Length correction for larval and early-juvenile Atlantic menhaden (*Brevoortia tyrannus*) after preservation in alcohol

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Body length measurement is an important part of growth, condition, and mortality analyses of larval and juvenile fish. If the measurements are not accurate (i.e., do not reflect real fish length), results of subsequent analyses may be affected considerably (McGurk, 1985; Fey, 1999; Porter et al., 2001). The primary cause of error in fish length measurement is shrinkage related to collection and preservation (Theilacker, 1980; Hay, 1981; Butler, 1992; Fey, 1999). The magnitude of shrinkage depends on many factors, namely the duration and speed of the collection tow, abundance of other planktonic organisms in the sample (Theilacker, 1980; Hay, 1981; Jennings, 1991), the type and strength of the preservative (Hay, 1982), and the species of fish (Jennings, 1991; Fey, 1999). Further, fish size affects shrinkage (Fowler and Smith, 1983; Fey, 1999, 2001), indicating that live length should be modeled as a function of preserved length (Pepin et al., 1998; Fey, 1999).

The goal of this study was to analyze the shrinkage of late-larval and early-juvenile Atlantic menhaden (*Brevoortia tyrannus*) during preservation in 95% alcohol. A length correction formula is presented that allows live standard length to be calculated from preserved standard length.

Materials and methods

Larval and early juvenile Atlantic menhaden were collected on three different occasions during January–March 2003 with a neuston net (2-m^2 opening and 947-μm mesh) deployed for 2-minute durations from a bridge to Pivers Island, located about 2 km inside Beaufort Inlet, North Carolina. Samples were placed in a cooler and transported to the laboratory. Live Atlantic menhaden larvae were sorted from the samples (n=100) and their standard lengths (SL) were measured to the nearest 0.01 mm with a caliper. All specimens (19.1–31.4 mm SL) were placed in individual vials filled with 95% ethyl alcohol. The fish were remeasured 3, 20, and 90 days after preservation.

Repeated measures ANOVA and Tukey HSD tests were used to analyze the significance of length changes during 90 days of preservation. The preserved length after 90 days was than compared with live length to test whether a single correction factor is appropriate for a calculation of live length (t-test analysis for the slope difference from one). Additionally, the precision of measurements was evaluated by two replicate measurements of all larvae three days after preservation. Linear regression analysis was then used to describe the relationship between the two length measurements. The possible deviation of intercept from zero and slope from one was estimated (t-test) to test for the possible significant differences between the two measurements.

Results

Time in preservative had a significant effect on measured length of Atlantic menhaden larvae (repeated measures ANOVA, P<0.0001). Fish were significantly larger prior to preservation compared to three days after preservation (Tukey HSD, P<0.001) and significantly larger three days after preservation compared to 20 and 90 days after preservation (Fig. 1) (Tukey HSD, P<0.001). When shrinkage is described as a relative value, the change in length that occurred during the first three days of preservation was 3.62%. Length decreased by an average of 0.22% during the following 17 days and by 0.073% during the remaining 70 days.

Although smaller fish shrank proportionally more than the larger ones (t-test for H_0: slope=0, P<0.001) (Fig. 2A), no size effect was observed when shrinkage was analyzed as absolute length (regression slope= 0.996, SE=0.008; H_0: slope=1; t-test of regression slope, P=0.605). However, the y-intercept of the regression of preserved length at 90 days on live length was significantly different from zero (regression intercept=1.17; SE=0.21; H_0: y-intercept=0; t-test of regression intercept, P<0.001) (Fig. 2B). Therefore, the significantly different from zero intercept can be used as a correction factor (i.e., $SL_{fresh}=SL_{preserved}+1.17$ mm). The shrinkage magnitude observed by Maillet and Checkley (1991) was compared to the results derived in our study (Fig 2B). Their formula indicated shrinkage of about 2% compared to approximately 4% in our study.

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The two readings performed to estimate the measurement error were not statistically different as indicated by the parameters of the regression line fitted to the first measurement versus second measurement data (SL_1=0.992 SL_2+0.21, r^2=0.998). The slope was not statistically different from one (regression slope=0.992, SE=0.005; H_0: slope=1; t-test of regression slope, P=0.106), and the intercept was not statistically different from zero (regression intercept=0.21; SE=0.12; H_0: y-intercept=0; t-test of regression intercept, P=0.418). Measurement precision, the absolute values of the difference between the two series of length measurements of the same specimens averaged 0.12 mm (SD=0.09), which corresponded to an average of 0.47% of length (SD=0.35). Thus, the error associated with measurement is an order of magnitude less than the change in length due to shrinkage within the first three days of preservation. Changes in length during following 87 days were below measurement error.

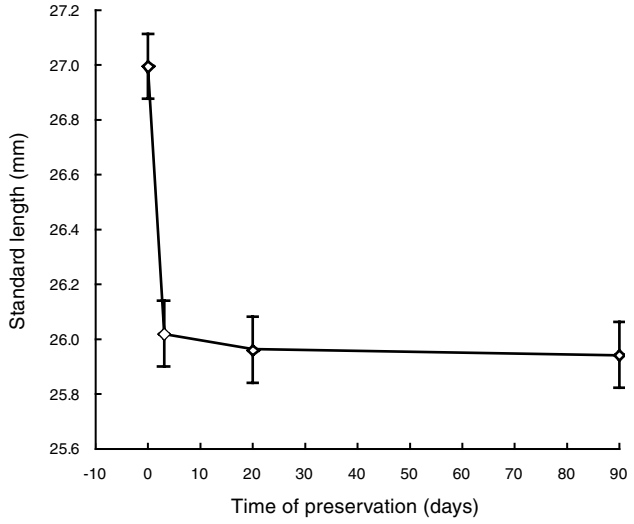

Figure 1

Change in standard length of Atlantic menhaden (*Brevoortia tyrannus*) (n=100) during 90 days of preservation in 95% alcohol. Mean values and standard error of length measurements obtained from repeated measurements of 100 fish.

Discussion

This research on late-larval and early-juvenile Atlantic menhaden shrinkage is the first for this species. Maillet and Checkley (1991) used a shrinkage correction formula (cited as unpubl. data) in their study on larval menhaden growth but did not provide additional information (e.g., range of fish sizes) to accompany their formula. Their correction formula differs from ours, and the discrepancy may be related to differences in experimental procedure and different developmental stages. In the present study live fish were used, but in Maillet and Checkley's study (1991) it was not indicated whether larvae were alive or dead prior to preservation. Further Maillet and Checkley (1991) examined larval menhaden (17–24.5 mm SL), whereas we examined late-larval to early-juvenile menhaden (19.1–31.4 mm SL).

The shrinkage of larval and early-juvenile Atlantic menhaden after the first three days of preservation was significant, but small in magnitude. Beyond 20 days of preservation significant additional shrinkage did not occur. In fact, the length changes after day 3 were below the estimated measurement

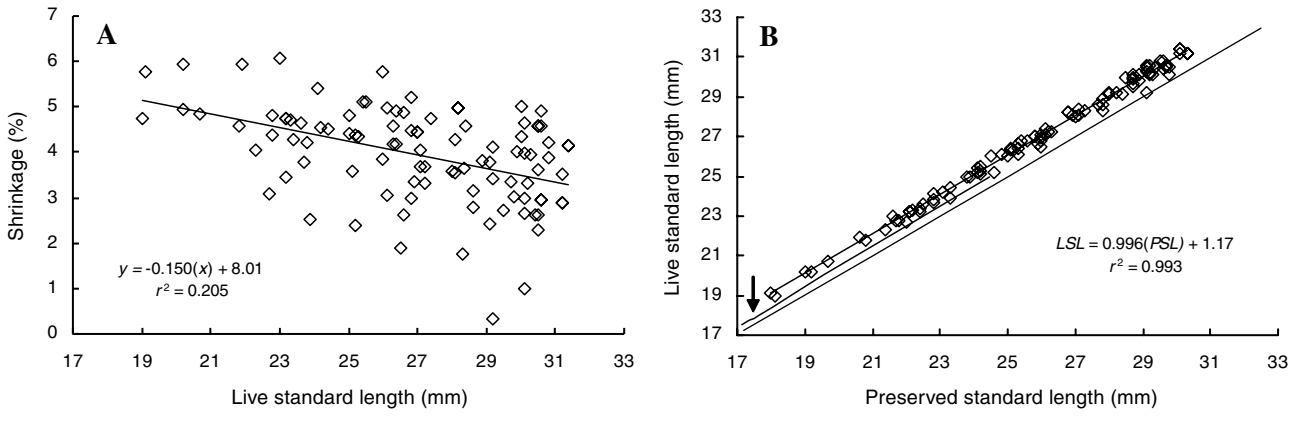

Figure 2

Length changes of Atlantic menhaden (*Brevoortia tyrannus*) during preservation for 90 days in 95% alcohol (n=100). (**A**) The relationship between live standard length (LSL) and relative (%) shrinkage magnitude; (**B**) the relationship between live and preserved standard lengths described with linear regression. The solid line indicates the 1:1 ratio. The arrow points to the correction curve obtained from Maillet and Checkley (1991): $SL_{live}=0.978(SL)e^{1.285e^{-0.578(SL)}}$.

error. Additionally, decreasing shrinkage as a function of increasing fish length was present when relative (%) shrinkage was analyzed. Similar results with regard to time and fish size effect were previously reported for other fish species preserved with formalin and alcohol (see Fey, 1999, for overview).

The effect of shrinkage on growth rate analysis was described by Fey (1999) for larval sprat. If growth rate is estimated by using a regression of length at age, the influence of shrinkage on growth estimates depends on the absolute value of length changes (i.e., expressed in mm) among small and large fish, and the error may be as high as 0.07 mm/d. However, if the absolute values of length decrease equally across fish lengths, even large shrinkage (on average) may have no effect on the results of growth rate analysis. In addition to length at age analysis, average growth rate (mm/d) may be calculated for individual fish. The potential error in growth estimates will then be directly proportional to both the relative and absolute magnitude of shrinkage. This potential bias in growth-rate calculations described by Fey (1999) for sprat emphasizes the importance of correcting for preservation. Although the relationship between otolith size and fish size may be used for length correction (Leak, 1986; Radtke, 1989), Fey (1999) showed that greater accuracy is provided when a fresh length–preserved length relationship is used. However, such a relationship may be supplemented by additional measurements (i.e., body depth and otolith size) to improve the accuracy of the correction model (Porter et al., 2001). In the current study, absolute changes in length (expressed in mm) of alcohol-preserved menhaden were not dependent on fish size and therefore a single correction factor was sufficient for a calculation of live length. The length correction factor provided in our study will benefit future studies on the ecology of early life stages of menhaden, similar to that conducted by Warlen et al. (2002), where preserved length measurements were used.

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