Effects of Formalin and Alcohol Preservation on Lengths and Weights of Juvenile Sockeye Salmon

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Reprinted from the
Alaska Fishery Research Bulletin
Vol. 3 No. 2, Winter 1996

Alaska Fishery Research Bulletin 3(2):81–93. 1996.

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ABSTRACT: Preserving fish can induce changes in fish tissue and structure that affect subsequent measurements of length and weight. We conducted 3 different experiments to quantify these changes and allow correction factors to be used as needed in analyses of fish growth and production. The experiments were designed to (1) determine temporal variation in the length and weight of juvenile sockeye salmon *Oncorhynchus nerka* as a result of storage in either 10% neutral freshwater-buffered formalin or 95% ethanol; (2) describe the amount of observed variation in length and weight measurements that can be attributed to reader variability; and (3) formulate conversion equations that allow for back-calculation to fresh lengths and weights. Fish preserved in alcohol for 70 d revealed a mean weight loss of 0.68 g after 16 d (19.7% of mean fresh weight; $P < 0.001$) and then appeared to stabilize through 70 d ($P > 0.05$). Length initially decreased by 1.41 mm after 16 d (2.19% of mean fresh weight; $P < 0.0001$), remained stable through 42 d, and then increased significantly ($P < 0.0001$) by day 70. Storage of fish in formalin produced various results. One experiment revealed a nonsignificant change in length ($P = 0.114$) after 70 d in the preservative; weight increased 0.24 g (7.12%; $P < 0.0001$) after 16 d of preservation, but then stabilized through 70 d. Additional formalin effects ranged from a minor length loss ($P < 0.0001$) and a substantial weight gain ($P < 0.0001$) after 106 d of storage to a significant ($P < 0.001$) loss in length after 30 d and continued loss through 99 d. Although a weight gain occurred after 30 d of preservation ($P < 0.0001$), no significant difference from fresh weight was found after 99 d. We examined the amount of variation in these results that may be attributed to reader differences. For the formalin group, maximum differences between readers were 3.5% ($P < 0.0001$) for lengths and 6.5% ($P < 0.0001$) for weights. For the alcohol group, differences were 1.9% ($P < 0.0001$) for lengths and 6.4% ($P < 0.0001$) for weights. Instructing readers on standardized blotting methods resulted in a maximum difference between readers of only 2.5% for weight measurements. Conversion equations that allow for back-calculation to original live lengths and weights were developed.

INTRODUCTION

Those investigating juvenile salmon growth rates and condition frequently cannot take, due to time or personnel constraints, length and weight measurements at capture. In these situations fish are often stored in a preservative for later examination. To effectively utilize these length and weight data, researchers must be able to isolate and quantify variation attributable to preservation.

Preservation techniques, however, have been reported to cause inconsistent effects on lengths and weights of fishes. For sockeye salmon *Oncorhynchus nerka* smolts Parker (1963) reported a gain of up to 20% of the live weight after 1 d in 3.8% formalin, but after 225 d smolt weights had gradually decreased to a gain of only 10%. Yeh and Hodson (1975) found that bluegill *Lepomis macrochirus* and white crappie *Pomoxis annularis* weights increased significantly when preserved in 10% formalin for 69 d. Yellow perch *Perca flavescens* increased in weight when placed in 10% formalin for 18 months (Stobo 1972).

Other researchers have reported weight losses of fish during preservation. Clutter and Whitesel (1956) found sockeye salmon smolts lost 2% of their live weight between 25 and 180 d after preservation in formalin, but original weights were not reported. Parker (1963) reported a live weight loss of 8.8% in pink salmon *O. gorbuscha* fingerlings and a 12.9% live weight loss in chum salmon *O. keta* fry 1 d after preservation in saltwater formalin. Billy (1982) reported dramatic changes in weight for *Sarotherodon mossambicus* after preservation in both formalin and ethanol.

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Acknowledgments: Alaska Department of Fish and Game personnel John Edmundson, Jennifer Brannen, Bill Glick, Morris Lambdin, and Dave Westerman — participated in collecting length and weight data. Gary Kyle — provided the incentive to conduct this work and reviewed an early version of the manuscript.

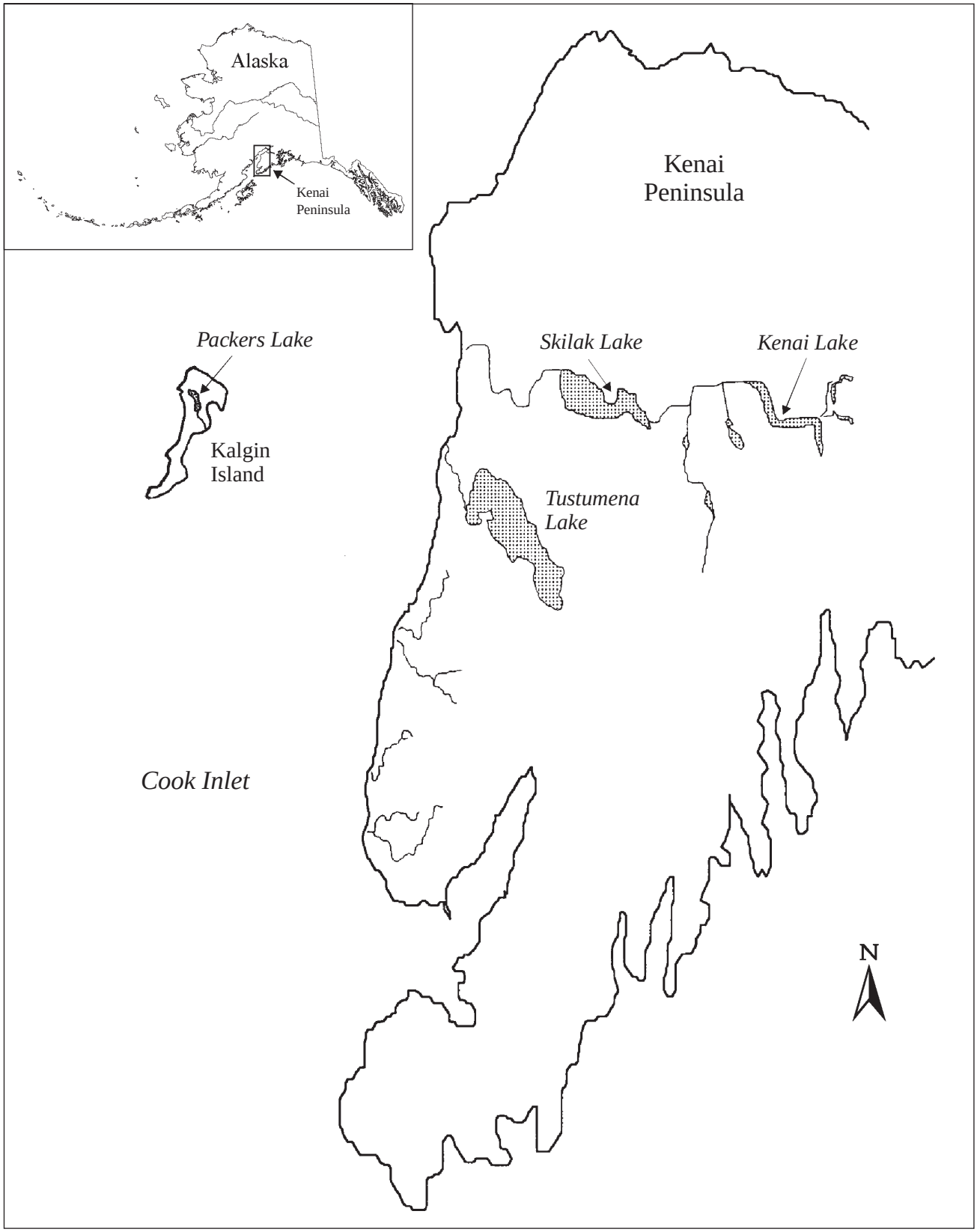

Figure 1. Location of Packers and Skilak Lakes on the Kenai Peninsula.

Reports of preservation effects on fish length also vary. Burgner (1962) determined that large sockeye salmon smolts after 5.5 months in 10% formalin shrank by 4.6% of their live length and by 6.8% for smaller specimens. Parker (1963) showed length shrinkage of 4.1% and 3.2% of live lengths in 2 groups of sockeye salmon after about 220 d in freshwater formalin. Billy (1982) found no shrinkage in *S. mossambicus* lengths after preservation in alcohol for 65 d.

Most preservatives act similarly: they replace water in the tissues soon enough after the fish dies to permit the tissues to retain their structure. This is referred to as becoming *fixed*, which refers to the specimen becoming rigid (Sturgess and Nicola 1975). This fixed position, which often leaves samples bent or twisted, may effect differences in length measurements between readers because returning the fish back into a flat plane to make the measurement can be a source of error.

Sturgess and Nicola (1975) describe 2 chemicals — formalin and ethyl alcohol — that are commonly used to preserve fish. Formalin is characterized as an inexpensive, effective preservative that rapidly penetrates tissue. It is composed of a solution of water saturated with formaldehyde gas (39–40% formaldehyde). Unbuffered formalin degenerates into formic acid, which decalcifies bones and will eventually render specimens soft, mushy, and unfit for morphometric or osteological use. Formic acid is easily neutralized by adding any of the following materials: borax, hexamine, potassium hydroxide, sodium carbonate, sodium hydroxide, chips of marble rock, shell fragments, or crushed coral. The preserving solution should be nearly neutral or slightly basic.

Ethyl alcohol (ethanol) is described as a chemical that penetrates tissue nearly as fast as formalin but is not as irritating as formalin to the skin and nasal passages of those working with it. Ethanol is more costly than formalin and also requires relatively more preservative per specimen to maintain firm tissue structure. However, because it dehydrates tissues but does not decalcify bone, ethanol is the preservative most often chosen when osteological studies are performed (e.g., otolith banding-pattern analyses). Although the dehydrating effects of alcohol are recognized, very little has been published that quantifies its effects on lengths and weights of juvenile salmon.

The literature indicates that preservation affects fish lengths and weight, as does the length of time that fish are stored in the preservative. Compounding the discrepancies observed in length and weight data is the need to also evaluate potential inter- and intra-reader measurement differences that may bias results and further mask real variation.

The objectives of this investigation were to (1) evaluate the effects of 2 commonly used preservatives, alcohol and formalin, on lengths and weights of sockeye salmon fry; (2) describe variation that can be attributed to reader measurement differences; and (3) examine the potential for conversion equations to model the relationship between live and preserved lengths and weights.

METHODS

Experiment A

Sockeye salmon fry were captured on October 4, 1993, using a monofilament trawl (2 m x 2 m) in Packers Lake on the Kenai Peninsula, Alaska (Figure 1). All fish were placed into coolers filled with chilled lake water and transported back to the laboratory. On October 5 (day 0), about 12 h after capture, 150 fish were selected without known bias, measured to the nearest 1 mm (fork length), and weighed to the nearest 0.1 g; at this time all fish were either dead or moribund. The fish were then placed into individual vials filled with either 10% freshwater-buffered formalin or alcohol (95% ethanol), resulting in 2 groups of 75 fish each.

Fish from each group were allocated among 4 individual readers for iterative measurements over the course of the experiment. Each reader used the same measuring device and electronic digital weighing scale throughout the entire experiment, thereby eliminating equipment effects among readers. Lengths and weights were remeasured after 16, 28, 42, and 70 d for each treatment. Previous measurements were not available to the readers to prevent those data from influencing the measurements.

To evaluate effects of preservation on fry lengths and weights and to assess temporal changes in lengths and weights, the results for each preservative composed a 1-group repeated measures design, in which fry were the experimental units, days preserved were the repeated factor, and lengths and weights were the dependent variables. A set of contrasts was applied to identify the nature of temporal patterns, where present. All tests were conducted at $\alpha = 0.05$. Linear regression analysis was used to develop the conversion equations to estimate fresh lengths and weights from the preserved measurements.

Experiment B

This experiment consisted of 2 separate collections and analyses on sockeye salmon fry collected from

Table 1. Packers Lake sockeye salmon fry means and standard errors (SE) of lengths and weights, and mean absolute (negative sign indicates a decrease) and percent changes from initial measurements before and after storage in 95% ethanol and 10% buffered formalin (Experiment A). Means of each variable that share the same superscript letter do not differ significantly ($P > 0.05$).

Variable	Day	Mean	SE	Change	Change (%)	% Fresh
Ethanol-Preserved (n = 75):						
Length (mm)	0	64.63[a]	0.078	NA	NA	100.0
	16	63.21[b]	0.078	-1.41	-2.19	97.8
	28	63.35[b]	0.078	-1.28	-1.98	98.0
	42	63.29[b]	0.078	-1.33	-2.06	97.9
	70	63.73[c]	0.078	-0.89	-1.38	98.6
Weight (g)	0	3.49[a]	0.020	NA	NA	100.0
	16	2.80[b]	0.020	-0.68	-19.7	80.3
	28	2.80[b]	0.020	-0.69	-19.7	80.5
	42	2.76[b]	0.020	-0.72	-20.8	79.2
	70	2.74[b]	0.020	-0.74	-21.3	78.7
Formalin-Preserved (n = 75):						
Length (mm)	0	60.83[a]	0.110	NA	NA	100.0
	16	60.72[a]	0.110	-0.11	-0.18	99.8
	28	60.76[a]	0.110	-0.07	-0.11	99.9
	42	61.07[a]	0.110	+0.24	-0.39	100.4
	70	60.69[a]	0.110	-0.13	-0.22	99.8
Weight (g)	0	3.14[a]	0.014	NA	NA	100.0
	16	3.38[b]	0.014	+0.24	+7.51	107.5
	28	3.33[c]	0.014	+0.19	+5.94	105.9
	42	3.38[b]	0.014	+0.24	+7.55	107.5
	70	3.37[bc]	0.014	+0.22	+7.12	107.1

Skilak Lake, located on the Kenai Peninsula, Alaska (Figure 1). All fish were obtained from trawl sampling and transported back to the laboratory in coolers filled with chilled lake water. Fresh length and weight measurements were made on mostly moribund fish approximately 12 h later, following the same protocol outlined in Experiment A. The fish were then placed into individual vials filled with 10% freshwater-buffered formalin.

The first experiment (B-1), based on 191 fry (183 age-0 and 8 age-1 fry) collected August 3, 1993, was designed to assess the long-term preservation effects of formalin. Fresh length and weight measurements were taken for each fish by a specific reader and subsequently repeated at 106 d. The second experiment (B-2) was designed to evaluate short (30 d) and extended (99 d) storage effects on 99 age-0 sockeye fry obtained from Skilak Lake on September 13 and 14, 1993. After the initial length and weight measurements were taken, they were repeated at 30 and 99 d. A different reader was used for each of the 2 collection dates.

To evaluate effects of formalin preservation on length and weight of the fry, Experiments B-1 and B-2 were each analyzed as a 1-group repeated measures design (B-1 reduced to a paired t-test); all hypotheses were tested at $\alpha = 0.05$. Linear regression analysis was used to develop conversion equations to estimate fresh length and fresh weight from measurements of preserved fry. In Experiment B-2 we also compared fry size after short (30 d) and extended (99 d) storage periods. Additionally, the 99-d results in Experiment B-2 were compared to the 106-d results in Experiment B-1.

Experiment C

Two sets of 50 sockeye salmon fry that had been previously preserved in formalin and alcohol for approximately 100 d were selected without known bias

Table 2. Skilak Lake sockeye salmon fry means and standard errors of lengths and weights, and mean absolute (negative sign indicates a decrease) and percent changes from initial measurements before and after storage in 10% buffered formalin (Experiments B-1 and B-2). Means of each variable that share the same letter do not differ significantly ($P > 0.05$).

Variable	Day	Mean	SE	Change	Change (%)	% Fresh
Experiment B-1 ($n = 191$):						
Length (mm)	0	50.41[a]	0.056	NA	NA	100.0
	106	49.75[b]	0.056	-0.660	-1.31%	98.7
Weight (g)	0	1.36[a]	0.006	NA	NA	100.0
	106	1.50[b]	0.006	+0.139	+10.20%	110.2
Experiment B-2 ($n = 99$):						
Length (mm)	0	50.88[a]	0.091	NA	NA	100.0
	30	49.80[b]	0.091	-1.08	-2.12	97.9
	99	49.32[c]	0.091	-1.56	-3.06	96.9
Weight (g)	0	1.18[a]	0.005	NA	NA	100.0
	30	1.24[b]	0.005	+0.62	+5.22	105.2

and placed into numbered vials (Experiment C-1). Four readers independently measured each set of fish for length and weight as described in Experiment A. Length and weight data were repeated 7 d (day 7) after the first set of measurements were taken (day 1). Again, identical measuring boards and calibrated scales were used by each reader throughout the experiment. A reader's day-1 measurements were not available when fish were remeasured at day 7, which minimized the potential for bias. Readers were not instructed on blotting procedures prior to weighing.

A follow-up experiment (C-2) involved standardization of weighing procedures. Heavy blotting, as described in Parker (1963), was practiced by all 4 readers. Each fish was placed on a paper towel and light pressure was applied to both sides of the fish to remove as much of the surface moisture as possible. On day 1 and day 5 weights were obtained by each reader on the same set of 50 alcohol-preserved fry.

The treatment structure of each experiment was a 4-by-2 factorial (4 readers, 2 d). For the statistical analysis, the design structure was a split-plot with blocking on the whole unit (fry were the blocks). Fisher's LSD test was used for planned pairwise comparisons and conducted at $\alpha = 0.05$. Because the fry had been preserved 100 d at the start of the experiment, we assumed that temporal effects between readings would not occur and that any significant differences would be attributable to individual readers.

RESULTS

Experiment A

Preserving sockeye salmon fry in alcohol resulted in a statistically significant loss in both mean length and mean weight ($P < 0.0001$; Table 1). The majority of loss (length = 2.19%; weight = 19.7%) occurred between days 0 and 16 and then appeared to stabilize until day 70 ($P > 0.05$ for all contrasts), although a nonsignificant downward trend was noted during that time. A minor but significant ($P < 0.0001$) increase in length occurred on day 70 compared to the other preserved measurements.

Storage in formalin resulted in a nonsignificant change in mean length ($P = 0.114$) and the preserved measurements appeared temporally stable (Table 1). After an initial gain (7.51%; $P < 0.0001$), weight appeared fairly stable ($P > 0.05$ for all contrasts) for the remainder of the experiment, except for a minor but significant ($P = 0.0062$) downward fluctuation on day 28 (Table 1).

Experiment B

After 106 d of storage in formalin (Experiment B-1), we found a minor but significant ($P < 0.0001$) loss in mean length of 1.31% and a substantial and significant ($P < 0.0001$) weight gain of 10.2% (Table

Table 3. Between- and within-reader effects (Experiment C-1) on length and weight measurements of formalin-preserved fry collected at Packers Lake (n = 50). Means of each variable that share the same letter do not differ significantly (P > 0.05). Absolute and percent differences are given for within-reader means (negative sign indicates a reduction from day 1 to day 7).

Variable	Reader	Day	Mean	Within Reader Diff.	Within Reader Diff. (%)
Length (mm)					
	1	1	65.8^{b}		
		7	65.0^{a}	-0.82	-1.3
	2	1	67.3^{e}		
		7	66.8^{d}	-0.54	-0.8
	3	1	66.0^{b}		
		7	65.9^{b}	-0.08	-0.1
	4	1	66.3^{cd}		
		7	66.3^{c}	-0.24	-0.4
Weight (g)					
	1	1	3.66^{e}		
		7	3.70^{f}	+0.040	+1.1
	2	1	3.55^{c}		
		7	3.53^{c}	-0.018	-0.5
	3	1	3.48^{ab}		
		7	3.47^{a}	-0.010	-0.3
	4	1	3.59^{d}		
		7	3.50^{b}	-0.088	-2.5

Table 4. Between- and within-reader effects (Experiment C-1) on length and weight measurements of alcohol-preserved fry collected at Packers Lake (n = 50). Means of each variable that share the same letter do not differ significantly (P > 0.05). Absolute and percent differences are given for within-reader means (negative sign indicates a reduction from day 1 to day 7).

Variable	Reader	Day	Mean	Within Reader Diff.	Within Reader Diff. (%)
Length (mm)					
	1	1	66.4^{a}		
		7	66.6^{ab}	+0.24	+0.4
	2	1	67.7^{d}		
		7	67.1^{c}	-0.62	-0.9
	3	1	66.6^{ab}		
		7	67.0^{c}	+0.42	+0.6
	4	1	66.9^{bc}		
		7	66.9^{bc}	+0.02	0.0
Weight (g)					
	1	1	3.38^{d}		
		7	3.39^{d}	+0.002	0.0
	2	1	3.23^{ab}		
		7	3.18^{a}	-0.054	-1.7
	3	1	3.29^{bc}		
		7	3.25^{b}	-0.038	-1.2
	4	1	3.33^{cd}		
		7	3.23^{ab}	-0.096	-2.9

2). In Experiment B-2, storage in formalin resulted in a significant (P < 0.0001) loss in mean length (2.12–3.06%); lengths observed after 99 d were less than they were at 30 d (P = 0.0003; Table 2). A minor but significant (P < 0.0001) weight gain occurred at 30 d (5.22%), but weight measurements at 99 d were nearly identical to those obtained originally (P = 0.89).

Experiment C

In the formalin-preserved fish (Experiment C-1), a significant (P < 0.0001) reader-day interaction occurred for both length and weight (Table 3). For mean lengths, readers 1 and 2 both reported a significant decrease on day 7, whereas measurements by readers 3 and 4 did not differ between days. Between-reader effects on length were generally of a larger magnitude than within-reader effects. For example, a mean difference of 2.3 mm occurred between reader 1 on day 7 and reader 2 on day 1. However, differences between reader 3 (both days) and reader 1 on day 1 were not significant. For weight measurements, significant within-reader differences occurred for readers 1 and 4, but not for readers 2 and 3. As with length, between-reader differences were generally of a higher magnitude than within-reader differences.

In the alcohol treatment group (Experiment C-1; Table 4) a significant (P < 0.0001) reader-day interaction was found for length measurements, whereas the interaction effect for weight measurements was not

Table 5. Largest mean absolute and percent differences in length and weight of Packers Lake sockeye salmon fry observed in 2 experiments (alcohol- and formalin-preserved) designed to evaluate between- and within-reader measurement effects (n = 50) for each experiment. These differences are all statistically significant ($P < 0.05$).

Experiment	Variable	Reader	Day	Mean	Difference	Difference (%)
Formalin	Length	1	7	65.00		
		2	1	67.30	2.30	3.5
	Weight	1	7	3.70		
		3	7	3.47	0.23	6.5
Alcohol	Length	1	1	66.40		
		2	1	67.70	1.30	1.9
	Weight	1	7	3.39		
		2	7	3.18	0.21	6.4

significant (P = 0.195). Significant differences in mean length occurred for reader 3, whose measurements were greater on day 7, and reader 2, whose measurements were lower on day 7. Mean lengths on day 7 did not differ significantly from day 1 for either reader 1 or 4. Between-reader effects were less on day 7 than on day 1. Except for reader 4, whose weight measurements were significantly lower, mean weight did not differ significantly between day 1 and day 7. Between-reader differences in mean weight remained relatively constant between days.

Table 5 provides a summary of the largest observed differences in length and weight for Experiment C-1. Note that the larger differences always occurred between readers, indicating that within-reader precision was higher than between-reader precision. Also, note that length differences were relatively less than weight differences.

Table 6 (Experiment C-2) provides a summary of the results of weight measurements after all 4 readers were instructed on standardized blotting procedures (Parker 1963). The largest observed difference within or between readers was 0.08 g (2.5%; $P < 0.0001$), which occurred between readers 2 and 4 on day 1. Therefore, by standardizing weight-measurement procedures, the largest absolute mean difference in weight collection was reduced by a factor of nearly 3.

Conversion Equations for Alcohol-Preserved Fry

Experiment A (Packers Lake fry in both preservatives) avoided between-reader effects by using the same reader for each group of fish for the entire experiment. Therefore, we postulated that observed changes in lengths and weights were primarily attributable to the preservative and within-reader variation and were not a function of bias or error introduced through between-reader effects. Length increases on day 70 for the alcohol-preserved samples were somewhat unexpected because they followed stabilized lengths between days 16 and 42 (Table 1). Length measurements were averaged to develop the length-conversion equation; no attempt was made to include time in the model. Two regression analyses were conducted: 1 with and 1 without the day-70 data (Figure

Table 6. Within- and between-reader effects on weight measurements of Packers Lake sockeye salmon fry, using standardized blotting procedures (n = 50). Means that share the same letter do not differ significantly ($P > 0.05$). Absolute and percent differences are given for within-reader means.

			Within Reader	
Reader	Day	Mean wt. (g)	Difference	Difference (%)
1	1	3.170[b]		
	5	3.164[b]	0.006	0.19
2	1	3.208[c]		
	5	3.156[b]	0.052	1.63
3	1	3.156[b]		
	5	3.154[b]	0.002	0.06
4	1	3.128[a]		
	5	3.164[b]	0.036	1.14

2). A simple linear regression model appeared adequate. Fresh length (*L*) can be computed from alcohol-preserved length (*AL*) using equation (1), which excludes day-70 measurements, or equation (2), which includes day-70 measurements:

$$L = 1.24 + 1.00(AL)\,, \qquad (1)$$

$$L = 1.00 + 1.00(AL)\,. \qquad (2)$$

In both of these equations the slope estimate is 1.0, which indicates that alcohol reduces the length of fry by a constant value regardless of fry size. For application purposes the difference in these equations is minimal because fry are large (40+ mm) compared to the 0.24-mm difference between the intercept estimates. Essentially, alcohol reduces fry length by only about 1 mm.

Because weight appeared to stabilize in alcohol after 16 d (Table 1), day-16 to day-70 data were averaged before developing the conversion equation. Again, the linear regression model was appropriate; Figure 3 shows this relationship and the results. Fresh weight (*W*) can be computed from alcohol-preserved weight (*AW*) using the following equation:

$$W = 0.225 + 1.174(AW)\,. \qquad (3)$$

The linear association between weight in alcohol and fresh weight is particularly strong ($r^2 = 0.999$); thus, the conversion equation should be very reliable.

Conversion Equations for Formalin-Preserved Fry

Because no significant difference in length was found between the fresh and formalin-preserved specimens in Packers Lake (Experiment A), a length-conversion equation was not developed. Weight changes in Packers Lake fry after 16 d in formalin were reasonably stable. Therefore, preserved weight data were averaged before developing the conversion equation. Figure 4 shows the relationship and results of the regression analysis; again, the linear model appeared appropriate. Fresh weight can be computed from formalin-preserved weight (*FW*) using the following equation:

$$W = 0.008 + 0\ .931(FW)\,. \qquad (4)$$

The linear association was very strong ($r^2 = 0.999$), indicating a very reliable conversion. Conversion equations using data from Experiment B-1 (Skilak Lake formalin-preserved fry) were also developed for length and weight. Note that if future studies reveal significant temporal trends, this factor could be added to the model, or perhaps more preferably, specimens could be stored until stability is reached. The length-weight relationship from this data set was explored and a regression model was developed. Figure 5 shows the results of a regression analysis applied to convert

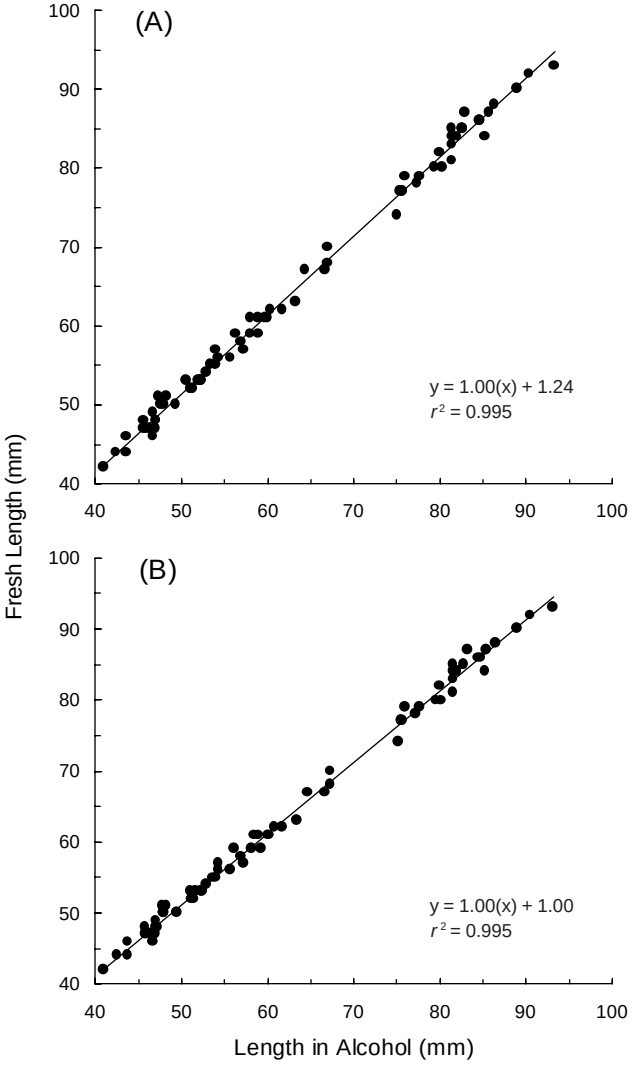

Figure 2. Regression analysis of alcohol-preserved length on fresh length measurements of sockeye salmon fry collected in Packers Lake, 1993. (A) Lengths in alcohol are averages of measurements at 16, 28, and 42 d of storage, which did not differ significantly ($P > 0.05$); (B) lengths in alcohol include day 70 in the average, which was significantly greater (about 0.5 mm; $P < 0.0001$) than days 16–42.

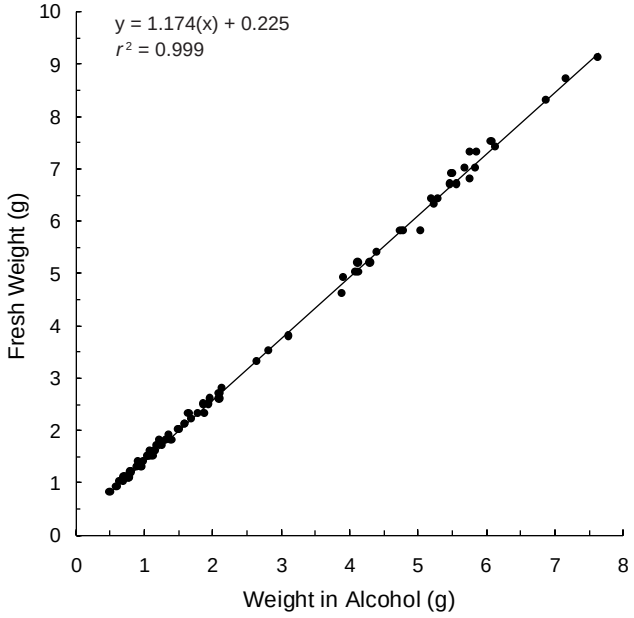

Figure 3. Regression analysis of alcohol-preserved weights on fresh weights of sockeye salmon fry collected in Packers Lake, 1993. Weights in alcohol are averages of measurements after 16, 28, 42, and 70 d of storage, which did not differ significantly ($P > 0.05$).

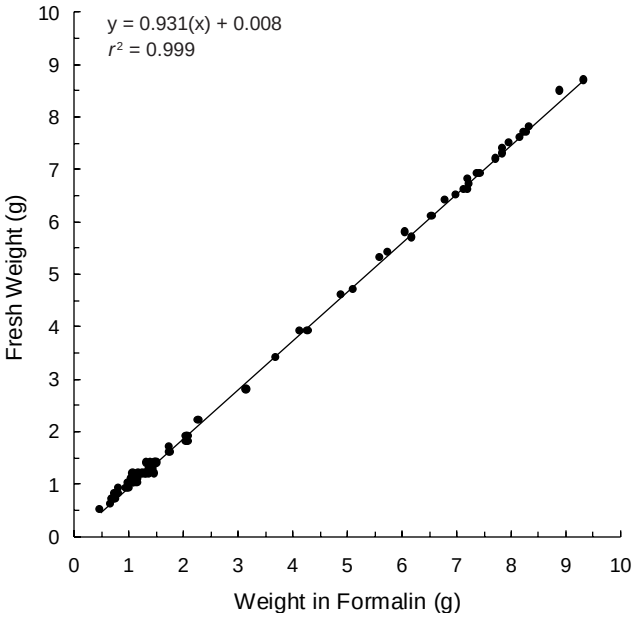

Figure 4. Regression analysis of formalin-preserved weights on fresh weights of sockeye salmon fry collected in Packers Lake, 1993. Weights in formalin are averages of measurements after 16, 28, 42, and 70 d of storage, which did not differ significantly ($P > 0.05$).

formalin-preserved length (FL) to fresh length (L), which is calculated as

$$L = 0.744 + 0.998(FL) . \qquad (5)$$

The estimate of fresh length, then, is about 0.74 mm greater than preserved length, with a slight adjustment for size. This relationship was very strong ($r^2 = 0.990$), and the linear model appeared adequate.

Figure 5 gives the results of a regression analysis used to convert formalin-preserved weight (FW) to fresh weight (W). Fresh weight is calculated as

$$W = 0.939(FW) - 0.048 . \qquad (6)$$

This relationship was also very strong ($r^2 = 0.993$), and again, the linear model appeared adequate.

DISCUSSION

Alcohol Effects

Choosing a preservative should depend on objectives of the study. In osteological research, preservation in alcohol is necessary because it does not decalcify bone. Some researchers also prefer alcohol because it is deemed less irritating and hazardous to those who handle the preserved fish (Sturgess and Nicola 1975). Consequently, these researchers must be prepared to account for the significant weight loss that occurs from using this desiccating solution.

Weight loss of fish preserved in alcohol is predictable because this preservative is known to dehydrate tissue (Glenn and Mathias 1987). Our findings on weight loss of samples stored in alcohol (Experiment A) are comparable to other studies where the majority of shrinkage occurred very soon after placement in the preservative. Glenn and Mathias (1987) showed that juvenile walleye *Stizostedion vitreum* shrunk by 8% of their live length after only 1 d in ethanol and by 11% after 3 d. Billy (1982) stated that weights of formalin-preserved fish were reduced considerably within days of being transferred to isopropyl alcohol.

Our results indicate that alcohol may not cause substantial changes in the weights of juvenile sockeye salmon, although Table 1 shows losses of about 20% of fresh weight. The mean weight loss appeared to stabilize from day 16 through day 70, which may indicate long-term stability with preservation in alcohol.

Glenn and Mathias (1987) also noted that specimens placed in ethanol became twisted and appeared more dehydrated than fish stored in other preservatives. Even though each fish in our study was stored in a sep-

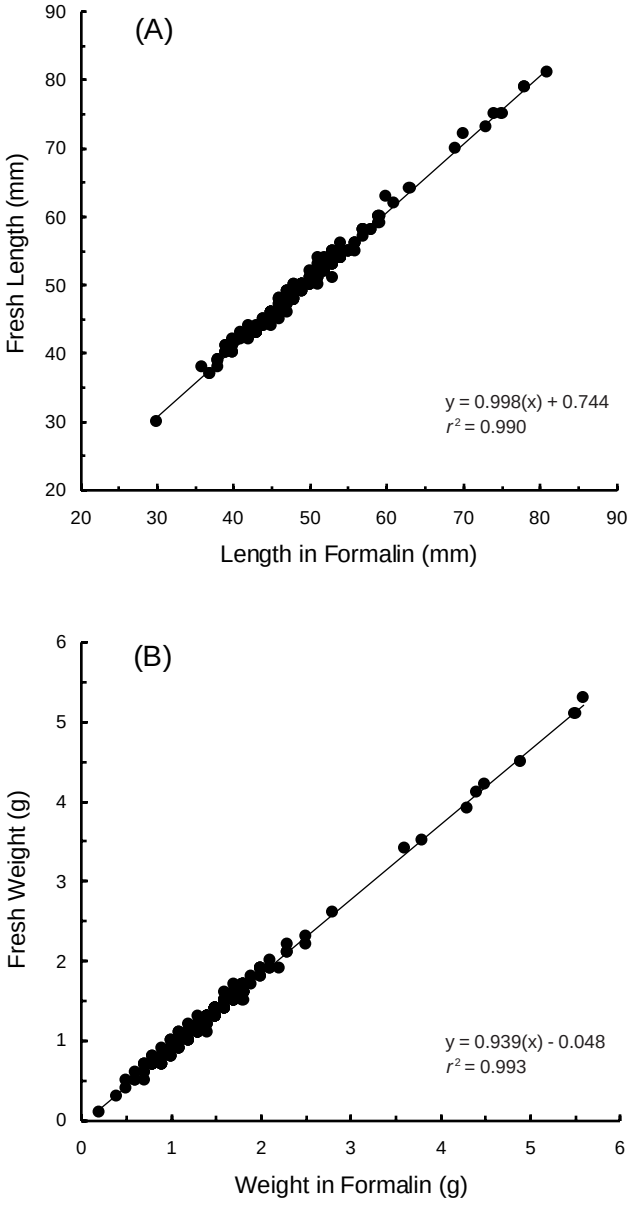

Figure 5. Regression analysis of (A) formalin-preserved length on fresh length measurements and (B) formalin-preserved weight on fresh weight measurements of sockeye salmon fry collected in Skilak Lake, 1993. The fry were stored in formalin for 106 d prior to remeasurement.

arate vial, they still tended to bend and twist, requiring the reader to hold them down with both hands to obtain length measurements. All of the readers commented that the irregular shape of the samples made length measurements difficult. Furthermore, caudal fins tended to split, making fork length measurements somewhat subjective. This may partially explain the increase in length measured on day 70 (Table 1), which could indicate length instability in alcohol but was probably due to a measurement bias that day, given the relatively stable pattern from day 16 to day 42.

Formalin Effects

As a fixing agent, formalin has been judged as the best solution to use (Sturgess and Nicola 1975). Based upon the minimal changes in length and weight observed in our preserved samples, we also recommend using formalin over alcohol when study objectives allow a choice. In Experiment A, samples stored in formalin revealed a nonsignificant change in length and significant gains in weight through 70 d of preservation (Table 1). Weights were notably more stable between days 16 and 70 than between days 1 and 16. Weight stabilization probably occurred before day 16, which was the first day of postpreservative measurement. Parker (1963) showed that fish stored in freshwater formalin shrank by 3% of their original length after 24 h, but subsequent measurements at 30 and 40 d revealed a reduction of only 4% of their original length. Parker also reported that weights of the specimens increased rapidly during the first 1 or 2 d, then decreased at a decelerating rate. Treasurer (1992) found that at 24 h after preservation in formalin, yellow perch increased in weight: 19.6% for small specimens and 16.8% for large specimens. Based on our results, additional experimentation may be warranted to examine how long it takes for weights to stabilize in formalin.

Conversion Equations

Regardless of the preservative used, researchers will need to decide whether the amount of change in length and weight measurements caused by the preservative warrants using conversion equations to backcalculate to live or fresh conditions. Billy (1982) discussed conversion equations and concluded that, although it was once common practice to use standardized correction factors to calculate live measurements from preserved fish, this practice was unreliable, except possibly to provide very rough approximations of the live condition. Conversely, Kruse and Dalley (1990) suggested using regression equations to convert preserved lengths to fresh lengths for size comparisons.

We believe that conversion equations can be safely used for weight data collected from alcohol-preserved specimens, especially when inter- and intra-annual weight comparisons are being made. However, we be-

lieve, given the potential problems in making such conversions, that it is far better to use live weights whenever possible. We also agree with Billy's (1982) recommendation to not use conversion equations for length measurements, unless researchers are willing to develop, systematically review, and update study-specific equations for each watershed and species. We believe this conclusion is warranted because our investigations showed that fry from different initial conditions or from different stocks can respond dissimilarly to preservation, and preservative effects between years within the same system can also differ.

Skilak Lake fry stored in formalin (Experiment B) provided somewhat different results than formalin-preserved fry from Packers Lake (Experiment A). Skilak Lake fry, for example, showed a significant, though minor, length loss and substantially less weight gain than Packers Lake fry. Likewise, the length-weight models for Packers Lake fry do not appear applicable to Skilak Lake fry. The Packers Lake sample contained a mixture of age-0 and age-1 fry. The age-1 fry were generally >70 mm in fresh length and >3.5 g in fresh weight. However, the age dichotomy did not appear to affect the statistical analyses, although this may not be the case in other systems where 1 age group may predominate in the sample.

More extensive studies are needed to determine if a universal, species-specific equation could be developed on a regional or statewide basis. Large representative samples of fish would be needed from multiple systems. All samples would have to be handled, preserved, and measured using standardized methods. In the meantime, if conversion equations are used, we suggest they be developed specifically for the watershed and stock in question and be reviewed periodically to ensure validity.

Reader Effects

The results of Experiments B-1 and B-2 were somewhat unexpected. The observed weight loss at 99 d of formalin storage in Experiment B-2 (Table 2) was surprising because fish gained weight in Experiment A (Table 1) and their weights were fairly stable between 16 and 70 d. Furthermore, the weight gain (Table 2) in Experiment B-1 was significant (about 12% at 106 d) and larger than any weight gain observed in Experiment B-2 (about 5% at 30 d) or Experiment A (about 7.6% at 70 d). Observed length loss between the 2 groups in Experiment B was also interesting; after storage in formalin for 99 d, the B-2 length loss was over 2 times the loss in the B-1 group at 106 d.

Disparities in these results are difficult to explain, but we offer several observations. Experiment C, in which we examined variation in length and weight measurements attributable to reader effects, clearly indicated that fairly substantial differences may occur within and especially between reader measurements of length and weight (Tables 3, 4). Because all readers commented about the difficulty of measuring the "curled-up" fish preserved in alcohol, we were somewhat surprised that differences in length measurements of the formalin-preserved group (Table 5) exceeded those of the alcohol-preserved group (Table 6). A possible explanation may be that because the alcohol-preserved specimens were more difficult to measure, more care was exercised when obtaining their lengths, resulting in increased accuracy. Weight differences among all samples were greater than anticipated and were probably due to individual differences in handling technique (i.e., blotting procedures).

Experiment C-2 was conducted to evaluate the effects of standardizing blotting procedures prior to obtaining weight data. In Experiment C-1, all 4 readers used a slightly different method of blotting prior to taking weight measurements. Parker (1963) compared results of weight measurements using 3 different blotting methods and determined there were significant differences in mean weight between the groups. However, within each treatment, standard deviations were quite small, demonstrating that a standardized method of blotting can yield precise results. In Experiment C-2, we followed Parker's "heavy" blotting procedure and had the same 4 readers collect weights from the same set of fish on 2 different days. Analyses of these data sets showed that the largest observed difference within or between readers was about one-third the average difference without weighing standardization (Table 6). These findings may provide further explanation for observed weight differences in our studies and perhaps in other data sets where readers have used different blotting procedures prior to weighing.

In general, length measurements appear to be more precise and reliable than weight measurements and are probably less affected by either preservative. How our findings are viewed and applied depends on the size of the differences the researcher hopes to detect. Between systems, where fry grossly differ in size, the problem may be almost inconsequential. Within a system, where yearly changes in size may be subtle (and of interest), this problem may cause an invalid inference. We may need to develop more rigorous techniques for measuring small fish. Our study also indicates the need to establish a set of guidelines for collecting weight data.

Sample Collection

A final explanation for incongruities in our analyses may be related to the condition of the fish prior to preservation. Parker (1963) indicated that observed variance in fish weights can be partly attributed to osmotic exchange of water. The state of osmoregulation at the onset of preservation may greatly affect the amount of initial change in weight. He reported that fish held captive in a gillnet for 12 h had increased more than 20% of their precaptive weight. Other factors may also affect the "fresh condition" of fish, such as holding them in water with insufficient oxygen or keeping fish in water after death and prior to obtaining weights. All of the fish used in our experiments were held about 12 h in chilled water before making fresh measurements. It is possible that actual live weights were slightly altered by the handling procedures prior to preservation.

We also offer the following thoughts regarding the use of preservatives. Researchers should carefully monitor the concentrations of the solutions or mixtures of their preservatives. We used 10% buffered (with sodium hydroxide to a pH of 7.0) formalin for all of our preservations. The final strength was obtained by mixing 9 parts of water to 1 part of full-strength formaldehyde (37.5%). For alcohol preservation, we used 95% ethanol, undiluted; mixing ethyl alcohol with water may affect its preserving and desiccating properties. Similarly, using 5 or 15% buffered formalin may also alter its preserving characteristics and effects on length or weight. A standardized set of guidelines should be strictly adhered to when choosing which mixture or solution to use.

Biological vs Statistical Significance

Our experiments revealed that relatively small changes in average length (about 1 mm) and weight (about 0.1 g) were statistically significant. However, a change in mean length from 69 to 68 mm, for example, may not be biologically significant to the researcher. Our results show that preserving sockeye salmon fry in ethanol has a statistically and biologically significant effect on weight, but weight changes in formalin-preserved fish ranged from no change to a reported high of only 0.24 g or 7.6%, which may or may not be biologically significant to the researcher. The largest observable changes in length occurred between readers for formalin-preserved fish and represented a 3.5% change. Again, these differences are statistically significant and should be reported as such, but the change may not be of practical importance for many research projects.

RECOMMENDATIONS

We recommend use of live or fresh lengths and weights wherever possible. When this is not feasible, we recommend the following:

1. If osteological research is not part of the study objective, buffered formalin should be used as the preserving agent.
2. Conversion equations should be used to account for significant weight loss of fish preserved in alcohol.
3. Do not use conversion equations to correct for lengths of fish preserved in either alcohol or formalin (length effects are minimal and not well-defined).
4. Develop and employ standardized methods prior to collecting length and weight measurements.
5. Adhere to strict guidelines for preservative preparation and use.

LITERATURE CITED

Billy, A. J. 1982. The effects of formalin and isopropyl alcohol on length and weight measurements of *Sarotherodon mossambicus* Trewavas. Journal of Fishery Biology 21: 107–112.

Burgner, R. L. 1962. Studies of red salmon smolts from the Wood River Lakes, Alaska. Pages 251–316 *in* T. S. Woods, editor. Studies of Alaska red salmon. University of Washington Publications in Fisheries.

Clutter, R. I., and L. E. Whitesel. 1956. Collection and interpretation of sockeye and salmon scales. Bulletin of the International Pacific Salmon Fisheries Commission 9.

Glenn, C. L., and J. A. Mathias. 1987. Body shrinkage in young walleye, *Stizostedion vitreum*, preserved with AFA, formalin, ethanol, and quick freezing. Canadian Field-Naturalist 101:408–414.

Kruse, G. H., and E. L. Dalley. 1990. Length changes in capelin, *Mallotus villosus* (Müller), larvae due to preservation in formalin and anhydrous alcohol. Journal of Fishery Biology 36:619–621.

Parker, R. R. 1963. Effects of formalin on length and weight of fishes. Journal of the Fisheries Research Board of Canada 20:1441–1455.

Stobo, W. T. 1972. Effects of formalin on the length and weight of yellow perch. Transactions of the American Fisheries Society 101:362–364.

Sturgess, J. A., and S. J. Nicola. 1975. Preparation of fish for identification and preservation as museum specimens. The Resources Agency of California, Department of Fish and Game, Inland Fisheries Informational Leaflet 29, Sacramento.

Treasurer, J. W. 1992. Length and weight changes in 0+ perch, *Perca fluviatilis* L., following fixation in formalin. Journal of Fishery Biology 41:1033–1036.

Yeh, C. F., and R. G. Hodson. 1975. Effects of formalin on length and weight of bluegill and white crappie from Lake Nasworthy, Texas. Southwest Naturalist 20:315–322.