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Total Length Reduction in Preserved Yellow Perch Larvae

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Abstract.—The high variability in reported lengths of larval yellow perch *Perca flavescens* at hatching, dietary shift, and morphometric transformation may be partly caused by shrinkage that occurs after preservation. Larval yellow perch were captured, randomly assigned to one of six preservative treatments (100%, 95%, 80%, and 50% ethyl alcohol and 5% and 10% formalin), and measured (before preservation) for total length (TL). Larval yellow perch total lengths were then recorded on days 1, 7, 14, and 21 after storage in each of the six preservatives. Significant reductions in TL (11.5–14.3%) occurred during the first 24 h after fixation and larvae continued to contract at a lesser rate through day 7 in all four ethyl alcohol treatments. Total length reductions of up to 2.5% also occurred during the first 24 h in each formalin concentration. Our findings report the total length reductions of larval yellow perch at a length range used by some biologists when indexing year-class strength and during studies of early life history. The length reductions of larval yellow perch that are associated with storage in different concentrations of preservative appear to be inconsistent with those noted for other species; therefore, our findings support the need to obtain species-specific data for larval shrinkage.

Yellow perch *Perca flavescens* typically hatch at a total length (TL) of 4.7–6.6 mm (Mansueti 1964; Holland-Bartels et al. 1990) and begin feeding at 6.0–10.0 mm TL (Swindoll 1981; Whiteside et al. 1985; Hinshaw 1986). However, Fisher and Willis (1997) found food items in stomachs of larval yellow perch that were shorter (4.0 mm TL) than the expected length at hatching. Variability in reported TLs at initial feeding, during dietary shifts, and at the time of morphometric changes are not surprising when populations of a given species are compared; however, substantial differences raise questions about the effect of handling and preservation on the accuracy and precision of larval fish measurements and the subsequent applicability of any associated research results.

Total length of larval fishes frequently declines up to 10% after fixation in formalin and varies with differing concentration, salinity, buffer, and taxon (Hay 1982; Tucker and Chester 1984; Morkert and Bergstedt 1990). Problems with length reduction have also been associated with alcohol preservatives. For example, preservation of larval fishes in 95% and 70% ethanol resulted in mean length reductions of approximately 7% and 4%, respectively (Fowler and Smith 1983; Jennings 1991); another study that used 80% ethanol reported shrinkage of less than 1% (Theilacker 1980). Preservation in 100% anhydrous alcohol produced mean larval fish shrinkage ranging from 2.3% to 14.4%, depending upon initial TL of larvae (Kruse and Dalley 1990).

Jennings (1991) suggested that the variability in shrinkage among larval fishes preserved in any one preservative is species specific, and several studies (e.g., Fowler and Smith 1983; Kruse and Dalley 1990) have also suggested that initial total length is an important factor. The effect of alcohol concentration on total length reduction has not been well documented for any fish species. Therefore, the objective of this study was to compare mean larval yellow perch total lengths preserved in four alcohol and two formalin concentrations.

Methods

Larval yellow perch were collected on 3 June 1997 from a eutrophic natural lake in eastern South Dakota. Yellow perch larvae were captured with an ichthyoplankton surface trawl (0.76-m diameter; 500-μm mesh), transported back to the laboratory, and randomly sorted into four groups of 50 fish and two groups of 100 fish. The larvae were transported in water from the lake in which they were captured, and total elapsed time between capture and placement into one of the selected preservatives was between 2 and 4 h. Larvae were dead at the time of placement into the preservatives. Each fish in the 50-larvae groups was measured to the nearest 0.1 mm TL with an ocular micrometer fitted to a dissecting microscope and then placed into one of four ethyl alcohol concentrations (100%, 95%, 80%, and 50%). Pure ethyl alcohol and distilled water were used to obtain appropriate alcohol concentrations. Each fish in the 100-larvae groups was also measured to the nearest 0.1 mm but was then placed into one of two unbuffered formalin concentrations (5% and 10%) that were obtained by diluting 40% formaldehyde with distilled water. An analysis of vari-

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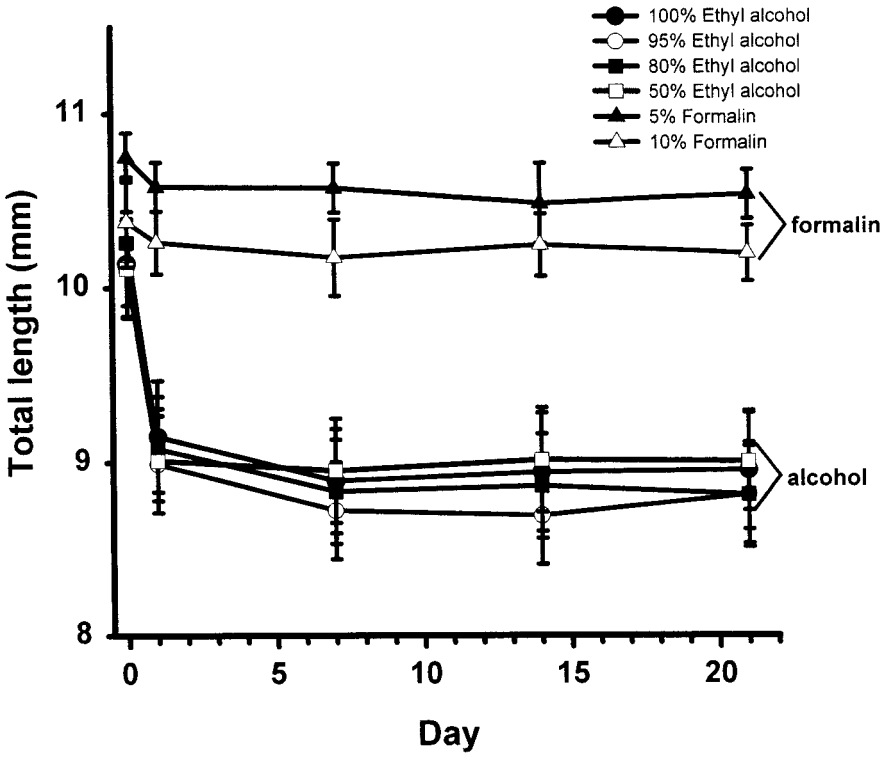

FIGURE 1.—Mean total length of larval yellow perch preserved in various concentrations of ethyl alcohol and formalin. Vertical lines indicate the 95% confidence limits. Measurements were completed on day 0 (before fixation) and then again on days 1, 7, 14, and 21 after fixation.

ance (ANOVA; GLM procedure; SAS Institute 1990) was used to determine if any significant differences in mean TL existed among the six treatments on day 0.

Each larval fish from each preservative treatment group was again measured to the nearest 0.1 mm TL at 24 h and 7, 14, and 21 d after fixation. Larval TL measurements were tested for normality with a Shapiro–Wilk test (UNIVARIATE procedure; SAS Institute 1990). Normally and independently distributed data within each preservative treatment were then analyzed with a two-way ANOVA (GLM procedure; SAS Institute 1990) to test for significant differences ($\alpha = 0.05$) among measurement days and various preservative concentrations; 95% confidence limits (CL) were also calculated. When the interaction between concentration and measurement day was not significant and a significant difference was detected in one of the main effects, a Tukey's multiple-range test was used to determine which days or concentrations were significantly different from one another (MEANS/TUKEY procedure; SAS Institute 1990).

Results and Discussion

Mean TL of yellow perch were similar ($F = 0.21$; df = 5; $P = 0.889$; Figure 1) among the six preservative groups on day 0, indicating that the larval perch had been randomly sorted. Larval yellow perch TL measurements were normally and independently distributed within each preservative group of perch larvae (Shapiro–Wilk $W > 0.8$; $P > 0.05$); therefore, ANOVA was appropriate. The interaction term between days and preservative concentrations with the two-way ANOVA was significant ($F = 5.62$; df = 20; $P < 0.001$), indicating that a pattern difference existed among the observations. Based on Figure 1, data for each preservative type (i.e., alcohol and formalin) were analyzed independently. Mean TL of yellow perch larvae placed into the alcohol preservatives varied significantly among days ($F = 55.00$; df = 4; $P < 0.001$), but not among concentrations ($F = 1.10$; df = 3; $P = 0.347$). There was no significant interaction term ($F = 0.28$; df = 12; $P = 0.993$). Within each alcohol concentration, mean TL on

day 0 was longer than the mean TL on days 1, 7, 14, and 21 (Figure 1). Mean TL of yellow perch larvae varied significantly between the two formalin concentrations ($F = 40.03$; df = 1; $P = <0.001$), but did not differ among days ($F = 2.22$; df = 4; $P = 0.065$), indicating that the relative length reductions from the initial length were negligible. The significant difference in yellow perch mean TL between the two formalin concentrations could be the result of differential initial mean lengths (10.75 and 10.38 mm TL; Figure 1) that may have been amplified over the measurement days.

Our analysis indicated that significant reductions in the TL of yellow perch larvae that had initial TL of approximately 10 mm occurred during the first 24 h after fixation in ethyl alcohol, regardless of alcohol concentration (Figure 1). The analysis also revealed that there was a reduction in mean TL of larval yellow perch during the first 24 h after preservation in 5% or 10% formalin; however, these mean lengths were not significantly reduced when compared with the mean lengths on day 0 (Figure 1). Mean TL was reduced in the alcohol concentrations from 10.14 mm (CL = 9.84, 10.44) on day 0 to 8.89 mm (CL = 8.59, 9.19) by day 7, whereas mean TL in the two formalin preservatives was reduced from 10.51 mm (CL = 10.21, 10.81) to 10.37 mm (CL = 10.18, 10.56) on day 7. Our findings are substantially different from the results of shrinkage studies completed on other larval fishes, some of which were of similar size (e.g., Theilacker 1980; Kruse and Dalley 1990; Morkert and Bergstedt 1990), and support the suggestion by Jennings (1991) that larval shrinkage be determined on a species-specific basis. However, Hay (1982) suggested that the TL at the time of fixation is another important length reduction factor to consider.

Early life history management and research (e.g., Fisher and Willis 1997; Anderson et al., in press) is occasionally questioned because of inconsistencies in reported total lengths. Wide variations in TL of larval yellow perch at important life stage events is probably due to many factors, such as genetic variation, food availability, and habitat quality, and attempts to determine causes of such variation can be confounded by length reductions resulting from specimen preservation. Length reductions caused by preservation should therefore be estimated before reporting initial feeding lengths, discussing dietary shifts, or when length is used as a descriptive or morphometric tool. The results of our study also indicate that formalin may be a better preservative if minimizing shrinkage of 8–12-mm larval yellow perch is important. However, alcohol preservative should be used if otolith analysis is planned because of the degenerative effects of formalin on calcified structures (Essig and Cole 1986).

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