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## Shrinkage of Inland Silverside Larvae Preserved in Ethanol and Formalin

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**Abstract.**—Length measurements of preserved larval fish are necessary in many types of larval fish surveys. If the fixative causes significant shrinkage, then the preserved lengths cannot be used to indicate accurate live lengths. The objective of this study was to determine how preservation in two different concentrations of formalin and ethanol affects the total length of larval inland silversides *Menidia beryllina*. Larvae were measured (nearest 0.1 mm) and individually fixed in one of four fixative treatments (80% ethanol, 100% ethanol, 5% buffered formalin, and 10% buffered formalin). Fish were remeasured (nearest 0.1 mm) at 15 min; 4 h; and 1, 7, 14, and 21 d after preservation. Most shrinkage occurred within the first day after preservation in all four fixatives. Initial length was positively correlated with absolute shrinkage, but percent shrinkage was not affected by initial length. We found no difference in percent shrinkage between the 80% and 100% ethanol concentrations. The 10% buffered formalin caused more percent shrinkage than 5% buffered formalin. Furthermore, ethanol (80% and 100% combined) caused greater percent shrinkage than either 5% or 10% buffered formalin.

Accurate length measurements are necessary in many types of larval fish surveys to determine factors such as age, growth, or size-selective mortality. When a large number of fish are collected in the field, it is often necessary to preserve them so they can be analyzed later. If the fixative causes significant shrinkage, then preserved lengths cannot be used to indicate accurate live lengths.

Past studies have documented that shrinkage of larval fishes preserved in formalin varies among species. For example, Pacific herring *Clupea pallasii* larvae preserved in 20–30% formalin shrank about 3% (Hay 1982), and silver hake *Merluccius bilinearis* preserved in 4% formalin shrank 4.3% (Fowler and Smith 1983). However, southern flounder *Paralichthys lethostigma* had no consistent change in length when preserved in 4% formalin (Tucker and Chester 1984). Although most studies found at least some shrinkage of larval fish

preserved in formalin, larval Mozambique mouth-brooder *Sarotherodon mossambicus* (=Mozambique tilapia *Tilapia mossambica*) actually increased 1.7% in length when preserved in 10% formalin (Billy 1982).

Different shrinkage rates between species also occurred with alcohol preservation. Fisher et al. (1998) found 12.3% shrinkage of yellow perch *Perca flavescens*, but no difference between ethanol concentrations of 50, 80, 95, and 100%. Silver hake larvae shrank 7% when preserved in 95% ethanol (Fowler and Smith 1983), and larval sea bass *Dicentrarchus labrax* (=European bass *Morone labrax*) shrank 4% in 70% ethanol (Jennings 1991). Contradicting most other studies, larval northern anchovy *Engraulis mordax* did not shrink when preserved in 80% ethanol, but shrank 8% when preserved in 5% formalin (Theilacker 1980). Because different amounts of shrinkage in formalin and ethanol have been reported for different species, assessments of shrinkage caused by preservation must be species-specific.

Our objective was to determine how preservation in two different concentrations of formalin and ethanol affects total length of larval inland silversides *Menidia beryllina*. We examined length change relative to initial length and compared the effects of the two fixative types.

### Methods

Larval inland silversides were collected with a 500- $\mu$ m-mesh ichthyoplankton trawl from Buffalo Springs Lake, a eutrophic west Texas reservoir. To ensure a wide range of initial lengths, fish were collected on 2 d a week apart. Live fish were transported to the laboratory. An 80% ethanol fixative was prepared by diluting ethanol (190 proof undenatured) with reverse-osmosis-filtered (RO) water. A 5% buffered formalin fixative was prepared by diluting 10% buffered formalin (40 mL formaldehyde, 960 mL RO water, 4.0 g sodium acid phosphate [monobasic], and 6.5 g anhydrous sodium phosphate) with RO water (1:1). We used 75 fish for each fixative (80% ethanol, 100% ethanol,

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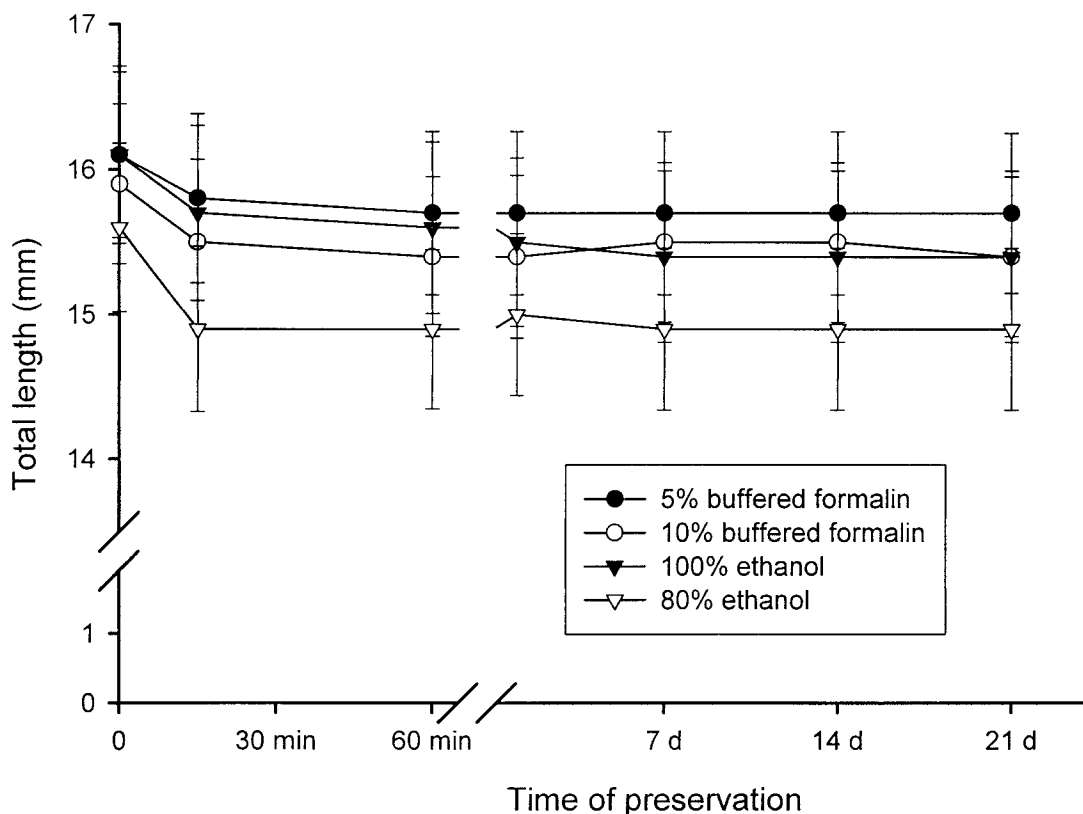


FIGURE 1.—Mean ( $\pm$ SE) total lengths of larval inland silversides preserved in two concentrations of ethanol and two concentrations of buffered formalin. Measurements were completed at 0 min (initial length); 15 min; 1 h; and 1, 7, 14, and 21 d after preservation.

5% buffered formalin, and 10% buffered formalin) with approximately 5 mL of fixative for each fish (300 total larvae). Each fish was euthanized with tricaine methanesulfonate (MS-222) and immediately measured (nearest 0.1 mm) with an ocular micrometer on a dissecting microscope to obtain initial total length (nearest 0.1 mm). Individual fish were randomly placed into individual vials containing the appropriate fixative. Fish were remeasured at 15 min; 4 h; and 1, 7, 14, and 21 d after preservation.

Analysis of variance (ANOVA) within fixatives was used to test for differences in initial and preserved lengths. Pearson's product-moment correlation was used to assess relations of initial length to absolute and percent shrinkage. An ANOVA was used to test for differences in percent shrinkage among the four fixatives.

**Results and Discussion**

Initial length of preserved larval inland silversides ranged from 6.3 to 29.7 mm (Table 1).

TABLE 1.—Range of initial total lengths and mean ( $\pm$  SE) percent shrinkage of larval inland silversides over 21 d of preservation in two concentrations of ethanol and two concentrations of buffered formalin.

Fixative	Range of initial total lengths (mm)	Mean shrinkage (%) after preservation for				
		4 h	1 d	7 d	14 d	21 d
80% ethanol	6.3–26.7	3.8 $\pm$ 0.3	3.6 $\pm$ 0.3	3.8 $\pm$ 0.3	3.7 $\pm$ 0.3	3.9 $\pm$ 0.4
100% ethanol	8.4–29.7	3.4 $\pm$ 0.4	3.9 $\pm$ 0.3	4.1 $\pm$ 0.3	4.5 $\pm$ 0.5	4.1 $\pm$ 0.3
5% buffered formalin	7.8–27.9	2.4 $\pm$ 0.3	2.5 $\pm$ 0.3	2.3 $\pm$ 0.3	2.3 $\pm$ 0.2	2.2 $\pm$ 0.2
10% buffered formalin	8.6–27.0	3.6 $\pm$ 0.4	4.3 $\pm$ 0.4	3.2 $\pm$ 0.3	3.2 $\pm$ 0.3	3.2 $\pm$ 0.3

Lengths of inland silverside larvae differed between the initial and 21-d measurements for all four fixatives: 80% ethanol ( $F = 8,216$ ,  $df = 1$ ,  $73$ ,  $P = 0.0001$ ), 100% ethanol ( $F = 13,486$ ,  $df = 1$ ,  $73$ ,  $P = 0.0001$ ), 5% buffered formalin ( $F = 22,380$ ,  $df = 1$ ,  $73$ ,  $P = 0.0001$ ), and 10% buffered formalin ( $F = 15,211$ ,  $df = 1$ ,  $71$ ,  $P = 0.0001$ ). Mean percent shrinkage after 21 d was 3.9% (80% ethanol), 4.1% (100% ethanol), 2.2% (5% buffered formalin), and 3.2% (10% buffered formalin). Most shrinkage occurred within the first day (Figure 1). Even though initial length was positively correlated (80% ethanol:  $r = 0.33$ ,  $P = 0.004$ ; 100% ethanol:  $r = 0.48$ ,  $P = 0.0001$ ; 5% buffered formalin:  $r = 0.40$ ,  $P = 0.0004$ ; 10% buffered formalin:  $r = 0.26$ ,  $P = 0.02$ ) with absolute shrinkage, percent shrinkage was not affected by initial length (all  $P > 0.4$ ).

Percent shrinkage differed among the four fixatives ( $F = 8.91$ ,  $df = 3$ ,  $294$ ,  $P = 0.0001$ ). There was no difference in percent shrinkage between the two ethanol treatments ( $F = 0.18$ ,  $df = 1$ ,  $148$ ,  $P = 0.67$ ). However, the 10% buffered formalin caused more percent shrinkage than the 5% buffered formalin ( $F = 7.03$ ,  $df = 1$ ,  $146$ ,  $P = 0.009$ ). Furthermore, ethanol (80% and 100% combined results) caused greater percent shrinkage than 5% buffered formalin ( $F = 25.3$ ,  $df = 1$ ,  $223$ ,  $P = 0.0001$ ) and 10% buffered formalin ( $F = 5.01$ ,  $df = 1$ ,  $221$ ,  $P = 0.026$ ).

As expected, greater shrinkage occurred in ethanol than in buffered formalin, but the difference between fixatives was not as great as that reported for other fish species (e.g., Fowler and Smith 1983; Kruse and Dalley 1990; Fisher et al. 1998). We also found no correlation between initial length and percent shrinkage, whereas other studies have documented increased percent shrinkage in shorter larvae (e.g., Hay 1982; Fowler and Smith 1983; Morkert and Bergstedt 1990).

When possible, total length of preserved specimens should be adjusted to account for length reductions caused by preservation. Formalin should be used in place of ethanol to preserve larval inland silversides when minimal shrinkage is important. However, ethanol should be used to preserve larval inland silversides if otolith analysis is planned because of the degenerative effects of

formalin on calcified structures (Essig and Cole 1986).

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