

## Validation of back-calculation equations for juvenile bluefish (*Pomatomus saltatrix*) with the use of tetracycline-marked otoliths

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In recent years, a decrease in the abundance of bluefish (*Pomatomus saltatrix*) has been observed (Fahay et al., 1999; Munch and Conover, 2000) that has led to increased interest in a better understanding the life history of the species. Estimates of several young-of-the-year (YOY) life history characteristics, including the importance and use of estuaries as nursery habitat (Kendall and Walford, 1979) and size-dependant mortality (Hare and Cowen, 1997), are reliant upon the accuracy of growth determination. By using otoliths, it is possible to use back-calculation formulae (BCFs) to estimate the length at certain ages and stages of development for many species of fishes. Use of otoliths to estimate growth in this way can provide the same information as long-term laboratory experiments and tagging studies without the time and expense of rearing or recapturing fish. The difficulty in using otoliths in this way lies in validating that 1) there is constancy in the periodicity of the increment formation, and 2) there is no uncoupling of the relationship between somatic and otolith growth.

To date there are no validation studies demonstrating the relationship between otolith growth and somatic growth for bluefish. Daily increment formation in otoliths has been documented for larval (Hare and Cowen, 1994) and juvenile bluefish (Nyman and Conover, 1988).

Hare and Cowen (1995) found age-independent variability in the ratio of otolith size to body length in early age bluefish, although these differences varied between ontogenetic stages. Furthermore, there have been no studies where an evaluation of back-calculation methods has been combined with a validation of otolith-derived lengths for juvenile bluefish.

This study uses tetracycline-marked YOY bluefish otoliths to achieve two objectives: 1) to validate the relationship between somatic and otolith growth for juvenile bluefish, and 2) to compare the effectiveness of the Dahl-Lea equation, Fraser-Lee equation, scale proportional hypothesis (SPH), and body proportional hypothesis (BPH) length back-calculation formulae.

### Materials and methods

Young-of-the-year bluefish were collected by beach seine (15 m × 1.2 m × 6 mm mesh) from Clarks Cove in Buzzards Bay, Massachusetts, in July and August 2005. The fish were anesthetized with eugenol (clove oil), measured for fork length (FL), weighed, injected with tetracycline hydrochloride (75 mg/kg body weight), and individually marked with visible implant elastomer (VIE) tags (Northwest Marine Technology, Shaw Island, WA). The fish were maintained in a

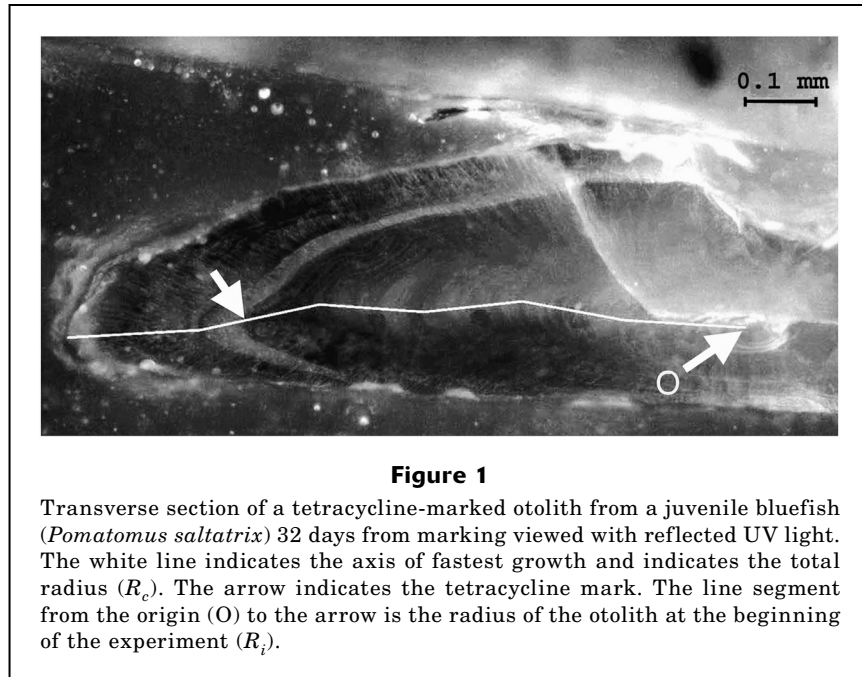
970 liter flow-through seawater tank for 1 month at ambient water temperature and fed with chopped squid and fish once daily to satiation. After 32 days, the fish were remeasured, reweighed, and their sagittal otoliths were removed for analysis. In addition, 417 YOY juvenile bluefish between 62 and 182 mm FL were collected from southeastern Massachusetts during the years 2004 and 2005 and were used to develop models of the relationship between otolith radius and fork length. ANCOVA was used to compare the slopes of the otolith-size–body-size relationship between wild fish and experimental fish to determine if the experimental conditions caused uncoupling of the relationship.

Otoliths were prepared for analysis as in Oliveira (1996) with two modifications; a 0.48-mm section was cut through the nucleus and visibility of the microstructure was enhanced by soaking the section in trypsin solution. Cross-sectioned bluefish otoliths have an occluded area in the center which obscures observation of the microstructure of the otolith. To clear this region, a procedure was developed in which the sections were placed in 2% trypsin solution for approximately 48 to 72 hours to remove excess protein. This procedure cleared the masked area and enhanced visibility of the rings.

Otoliths were observed on a computer monitor at 100× magnification so that the entire otolith from primordia to ventral edge could be viewed. Measurements were taken by using the Image-Pro Plus 5.0 image analysis software (Media Cybernetics, Silver Spring, MD). Two radial measurements were made on each otolith. The first measurement,  $R_c$ , was defined as the distance from the primordium to the ventral edge of the otolith (Fig. 1) and represents the radius of the otolith at the end of the experiment. Because bluefish otoliths develop a curvature that increases

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**Figure 1**

Transverse section of a tetracycline-marked otolith from a juvenile bluefish (*Pomatomus saltatrix*) 32 days from marking viewed with reflected UV light. The white line indicates the axis of fastest growth and indicates the total radius ( $R_c$ ). The arrow indicates the tetracycline mark. The line segment from the origin (O) to the arrow is the radius of the otolith at the beginning of the experiment ( $R_i$ ).

with age, a straight line measurement from nucleus to edge would not reflect the true radius and therefore would bias the estimation of growth increments associated with the curvatures. In order to account for this curvature, radial measurements were made along the axis of fastest growth, which was the sum of several straight line measurements from the nucleus to the edge. The second measurement,  $R_i$ , was defined as the distance from the primordium to the inner edge of the tetracycline mark deposited on the otolith, along the axis of fastest growth, and represents the radius of the otolith at the time of injection.

The frequency of ring deposition was also determined. The sectioned otoliths were observed at 400 $\times$  magnification under reflected UV light. When necessary, computer images were enhanced by alternating changes in contrast, and further digital magnification was used to observe rings. The tetracycline mark was located and then the UV light was turned off and the number of rings between the tetracycline-marked ring to the edge of the otolith were counted in normal transmitted light. Two replicates were made for otolith daily ring counts.

#### Back-calculation formulae

Growth rates from bluefish were estimated by using four back-calculation growth models as described in Francis (1990). In these equations,  $L_i$  is the initial fork length of the fish and  $L_c$  is the fork length of the fish at the end of the experiment.

The Dahl-Lea equation (based on the study of Lea [1910]) is a simple linear ratio of scale growth to body growth, with the assumption that the two are in exact proportion.

$$L_i = L_c \left( \frac{R_i}{R_c} \right). \quad (1)$$

The Fraser-Lee equation (based on the approach described by Fraser [1916]) is similar to the Dahl-Lea equation but with the Fraser-Lee equation there is the assumption that each back-calculation line passes through the point  $c$ , resulting in Equation 2. The value  $c$  was calculated as the intercept of the regression of otolith radius and body length (Fig. 2).

$$L_i = c + (L_c - c) \left( \frac{R_i}{R_c} \right). \quad (2)$$

With the SPH (Whitney and Carlander, 1956) there is the assumption of a constant proportional deviation from the mean in scale size such that if the scale is ten percent larger than average for a fish of that length, then it will be ten percent larger throughout the life of the fish. The  $a$  and  $b$  values for the SPH were calculated as the intercept and the slope of the regression of otolith radius ( $y$ ) against fork length ( $x$ ) (Fig. 2). In its linear form, the SPH is expressed as follows:

$$L_i = \left( \frac{-a}{b} \right) + \left( L_c + \frac{a}{b} \right) \left( \frac{R_i}{R_c} \right). \quad (3)$$

The BPH (Whitney and Carlander, 1956) is similar in principal to the SPH but carries that assumption that if a fish is ten percent smaller than an average fish with that size scale, it will be ten percent smaller throughout its life. For the BPH,  $c$  is as in Equation 2 and  $d$  was

calculated as the slope of fork length ( $y$ ) plotted against otolith radius ( $x$ ). In linear form the BPH is expressed as follows:

$$L_i = L_c \left( \frac{c + dR_i}{c + dR_c} \right) \quad (4)$$

The otolith radii and fork lengths were fitted to linear, quadratic, and cubic regression models. The data fitted all the models similarly:  $r^2$  for the linear and quadratic functions were 0.86 and the cubic function had an  $r^2$  of 0.85. A visual comparison of residuals between linear and nonlinear models showed the data to be homoscedastic and therefore the linear model was chosen because of its parsimony. Using these formulae, we estimated the lengths of individual bluefish at the start of the experiment and then compared our estimates to the actual measured lengths. The best model for estimating growth was determined by using paired  $t$ -tests. All statistical analyses were made with SPSS, vers. 13.0 (SPSS, Inc., Chicago, IL). For all comparisons  $P < 0.05$  was considered significant.

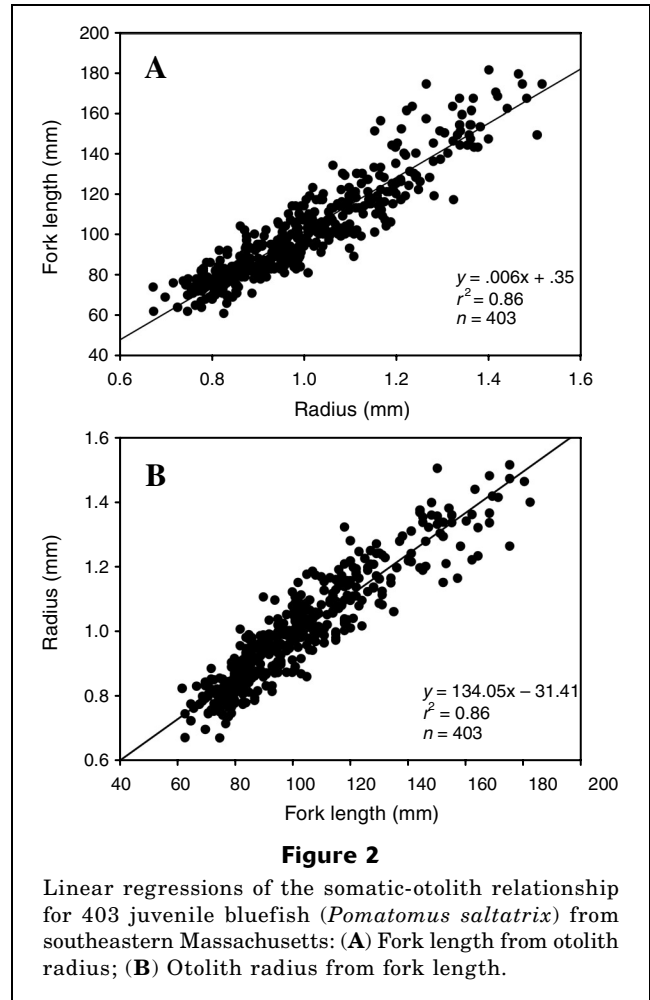
## Results

Seventy-seven fish were injected with tetracycline and 13 produced readable otoliths that could be used for age and growth analyses. Mortality was high in the first day of the experiment when 26 fish died. Five fish that appeared to be diseased were also removed from the experiment. An additional 21 fish that survived did not show the tetracycline mark and 12 fish had unreadable otoliths. Growth increments were distinguishable and the number of observed rings after the tetracycline injection was within 5% of the number of days in captivity for all tetracycline-injected fish. All replicate counts were within two days of the number of days in captivity.

Some fish died before the 32-day intended experimental time period, and two distinct groups of fish that died were evident. Group 1 included the seven fish that survived at least three weeks (23–32 days) and group 2 included the six fish which lived one week or less (3–7 days). Group 2 did not survive the entire study period but did have the same growth patterns as group-1 fish. For this reason, the data were analyzed as 1) pooled data (all 13 fish), and 2) data from group 1 (7 fish).

Measured growth estimates from all fish varied between 0.4 and 3.0 mm FL/day (Table 1). There was no significant difference ( $P=0.13$ ) in otolith growth rates and body growth rates for the wild fish used to develop the BCFs and the experimental fish, indicating that uncoupling of the relationship did not occur under experimental conditions.

The closest estimate of the measured  $L_i$  came from the Dahl-Lea equation, showing no significant difference ( $P=0.48$ ) between the estimates and the initial lengths of the bluefish. The estimates from the Fraser-Lee equation, BPH, and SPH were all significantly different from the measured initial length ( $P < 0.01$ ). The



Dahl-Lea equation estimated the  $L_i$  by a mean absolute difference of  $2.0\% \pm 0.6$  standard error (SE) and had a tendency to over-estimate the initial lengths. The Fraser-Lee equation and the BPH were similar in their estimations, tending to under-estimate length by  $2.9\% \pm 0.9$  SE and  $3.0\% \pm 0.9$ , respectively. The SPH under-estimated lengths by a mean of  $5.3\% \pm 1.4$  SE.

Our analysis of the same data without group-2 fish produced results similar to the results with the pooled data. The Dahl-Lea equation estimations were not significantly different from the  $L_i$  ( $P=0.41$ ), whereas the other three equations were significantly different from the measured length ( $\leq 0.02$ ). Percent differences for all the equations were slightly higher for group-1 fish alone than for the pooled fish. Dahl-Lea estimates differed from the measured lengths by  $3.1\% \pm 0.9$  SE, the Fraser-Lee and BPH estimates both differed by  $4.7\% \pm 1.3$  SE, and the SPH differed by  $8.6\% \pm 1.7$  SE.

## Discussion

The agreement between the number of daily rings and the days after injection indicates that the observed ring

**Table 1**

Summary of variables and back-calculation equation results for tetracycline-injected juvenile bluefish (*Pomatomus saltatrix*). BPH = body proportional hypothesis, SPH = scale proportional hypothesis,  $L_i$  = initial fork length of fish,  $L_f$  = final fork length of fish,  $R_i$  = initial radius of otolith, and  $R_f$  = final radius of otolith. \* Indicates a significant difference from the measured initial fork length ( $L_i$ ) ( $P < 0.05$ ).

| Fish number | Days after injection | $L_i$ | $L_f$ | Growth (mm) | Growth rate (mm/day) | $R_f$ | $R_i$ | Dahl-Lea | Fraser-Lee* | BPH*  | SPH*  |
|-------------|----------------------|-------|-------|-------------|----------------------|-------|-------|----------|-------------|-------|-------|
| 1           | 32                   | 84.0  | 116.0 | 32.0        | 1.0                  | 1.0   | 0.8   | 86.1     | 78.0        | 77.3  | 72.2  |
| 2           | 32                   | 89.0  | 120.0 | 31.0        | 1.0                  | 1.2   | 0.9   | 94.6     | 87.9        | 88.1  | 83.1  |
| 3           | 32                   | 89.0  | 112.0 | 23.0        | 0.7                  | 0.9   | 0.7   | 94.6     | 89.6        | 88.2  | 86.1  |
| 4           | 25                   | 91.0  | 105.0 | 14.0        | 0.6                  | 1.1   | 0.9   | 88.7     | 83.7        | 84.3  | 80.3  |
| 5           | 23                   | 93.0  | 102.0 | 9.0         | 0.4                  | 1.0   | 0.9   | 92.9     | 90.1        | 90.1  | 88.1  |
| 6           | 23                   | 93.0  | 108.0 | 15.0        | 0.7                  | 1.0   | 0.8   | 89.7     | 84.4        | 84.0  | 80.6  |
| 7           | 23                   | 101.0 | 115.0 | 14.0        | 0.6                  | 1.1   | 0.9   | 101.6    | 97.9        | 97.8  | 95.3  |
| 8           | 7                    | 118.0 | 121.0 | 3.0         | 0.4                  | 1.2   | 1.2   | 118.2    | 117.5       | 117.6 | 117.0 |
| 9           | 4                    | 122.0 | 125.0 | 3.0         | 0.8                  | 1.2   | 1.1   | 122.2    | 121.5       | 121.5 | 121.0 |
| 10          | 4                    | 128.0 | 130.0 | 2.0         | 0.5                  | 1.1   | 1.1   | 127.7    | 127.1       | 127.1 | 126.7 |
| 11          | 3                    | 127.0 | 129.0 | 2.0         | 0.7                  | 1.2   | 1.2   | 126.4    | 125.7       | 125.7 | 125.2 |
| 12          | 3                    | 118.0 | 127.0 | 9.0         | 3.0                  | 1.2   | 1.1   | 119.7    | 117.8       | 117.8 | 116.6 |
| 13          | 3                    | 133.0 | 136.0 | 3.0         | 1.0                  | 1.1   | 1.0   | 130.8    | 129.6       | 129.3 | 128.7 |
| Mean        |                      | 106.6 | 118.9 | 12.3        | 0.9                  |       |       | 107.2    | 103.9       | 103.8 | 101.6 |

formation occurred daily. This is in agreement with Nyman and Conover (1988) who used tetracycline to validate that rings were formed daily in juvenile bluefish. This finding indicates that the tetracycline mark was formed directly after the injection and that the number of rings, after tetracycline marking, corresponded with the number of days in the study period.

The Dahl-Lea equation provided the most accurate estimate of the initial size of the bluefish at the start of the experiment. This is the simplest equation and is based solely on the linearity of the otolith-size-body-size relationship. The accuracy of the estimation may be due to bluefish being a fast growing species. Wright et al. (1990) found that faster growing salmon smolts fit a linear growth model more favorably than slower growing conspecifics. It is possible that daily otolith growth is conservative, to a certain extent, as found by Panfili and Tomás (2001), but that the width of the daily growth increments may be a function of metabolism as postulated by Wright et al. (1990) or a function of temperature (Mosegaard et al., 1988). Therefore, species with high metabolisms, such as bluefish, may be expected to show a more direct relationship between otolith growth and somatic growth than a fish with a slower metabolism.

The Fraser-Lee equation underestimated the true initial lengths of the fish by a mean of 2.8%. This underestimate may be due the length-intercept constant  $c$  in the to the Fraser-Lee equation. When this constant is given a biological interpretation of zero, the equation is the same as the Dahl-Lea equation; therefore the interpretation of  $c$  is important in determining the accuracy of the Fraser-Lee and BPH equations. The  $c$  calculated by the linear regression was a negative

value,  $-31.41$ ; the underestimation was likely caused by changes in the otolith-size-body-size relationship during different life stages.

Hare and Cowen (1997) found significant differences between different ontogenetic stages during the bluefish larval period. These differences would explain why the regression of the otolith radius on fork length data did not predict a biologically reasonable  $y$ -intercept, although the data were strongly linear: the ratios during the juvenile and larval stages were different. When applying BCFs a biological intercept may be more useful and could make the Fraser-Lee BCF the more accurate equation. However, because otolith formation occurs during the early egg stage (0–24 hours) (Hare and Cowen, 1994), it would be problematic to get an accurate mean length at time of formation. Given that this measurement is likely to be less than the 2.0–2.4 mm hatching size (Klein-MacPhee, 2002) and that the otolith growth to somatic growth relationship is linear, forcing an intercept of zero should provide reasonably accurate length estimations.

In summary, this study was designed to validate and compare back-calculation methods for a fast growing juvenile bluefish, rather than to characterize bluefish growth. All four back-calculation formulae did result in close estimates ( $\leq 8.6\%$ ) of the true initial length regardless of growth rate (0.4 to 3.0 mm FL/day). Although the sample sizes were not adequate to properly explore the observed variability in growth rate the fact that the precision of the BCFs was not affected by growth rate illustrates a link between the rate of otolith growth and body growth, at least in the short term. In the case of bluefish, the results of this study indicate that the relationship between otolith growth

and body growth is linear for juveniles. The Dahl-Lea equation is the most parsimonious BCF equation and best approximated the true growth rate of juvenile bluefish, therefore making it the best suited method for estimating growth rate during the juvenile phase of this species. However it should be noted that the linear model used to develop the BCFs may not hold true for larval development or for annual growth of adults; more complex models may be needed to accurately estimate the amount of growth beyond year one. Caution must also be used in applying the equation because the lack of significant difference between the Dahl-Lea-predicted lengths and true initial lengths of the fish may have been due to the small sample size. If the linear relationship between somatic and otolith growth is, in fact, influenced by growth rate, then the Dahl-Lea equation may prove to be useful in back-calculating length for other fast-growing species of fishes.

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