Changes in the probability density function of larval fish body length following preservation

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The influence of body size on physiological and developmental processes during the early life history of fishes has been clearly demonstrated (Miller et al., 1988; Houde, 1989; Pepin, 1991). Because of this and to provide a direct comparison of field collections with laboratory observations that often use measurements of fresh specimens, numerous studies have quantified the effects of preservation and handling on the length of larval fish (Table 1). Most of the research has used laboratory-reared animals for which changes in length are assessed using either comparisons among treatments (i.e. ANOVA) or departures from a one-to-one relationship (i.e. regression) (Table 1). Shrinkage in length is the predominant response to preservation (Table 1), and the amount of shrinkage is influenced by handling (Theilacker, 1980, 1986; Theilacker and Porter, 1995; Fox, 1996).

Changes in larval body length due to preservation are relatively small (3–15%) although variations of up to 1 mm are not uncommon (Table 1). The contrast among species led Jennings (1991) to suggest that specific correction factors would be required. An alternative was Hjörleifsson and Klein-Macphee's (1992) simple model of the relative change in larval lengths, based on a review of previous studies, which showed clear evidence of the effects of body length and preservation time on shrinkage. Of course, as with any such review, the effects of unaccounted for or confounding variables are unknown (Pepin and Miller, 1993). However, Hjörleifsson and Klein-Macphee (1992) predicted a maximum mean shrinkage of ~15% for the smallest larvae (~2 mm) followed by an exponential decrease in relative shrinkage with increasing length. This raises an important point. Although differences in mean larval body length may appear significant from a statistical perspective, the importance of correcting for the effects of preservation are most pronounced at the level of the individual because morphological measurements are used in the analysis of each larva's physiological condition (e.g. Theilacker and Porter, 1995) to assess the relative state of a population. Even small changes in length resulting from shrinkage can create important biases at this level of analysis. In contrast, the small relative effects of preservation on body length (Hjörleifsson and Klein-Macphee, 1992) is unlikely to substantially influence estimates of length-frequency distributions.

Fundamental issues yet to be addressed in the assessment of larval shrinkage include 1) the effect of preservation on the distribution of larval length measurements within a given length interval rather than the mean, and 2) the contribution of investigator-induced error on that distribution. The former point is of particular importance because the application of correction factors to individual larvae must maintain the status of that animal in relation to others within the population. Investigator-induced error may be equally important. Few studies have attempted to quantify the variance in repeated estimates for a given operator (Jennings, 1991; Hjörleifsson and Klein-Macphee, 1992; Fox, 1996) and none have contrasted bias and variance among operators. Our failure to address these questions to date probably results from 1) the small sample sizes (i.e. generally <100) presented in most studies of the effects of preservation (Table 1), and 2) the fact that usually only a single investigator is involved in making the measurements of the larvae. Nonetheless, these issues are important because they provide the basis for narrowing the possible sources of error in interpretations of physiological processes that influence ichthyoplankton population dynamics.

In this study we report on an evaluation of the shrinkage effects of preservation on several species of larval fish collected as part of a field study. We consider changes in both the mean and variance of the distribution of individuals within narrow length intervals and assess the null hypothesis that there are no differences among species. We also

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Table 1

Summary of studies dealing with the effect of formaldehyde preservation on the length of larval fish. Under results the various symbols are S = shrinkage in length (mm); L = length before preservation; P = length following preservation; and CI = 95% confidence interval. Levels of precision recorded in either length range or results are those reported by the authors. With one exception, our summary of data reported from laboratory studies did not include simulated capture.

Species	Length range (mm)	Formaldehyde solution	Measurement method	Analysis	Sample size	Result	Source of larvae	Reference
Clupea harengus pallasi	8–26	3.7% with calcium borate or sodium borate	Micrometer	ANCOVA	$n = 120$	$S = 0.564 + 0.016\,L$	Laboratory	Hay, 1982
Clupea harengus pallasi	12–22 8–10	4%	Micrometer	ANOVA	$n = 326$ $n = 88$	Significant shrinkage	Sea–caught (dip net) and laboratory	Schnack and Rosenthal, 1978
Clupea harengus	9–20	4% with 2.5% sodium acetate trihydrate	Image analysis	ANOVA and regression	$n = 49$	$L = 1.765 + 0.867\,P$	Laboratory	Fox ,1996
Clupea harengus	7.75–9.17 13.1–17.4	0.75–3.7% in 15 or 34 ppt sea water	Micrometer	None	$n = 20$–50	7–14% shrinkage 3–7% shrinkage	Laboratory Sea–caught	Blaxter, 1971
Coregonus albula	8–10	4% neutralized with hexametylentetramin	Micrometer	ANOVA	$n = 9$–15	Significant shrinkage	Laboratory	Karjalainen, 1992
Dicentrarchus labrax	10–18	4% with 3 g/l sodium acetate trihydrate	Micrometer	ANOVA	$n = 109$	7% shrinkage (CI = –2 to 16%)	Laboratory (simulated capture)	Jennings, 1991
Engraulis mordax	3.9–21.6	2.2% buffered	Micrometer	Regression	$n = 61$	8% shrinkage (SD = 3%)	Laboratory	Theilacker, 1980
Gadus morhua	4–5	3.7% in 50% sea water	Micrometer	None	$n = 10$	12% shrinkage	Laboratory	Yin and Blaxter, 1986
Mallotus villosus	4.6–19.6	2.2% buffered	Micrometer	Regression	$n = 113$	$L = 0.36 + 1.016\,P$	Field (bongo)	Kruse and Dalley, 1990
Merluccius bilinearis	3.7–14.4	1.5% neutralized	Micrometer	Repeated measures ANOVA	$n = 83$	4.3% shrinkage	Sea–caught (bongo)	Fowler and Smith, 1983
Merluccius productus	4–5	1.1%	Micrometer	None	$n = 3$	8.9–40% shrinkage mean ≈15–20%	Laboratory	Bailey, 1982
Pagrus major	5–7 13–19	3.7% at pH 4.3	Micrometer	None	$n = 7$ $n = 16$	7–12 % shrinkage 3–7% shrinkage	Laboratory	Takizawa et al., 1994
Paralichthys olivaceus	7–11 12–19	3.7% at pH 4.3	Micrometer	None	$n = 18$ $n = 4$	9–16% shrinkage 2–12% shrinkage	Laboratory	Takizawa et al., 1994
Paralichthys lethostigma	9.49–13.05	1.48% buffered	Micrometer	Repeated measures ANOVA	$n = 72$	5–10% shrinkage in sea water	Sea–caught (Live trap)	Tucker and Chester, 1984
Platichthys flesus	3–4	3.7 % in 50% sea water	Micrometer	None	$n = 11$	11% shrinkage	Laboratory	Yin and Blaxter, 1986
Pleuronectes americanus	3–8.5	0.75%	Image analysis	Pairwise and regression	$n = 102$	$P = -0.20 + 0.92\,L$	Laboratory	Hjörleifsson and Klein–MacPhee, 1992
Sardinops caerulea	3.45–6.19	1.1% buffered	Micrometer	Regression	$n = 75$	$P = -0.26 + 0.9724\,L$	Sea–caught	Farris, 1963
Stizostedion vitreum	9–21	1.9% with sodium borate	Micrometer	Regression	$n = 80$	$L = 0.087 + 1.014P$	Laboratory	Johnston and Mathias, 1993
Theragra chalcogramma	4.2–20.0	1.85%	Micrometer	ANCOVA	$n = 42$	$L = 0.344 + 1.021\,P$	Laboratory	Theilacker and Porter, 1995
Trachurus symmetricus	3.35–4.10	1.85% buffered	Micrometer	None	$n = 13$	4% shrinkage	Laboratory	Theilacker, 1986

evaluate the magnitude of intra- and interoperator differences in performance for repeated measurements of the same specimens.

Materials and methods

The study was conducted on Conception Bay, Canada (47°45'N, 53°00'W), during the period of 12 July to 4 August 1995. Sampling was performed daily from CSS *Shamook* (23-m boat length) at a single site near the head of the bay.

Larvae from a number of species were obtained from vertical plankton hauls made with a square trawl 4 m long with a 4-m^2 mouth fitted with 333-μm mesh nitex and an oversize codend 20 cm in diameter and 30 cm long. The net was lowered to a depth of 20–30 m and retrieved at a rate of 1 m/s. Net design and deployment protocol were chosen to minimize trauma to larvae. On deck, the net was washed and the codend contents poured into a 20-L plastic bucket. Live and freshly dead ichthyoplankton were immediately sorted with flexible forceps and transferred to petri dishes filled with chilled seawater. Moribund larvae, indicative of death or extremely poor condition prior to capture (O'Connell, 1981; Otto and Boggs, 1983; Takizawa et al., 1994), were excluded from our samples to avoid possible bias. Each larva was assigned an indentification number, tentatively identified to the lowest taxonomic level possible, and recorded on videotape with a camera mounted on a Wild M3C dissecting microscope (S-type mount, 0.5× objective). Individual larvae were immediately transferred into 1.5 mL microcentrifuge tubes filled with 2% buffered formaldehyde. Identifications were confirmed in the laboratory and standard lengths were determined to the nearest 0.1 mm using an Optimas© image analysis system. Measurements of the video-recorded fresh standard lengths were performed at the Northwest Atlantic Fisheries Centre by an operator with more than 10 years of experience in the study of larval fish. All laboratory analyses performed on preserved larvae were conducted approximately five months after the sampling cruise at Queen's University by a novice operator with less than 1 year of experience in the study of larval fish. Each preserved larva was extracted from its microcentrifuge tube, videotaped in a manner identical to that employed for recently captured larvae, and measured (standard length) with an Optimas© image analysis system. All measurements were performed by clicking on a series of points through the centre of the head and along the notochord.

To contrast the precision and accuracy of length measurements, the experienced operator repeated a set of measurements on a sample of randomly selected larvae from the initial videotape records. In addition, a random subsample of 72 preserved capelin larvae (*Mallotus villosus*) were selected from the collection for replicate measurements by the experienced and novice operators. In each instance, both operators independently videotaped each larva for both the initial and repeated measurements. In addition, the experienced operator performed duplicate measurements on approximately one half of the samples measured by both operators. All measurements were performed with only knowledge of specimen number and magnification level.

We evaluated individual differences in body length following preservation ($\Delta l = l_{preserved} - l_{fresh}$). For analysis, data were binned into 1-mm length intervals for fresh specimens (e.g. $1 \leq \times < 2$). The hypothesis that there was no significant difference in mean body length ($H_0: \Delta\bar{l} = 0$) was evaluated by using a two-sided t-test. Comparison among species was accomplished by using an ANOVA ($H_0: \Delta\bar{l}_1 = \ldots = \bar{l}_n$, for species 1 to n). A species was included in the analysis only if there were at least three specimens within a length interval. Homogeneity of the variances in fresh (f) and preserved (p) larval lengths within each length interval was assessed by using a one-sided F-test of the null ($H_0: s_f^2 >= s_p^2$) and alternative ($H_A: s_f^2 < s_p^2$) hypotheses. Changes in individual rank, within a length interval, were assessed by using Kendall's rank correlation coefficient (τ). Operator precision and accuracy were evaluated by using general linear models.

Results

Operator accuracy

The experienced operator exhibited consistent performance in repeated measurements of both fresh and preserved capelin larvae (Fig. 1; Table 2). Regression analysis of the independent measurements by the experienced operator showed no significant departure from an intercept of 0 and a slope of 1 for both fresh and preserved specimens (Table 2). Residual variances for the two treatments were not significantly different ($F_{40, 38}$=1.1, P>0.05).

Comparison of measurements by experienced and novice operators revealed no significant bias on the part of either operator although the results did approach significance (0.1>P>0.05) and the slope was not significantly different from 1 (Table 2). However, the residual variance about the novice-experienced operator regression was significantly greater than the residual variance of repeated measurements

made by the experienced operator ($F_{72, 38}$=4.61, P<0.001).

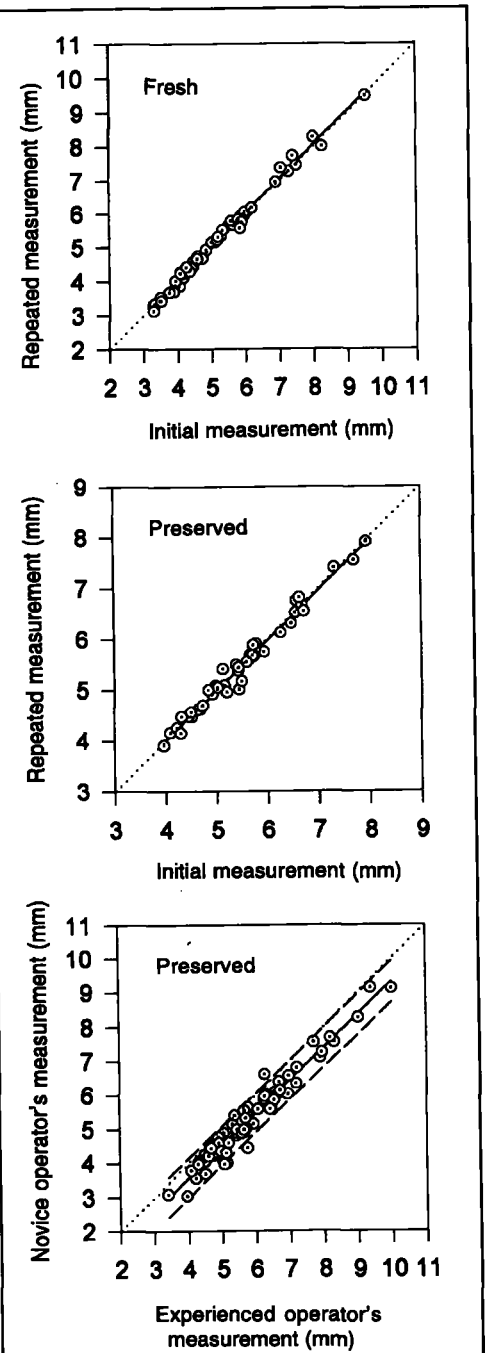

Figure 1

Comparison of repeated measurements of the experienced operator on fresh (top panel) and preserved (center panel) specimens as well as the comparison between experienced and novice operators (bottom panel). Solid lines represent least squares regressions (Table 2). Dotted lines show the 1:1 relationship. Dashed lines in the lower panel show the 95% prediction intervals.

Changes in body length

A total of 1179 larvae representing 9 different species were used in our analyses. Within each length interval, the total number of specimens available ranged from 10 to 281 individuals, representing 1 to 5 species (Table 3).

Within 1-mm length intervals, preservation resulted in significant increases in body length for individuals 3–6 mm fresh standard length and significant decreases in body length for individuals >7 mm fresh standard length (Fig. 2, Table 3). In six instances there were also significant differences among species in preserved body length (Table 3). For fresh length intervals between 5 and 9 mm there was consistency in the order of species; preserved specimens of *Hippoglossoides platessoides* were larger for a given length interval than *Ulvaria subbifurcata* and *Mallotus villosus*. However, above and below the 5–9 mm length intervals there was variation in the order of species. In the larger size classes (≥12 mm), preserved *Stichaeus punctatus* and *Gadus morhua* were larger than *Clupea harengus* and *Hippoglossoides platessoides*.

The initial variance of fresh standard lengths within each length interval ranged from 0.043 to 0.088 mm^2, whereas the variance of preserved standard lengths of these same individuals was significantly greater (Table 3) and ranged from 0.18 to 3.5 mm^2 (Fig. 3). Furthermore, the variance of the preserved length measurements increased significantly with increasing fresh length (r^2=0.70, P<0.01, n=15) (Fig. 3).

Kendall's correlation coefficient (τ) revealed that individual larvae remained at the same rank about 1 time in 3 (Fig. 4). This effect increased (i.e. 1 time in 4 or 5) with increasing fresh length.

Discussion

Changes in body length of larval fish due to handling and preservation are neither uniform nor consistent among individual animals: there is substantial variation in reaction to both handling (no matter how gentle) and preservation and variation is greatest in absolute terms for larger larvae.

No previous study (Table 1) has employed sample sizes large enough to permit the evaluation of changes in the distribution or rank of individual larvae within small length intervals. We found clear evidence that changes in body length can be more substantial than previously estimated by means of analyses applied to a broad range of sizes. Despite significant changes in body length for most length intervals, our results show that 1) variation about

Table 2

Results of the comparison of initial (I) and repeated (R) measurements on fresh and preserved larvae by the experienced (E) and novice (N) operators. Numbers in square brackets represent the standard error of the intercept (α) and slope (β). The sixth and seventh columns show the test of the hypotheses that the intercept is not significantly different from 0 and that the slope is not significantly different from 1.

Treatment	Initial	Repeat	Regression ($y = \alpha + \beta x$)	F-Value	t-value $H_0: \alpha = 0$	t-value $H_0: \beta = 1$	Residual variance
Fresh	E	E	R = 0.074 [0.128] + 0.983 [0.0229] I	$F_{1,40}$=1848, $P < 0.001$	0.58, $P > 0.05$	0.74, ns	0.020
Preserved	E	E	R = 0.113 [0.126] + 0.977 [0.0228] I	$F_{1,38}$=1833, $P < 0.001$	0.90, $P > 0.05$	1.01, ns	0.018
Preserved	E	N	R = -0.271 [0.149] + 0.959 [0.0263] I	$F_{1,72}$=1414, $P < 0.001$	1.81, $0.1 > P > 0.05$	1.56, ns	0.083

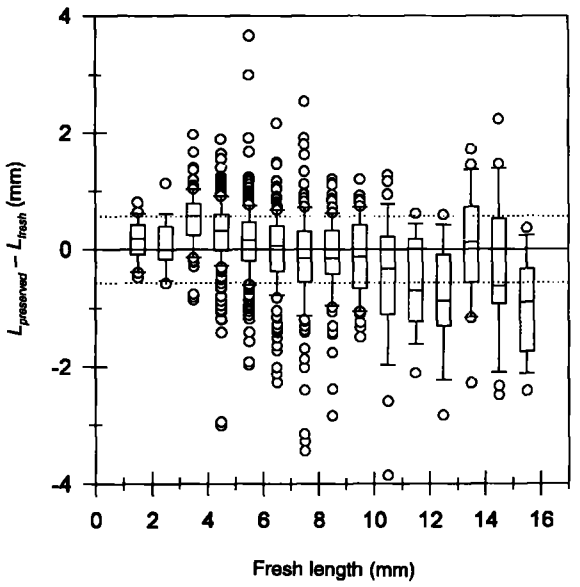

Figure 2

Box plot of the 25th, 50th, and 75th percentile of the difference in mean individual length of larval fish within each millimeter fresh length interval. Capped whiskers show the 10th and 90th percentiles and the open circles show the distribution of observations outside those confines. The dotted lines represent the 95% confidence intervals of the residual population variance from the comparison of measurement error between operators.

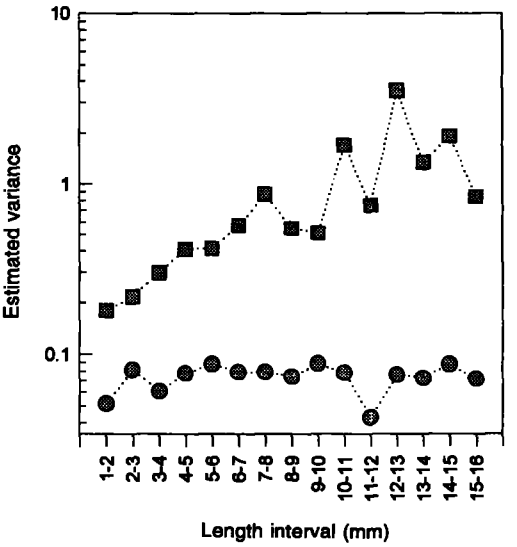

Figure 3

Variance in body length of fresh (circles) and preserved (squares) specimens within each millimeter interval.

the mean can be substantial and that 2) the relative position of an individual larva within a length interval may shift substantially.

Our findings are consistent with those of Hay (1982), Theilacker (1986), and Fox (1996) who all found that postpreservation changes in body length were greatest for large larvae. However, the marked increase in variance of preserved larval lengths in relation to the initial measurements was not caused by operator error. The residual variance of repeated measurements among operators was of the same order as the variance in fresh lengths within individual length intervals and was comparable to that obtained by Jennings (1991), Hjörleifsson and Klein-Macphee (1992), and Fox (1996). However, Fox (1996) found some evidence of increased variance with increased length, in contrast with our results. We conclude that remeasurement of larvae per se is unlikely to be a major contributor to our observation that the order of individual larvae, in relation to population estimates, is not maintained following preservation.

Our finding of postpreservation increases in body length, for individuals <6 mm SL, contrasts with most studies of the effects of formaldehyde preservation on larval fish. Fox (1996) and studies of other meth-

Table 3

Species specific mean differences in length following preservation within 1-millimeter length intervals. The number of specimens is indicated by n. Subscripts in the fourth and seventh columns refer to the species for which information was available within each length interval (i=1, . . . , n). The standard deviation of the species specific difference in length (SD) is also provided. A positive difference in length indicates expansion whereas a negative value indicates shrinkage. The last three columns present the analytical results for overall changes in length following preservation, differences among species, and homogeneity of variance between fresh and preserved specimens within each length interval.

Length Interval (mm)	Species	n	$\bar{l}_i$	SD (Δl_i)	t-value H_0:$\Delta\bar{l}=0$	F-value H_0:$\Delta\bar{l}_1=\ldots=\bar{l}_n$	F-value H_0:$s_f^2>=s_p^2$
1–2	*Pleuronectes americanus*	11	0.18	0.43	1.86, $P>0.05$	0.02, $P>0.5$	3.21, $P<0.01$
	Tautogolabrus adspersus	5	0.15	0.19			
2–3	*Pleuronectes americanus*	13	0.12	0.46	0.92, $P>0.2$	N/A	2.45, $P<0.05$
3–4	*Liparis* sp.	8	0.07	0.41	9.74, $P<0.001$	24.6, $P<0.001$	4.49, $P<0.001$
	Mallotus villosus	73	0.63	0.39			
	Pleuronectes americanus	6	–0.39	0.35			
4–5	*Hippoglossoides platessoides*	3	–0.33	0.75	6.96, $P<0.001$	2.78, $P<0.05$	4.87, $P<0.001$
	Liparis sp.	15	–0.07	0.99			
	Mallotus villosus	136	0.26	0.56			
	Pleuronectes americanus	3	0.09	0.77			
	Ulvaria subbifurcata	75	0.37	0.47			
5–6	*Hippoglossoides platessoides*	5	0.48	0.71	3.38, $P<0.001$	4.38, $P<0.01$	4.33, $P<0.001$
	Liparis sp.	7	–0.76	0.43			
	Mallotus villosus	119	0.14	0.53			
	Pleuronectes americanus	3	–0.10	0.49			
	Ulvaria subbifurcata	147	0.14	0.67			
6–7	*Hippoglossoides platessoides*	9	0.23	0.52	0.33, $P>0.5$	0.76, $P>0.2$	6.63, $P<0.001$
	Mallotus villosus	51	–0.07	0.65			
	Ulvaria subbifurcata	122	–0.01	0.70			
7–8	*Hippoglossoides platessoides*	6	0.73	0.67	2.70, $P<0.01$	4.61, $P<0.05$	10.1, $P<0.001$
	Mallotus villosus	33	–0.41	1.10			
	Ulvaria subbifurcata	96	–0.19	0.76			
8–9	*Hippoglossoides platessoides*	4	0.34	0.66	2.13, $P<0.05$	3.59, $P<0.05$	6.80, $P<0.001$
	Mallotus villosus	6	–0.79	1.54			
	Ulvaria subbifurcata	77	–0.14	0.59			
9–10	*Clupea harengus*	3	–0.12	1.08	1.71, $P>0.05$	0.13, $P>0.5$	5.35, $P<0.001$
	Hippoglossoides platessoides	7	–0.06	0.65			
	Ulvaria subbifurcata	36	–0.20	0.66			
10–11	*Hippoglossoides platessoides*	4	–1.00	2.11	2.68, $P<0.05$	0.42, $P>0.5$	19.9, $P<0.001$
	Ulvaria subbifurcata	28	–0.56	1.18			
11–12	*Hippoglossoides platessoides*	4	–0.68	0.69	2.58, $P<0.05$	0.03, $P>0.5$	16.3, $P<0.001$
	Ulvaria subbifurcata	8	–0.58	0.93			
12–13	*Clupea harengus*	3	–0.13	0.73	2.75, $P<0.05$	1.26, $P>0.5$	42.7, $P<0.001$
	Hippoglossoides platessoides	3	–1.25	0.39			
	Ulvaria subbifurcata	4	–1.09	1.29			
13–14	*Clupea harengus*	10	–0.13	0.78	0.1, $P>0.5$	2.64, $P>0.1$	16.9, $P<0.001$
	Hippoglossoides platessoides	3	–0.63	1.44			
	Stichaeus punctatus	4	0.88	0.87			
14–15	*Clupea harengus*	11	–0.81	0.97	0.8, $P>0.2$	4.74, $P<0.05$	20.2, $P<0.001$
	Gadus morhua	3	0.87	0.72			
	Stichaeus punctatus	3	0.72	1.50			
15–16	*Clupea harengus*	8	–0.80	1.00	3.78, $P<0.01$	0.82, $P>0.2$	10.8, $P<0.001$
	Hippoglossoides platessoides	4	–1.30	0.58			

ods of preservation (e.g. alcohol, freezing [Theilacker, 1980; Fowler and Smith, 1983; Kruse and Dalley, 1990; Hjörleifsson and Klein-Macphee, 1992]) have shown that individual animals may increase in

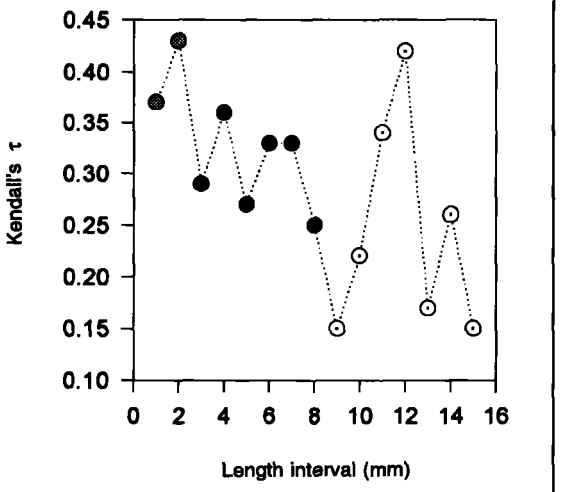

Figure 4

Rank correlation (Kendall's τ) of preserved versus fresh lengths, within millimeter intervals of fresh lengths. Open symbols represent values not significantly different from 0, grey symbols present significant values ($P<0.05$), and black symbols represent highly significant values ($P<0.01$).

length following preservation. It is important to note, however, that although our results show that the mean change in length for larvae <6 mm SL was positive, there were also numerous individuals in these same length intervals that either shrank or showed no change in body length after preservation. This finding underscores our contention that responses to preservation vary significantly at the level of the individual as well as among species.

It is possible that the capture, sorting, and preservation approaches used in this study may have led to variations in the general pattern of changes in body length. Following capture, sorting on deck may have caused a change in water temperatures. This procedure may have precipitated the death of individual larvae and resulted in contraction of body tissue in the "fresh length" measurement and consequently may have produced an apparent increase in body length following remeasurement. The source of larvae may also be an important feature to consider. Most preservation studies are based on laboratory-reared animals rather than on field-caught specimens (Table 1). Laboratory-reared animals show greater changes in length than field caught specimens (Table 1), although there is some variation about this general pattern (e.g. Theilacker, 1986).

Change in body length of marine fish larvae following capture and preservation has been attributed mainly to a breakdown of the osmoregulatory capacity of larvae, leading to a net loss of water (Ahlstrom, 1976). Simple laboratory-based studies of shrinkage have concluded that preservation results in relatively small changes in body length (see Hjörleifsson and Klein-Macphee, 1992) but that the impact of net-capture and handling is likely to have the greatest impact on changes in body length (Theilacker, 1980; Jennings, 1991; Fox, 1996). Most laboratory simulations of capture used fixed handling times; however, under field conditions it is unknown when a larva enters the net. Thus correction factors for a fixed handling time may introduce substantial error. Our sampling methods differed dramatically from those normally employed in field situations in both the duration of the residence time of larvae in the net and in the aggressiveness of capture and handling. It is therefore likely that, in comparison with other studies, the effect of capture and handling in our study was small. However, despite the possible importance of capture and handling, the interpretation of the condition of individual larvae or the distribution of population characteristics must be approached with caution.

Our results clearly show that the relative position of an individual within the size distribution of the sample is likely to change considerably following handling and preservation. Thus, attempts to correct for the effects of preservation or handling on fresh larval lengths can, in certain types of analysis (e.g. individual-based approaches to the use of morphometric measurements), introduce significant and unpredictable bias in both the data and their interpretation. There appears to be no simple solution to this problem. Conclusions as to the overall vulnerability of a fractional portion of a population to a given factor must consider the inherent variability in the distribution of measurements caused by operator, handling, and preservation-induced error.

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