Estimating live standard length of net-caught walleye pollock (*Theragra chalcogramma*) larvae using measurements in addition to standard length*

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Accurate measurement of larval fish lengths from ichthyoplankton samples is critical in the estimation of growth, birthdate and mortality. It is well known that larvae shrink in length 1) when caught in plankton nets (Theilacker, 1980; Hay, 1981), 2) between the time of collection and preservation, and 3) afterwards from the effects of preservation in chemicals or from freezing (Theilacker, 1980; Fowler and Smith, 1983; Yin and Blaxter, 1986). Shrinkage caused by the plankton net (the net can damage a larva's integument; Holliday and Blaxter, 1960) and by delays in preservation can be up to 40% of the initial live length (Hay, 1981). In some cases, measurements of larval body length may be unsuitable for growth estimation (Jennings, 1991). In other cases, algorithms have been developed to estimate live lengths from preserved larvae (Bailey 1982, Hjörleifsson and Klein-MacPhee, 1992; Fey, 1999) and from net-caught and preserved larvae (Theilacker, 1980; Theilacker and Porter, 1995; Fox, 1996). It has also been noted that constant shrinkage-correction factors should be applied cautiously (Pepin et al., 1998).

Shrinkage of the standard length of fish larvae is species-specific (Jennings, 1991; Fey, 1999), size-dependent (Fowler and Smith, 1983; Theilacker and Porter, 1995; Fey, 1999), solution-dependent (Hay, 1982; Tucker and Chester, 1984), and may also depend to some degree on the ambient temperature at the time of preservation (Hay, 1982). Other body measurements may shrink due to effects of collection and preservation. In fact, many corrections for larval shrinkage have been developed with respect to estimating morphometric-dependent condition factors (Theilacker, 1980; McGurk, 1985; Yin and Blaxter, 1986).

Measurement of otoliths has been proposed as a method to correct for shrinkage in the length of fish larvae (Leak, 1986; Radtke, 1989), but this method may not be generally applicable because of systematic variations in the relationship between fish size and otolith size (Neilson and Campana, 1990). In addition, this relationship can be nonlinear and unpredictable. Another approach to obtain shrinkage correction factors is by controlled experiments in which larval shrinkage is recorded from the time of death, or for the period of time larvae are in a plankton net, and again after preservation. The problem with this approach is that during most sampling at sea, the time larvae enter the net to the time of their preservation is unknown and can vary from several minutes to a half hour or more, depending on their depth of capture and the duration of the tow.

In our study, we used measurements of other body parts as ancillary data to estimate live lengths of preserved fish larvae caught at sea. We report on two common preservatives: ethanol and formalin. To avoid the problem of duration of time in the net, shrinkage correction equations were formulated by pooling data of known-length larvae that were treated for varying durations in simulated plankton tows in the laboratory and then preserved. Morphometric measurements were collected on preserved larvae that could be used in equations to obtain more accurate and precise estimates of live length. Rather than use alternative body part measurements as a substitute for length, we hypothesized that other body parts, nonshrinking or otherwise, could be used to correct for shrinkage, in spite of how long larvae had been in nets.

Materials and methods

Rearing protocol

Spawning walleye pollock (*Theragra chalcogramma*) were collected by trawl in the eastern Bering Sea by the NOAA ship *Miller Freeman* during April 1999. Fertilized eggs were transported to the Alaska Fisheries Science Center, Seattle, Washington, where they were incubated at 6°C in the dark in 4-L glass jars filled with 3 L of filtered seawater at a salinity of 33‰. Larvae were reared in 120-L circular, black fiberglass tanks (62 cm diameter, 43 cm deep) filled with 90-L filtered seawater, and maintained at a temperature of 6°C. Two replicate tanks with approximately 1000 eggs each were maintained. A 16:8 hour light:dark cycle was started at hatching and the amount of light at the surface of the water varied from 3.0 to 3.5 µ mol photon/m^2/s. For the first two weeks of feeding, prey consisted of rotifers, *Brachionus plicatilis,* at a concentration of approximately 10/mL, and wild zooplankton (a mixture of *Acartia* sp. copepod nauplii and copepodites, and gastropod and polychaete larvae; at approximately

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1/mL) collected from local lagoons. After two weeks of feeding, *Artemia* sp. brine shrimp (at approximately 10/mL) were added as well. Further details on larval rearing are described in Porter and Theilacker.[1]

Shrinkage experiments

Larval walleye pollock shrinkage was examined for three age groups of larvae (8, 15, and 33 days after hatching) with treatment with preservative only (no net treatment), with 5-minute net treatment and then treatment with preservative, and 15-minute net treatment and then treatment with preservative. The preservatives used were 5% buffered formalin in 33‰ seawater, and 95% ethanol buffered with 0.6 mM sodium carbonate, and 0.6 mM sodium bicarbonate. For each age group, 10 larvae were sampled for each treatment and preservative combination. A pipette was used to place a live larva in seawater on a microscope slide and the larva was quickly recorded on videotape with a Panasonic 5100 digital video camera mounted on a dissecting microscope. Next, the larva was either placed directly into preservative or into the net-treatment apparatus, after which it was preserved. The net-treatment apparatus consisted of a small net submerged in a tank of 6°C seawater. A submersible pump was used to circulate seawater through the net to simulate a plankton tow (Theilacker, 1980). Water flow through the net was adjusted so that a larva would come in contact with the mesh as it might during a tow at sea. One month was chosen as the length of time walleye pollock larvae would remain in preservative before they were recorded on videotape. The length of time for standard length to stabilize in preservative varies depending on the species of fish. For herring (*Clupea harengus*) larvae, standard length was stable after 30 days (Fox, 1996); for red sea bream (*Chrysophrys major*) larvae, most of the shrinkage occurred during the first 2–3 days in presevative (Rosenthal et al., 1978). Optimus version 5.0 image analysis software was used to measure standard length (SL), eye diameter, head length (tip of snout to pectoral fin), and body depth at the anus of both live and preserved larvae.

Otoliths were dissected from the preserved larvae, and maximum diameter of the sagittae were measured at 1000× magnification with an ocular micrometer. Some of the otoliths from larvae preserved in formalin had eroded edges and their diameters could not be measured reliably; therefore, otoliths from formalin-preserved larvae were not used in the shrinkage correction model.

Myomere width measurements taken from formalin preserved larvae were used in the shrinkage correction model. The width of five consecutive myomeres located between the two pigment bands on the tail were measured at 50× with an ocular micrometer and polarized light. For ethanol-preserved larvae, myomere measurements could not be made because the tissue had turned opaque during preservation.

Data analysis

For each preservative, data from all larval ages and shrinkage treatments were pooled and backward stepwise regression (SPSS, Inc., 1998) was used to determine which variables produced the best model for estimating live SL. The data were pooled because the regression is used to estimate live SL of larvae caught at sea and because the amount of time larvae spend in the sampling net is unknown; therefore a general relationship was sought.

For each preservative, the growth rate of walleye pollock larvae between the first (8 days after hatching) and last sampling days (33 days after hatching) was determined by using linear regression. Only the first and last sampling days were used because there is a lag in growth of walleye pollock larvae that occurs for about a week during the transition from endogenous to exogenous feeding (Yamashita and Bailey, 1989). Dunnett's test (Zar, 1996) was used to compare growth rates calculated with live SL (which was considered to be the control) against growth rates calculated from the shrinkage correction models.

Results

Shrinkage with ethanol

Walleye pollock larvae shrank an average of 6% in 95% ethanol when all shrinkage treatments were pooled (Fig. 1A). Larvae used in the shrinkage experiments ranged in live SL from 5.41 to 9.25 mm. The best model to correct for shrinkage contained preserved SL, body depth, and otolith diameter, r^2 = 0.90 (Table 1, Fig. 1B). Results indicated that there was borderline multicollinearity in the data (the variance inflation factor for otolith diameter was 4.79), probably due to correlation between preserved SL and otolith diameter. The r^2 with only preserved SL was 0.82. The use of additional variables reduced the mean square error (MSE) by 44% (Table 1).

Residuals from the best model increased as the percentage of shrinkage increased, which suggests that the shrinkage correction model does not work well for larvae that shrink greatly. At largest values of shrinkage, 17% and 19%, the best model underestimated SL by 10%, but at 15% shrinkage the error was 3%.

Shrinkage with formalin

For larvae preserved in 5% formalin, the overall mean shrinkage was 10%. Larvae ranged in live SL from 5.61 to 9.43 mm (Fig. 2A). When compared with the model using only preserved SL (r^2=0.81, MSE=0.112; Table 1), the model containing both preserved SL and body depth at the anus produced the best model for estimating live SL (r^2=0.88, MSE=0.073; Table 1, Fig. 2B).

[1] Porter, S. M., and G. H. Theilacker. 1996. Larval walleye pollock, *Theragra chalcogramma*, rearing techniques used at the Alaska Fisheries Science Center, Seattle, Washington. AFSC Processed Report 96-06, 26 p. U.S. Dep. Commerce, NOAA, NMFS, Alaska Fisheries Science Center, 7600 Sand Point Way NE, Seattle, WA, 98115.

Table 1

Shrinkage correction equations used to estimate the live standard length (SL) of net-treated and preserved laboratory-reared walleye pollock (*Theragra chalcogramma*) larvae. Best model and model containing only preserved standard length (PSL) are shown. For 95% ethanol, results indicated that there was borderline multicollinearity in the data (see "Results" section). All variables were measured in millimeters. Body depth was measured at the anus. Maximum diameter of the sagitta otolith was used.

95% ethanol (n=91; SL range: 5.41 to 9.25 mm[1])	r^2	MSE
$Live\ SL = 2.18 + 0.464(PSL) + 1.71(body\ depth) + 30.53(otolith\ diameter)$	0.90	0.073
$Live\ SL = 0.230 + 1.02(PSL)$	0.82	0.130
5% formalin (n=90; SL range: 5.61 to 9.43[1])		
$Live\ SL = 2.52 + 0.509(PSL) + 3.18(body\ depth)$	0.88	0.073
$Live\ SL = 1.88 + 0.807(PSL)$	0.81	0.112

[1] Live standard length of larvae used to formulate regression equations.

Table 2

Growth rates of laboratory-reared walleye pollock (*Theragra chalcogramma*) larvae between the first and last sampling days determined by using live standard length (LSL), and standard length corrected for shrinkage (for shrinkage correction equations see Table 1).

95% ethanol (n=61)	Growth rate (mm/day)	% Error from LSL
Live standard length	0.056	
Standard length corrected with only preserved standard length	0.048	14
Standard length corrected with best model	0.058	4
5% formalin (n=60)		
Live standard length	0.050	
Standard length corrected with only preserved standard length	0.043	14
Standard length corrected with best model	0.045	10

For 5% formalin, residuals for the best shrinkage correction model did not increase as the amount of shrinkage increased; therefore the model worked well for all shrinkage up to the maximum amount of 27%.

Growth rates

Growth rates were calculated with live SL, corrected SL from the preserved SL only models, and corrected SL from the multivariate models. For each preservative there was no significant difference between the growth rate for live SL and growth rates calculated with the models (Dunnett's test, P>0.05). Although the growth rates were not significantly different, growth rates calculated from the multivariate models were more similar to growth rates calculated from live SL (Table 2); therefore additional morphometric variables improve growth rate estimates over using preserved SL alone.

Discussion

Fish larvae shrink considerably when they are caught in nets and preserved. Shrinkage during collection is most likely caused by the plankton net, which can damage the larva's integument (Holliday and Blaxter, 1960). Shrinkage also varies between preservatives because of the differing ionic strengths of these solutions (Parker, 1963; Hay, 1982; Tucker and Chester, 1984). We found that shrinkage of walleye pollock larvae was greater in 5% formalin than in 95% ethanol—a finding similar to results from other species (Bailey, 1982; Fey, 1999). Often only preserved SL has been used to correct for shrinkage in length (Theilacker and Porter, 1995; Fox, 1996; Kristoffersen and Gro Vea Salvanes, 1998), although other measurements, such as otolith size, have been suggested for use to correct for larval fish shrinkage (Leak, 1986; Radtke, 1989,1990). By using measurements that are easily made and readily adapted to

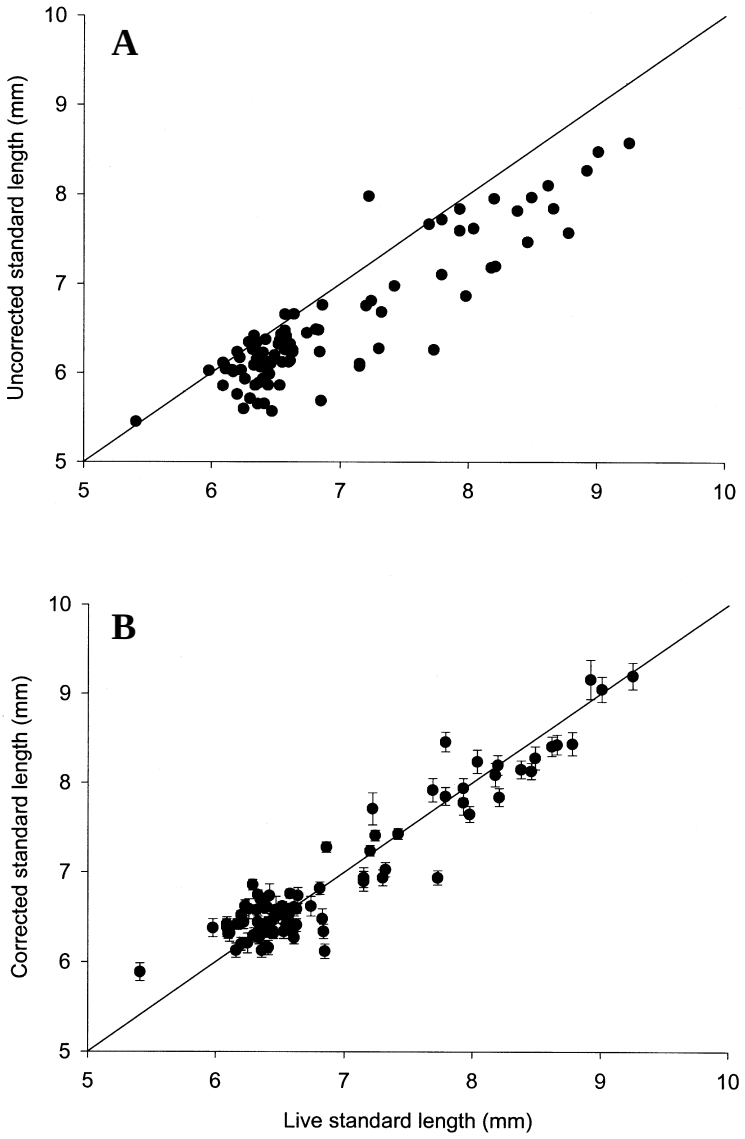

Figure 1

Uncorrected (**A**) and corrected (**B**) standard length determined by using best shrinkage correction model, see Table 1) for laboratory-reared walleye pollock (*Theragra chalcogramma*) larvae subjected to various shrinkage treatments and preserved in 95% ethanol (n=91). The 95% confidence limits are shown for each predicted value, and the diagonal line shows a 1:1 ratio.

routine data collection, we have shown improved accuracy of shrinkage correction models by use of measurements in addition to preserved SL: for 5% formalin, 7% more of the variance was explained by adding body depth; and for 95% ethanol, 8% more of the variance was explained by including body depth and otolith diameter. For 95% ethanol, the shrinkage correction model formulated in our study may significantly underestimate SL when shrinkage is high, but because there were only two larvae in this category, results were inconclusive.

Shrinkage correction models are usually formulated by using fed, laboratory-reared larvae (Jennings, 1991; Theilacker and Porter, 1995; Fox, 1996), but individual larvae response to handling and preservation can vary significantly (Pepin et al., 1998). These correction models were developed to allow more accurate calculation of the growth rate of fish larvae in the sea and to apply laboratory-derived indices to the field. We have shown factors in addition to preserved SL that may improve the accuracy of live-length estimates for walleye pollock.

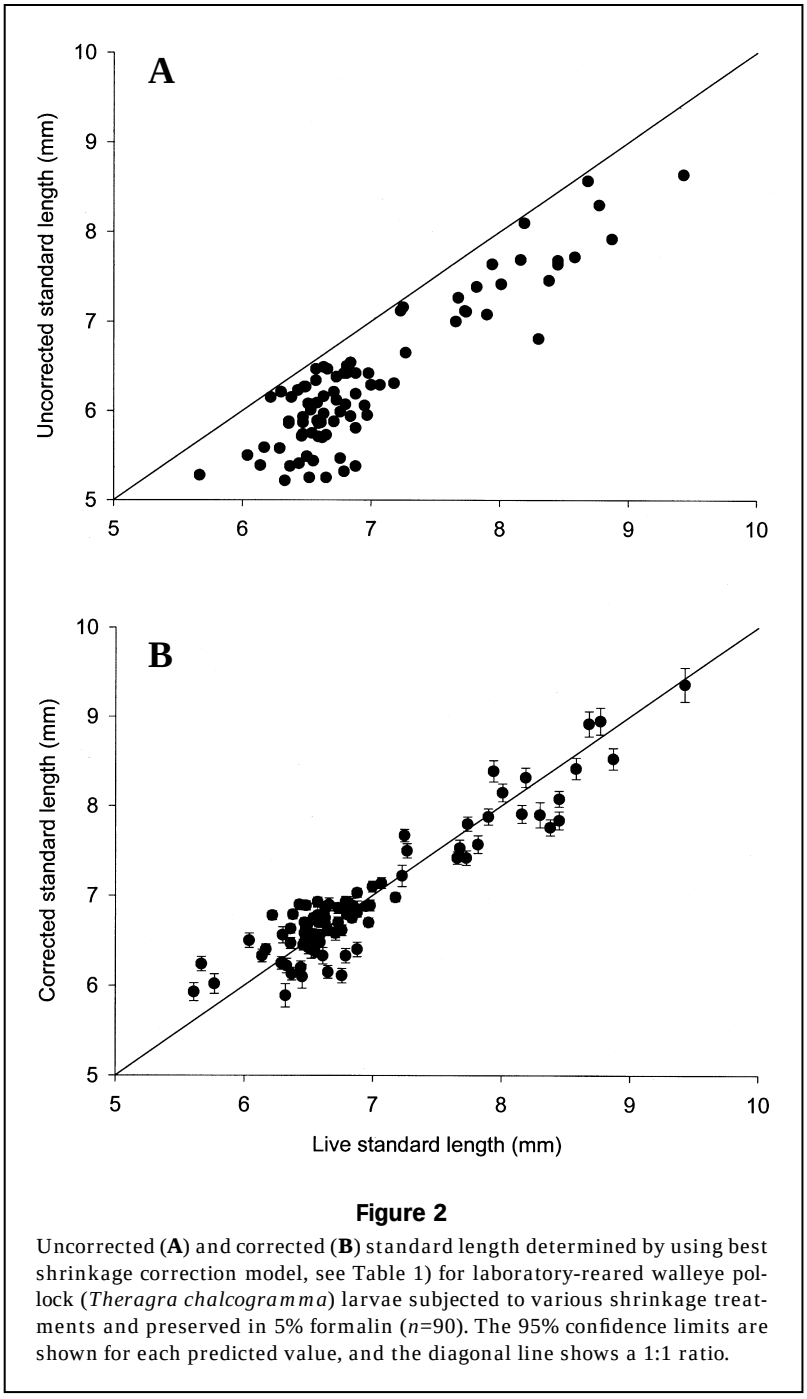

Figure 2

Uncorrected (**A**) and corrected (**B**) standard length determined by using best shrinkage correction model, see Table 1) for laboratory-reared walleye pollock (*Theragra chalcogramma*) larvae subjected to various shrinkage treatments and preserved in 5% formalin (n=90). The 95% confidence limits are shown for each predicted value, and the diagonal line shows a 1:1 ratio.

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