

## Length and Weight Reduction in Larval and Juvenile Yellow Perch Preserved with Dry Ice, Formalin, and Ethanol

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**Abstract.**—Due to the increasing interest in biochemical indices such as the RNA–DNA ratio used to measure fish growth, fish often need to be stored frozen with dry ice (i.e.,  $-80^{\circ}\text{C}$ ). The objectives of this study were to (1) quantify the effects of dry ice on both the length and weight of larval and juvenile yellow perch *Perca flavescens* preserved for storage periods of 15 d and 7–8 months, (2) compare these effects with those of two commonly used preservatives (a 10% solution of formalin and a 75% solution of ethanol), and (3) provide equations to convert the lengths and weights of larval and juvenile yellow perch preserved with dry ice, formalin, and ethanol back to their initial unpreserved values. For all preservation methods, fish weight was more affected than length. The smallest length reduction was observed with formalin (short term: 2.1% and 0.1% for larvae and juveniles, respectively; long term: 10.1% and 1.2%), followed by dry ice (short term: 4.0% and 1.4%; long term: 7.2% and 3.9%) and ethanol (short term: 9.6% and 1.2%; long term: 11.7% and 1.2%). The smallest weight reduction was also observed with formalin (short term: 21.9% and 2.2%; long term: 23.2% and 3.9%), followed by dry ice (short term: 54.0% and 11.1%; long term: 52.8% and 8.4%) and ethanol (short term: 61.1% and 22.0%; long term: 66.0% and 26.0%). Except for one case, all of the regression equations that were built to convert the lengths and weights of larval and juvenile yellow perch preserved with dry ice, formalin, and ethanol back to initial measurements were highly significant.

Accurate length measurements are fundamental when investigating the population dynamics of larval

and juvenile fish (Jennings 1991). It is rarely possible to measure lengths and weights during fieldwork, so fish are preserved for later measurements in the laboratory. Fixation and preservation techniques are known to change larval length and weight for a number of fish species (Parker 1963; Leslie and Moore 1986; Kelso and Rutherford 1996) and to introduce bias in morphometric analyses (Sagnes 1997). For yellow perch *Perca flavescens* and European perch *P. fluviatilis*, the effects of formalin and ethanol on larval length have been documented (Treasurer 1992; Fisher et al. 1998), and the impact of formalin and freezing at  $-18^{\circ}\text{C}$  and  $-25^{\circ}\text{C}$  have been evaluated for age-1 juveniles and adults (Stobo 1972; Engel 1974; Treasurer 1990). To date, the effects of formalin and ethanol on the weight of small, posthatch yellow perch larvae are still not known. The effect of dry ice preservation ( $-80^{\circ}\text{C}$ ) also remains undocumented, even though an increasing number of biochemical methods require that fish be preserved with dry ice (Weber et al. 2003). Dry ice preservation avoids the denaturation of nucleic acids, which are necessary, for example, to measure instantaneous fish growth with the RNA–DNA ratio approach (Bergeron 1997; Pepin et al. 1999; Tardif et al. 2005). Tardif et al. (2005) suggested that a correction factor be developed to calculate the initial fish length from preserved larvae to avoid misinterpreting the biochemical growth indices.

The objectives of this study were to (1) quantify the effect of dry ice on both the lengths and weights of larval and juvenile yellow perch preserved for storage periods of 15 d and 7–8 months, (2) compare the

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effects of dry ice with two commonly used preservatives (a 10% solution of formalin and a 75% solution of ethanol), and (3) provide equations to convert the lengths and weights of larval and juvenile yellow perch preserved with dry ice, formalin, and ethanol back to their initial unpreserved values.

### Methods

Fish samples were collected in shallow wetlands of Lake Saint-Pierre (46°15'N, 72°50'W) in Quebec. Larval yellow perch (7.1–19.0 mm) were collected with push nets (0.40-m square mouth opening; 500- $\mu$ m mesh) in May 2003 and June 2004, while juveniles (33.9–58.7 mm) were caught with beach seines (12.5  $\times$  4 m; 3.2-mm stretched mesh) in July 2003 and August 2004. Fish samples were transported in freshwater to the laboratory and divided into three subsamples. After being blotted dry with paper towels, each fish was weighed ( $\pm 0.001$  g) and measured ( $\pm 0.01$ -mm total length); length measurements were made using an ocular micrometer mounted on a dissecting microscope for larvae and a digital caliper for juveniles. Fish were then individually preserved in vials using one of the following preservatives: 10% formalin, 75% ethanol (anhydrous ethyl alcohol), or dry ice (freezing at  $-80^{\circ}\text{C}$ ). For the dry ice treatment, fish were placed flat and straight in vials and frozen dry. The elapsed time between capture and preservation never exceeded 12 h. Larvae and juveniles were dead at the time of placement into the preservatives. To investigate the effect of each preservation method on yellow perch length and weight, fish were remeasured and reweighed after a period of 15 d (hereafter "short preservation period") and after 8 months for larvae and 7 months for juveniles (hereafter "long preservation period"). Before the second measurement, frozen fish were thawed at room temperature and individuals from all preservation methods were blotted with paper towels. The number of specimens used in each treatment ranged from 24 to 49 for larvae and from 19 to 35 for juveniles. Independent samples were used for the short-term and long-term effect of preservation.

Changes in length were expressed in terms of the percent shrinkage (Fowler and Smith 1983) for each specimen, namely,

$$[1 - (L_t/L_0)] \cdot 100,$$

where  $L_0$  is the initial length and  $L_t$  the length at time  $t$  (the same calculation was done for weight measurements). Paired  $t$ -tests were used to compare initial and preserved measurements of larval and juvenile yellow perch for the short and long preservation periods. Analyses of covariance (ANCOVA) were performed to compare the effects of the three preservation methods

on length and weight changes (Johnston and Mathias 1993). The initial length or weight was used as a covariable in the model. The interaction term (e.g.,  $L_0 \times$  preservation method) was also included in the analyses to test for differences in the slope of relationships between fresh and preserved lengths (and weights). When significant differences occurred between preservation methods, pairwise comparisons (least-squares means) with Bonferroni corrections were conducted to determine which preservation methods differed from the others. The same statistical procedure was used for weight comparisons. When significant shrinkage occurred, equations were developed to convert preserved measurements into initial unpreserved ones. The relationship between initial and preserved measurements was calculated using a least-squares linear regression (Treasurer 1990). For example, the relationship for length was

$$L_0 = a + b \cdot L_t,$$

where  $a$  is the intercept and  $b$  the slope (the same calculation was done for weight measurements). All statistical analyses were performed in SYSTAT 10.2.

### Results

#### *Larvae: Short Preservation Period*

After a preservation period of 15 d, larval yellow perch exhibited a significant average length reduction of 9.6% in ethanol, 2.1% in formalin, and 4.0% with dry ice (ethanol:  $t = 19.58$ ,  $P < 0.001$ ; formalin:  $t = 3.40$ ,  $P < 0.01$ ; dry ice:  $t = 8.65$ ,  $P < 0.001$ ; Table 1). An ANCOVA revealed that the slopes of the regressions between fresh and preserved lengths were not significantly different among the three preservation methods ( $F = 0.08$ ;  $P = 0.923$ ) but that the intercepts differed significantly ( $F = 55.14$ ;  $P < 0.001$ ). Shrinkage was significantly higher with ethanol than with formalin ( $P < 0.001$ ) and dry ice ( $P < 0.001$ ), but it did not significantly differ between formalin and dry ice ( $P = 0.051$ ). Weight loss was also significant among the three preservation methods (Table 1), reaching 61.1% in ethanol ( $t = 18.27$ ;  $P < 0.001$ ), 21.9% in formalin ( $t = 10.61$ ;  $P < 0.001$ ), and 54.0% with dry ice ( $t = 23.62$ ;  $P < 0.001$ ). Weight loss differed significantly between preservation methods (ANCOVA; slope:  $F = 29.17$ ,  $P < 0.001$ ). Post hoc comparisons revealed significant differences in the slope of the regression lines between ethanol and formalin ( $P < 0.001$ ) and between formalin and dry ice ( $P < 0.05$ ), but not between dry ice and ethanol ( $P = 0.137$ ).

#### *Larvae: Long Preservation Period*

After 8 months of storage, larval yellow perch exhibited a significant average length reduction of

TABLE 1.—Lengths (mm) and weights (g) of fresh larval yellow perch and larval yellow perch stored for 15 d and 8 months in 75% ethanol, 10% formalin, or dry ice (−80°C). The parameters (*a* and *b*) of the bivariate regression equations used to convert preserved lengths and weights to their fresh counterparts are also shown. All regression equations were significant ( $P < 0.001$ ).

15-d preservation period								
Preservative	<i>n</i>	Range of fresh values	Mean ± SD fresh value	Mean ± SD preserved value	Parameter		<i>R</i> <sup>2</sup>	
					<i>a</i>	<i>b</i>		
<b>Length</b>								
Ethanol	44	12.1–18.1	13.89 ± 1.48	12.61 ± 1.43	1.415	0.993	0.91	
Formalin	43	10.8–18.0	13.98 ± 1.64	13.61 ± 1.58	1.273	0.928	0.87	
Dry ice	24	10.9–14.0	12.35 ± 0.69	11.88 ± 0.73	1.548	0.912	0.86	
<b>Weight</b>								
Ethanol	48	0.012–0.061	0.025 ± 0.011	0.010 ± 0.006	0.008	1.658	0.90	
Formalin	49	0.013–0.080	0.029 ± 0.014	0.023 ± 0.013	0.004	1.077	0.92	
Dry ice	32	0.008–0.021	0.013 ± 0.003	0.006 ± 0.002	−0.001	0.537	0.87	

11.7% in ethanol, 10.1% in formalin, and 7.2% with dry ice (ethanol:  $t = 15.69$ ,  $P < 0.001$ ; formalin:  $t = 17.07$ ,  $P < 0.001$ ; dry ice:  $t = 6.68$ ,  $P < 0.001$ ; Table 1). The ANCOVA indicated that the slopes of the regression between fresh and preserved lengths were not significantly different among the three preservation methods ( $F = 1.80$ ;  $P = 0.169$ ) but that the intercepts were significantly different ( $F = 7.95$ ;  $P < 0.001$ ). Shrinkage was significantly lower with dry ice than in ethanol ( $P < 0.05$ ) and formalin ( $P < 0.05$ ), but it did not differ between ethanol and formalin ( $P = 0.197$ ). The weight of larval yellow perch showed a mean shrinkage of 66.0% in ethanol ( $t = 15.69$ ;  $P < 0.001$ ), 23.2% in formalin ( $t = 6.68$ ;  $P < 0.001$ ), and 52.8% with dry ice ( $t = 17.07$ ,  $P < 0.001$ ; Table 1). The ANCOVA revealed that the slopes of the regressions between fresh and preserved weights differed among the three preservation methods ( $F = 57.30$ ;  $P < 0.001$ ),

and the post hoc comparisons indicated significant differences among all methods ( $P < 0.05$ ).

*Juvenile: Short Preservation Period*

After a storage period of 15 d, juvenile yellow perch exhibited a mean length reduction of 1.2% in ethanol ( $t = 5.38$ ;  $P < 0.001$ ), 0.1% in formalin ( $t = 0.40$ ;  $P = 0.694$ ), and 1.4% with dry ice ( $t = 8.43$ ,  $P < 0.001$ ; Table 1). The ANCOVA revealed that the relationships between fresh and preserved lengths in ethanol and dry ice were not significantly different (slope:  $F = 0.47$ ,  $P = 0.497$ ; intercept:  $F = 0.80$ ,  $P = 0.374$ ). Weight loss was 22.0% in ethanol, 2.2% in formalin, and 11.2% with dry ice (ethanol:  $t = 25.03$ ,  $P < 0.001$ ; formalin:  $t = 5.85$ ,  $P < 0.001$ ; dry ice:  $t = 31.63$ ,  $P < 0.001$ ; Table 1). The ANCOVA and post hoc analyses revealed significant differences among all preservation methods (slope:  $F = 78.60$ ,  $P < 0.001$ ; post hoc between all preservatives:  $P < 0.001$ ).

TABLE 2.—Lengths (mm) and weights (g) of fresh juvenile yellow perch and juvenile yellow perch stored for 15 d and 7 months in 75% ethanol, 10% formalin, or dry ice (−80°C). The parameters (*a* and *b*) of the bivariate regression equations used to convert preserved lengths and weights to their fresh counterparts are also shown. All regression equations were significant ( $P < 0.001$ ) except where indicated otherwise.

15-d preservation period								
Preservative	<i>n</i>	Range of fresh values	Mean ± SD fresh value	Mean ± SD preserved value	Parameter		<i>R</i> <sup>2</sup>	
					<i>a</i>	<i>b</i>		
<b>Length</b>								
Ethanol	35	37.0–58.7	47.66 ± 4.50	47.09 ± 4.42	0.733	0.973	0.98	
Formalin	30	39.0–56.5	48.83 ± 4.25	48.79 ± 4.21	<sup>a</sup>	<sup>a</sup>	<sup>a</sup>	
Dry ice	32	36.0–57.0	46.10 ± 4.53	45.20 ± 4.64	−0.331	0.993	0.99	
<b>Weight</b>								
Ethanol	35	0.835–2.686	1.465 ± 0.412	1.129 ± 0.353	0.087	1.202	0.99	
Formalin	30	0.789–2.555	1.606 ± 0.442	1.574 ± 0.443	0.038	0.996	0.99	
Dry ice	35	0.572–2.392	1.299 ± 0.391	1.161 ± 0.372	0.079	1.051	0.99	

<sup>a</sup> No significant change.

TABLE 1.—Extended.

8-month preservation period						
n	Range of fresh values	Mean ± SD fresh value	Mean ±SD preserved value	Parameter		R <sup>2</sup>
				a	b	
<b>Length</b>						
42	7.1–11.9	9.51 ± 1.28	8.31 ± 1.26	1.908	0.901	0.98
40	12.7–19.2	16.01 ± 1.97	14.37 ± 1.71	0.998	0.836	0.91
36	11.4–19.0	15.53 ± 2.11	14.37 ± 1.75	2.337	0.917	0.71
<b>Weight</b>						
41	0.001–0.017	0.006 ± 0.003	0.002 ± 0.001	0.003	1.901	0.52
40	0.012–0.062	0.036 ± 0.015	0.028 ± 0.012	0.002	1.221	0.96
33	0.001–0.061	0.031 ± 0.014	0.015 ± 0.006	0.004	1.903	0.74

*Juvenile: Long Preservation Period*

Juvenile yellow perch stored for 7 months showed significant length reduction in ethanol (1.2%), formalin (1.2%), and dry ice (3.9%) (ethanol:  $t = 2.77$ ,  $P < 0.05$ ; formalin:  $t = 2.81$ ,  $P < 0.05$ ; dry ice:  $t = 6.46$ ,  $P < 0.001$ ; Table 1). The ANCOVA revealed that the slopes of the regressions between fresh and preserved lengths differed among the preservation methods ( $F = 0.73$ ,  $P = 0.485$ ; intercept:  $F = 7.78$ ,  $P < 0.05$ ). The post hoc tests indicated that dry ice induced more shrinkage than ethanol ( $P < 0.05$ ) and formalin ( $P < 0.05$ ), while no difference was found between ethanol and formalin ( $P = 0.99$ ). Juveniles also showed a significant weight reduction in ethanol (26.0%), formalin (3.9%), and dry ice (8.4%) after the 7-month storage (ethanol:  $t = 16.96$ ,  $P < 0.001$ ; formalin:  $t = 5.11$ ,  $P < 0.001$ ; dry ice:  $t = 7.91$ ,  $P < 0.001$ ; Table 1). The ANCOVA revealed that the slopes of the regressions between fresh and preserved weights

differed among the three preservation methods (slope:  $F = 8.40$ ,  $P < 0.001$ ). Post hoc analysis revealed that the three preservatives differed ( $P < 0.001$ ), ethanol inducing the greatest weight lost followed by dry ice and formalin.

*Conversion Equations*

Equations to convert preserved lengths and weights of larval and juvenile yellow perch in ethanol, formalin, and dry ice into unpreserved specimens are given in Tables 1 and 2, respectively. All the regression equations but one (larval weight in ethanol) were highly significant,  $R^2$  values ranging from 0.52 to 0.98 for larvae and from 0.89 to 0.99 for juveniles.

**Discussion**

The present study shows that the length shrinkage and the weight loss of yellow perch differed according to preservation method, ontogenetic stage (larval

TABLE 2.—Extended.

7-month preservation period						
n	Range of fresh values	Mean ± SD fresh value	Mean ± SD preserved value	Parameter		R <sup>2</sup>
				a	b	
<b>Length</b>						
19	33.9–51.6	45.39 ± 1.18	44.86 ± 4.13	0.852	0.993	0.96
20	29.6–57.6	42.82 ± 7.52	42.22 ± 7.06	2.267	0.993	0.98
27	36.7–54.6	46.64 ± 4.61	44.76 ± 4.29	1.091	1.017	0.89
<b>Weight</b>						
19	0.433–1.540	1.139 ± 0.307	0.851 ± 0.257	0.139	1.174	0.96
20	0.308–1.939	0.939 ± 0.464	0.911 ± 0.468	0.037	0.990	0.99
27	0.577–2.022	1.216 ± 0.350	1.117 ± 0.333	0.061	1.035	0.97

TABLE 3.—Synopsis of studies reporting length and weight changes of preserved larval, juvenile, and adult yellow perch (European perch in Treasurer 1990, 1992). The studies are listed by preservation method, fresh size of preserved specimens, and storage duration.

Preservation method	Stage; fresh size (mm)	Duration	% Change		Source	
			Length	Weight		
Ethanol	Larvae; 12.1–18.1	15 d	–9.6	–61.1	Present study	
		8 months	–11.7	–66.0	Present study	
	Larvae; 10.14 (mean) Juveniles; 37.0–58.7	1, 7, 14, or 21 d	–12.3		Fisher et al. (1998)	
		15 d	–1.2	–22.0	Present study	
		7 months	–1.2	–26.0	Present study	
Formalin	Larvae; 10.8–18.0	15 d	–2.1	–21.9	Present study	
		8 months	–10.1	–23.2	Present study	
	Larvae; 10.51 (mean) Juveniles; 39.0–56.5	1, 7, 14, or 21 d	–2.0		Fisher et al. (1998)	
		15 d	–0.1	–2.2	Present study	
		7 months	–1.2	–3.9	Present study	
	4–10%	Juveniles; 37.8 ± 0.6	24 h to 72 weeks	–1.7	+18	Treasurer (1992)
	4–10%	Juveniles; 54.9 ± 1.3	24 h to 72 weeks	–3.5	+16	Treasurer (1992)
	10%	Adults; 127–160	72 h	–0.7	+0.5	Engel (1974)
	10%	Adults; 68–240	250 d	–1.4	+7.5	Stobo (1972)
	Dry ice (–80°C)	Larvae; 10.9–14.0  Juveniles; 36.0–57.0	15 d	–4.0	–54.0	Present study
8 months			–7.2	–52.8	Present study	
15 d			–1.4	–11.1	Present study	
		7 months	–3.9	–8.4	Present study	
Freezing	Adults; 133–171 Adults; 110–340	72 h	–0.7	–1.7	Engel (1974)	
		10–14 weeks	–1.7	–2.7	Treasurer (1990)	

versus juvenile), and, to a lesser extent, the duration of the storage period. For all three preservation methods, weight was more affected than length for both larvae and juveniles. Shrinkage occurred mainly within the first 15 d of preservation and generally increased slightly after 7–8 months. This agrees with the observations of Fisher et al. (1998), who suggested that most length reduction in larval yellow perch occurred within the first 24 h of preservation in ethanol and formalin. For both larvae and juveniles, length and weight reductions showed the same trend among the preservation methods over short and long storage periods: formalin reduced length and weight to a lesser extent than ethanol. These findings are consistent with those of Fisher et al. (1998), who showed that ethanol at several different concentrations varying between 50% and 100% caused greater length shrinkage than formalin in yellow perch larvae. The mean length reductions with formalin and ethanol reported in our study are comparable to those reported in the literature for a given fish size (Table 3). The weight of juvenile and adult yellow perch stored in formalin is known to increase immediately after preservation and then to gradually decrease (Stobo 1972; Treasurer 1992). The initial increase in fish weight is likely due to disrupted osmoregulation (Parker 1963). We did not observe this increase in weight because larval size was relatively small and the duration of the shortest preservation

period ( $\geq 15$  d) largely exceeded the time scale at which such an increase could be detected.

Our results revealed that the impact of dry ice differed from those of ethanol and formalin for larval and juvenile yellow perch. The decreases in length and weight were generally higher with ethanol than dry ice and lower with formalin. We could not compare the effect of freezing with dry ice to freezing at other temperatures (–15°C and –25°C) because of a lack of data in the literature on the effect of freezing on small, posthatch larvae of similar sizes (Table 3). It is noteworthy that keeping the specimen as flat as possible (i.e., maximizing the distance between the head and tail) and avoiding contact with air when freezing were shown to reduce damage, body shrinkage, and weight loss through desiccation (Boyd et al. 1967; Armstrong and Stewart 1997).

The choice of the preservation method is determined by the study objectives. Formalin can be the best choice for minimizing fish shrinkage, but it is not recommended for studies with stable isotopes (Sarakinis et al. 2002) or otolith analysis (Essig and Cole 1986). Given that studies often focus on multiple objectives (e.g., analyzing stomach contents, otoliths, stable isotopes, contaminants, or RNA–DNA ratios), it is often necessary to store samples using different preservation methods (Kruse and Dalley 1990). Biochemical growth indices such as RNA–DNA ratios are increasingly used as indices of instantaneous

growth rate and nutritional condition of larval fish (Bergeron 1997). Larval specimens need to be stored frozen with dry ice (i.e.,  $-80^{\circ}\text{C}$ ) for these analyses to avoid nucleic acid denaturation (Weber et al. 2003).

The slope of the relationships between initial and preserved fish differed among preservation methods (interaction terms were significant). In this context, percent shrinkage is inadequate to compare initial lengths or weights of fish stored in different preservatives. We thus recommend that the initial fresh measurements be estimated using the equations presented in Tables 1 and 2. These equations should be used only for larval and juvenile yellow perch within similar size ranges.

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