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The Effect of Fixative on Total Length of Small-Bodied Stream Fishes

ABSTRACT

Longnose dace (*Rhinichthys cataractae*), red shiner (*Cyprinella lutrensis*), and green sunfish (*Lepomis cyanellus*) were fixed in 5% and 10% formalin and 70% and 95% ethyl alcohol to determine fixative effects on total length (TL). Total length reduced over the first 24h for all species ($P < 0.0001$) but then stabilized. Longnose dace and green sunfish TL reduction was less for 5% formalin than for either 70% or 95% ethanol (both $P < 0.0001$), whereas the fixative solution had no effect on red shiner TL ($P = 0.347$). A greater percentage of change in TL was observed in green sunfish and red shiner than in longnose dace, suggesting that body form (compressiform vs. fusiform) may affect shrinkage rate among adult stream fishes.

Differences in length change of fixed fish have been shown between formalin and alcohol, and also for different concentrations of these preservatives. Ethyl alcohol causes greater reduction in preserved larval fish length than formalin (Kruse and Dalley 1990, Fisher et al. 1998, Cunningham et al. 2000, Moku et al. 2004, Buchheister and Wilson 2005). However, these studies have focused on larval fishes and little information is available on the effects of fixative on adult and juvenile stream fish or different body forms. Therefore we evaluated the effects of fixative solution and time on the total lengths (TL) of three stream fish species - longnose dace (*Rhinichthys cataractae*), red shiner, (*Cyprinella lutrensis*), and green sunfish, (*Lepomis cyanellus*).

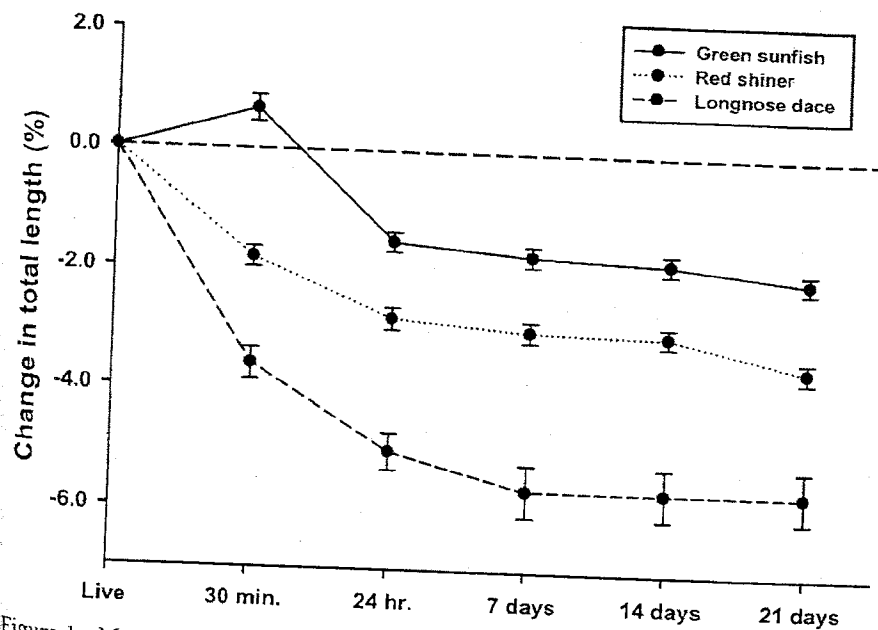


Figure 1. Mean percentage change in total body length over time for fish species in four fixative solutions. Bars represent standard error. Results are for all fixative solutions combined for each species.

We collected 48 longnose dace (49 - 75 TL) from the North Loup River, Cherry County, Nebraska on 8 July 2005; fifty green sunfish (59 - 99 TL) were collected from Mill Creek, Riley County, Kansas on 21 October 2005; and sixty red shiners (42 - 60 TL) were collected from the Kansas River, Riley County Kansas on 10 November 2005. The fish were visually sorted to obtain samples of similar total lengths (measured to the nearest millimeter), and then were placed into individually numbered sample bags containing 100 ml of one of the fixative solutions (5% and 10% formalin and 70% and 95% ethyl alcohol). Subsequently, TLs were measured at 30 min, 24 h, 7, 14, and 21 days following the initial live measurement. All measurements were done by one individual to reduce bias. A repeated measures analysis of covariance was used to determine the effects of time and fixative solution on TL with initial length being the covariate. All statistical analyses were conducted using Statistical Analysis System (SAS 9.1.3, SAS Institute 2006), and the alpha level for significance was set at 0.05.

For all three species, TL was significantly different from live length after 30 min in all fixative solutions (all $P_s < 0.05$). Since there was no time by fixative interaction for any species ($P_s \geq 0.26$), results were consistent across time for each fixative and consistent among fixatives across time. After 24 h, the length reduction rates stabilized, with mean reduction in TL of 2.4% for green sunfish, 5.3% for longnose dace, and 3.6% for red shiners for all fixatives combined (Fig. 1). For green sunfish and longnose dace, length reduction was less for formalin concentrations than either 70% or 95% ethanol ($P < 0.05$, Fig. 2). Red shiner length reduction did not differ among fixatives ($P = 0.35$). There was no difference in length reduction between 5% and 10% formalin for any of the three species.

Our results are consistent with those from other studies that indicated greater shrinkage in alcohol for larval fish (Fisher et al. 1998, Cunningham et al. 2000). Similarly, Distefano et al. (1994) found preservative had no effect on the percentage of change in the overall length of central stoneroller (*Camptostoma anomalum*) after 90 and

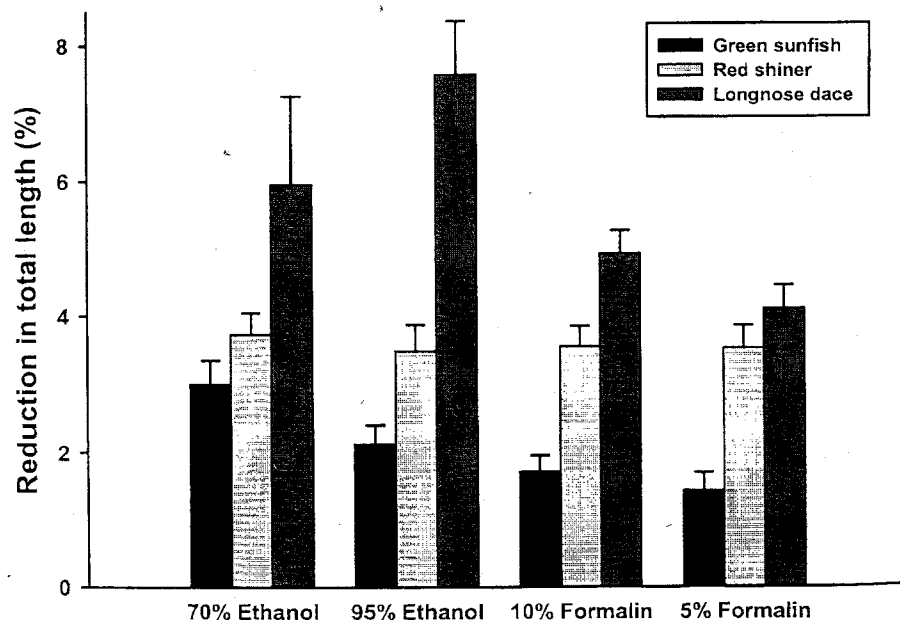


Figure 2. Comparison of mean length reduction for four different fixative solutions used to preserve stream fish species. Bars represent standard error. Results are for final length attained after 21 days in the fixative solution.

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180 day intervals. The overall percentage of changes (< 3%) from Disterano et al. (1994) study was also similar to the results from red shiner and green sunfish but less than that of longnose dace. However, we are aware of no studies that indicated changes in length in adult stream fish. Overall, slender-bodied fish may be more susceptible to proportionally greater length reduction from fixation when compared to more deep-bodied fish.

Our results suggest that in studies of stream fish communities that include collecting small-bodied fish for later analysis, fixation in either the standard 10% formalin will cause less total length reduction than ethanol. However, our results also suggest that shrinkage due to fixative is consistent neither among body forms of adult small-bodied stream fish nor among preservatives. If length measurements from fixed fish must be obtained it may be necessary to create adjustment factors specific to individual body forms and length categories to prevent bias in comparisons to live lengths.

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