Vol. 82: 13–19, 1992 | MARINE ECOLOGY PROGRESS SERIES Mar. Ecol. Prog. Ser. | Published May 14

Estimation of live standard length of winter flounder *Pleuronectes americanus* larvae from formalin-preserved, ethanol-preserved and frozen specimens

Einar Hjörleifsson, Grace Klein-MacPhee

University of Rhode Island, Graduate School of Oceanography, South Ferry Road, Narragansett, Rhode Island 02882-1197, USA

ABSTRACT: Changes in standard length of laboratory-reared winter flounder *Pleuronectes americanus* Walbaum larvae, due to preservation in 2‰ formalin, 95‰ ethanol and frozen at −70 °C in seawater, were studied in larvae ranging from 3.0 to 8.5 mm long. Shrinkage is significant in all media except for larvae > 5 mm preserved in ethanol. The amount of shrinkage is related to size, longer larvae generally shrinking proportionally less than shorter larvae. Shrinkage is highest in 2 % formalin, intermediate in −70 °C seawater and lowest in 95 % ethanol. Linear regressions of preserved standard length (L_t) on live standard length (L_0) for all 3 preservation methods are: $L_t = -0.20 + 0.92\,L_0$ ($r^2 = 0.99$, n = 102), $L_t = -0.43 + 1.06\,L_0$ ($r^2 = 0.98$, n = 116), and $L_t = -0.15 + 0.95\,L_0$ ($r^2 = 0.99$, n = 112) for formalin, ethanol and freezing, respectively. The relationships can be used to obtain an estimator of live length of laboratory-reared winter flounder larvae and are approximations of live length of field-collected larvae. A nonlinear multiple regression of percent shrinkage (S) due to formalin preservation as a function of live length (L_0 [mm]) and logarithm of preservation duration (D) in days is calculated for our data and for published studies of other species where shrinkage is reported as a function of live length: $S = 11.22e^{(-0.463\,L_0 + 0.071\,D)}$ ($r^2 = 0.79$, n = 32). Live length (L_0) as a function of preserved length (L_t) and duration of preservation is: $L_0 = 0.13 + 1.03\,L_t + 0.07\,D$ ($r^2 = 0.99$, n = 32). This relationship can be used to predict live length from formalin-preserved larvae in species where size-related shrinkage is unknown.

INTRODUCTION

The measurement of length of larval fishes is a common way to describe size, and therefore, growth. Since the measurement of live larval length is often impractical the larvae are preserved for later length determination. The type of preservative used to fix larvae before length measurement depends on the purposes of the study. Preservation in formalin is most commonly used and is recommended for standard ichthyoplankton surveys (Ahlstrom 1976, Smith & Richardson 1977, Snyder 1983), and feeding studies (Bowen 1983). However, because of decalcification of skeletal structures in formalin, ethanol or freezing is recommended for the preservation of larvae for age estimation from daily rings in otoliths (Brothers 1987). For biochemical analysis, larvae are generally frozen at −70 °C (Buckley & Bulow 1987).

However, both handling and preservation result in shrinkage of larvae (Theilacker 1980, Hay 1981, 1982, Jennings 1991), and thus lead to an underestimation of live length. Factors affecting shrinkage include size of larvae; whether the larvae were captured from the field or aquaria in the lab; duration of net retention; time elapsed before fixation; type, strength and buffering of fixative; duration of preservation; and temperature during storage of preserved samples.

Comparative studies on the effect of formalin and ethanol on larval length are few and contradictory. Theilacker (1980) reported that shrinkage of *Engraulis mordax* reared in the laboratory and placed in preservative while they were still alive was negligible in ethanol, but ca 8 % in formalin. In contrast, shrinkage has been reported to be greater in ethanol than in formalin in net-collected *Merluccius bilinearis* (Fowler & Smith 1983), *Stizostedion vitreum* (Glenn & Mathias 1987) and *Mallotus villosus* (Kruse & Dalley 1990) larvae.

0171-8630/92/0082/0013/$ 03.00

Only 2 published studies have investigated the effect of freezing on larval length. Fowler & Smith (1983) and Glenn & Mathias (1987) found shrinkage to be greater in larvae frozen on filter paper at −15 °C and in ethanol at −70 °C, respectively, than when preserved in either ethanol or formalin at room temperature. The former also concluded that predicting live length from frozen samples was impossible due to high variability in shrinkage.

The objectives of this study were to estimate shrinkage of winter flounder (*Pleuronectes americanus* Walbaum; formerly *Pseudopleuronectes americanus* Walbaum; Robins et al. 1991) larvae after preservation in 2% formalin, 95% ethanol and −70 °C seawater and to determine the effect of size on the amount of shrinkage. The relationship between preserved and live standard length can be used to predict the latter from preserved, field-collected larvae, when additional effects of net collection upon body size are either small or can be estimated.

MATERIAL AND METHODS

Adult winter flounder were obtained by trawling in Narragansett Bay, Rhode Island, USA, in early March 1989. The 2 sexes were maintained in the laboratory for ca 2 wk (until ripe) in separate flow-through fiberglass tanks supplied with filtered ambient seawater. No food was provided. Fertilized eggs were obtained by mechanical stripping of 1 ripe female and 2 ripe males (Smigielski & Arnold 1972). The eggs were incubated in filtered seawater, in 7 l flow-through fish hatching jars (Buss 1959) that were maintained at an average temperature of 3 to 4 °C. The flow supplied was just sufficient to maintain the demersal eggs in partial suspension. Approximately 1200 newly hatched larvae were transferred to a 200 l black polypropylene barrel, maintained at 6 to 7 °C in a constant temperature room. Water in the barrel was gently aerated, providing mixing. Light was supplied with 'cool-white' fluorescent lights mounted overhead. The irradiance level was 27 $\mu E\ m^{-2}\ s^{-1}$ and the light period was 12 h. The larvae were fed *ad libitum* with local wild plankton collected with a 64 µm mesh, 0.5 meter diameter net at 2 to 3 d intervals. Large zooplankton were removed by filtration through a 202 µm mesh screen; the remaining assemblage consisted primarily of copepod nauplii and rotifers.

At weekly intervals groups of ca 10 to 20 larvae were collected and preserved in either 2% formalin (ca 0.75% formaldehyde in seawater) or 95% ethanol, or frozen at −70 °C in seawater. Larvae were collected with a ladle, anesthetized by addition of a small amount of MS 222 (3-aminobenzoic acid ethyl ester metanesulfonate salt) and stored in a refrigerator at 3 to 4 °C until further processing. Individual live larvae were then pipetted from the anesthetic solution, submerged in a small amount of filtered seawater retained in a 0.1 ml depression on a microscopic slide, and viewed through a stereo dissecting microscope. During viewing under the microscope, the slide was kept cold by placing it on top of a cooled (0 to 4 °C) 1 cm thick aluminum block with a 1.5 cm hole allowing passage of light through the specimen. A digitized image of each larva was obtained using a video camera connected to the microscope and a computerized image analysis system (Image Measure) and stored on a computer (Compaq Deskpro 286). Larvae were pipetted from the depression slides into separate vials containing the appropriate medium. For larvae to be frozen, a coverslip was placed over the depression and the whole slide quick frozen with dry ice and stored at −70 °C. Images of larvae which had been preserved in ethanol and formalin were obtained by retransferring the larvae from the storage vials to depression slides containing proper medium.

Larvae preserved in ethanol and formalin exhibited a slight degree of lateral bending. For length measurements, most of the medium in the depression was removed before the image was recorded, causing the larvae to straighten and adhere to the underlying glass slide. Larvae preserved frozen showed no lateral bending. Images of larvae that had been frozen were obtained immediately after the specimen had thawed in the depression slide without any removal of medium. Larvae preserved in formalin and ethanol were analyzed after ca 12 mo of storage while larvae preserved frozen were analyzed after 8 wk of storage.

Larval size was determined from the stored digitized images displayed on a 9 inch television monitor (Sony Trinitron). Standard length was measured by drawing a line from the tip of the snout to the posterior end of the notochord, with the aid of a computer mouse (Estep et al. 1986). Thirty replicate measurements of standard length of the same larva gave mean and 95% confidence limit of 3.822 ± 0.004, with the coefficient of variation 0.31%. The precision in measurements was thus < 0.01 mm. Precent shrinkage [S] for individual fish was calculated as: $S = 100\,(L_0 - L_t)/L_t$ where L_0 is the initial live length and L_t is the preserved length. All statistical analyses were done using the SYSTAT statistical program (Wilkinson 1989) on an Apple Macintosh II computer.

RESULTS

A total of 116, 102 and 112 larvae ranging in age and size from 0 to 39 d posthatch and 3.0 to 8.5 mm in standard length were measured following preservation

in ethanol, formalin and frozen seawater, respectively. For analysis, larvae were divided into 1 mm size classes (except the largest size class of 7–9 mm due to small sample size). Results are presented in Table 1 Shrinkage was observed in all the formalin-preserved and frozen larvae and in most of the ethanol-preserved larvae <5 mm. Length changes were negligible in ethanol-preserved larvae 5 mm and longer Shrinkage is related to size, longer larvae generally shrinking proportionally less than shorter larvae within each preservation method (Table 1).

In almost all cases shrinkage within each size class differed significantly with method of preservation (multiple HSD Tukey test, $p < 0.001$). An exception was between the 3–4 mm sized larvae preserved in ethanol and those preserved in frozen seawater, where the average shrinkages of 6.2 % and 6.4 %, respectively, were not significantly different ($p > 0.05$; Table 1).

The average shrinkage in formalin was 15 % in newly hatched 3–4 mm larvae, and decreased with increasing size to ca 11 to 12 % in larvae larger than 5 mm (Table 1). Shrinkage of the 3–4 mm larvae was significantly different from the larger size classes ($p \leq 0.02$) but there was no significant difference between the larger size classes (Table 2). Variability in shrinkage was highest in larvae in the 4–5 mm size range, which consisted mostly of late yolk sac and first feeding larvae.

Shrinkage of larvae preserved in ethanol was highest (6.2 %) in the 3–4 mm size class and decreased to a negligible amount in larvae larger than 5 mm (Table 1). The shrinkage of the newly hatched larvae was significantly different from all of the larger size classes ($p \leq 0.02$; Table 2). In addition, shrinkage in 4–5 mm larvae was significantly different from the 6–7 mm larvae ($p < 0.001$). The variability in shrinkage was highest in larvae in the 4–5 mm size range.

Shrinkage of frozen larvae was intermediate between those preserved in formalin and ethanol (Table 1). The average shrinkage of newly hatched larvae (6.4 %) was lower than in 4–5 mm sized larvae (8.8 %). Larger larvae tended to shrink less, the average shrinkage in the largest size class (7–9 mm) being 5.3 % (Table 1). Shrinkage of frozen larvae of size classes 3–4 mm vs 4–5 mm, 4–5 mm vs 7–9 mm, and 5–6 mm vs 7–9 mm were significantly different ($p \leq 0.04$; Table 2).

The relationships between live (L_0) and preserved standard lengths (L_t) were described by a linear regression for all 3 preservatives (Fig. 1). The slopes and the intercepts are highly significantly different from zero ($p \leq 0.002$). The relationships between live and preserved lengths among the different preservatives used are significantly different (pairwise analysis of covariance, $p \leq 0.004$). Thus, the amount of length change as a function of size is different for each type of preservative. The 95 % confidence interval of an inverse prediction of live length from preserved length (Neter et al. 1985) is ± 0.03 mm, ± 0.05 mm and ± 0.03 mm for frozen, ethanol and formaldehyde preserved larvae within the size range studied.

Table 1. *Pleuronectes americanus*. Average percent shrinkage and standard deviation of live larvae within size categories preserved in 2 % formalin, 95 % ethanol, and seawater frozen at −70 °C. Number of individuals analyzed are given in parenthesis

Size class (mm)	Formalin	Ethanol	Frozen seawater
3–4	15.0 ± 1.0 (18)	6.2 ± 2.5 (18)	6.4 ± 2.2 (20)
4–5	12.5 ± 2.6 (45)	3.3 ± 3.9 (56)	8.8 ± 2.3 (50)
5–6	11.8 ± 1.5 (20)	1.5 ± 3.1 (20)	7.7 ± 2.1 (25)
6–7	11.2 ± 1.2 (14)	−0.7 ± 1.8 (16)	7.0 ± 1.5 (9)
7–8	11.8 ± 1.4 (5)	0.9 ± 2.5 (5)	5.3 ± 1.5 (8)

DISCUSSION AND CONCLUSIONS

We have summarized published estimates of length shrinkage due to formalin preservation of live fish larvae in Table 3. Data where length was not reported or where live larvae were pretreated by netting prior to fixation were not included in the summary. Theilacker (1980) reported preservation shrinkage of 4 to 22 mm *Engraulis mordax* larvae to be constant, irrespective of size. However, decrease in average shrinkage with increasing length after formalin preservation has been observed in *Sardinops caerulea* (Farris 1963), *Clupea harengus* (Schnack & Rosenthal 1978, Hay 1981, 1982),

Table 2. *Pleuronectes americanus*. Multiple pairwise comparison probabilities between shrinkage of larvae of different size classes within each type of preservative, obtained using a post hoc Tukey HSD test. ns: nonsignificant differences ($p > 0.05$)

Size classes compared (mm)	Formalin	Ethanol	Frozen seawater
3–4 vs 4–5	< 0.001	0.022	< 0.001
3–4 vs 5–6	< 0.001	< 0.001	ns
3–4 vs 6–7	< 0.001	< 0.001	ns
3–4 vs 7–9	0.018	0.016	ns
4–5 vs 5–6	ns	ns	ns
4–5 vs 6–7	ns	< 0.001	ns
4–5 vs 7–9	ns	ns	< 0.001
5–6 vs 6–7	ns	ns	ns
5–6 vs 7–9	ns	ns	0.041
6–7 vs 7–9	ns	ns	ns

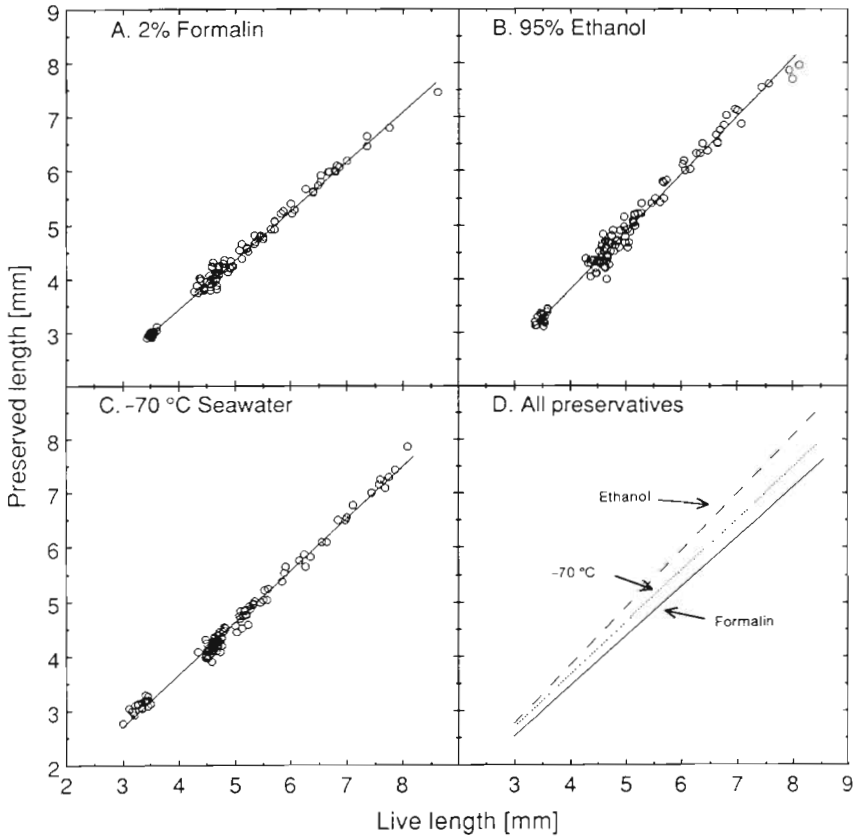

Fig. 1. *Pleuronectes americanus*. Preserved standard length (L_t) *as a function of live standard length* (L_0) of larvae due to preservation in (A) 2% formalin; $L_t = -0.20 + 0.92 L_0$ ($r^2 = 0.99$, n = 102); (B) 95% ethanol; $L_t = -0.43 + 1.06 L_0$ ($r^2 = 0.98$, n = 116); (C) −70 °C seawater; $L_t = -0.15 + 0.95 L_0$ ($r^2 = 0.99$, n = 112); and (D) linear regression of all 3 preservatives

and *Stizostedion vitreum* (Glenn & Mathias 1987) larvae. Fowler & Smith (1983) and Kruse & Dalley (1990) found a similar pattern of decreasing shrinkage with increasing size of *Merluccius bilinearis* and *Mallotus villosus* larvae when length changes of preserved, netted larvae were calculated as a function of unpreserved but netted larvae. However, since pre- and postfixation shrinkage interact in a nonadditive way (Blaxter 1971, Theilacker 1980) the 2 latter studies are not comparable with the data in Table 3.

The amount of shrinkage due to formalin preservation of winter flounder larvae is among the highest reported in the literature (Table 3), but can be explained because of the small size of the larvae. By combining our data with published estimates of shrinkage in formalin calculated as a function of live untreated larval length, we can show that the proportional decrease in length is a function of initial live length (L_0 [mm]). A nonlinear exponential model was fitted to the data (Table 3; except Theilacker's) giving the following equation (Fig 2A):

$$\text{Shrinkage [\%]} = 15.69e^{(-0.066 L_0)} \quad (r^2 = 0.70, n = 33). \quad (1)$$

A linear regression of the residuals of Model (1) on the natural logarithm of the storage duration in days is significant ($p < 0.01$; Fig. 2B). Incorporating the natural logarithm of the time [days] of storage (D) into the above model gives:

$$\text{Shrinkage [\%]} = 11.22e^{(-0.063 L_0 + 0.071 D)} \quad (r^2 = 0.79, n = 32). \quad (2)$$

The unexplained variability of 21% is low considering the different taxa, formaldehyde strength, and handling in the different studies. The relationship between live length (L_0) and preserved length (L_t) for the data is (Fig. 2C):

$$L_0 = 0.47 + 1.02 L_t \quad (r^2 = 0.99, n = 33). \quad (3)$$

Live length (L_0) as a function of both preserved length and natural logarithm of storage duration in days (D) is:

$$L_0 = 0.13 + 1.03 L_t + 0.07 D \quad (r^2 = 0.99, n = 32). \quad (4)$$

We suggest that Model (4), or Model (3) if storage duration is unknown, could be used to predict live length from formalin-preserved larvae in species where size related shrinkage is unknown; or in species where shrinkage has not been documented, although it should be used with caution.

Shrinkage of winter flounder larvae preserved in ethanol is inversely related to size, being 6.2% in 3–4 mm sized larvae and becoming negligible in larvae

Table 3. Summary of published estimates of length shrinkage due to formalin preservation in fish larvae

Species	Live length (mm)	Preserved length (mm)	Shrinkage (%)	Formalin (%)	n	Storage duration (d)
Sardinops caerulea[1]	3.7[a]	3.3[a]	10.4[e]	3	19	42
Sardinops caerulea[1]	4.6[a]	4.2[a]	10.2[e]	3	30	42
Sardinops caerulea[1]	5.8[a]	5.4[a]	7.4[e]	3	26	42
Clupea harengus harengus[2]	8.2[a]	7.1[a]	13.4[e]	3	110	90
Clupea harengus harengus[2]	9.2	8.6	7.0	10	20	90
Chrysophrys major[3]	3.0	2.5	14.8	3	18	50
Chrysophrys major[3]	2.2	1.9	15.3[e]	3.5	100	50
Clupea harengus pallasi[4]	9.0	8.2	8.9	10	22	547
Clupea harengus pallasi[4]	13.0[b]	12.0[f]	8.3	10	21	400
Clupea harengus pallasi[4]	15.0[b]	14.0[f]	7.4	10	99	400
Clupea harengus pallasi[4]	17.0[b]	16.0[f]	6.3	10	128	400
Clupea harengus pallasi[4]	20.0[b]	18.7[f]	6.7	10	78	400
Engraulis mordax[5]	4–22[c]		8	5	61	
Clupea harengus pallasi[6]	9.6[d]	8.8[d]	8.6	10	10	5
Clupea harengus pallasi[6]	11.8[d]	11.1[d]	5.7	10	20	60
Clupea harengus pallasi[6]	12.2[d]	11.6[d]	5.3	10	77	
Merluccius productus[7]	4.6	4.2	8.9	3	3	17
Coregonus albula[8]	9.8	9.0	8.2	10	22	285
Clupea harengus pallasi[9]	9.0[bd]	8.4[f]	7.5[d]	10	29	15
Clupea harengus pallasi[9]	12.0[bd]	11.3[f]	6.3[d]	10	55	15
Clupea harengus pallasi[9]	16.0[bd]	15.2[f]	5.0[d]	10	6	15
Clupea harengus pallasi[9]	21.0[bd]	20.2[f]	3.9[d]	10	25	15
Clupea harengus pallasi[9]	26.0[bd]	25.0[f]	4.0[d]	10	4	15
Paralichthys lethostigma[10]	11.3[b]	10.5[f]	7.3[b]	10	24	2190
Gadus morhua[11]	4.8	4.2	12.3	10	10	180
Platichtys flesus[11]	4.2	3.8	10.6	10	11	135
Stizostedion vitreum[12]	9.8	9.1	7.0	5	10	3
Stizostedion vitreum[12]	10.9	10.6	3.0	5	10	3
Stizostedion vitreum[12]	30.9	30.0	3.0	5	10	3
Pleuronectes americanus[13]	3.5	3.0	15.0	2	18	365
Pleuronectes americanus[13]	4.6	4.0	12.5	2	45	365
Pleuronectes americanus[13]	5.5	4.8	11.8	2	20	365
Pleuronectes americanus[13]	6.5	5.8	11.2	2	14	365
Pleuronectes americanus[13]	7.6	6.7	11.8	2	5	365

[1] Farris (1963), [2] Blaxter (1971), [3] Rosenthal et al. (1978), [4] Schnack & Rosenthal (1978), [5] Theilacker (1980), [6] Hay (1981), [7] Bailey (1982), [8] Dabrowski & Bardega (1982), [9] Hay (1982), [10] Tucker & Chester (1984), [11] Yin & Blaxter (1986), [12] Glenn & Mathias (1987), [13] this study

[a] Estimated from tabulated values; [b] midpoint in range; [c] size range; [d] estimated from figure; [e] calculated from averages; [f] preserved length calculated from live length and percent shrinkage

greater than 5 mm. Studies of ethanol shrinkage are relatively few (*Gadus morhua*: Radtke & Waiwood 1980, Radtke 1989; *Meluccius productus*: Bailey 1982; *Merluccius bilinearis*: Fowler & Smith 1983; *Stizostedion vitreum*: Glenn & Mathias 1987; *Mallotus villotus*: Kruse & Dalley 1990) and show no consistent trend of size-related shrinkage between different species unlike that described above for formalin-preserved larvae. For example, within the narrow size range of 4 to 6 mm, shrinkage due to preservation ranges from negligible in *Engraulis mordax* (Theilacker 1980) preserved in 80 % ethanol to 14 % in *Gadus morhua* (Radtke & Waiwood 1980) preserved in 60 % and then transferred to 95 % ethanol. The reason for this variability could be species-specific response to fixation in ethanol; but it could also be due to other factors such as different handling prior to and during preservation, different initial strength of ethanol, and stepwise transfer of larvae to higher strength ethanol.

Shrinkage of winter flounder larvae preserved in 2 % formalin was greater than larvae preserved in 95 % ethanol. Theilacker (1980) obtained the same order in *Engraulis mordax* using 5 % formalin and 80 % ethanol and Bailey (1982) in *Merluccius productus* using 3 % formalin and 80 % ethanol. However the reverse order was found in *Merluccius bilinearis* (Fowler & Smith 1983) and *Mallotus villosus* (Kruse & Dalley 1990). In the 2 latter studies the larvae were net-treated preceding preservation but in the former they were preserved live without net treatment. It is thus unclear whether

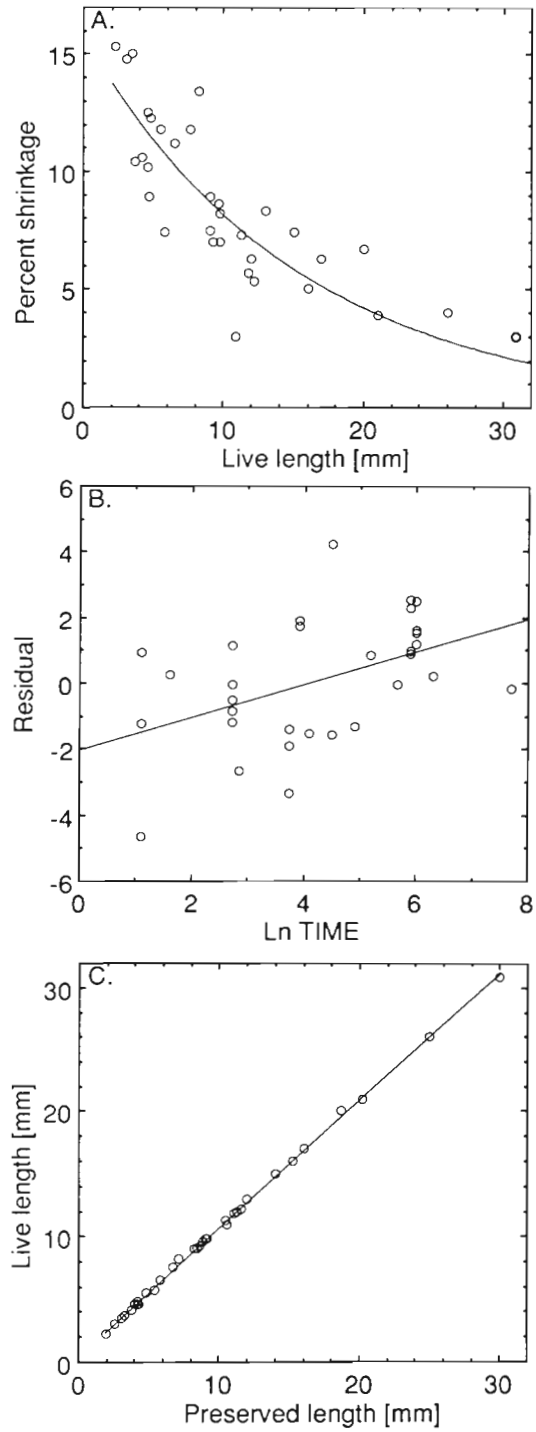

Fig. 2. Shrinkage in length due to formalin preservation of fish larvae based on data in Table 3, excluding *Engraulis mordax*. (A) Percent shrinkage [S] as a function of live length (L_0 [mm]). A nonlinear exponential model is fitted: S [%] = $15.69e^{(-0.066 L_0)}$ (r^2 = 0.70, n = 33). (B) Correlation between residuals ($S_{predicted} - S_{observed}$) of model in (A) and natural logarithm of storage duration (D) in days (r^2 = 0.22, $p < 0.01$, n = 32). (C) Live length as a function of preserved length (L_t [mm]). A linear regression is fitted: $L_0 = 0.47 + 1.02 L_t$ (r^2 = 0.99, n = 33)

this difference in the amount of shrinkage in the 2 media is species specific or because of difference in treatments of larvae preceding preservation.

The average shrinkage of winter flounder larvae preserved in seawater frozen at −70 °C is inversely related to size except in newly hatched larvae where percent shrinkage is comparable to that of the larger size classes. One possible reason for the lower shrinkage in the smallest size class could be that the yolk sac of newly hatched larvae maintains a certain rigidity during the freezing and thawing process. Using our method, live length of winter flounder larvae can be satisfactorily predicted from frozen larvae length. Fowler & Smith (1983) found highly variable and unpredictable shrinkage in *Merluccius bilinearis* larvae stored on wetted filter paper at −15 °C. The reason could have been due to variable dehydration of exposed larvae and continuous autolysis at −15 °C. Glenn & Mathias (1987), quantifying shrinkage of *Stizostedion vitreum* larvae frozen in −70 °C ethanol, reported 13 and 8% shrinkage in 9.6 and 11.5 mm larvae respectively. This is higher than the 5.2% average shrinkage in the largest size range (7–9 mm) of winter flounder. Because of the high shrinkage, lateral bending and fin breakage, they concluded that ethanol was not a satisfactory preservative.

The winter flounder larvae were straight during the freezing process and after thawing they remained flexible and transparent so that even the small otoliths (20 µm in newly hatched larvae) were visible in intact larvae. Larvae preserved in formalin and ethanol were relatively less flexible and more opaque. Some lateral bending occurred in larvae preserved in formalin and ethanol, but the effect on the standard length measurements was minimized by removing most of the medium from the depression, the larvae adhering and being straightened by the underlying glass slide.

The most important use of shrinkage studies is to predict live size. The type of media used depends on the purpose of the study as well as the precision with which live length can be estimated from preserved length. We have shown that length shrinkage in winter flounder larvae is a function of the type of preservative used as well as the initial live length. The relationship between live and formalin-preserved, ethanol-preserved and frozen larvae were all linear with a precision of ± 0.05 mm live length or less. These relationships can be used to obtain an estimator of live standard length of laboratory-collected winter flounder larvae from preserved lengths and can be a first approximation of live length estimation of field-collected larvae.

Acknowledgements. We thank Larry Buckley and Al Smigielski of NMFS, Narragansett for technical assistance. We thank Ann Durbin, Ted Durbin and William Krueger for

revision and comments on an earlier version of this manuscript and 2 anonymous reviewers for comments on the submitted manuscript. This research was supported by the University of Rhode Island Sea Grant Program (under grant No. NA89AA-D-SG082), the Rhode Island Department of Environmental Management and the Icelandic Student Loan Fund, Reykjavik, Iceland.

LITERATURE CITED

Ahlstrom, E. H. (1976). Maintenance of quality in fish eggs and larvae collected during plankton hauls. In: Steedman, H. F. (ed.) Zooplankton fixation and preservation. Unesco Press, Paris, p. 313–318

Bailey, K. M. (1982). The early life history of the Pacific hake, *Merluccius productus* Fish. Bull. U.S. 80: 589–598

Blaxter, J. H. S. (1971). Feeding and condition of Clyde herring larvae. Rapp. R.-v. Réun. Cons. int. Explor. Mer 160: 128–136

Bowen, S. H. (1983). Quantitative description of the diet. In: Nielsen, L. A., Johnson D. L. (eds.) Fisheries techniques. American Fisheries Society, Bethesda, Maryland, p. 325–336

Brothers, E. B. (1987). Methodological approaches to the examination of otoliths in aging studies. In: Summerfelt, R. C., Hall, G. E. (eds.) Age and growth of fish. Iowa State University Press, Ames, Iowa, p. 319–330

Buckley, L. J., Bulow, F. J. (1987). Techniques for the estimation of RNA, DNA, and protein in fish. In: Summerfelt, R. C., Hall, G. E. (eds.) Age and growth of fish. Iowa State University Press, Ames, Iowa, p. 345–354

Buss, K. (1959). Jar culture of trout eggs. Prog. Fish. Cult. 1. 26–29

Dabrowski, K., Bardega, R. (1982). The changes of fish dimensions due to fixation in different preservatives. Zool. Jb. Anat. 108: 509–516

Estep, K. W., MacIntyre, F., Hjörleifsson, E., Sieburth, J. M. (1986). MacImage: a user-friendly image-analysis system for the accurate mensuration of marine organisms. Mar. Ecol. Prog. Ser. 33: 243–253

Farris, D. A. (1963). Shrinkage of sardine (*Sardinops caerulea*) larvae upon preservation in buffered formalin. Copeia: 185–186

Fowler, G. M., Smith, S. J. (1983). Length changes in silver hake (*Merluccius bilinearis*) larvae: effect of formalin, ethanol, and freezing. Can. J. Fish. Aquat. Sci. 40: 866–870

Glenn, C. L., Mathias, J. A. (1987). Body shrinkage in young walleye, *Stizostedion vitreum*, preserved with AFA, formalin, ethanol and quick freezing. Can. Field-Nat. 101. 408–414

Hay, D. E. (1981). Effects of capture and fixation on gut contents and body size of Pacific herring larvae. Rapp. R.-v. Réun. Cons. int. Explor. Mer 178: 395–400

Hay, D. E. (1982). Fixation shrinkage of herring larvae: effects of salinity, formalin concentration, and other factors. Can. J. Fish. Aquat. Sci. 39: 1138–1143

Jennings, S. (1991). The effect of capture, net retention and preservation upon lengths of larval and juvenile bass, *Dicentrarchus labrax* (L.). J. Fish Biol. 38: 349–357

Kruse, G. H., Dalley, E. L. (1990). Length changes in capelin, *Mallotus villosus* (Müller), larvae due to preservation in formalin and anhydrous alcohol. J. Fish Biol. 36: 619–621

Neter, J., Wasserman, W., Kulner, M. H. (1985). Applied linear statistical models. Regression, analysis of variance, and experimental designs. Irwin, Homewood, Illinois

Radtke, R. L. (1989). Larval fish age, growth, and body shrinkage: information available from otoliths. Can. J. Fish. Aquat. Sci. 46: 1884–1894

Radtke, R. L., Waiwood, K. G. (1980). Otolith formation and body shrinkage due to fixation in larval cod (*Gadus morhua*). Can. Tech. Rep. Fish. Aquat. Sci. 929

Robins, R. C., Bailey, R. M., Bond, C. E., Brooker, J. R., Lachner, E. A., Lea, R. N., Scott, W. B. (1991). Common and scientific names of fishes from the United States and Canada, 5th edn. American Fisheries Society Special Publication 20, 183 p.

Rosenthal, H., Kuhlmann, D., Fukuhara, O. (1978). Shrinkage of newly hatched larvae of the Red Sea bream (*Chrysophrys major* Temminck & Schlegel) preserved in formalin. Arch. FischWiss. 29: 59–63

Schnack, D., Rosenthal, H. (1978). Shrinkage of Pacific herring larvae due to formalin fixation and preservation. Meeresforsch. 26: 222–226

Smigielski, A. S., Arnold, C. R. (1972). Separating and incubating winter flounder eggs. Prog. Fish Cult. 34: 113

Smith, P. E., Richardson, S. L. (1977). Standard techniques for pelagic fish egg and larva surveys. FAO Fish. Tech. Pap. 175

Snyder, D. E. (1983). Fish eggs and larvae. In: Nielsen, L. A., Johnson, D. L. (eds.) Fisheries techniques. American Fisheries Society, Bethesda, Maryland, p. 165–197

Theilacker, G. H. (1980). Changes in body measurements of larval northern anchovy, *Engraulis mordax*, and other fishes due to handling and preservation. Fish. Bull. U.S. 78: 685–692

Tucker, J. W., Chester, A. J. (1984). Effect of salinity, formalin concentration and buffer on quality of preservation of southern flounder (*Paralichthys lethostigma*) larvae. Copeia: 981–988

Wilkinson, L. (1989). SYSTAT: the system for statistics. SYSTAT, Inc., Evanston, Illinois

Yin, M. C., Blaxter, J. H. S. (1986). Morphological changes during growth and starvation of larval cod (*Gadus morhua* L.) and flounder (*Platichthys flesus* L.). J. exp. mar. Biol. Ecol. 104: 215–228

This article was presented by K. Sherman, Narragansett, Rhode Island, USA

Manuscript first received: August 1, 1991
Revised version accepted: January 29, 1992