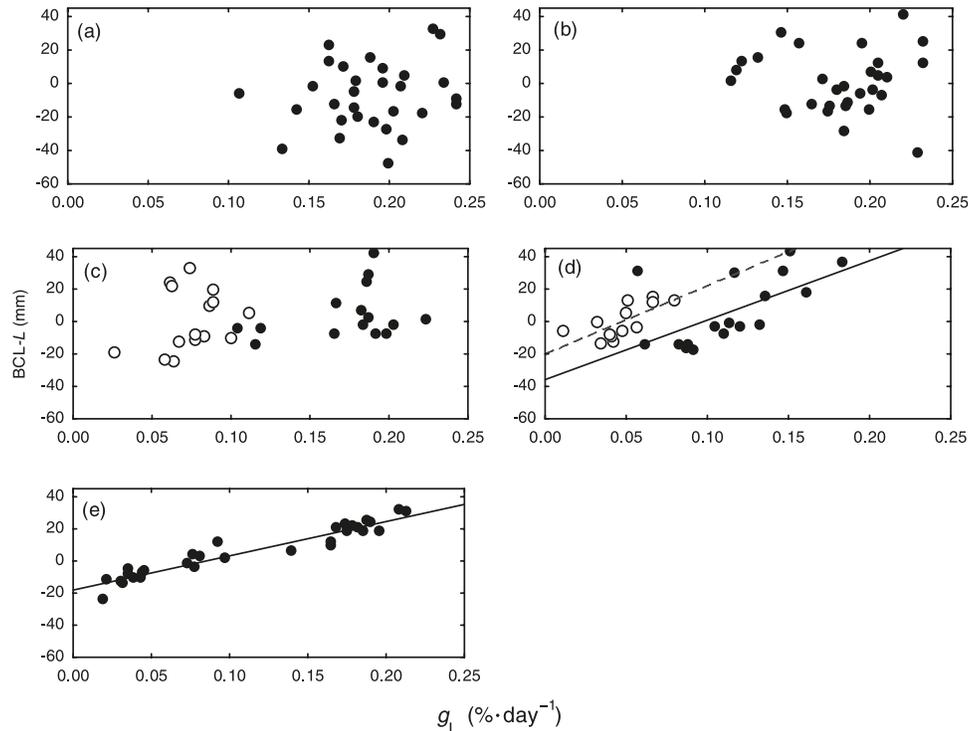
The choice of otolith dimension for size measurements influenced the results of the B-C. The ventral radius, usually the longest axis, is widely used (e.g., Smedstad and Holm 1996; Aubin-Horth and Dodson 2002; Roemer and Oliveira 2007), but it actually produced inaccurate BCL estimates. Width, or diameter (Secor and Dean 1992; Holmgren 1996; Roberson et al. 2005), consistently produced overestimated fish lengths but generally with the highest precision. Dorsal

and distal radii have rarely been used in B-C, probably because the measured distances are smaller, but they produced very accurate estimates with SPH and BI. Dorsal radius was the most accurate with BPH. The most surprising finding was that the regression between L and distal radius had the lowest multiple R^2 among all the regressions, yet the distal measurements with SPH resulted in very accurate BCLs. This challenges the assumption that a good regression relationship between otolith size and fish length is required to produce accurate BCLs (e.g., Panfili and Tomas 2001). The reason could be that the presence of distal lobes adds to the variation in the distal radii and leads to a low value of multiple R^2 in the regression. However, it seemed that the general proportionality between distal radius and fish length within a given section was maintained no matter how the sections were cut. Alternatively, multiple R^2 may be more correlated with precision instead of accuracy, because the regression between L and otolith width always had the highest multiple R^2 and otolith width demonstrated the highest precision with any method.

BI is the only method that does not rely on a regression between fish lengths and otolith sizes, but the choice of the point of biological intercept is crucial. Secor and Dean (1992) found that there is no clear point at which larval size and otolith size become “proportional”. However, BI performed very well for adult fish when using the values from Sinclair et al. (2002). The biological intercept nearly corresponded to fish length and otolith size at emergence for white spotted char (Morita and Matsuishi 2001), at swim-up for hybrid sunfish (Klumb et al. 2001), and the beginning of the juvenile stage for cod (Sinclair et al. 2002). Francis (1995) pointed out that individual trajectories are nonlinear in the early life stages but then become linear and therefore suggested a common convergence point, which is similar to the biological intercept, if length estimates are required only for the region where the trajectories are linear. Least sum of squares of the difference between BI-BCL and L for the 30 fish at each marking time produced statistically ideal biological intercept points that differed from the starting points reported by Sinclair et al. (2002), but the precision and accuracy were not altered markedly. As an example, statistically ideal biological intercept points of $L_0 = 163.5$ mm and dorsal radius $O_0 = 1.29$ mm yielded only slightly better accuracy and precision estimates (LME, mean Res improved from 0.008 to 0.006; standard deviation (SD) between fish from 0.043 to 0.041; SD within fish from 0.030 to 0.029). We therefore conclude that the biological intercept values from Sinclair et al. (2002) were adequate for this study.

A novel aspect of this study is that the deviation of estimated fish length was traced over a relatively long time interval with three potential factors investigated. As in other species, food ration has a stronger influence on somatic growth than on otolith growth (Secor and Dean 1992; Barber and Jenkins 2001; Fey 2005). We observe a positive correlation between growth rate and absolute residuals (BCL - L) as in Panfili and Tomas (2001), and in addition to the growth rate effect (Hare and Cowen 1995), this is partly an artifact because estimated growth rate and residual of the BCL are not truly independent (both depend on the proportionality at capture). The growth rate effect is visible only over the short term (Fig. 9) and disappears as the

Fig. 9. Residuals ($BCL - L$) using linear scale proportional hypotheses (SPH) on dorsal measures at marking times (a) 1, (b) 2, (c) 3, (d) 4, and (e) 5 versus length growth (g_L ; $\% \cdot \text{day}^{-1}$) in period following marking. Open symbols at marking times 3 and 4 are from fish in low (Lo) food availability treatments, solid symbols are from fish in high (Hi) food availability treatments. Significant linear regression lines for respective feeding groups and time periods are presented to show general trends.



back-calculation time interval increases. In Panfili and Tomas (2001), the fish were starved before the beginning of the experiments and experienced high feeding and growth rates during the experiments (about 1–2 months). This food regime from marking time to capture time introduced a short-term food effect in B-C. This effect should be considered only when B-C is to be used to estimate previous lengths over a short time interval — not far into the past. The effect was minor, as shown in our study, when back-calculating fish length over a time interval of nearly a year.

Back-calculation time interval and temperature effects are the main concerns for B-C of lengths over a long time interval. It is somewhat surprising that the time and temperature effects vary between otolith dimensions and between B-C methods. This may, however, be the underlying reason for differences in otolith shape observed between individuals from different populations (Cardinale et al. 2004). Because Res with distal radius is influenced significantly by the back-calculation time interval, the underestimation of BCLs will increase for earlier ages. This deviation should be accounted for, and width measurements might be a better choice of otolith size for B-C. On the other hand, because Res with width is more sensitive to temperature, it may not be the optimal choice in a temperature-varying environment.

More importantly, our results demonstrated that dorsal and distal measurements did provide accurate estimates of fish length and that otolith width measurements provided precise estimates of fish length. Although our results and conclusions are based on this particular experiment and the statistical procedure stated above, it is likely that the application of B-C depends on the relative importance of accu-

racy and precision in each situation, which will in turn influence the choice of otolith dimension and B-C model to be used. We therefore recommend that marking and tagging experiments similar to those used in this study be undertaken for other species of interest to improve reliability of B-C procedures.

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