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The Shrinkage of Sockeye Salmon Fry Fixed in 10% Formalin and Preserved in
37.5% Isopropanol

by

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ABSTRACT

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Formalin fixation is generally known to cause a reduction in the length of fishes. However, the effects of preservation in isopropanol following formalin fixation are not well described. We have found that formalin does indeed shrink the fork-length of sockeye salmon fry (*Oncorhynchus nerka*). However, the effect of formalin was not equal on all lengths and samples of fish. Isopropanol preservation did not produce any significant change in the length of fry after fixation in formalin. The reproducibility of fork-length observations was tested by comparing measurements made by four workers and found no statistical difference.

RÉSUMÉ

Macdonald, J. S., C. J. Williamson, D. A. Patterson et H. E. Herunter 1997. Le rénéissement de l'alevin de saumon rouge fixé dans la formaline à 10% et préservé dans l'isopropanol à 37.5 %. Can. Tech. Rep. Fish. Aquat. Sci. 2198: 11 p.

On sait généralement que la fixation dans la formaline réduit la longueur des poissons. Cependant, on connaît moins bien les effets de la préservation dans l'isopropanol après la fixation dans la formaline. Nous avons constaté qu'en fait la formaline ne réréit pas la longueur à la fourche de l'alevin de saumon rouge (*Oncorhynchus nerka*). Mais, l'effet de la formaline n'était pas égal pour toutes les longueurs et échantillons de poissons. La préservation à l'isopropanol n'a entraîné aucun changement significatif dans la longueur de l'alevin après fixation dans la formaline. Finalement, nous avons évalué la reproductibilité des observations de longueur à la fourche en comparant les mesures faites par quatre employés et n'avons observé aucune différence statistique.

INTRODUCTION

Fixation and preservation are generally known to affect the morphology of fishes. Much has been published regarding the morphological changes associated with a variety of handling and preservation techniques. The nature of the morphological change is influenced by many factors including: method of preservation (e.g. fixation vs. freezing) (Hjörleifsson and Klein-MacPhee 1992, Karjalainen 1992, Kruse and Dalley 1990, Leslie 1983, Billy 1982, Hay 1981, 1982), concentration and type of chemical preservation agents (Tucker and Chester 1984, Hay 1982, Rogers 1964), length of preservation period (Leslie and Moore 1986, Hay 1982, Schnack and Rosenthal 1978), and salinity and temperature of the preservative (Hay 1982, Parker 1963). Factors specific to the fish being preserved are also important including: species, age, size and developmental state (Radtke 1989, Hjörleifsson and Klein-MacPhee 1992, Hay 1982, Heming and Preston 1980, Schnack and Rosenthal 1978, Hjort 1977, Jones and Geen 1977, Parker 1963), the presence of rigor mortis, and the osmoregulatory state of the fish at death (Jennings 1991, Radtke 1989, Theilacker 1980, Blaxter 1971, Parker 1963, Shetter, 1936). However, despite the quantity of information available, little is known about the morphological changes associated with the combined influence of formalin as a fixative followed by alcohol for preservation. This preservation method is recommended by (Knudsen 1972, Bagenal 1978) and is commonly used.

Fish production assessment and life history studies frequently use preserved specimens of fish to provide morphometric information when fresh samples are not available. Measurement errors associated with preservation techniques can be corrected if a factor specific to the preservation method is available (Parker 1963). The purpose of this report is to develop a correction factor that can be used to obtain an approximation of the pre-preservation length of juvenile sockeye salmon (*Oncorhynchus nerka*) that have been preserved in isopropyl alcohol after a period of fixation in formalin. These fish have been collected to assess stock size and survival, and to document alternate life history patterns as an element of the Takla Fish/Forestry Interaction Project; a multi-disciplinary, long-term research project investigating the effects of forestry activities on ecosystem processes in northern watersheds. Additional information pertaining to this project including the study design, is provided in Macdonald et al. (1992) and Macdonald (1994). Results from this study in conjunction with many other research components associated with this study, will assist in the revision of fisheries/forestry management guidelines for interior British Columbia and will allow the evaluation of the effectiveness of BC's Forest Practices Code.

METHODOLOGY

Sockeye fry were captured from the main channel and off-channel areas of Forfar, Gluskie and O'Ne-eil (Kynoch) creeks and from the main channel of Middle River between April 30 and July 31, 1995 (Table 1). Live fish forklengths ranged from 24.0 mm to 46.0 mm. Most were captured at the mouths of each creek using 2' X 3' (0.6 X 0.9 m) inclined-plane traps (IPT's). As the fry out-migration diminished and creek water levels dropped in late spring and summer, the IPT capture rate declined. Additional fry were sampled from off-channel areas in the flood plains of O'Ne-eil and Forfar creeks using a large dipnet (1 x 1 m opening) and a two-directional box fyke trap (0.75 x 0.375 X 0.75 m per direction) placed at the mouth of an off-channel area. A floating fyke trap with a 1 x 1m net opening and a baffled live box, was used to capture fry from the center of Middle River near the outlet of Takla Lake. Twenty-one samples each with 5 - 20 fish, were taken from river, creek or off-channel sites through the spring and summer of 1995, and sorted by collection date into seven batches (Table 1). Mean fish size among the samples increased with collection time. Fish were anesthetized immediately after capture in a 1 ppt solution of Alka-seltzer®(active component sodium bicarbonate) and ambient water and measured to fork length to the nearest one-half (1/2) mm on a wetted measuring board. Each fish was then transferred to a solution of 10% fresh-water formalin. After 70 ± 6 days in formalin, each sample was drained and immersed in running tap-water for five minutes before being transferred to 37.5% isopropanol. This fixative/preservation technique has been recommended by Knudsen (1972) and Lowe-McConnell (1968).

During the period of fixation in formalin, each group of sockeye fry was measured at least twice and as frequently as 7 times. Once transferred to alcohol for preservation, measurements continued following a predetermined schedule (Table 1). Fork length measurements were to the nearest one-half (1/2) mm on a measuring board similar to the one used in the field. Excess alcohol or formalin was blotted off the fish with paper towel before measurement to prevent visual distortion of the measuring board by liquid droplets.

Mean fork-lengths of each sample were calculated for each measurement date throughout the fixation and preservation periods. For the purposes of further data analysis and presentation, mean fork-lengths from three exposure periods were used (t_0 = time 0, t_{70} = final day in formalin, t_{350} = final day in alcohol) (Table 2). A non-parametric sign test using percent change in length on days 70 and 350, was used to examine the effects of formalin and alcohol on fish length (Sokal and Rohlf 1981). Regression coefficients of weighted simple linear regressions between sample mean fork-lengths at t_0 and t_{70} , and t_0 and t_{350} were compared to a null model where the regression coefficient was equal to

one (Fig. 1). Significance of hypothesis tested by the sign test and the regression coefficients indicate a proportional change in length occurs in association with our fixation or preservation methods. The effect of alcohol preservation on length after formalin fixation was tested with an ANCOVA where the variation in fish length at t_0 (the covariate) was removed. The regression model provides a method to obtain a live length from fixed and preserved fish.

To test the error variation associated with measurements taken repeatedly by different researchers, a sample of 20 fry were measured by 4 researchers on two separate days. Variation in observed fry length among researchers, between days was examined with an ANOVA test.

RESULTS

Fixation in formalin for period of 70 days caused a proportional reduction in mean length of sockeye fry (sign test: $p < 0.01$, $b \neq 1$, $p < 0.01$). Shrinkage ceased by day 70 and no significant loss or gain in length was observed through the period of preservation in alcohol (ANCOVA: $p > 0.01$). The pre-preservation lengths of sockeye salmon fry that have been fixed and preserved following this method can be calculated using the formula:

$$(\text{live length mm}) = -8.5081 + 1.3441 (\text{preserved length})$$

This formula provides an estimate for fry 24-40 mm only. The effect of formalin on fry length in individual samples ranged from a 3% gain in length to a 12% length reduction (Table 2). The mean and median shrinkage in length were 3.9% (SE of the mean = 0.92) and 4.0 % respectively. Differences in fry lengths among measurements taken by four researchers were statistically undetectable (ANOVA: $p > 0.05$).

DISCUSSION

Our results agree with several other studies that have demonstrated length reductions in salmonids in association with formalin fixation. Parker (1963) reports 3.2 - 5.4% length shrinkage in samples of sockeye salmon smolts, pink salmon (*Oncorhynchus gorbuscha*), and chum salmon (*Oncorhynchus keta*) fry preserved in 10% formalin solutions. Heming and Preston (1980) found an average of 5.3% shrinkage of chinook salmon fry (*Oncorhynchus tshawytscha*, length range: 25.5-39.5 mm) stored in formalin. Hjort (1977) calculated 3.7-4.0 % shrinkage of salmon fry (length range 50-75 mm) that were preserved in formalin. He examined fish larger than 75 mm and found proportionally less shrinkage. Fewer studies have examined the cumulative impacts of formalin

fixation followed by preservation in isopropanol despite the wide acceptance of this technique (Knudsen 1972, Bagenal 1978). Our results support those of Shetter (1936) who found that shrinkage of brook (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) ceased once they were removed from a 10% solution of formalin and placed in a 70% alcohol solution. However, Billy (1982) detected additional weight loss when formalin-preserved fish were placed in isopropanol. Alcohol and possibly formalin cause sample dehydration, and after oxidation to make formic acid, formalin causes bone decalcification (Shields and Carlson 1996, Haedrich 1983).

The effects of formalin fixation on salmonid lengths has been reported to depend on a variety of factors including size of fish and measurement error among workers. Larger fish have been reported to shrink proportionally less than smaller fish (Burgner 1962 - sockeye smolts, Hjort 1977 - chinook and coho fry). Our results provided evidence of shrinkage proportional to fish length, thus the larger fish shrank a greater degree in an absolute sense, than the smaller fish. However, our experiment was not designed to investigate the effect of fish size on shrinkage rates. Fish sizes and size range in our experiment were smaller than in those used by Burgner (86.3-106.3 mm) or Hjort (58-146 mm).

Variability in length measurements among individual workers was a factor that contributed to the standard error of mean lengths in an experiment reported by Shields and Carlson (1996). Reader effects were not considered to be a large factor by Rogers (1964) and variability among four readers in this experiment were not statistically detectable. Other readers that were involved in this experiment were less experienced, and were not available to be tested, thus reader biases may still have been a source for error.

The effect on samples fixed in formalin followed by preservation in alcohol is reaffirmed by the results of our study. The reduction in length that we observed is consistent with previous studies and therefore predictable, albeit with a modest error associated with the estimate. The decision whether to convert preserved lengths to fresh lengths will depend on the individual researchers' choice. We recommend the use of the correction factor provided in this report when examining lengths of sockeye fry from the Stuart Lake stock; although fresh samples should be used whenever possible. Caution must be exercised when other stocks and species are considered. Many researchers have recommended the use of geographically specific correction equations (e.g. Billy 1982) as preservation effects can vary among stocks and location within the same species (Shields and Carlson 1996).

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Table 1. Summary of sockeye fry samples captured in 1995 and the number of days from capture that length measurements were collected. For description of capture methods see methodology section. IPT = Inclined plane trap. O/C= off channel site. n = sample size. Live = measurement taken when fish were alive. Formalin = the time period from capture to day 70. Isopropanol = the time period from last day in formalin to the end of the experiment.

Location and Capture Method.	Batch	Collection Date	n	Live	Formalin	Isopropanol
Forfar Cr. IPT	1	April 30, 1995	20	0	67	78 82 93 100 110 165 227 342
Gluskie Cr. IPT	1	April 30, 1995	20	0	67	78 82 93 100 110 165 227 342
Middle R. FL. FYKE	1	May 1, 1995	10	0	66	77 81 92 99 109 164 226 341
Middle R. FL. FYKE	2	May 11, 1995	10	0	56 67	71 82 89 99 154 216 361
Gluskie Cr. IPT	2	May 8, 1995	20	0	60 71	74 85 92 102 158 220 369
O'Ne-ail Cr. IPT	2	May 9, 1995	20	0	58 69	73 84 91 101 156 218 368
Forfar Cr. IPT	2	May 9, 1995	20	0	59 70	74 85 92 102 157 219 369
Gluskie Cr. IPT	3	May 16, 1995	20	0	51 62 66	77 84 94 149 211 361
Middle R. FL. FYKE	3	May 16, 1995	10	0	51 62 66	77 84 94 149 211 361
O'Ne-ail Cr. IPT	3	May 14, 1995	20	0	49 60 64	147 209 363
O'Ne-ail Cr. IPT	4	May 23, 1995	20	0	44 55 59 70	142 204 350
Gluskie Cr. IPT	4	May 23, 1995	20	0	44 55 59 70	142 204 350
Forfar Cr. O/C Trap	4	May 24, 1995	10	0	43 54 58 69	141 203 349
Forfar Cr. IPT	4	May 23, 1995	20	0	44 55 59 70	142 204 350
Gluskie Cr. IPT	5	May 28, 1995	13	0	39 50 54 65 72	137 199 345
Forfar Cr. O/C Trap	6	June 8, 1995	14	0	28 39 43 54 61 71	116 178 335
Forfar Cr. O/C Trap	6	June 7, 1995	5	0	29 40 44 55 62 72	117 179 336
Forfar Cr. O/C Trap	6	June 8, 1995	15	0	28 39 43 54 61 71	116 178 335
Gluskie Cr. IPT	6	June 8, 1995	8	0	28 39 43 54 61 71	116 178 335
O'Ne-ail Cr. Pole Seine	6	June 8, 1995	14	0	28 39 43 54 61 71	116 178 335
Gluskie Cr. O/C	7	July 31, 1995	11	0	8 18	135 339

Table 2. Location, sample size, mean lengths (mm) of each sample at: day 0, day 70 and day 350, percent shrinkage from day 0 to day 70 and from day 0 to day 350. Day 70 was the last day in 10% formalin and day 350 was the last measurement taken in 37.5% isopropanol. Note that shrinkage of length ranged from - 3% to 12 %.

Location	n	Batch	Day 0	SE	Day 70	Mean Fork-length (mm)		SE	Percent Shrinkage Day 70	Percent Shrinkage Day 350
						SE	Day 350			
Forfar Cr.	20	1	28.6	0.20	27.3	0.21	26.0	0.15	5	9
Gluskie Cr.	20	1	28.0	0.17	26.2	0.15	25.6	0.13	7	8
Middle R.	10	1	26.6	0.45	26.2	0.28	25.8	0.30	2	3
Middle R.	10	2	27.2	0.24	26.8	0.27	26.3	0.32	1	2
Gluskie Cr.	20	2	29.2	0.18	27.9	0.17	27.2	0.25	4	7
O'Ne-eil Cr.	20	2	26.9	0.30	27.7	0.17	26.9	0.18	-3	0
Forfar Cr.	20	2	27.8	0.18	27.1	0.16	26.4	0.19	3	5
Gluskie Cr.	20	3	26.9	0.25	27.4	0.16	26.5	0.17	2	2
Middle R.	10	3	28.3	0.40	27.1	0.34	26.0	0.36	4	8
O'Ne-eil Cr.	20	3	27.7	0.21	25.5	0.17	27.0	0.17	8	3
O'Ne-eil Cr.	20	4	27.1	0.27	27.5	0.22	26.0	0.22	-1	4
Gluskie Cr.	20	4	26.8	0.30	27.2	0.21	26.1	0.21	-1	3
Forfar Cr.	10	4	28.7	0.37	27.9	0.28	26.3	0.21	3	8
Forfar Cr.	20	4	27.0	0.23	27.5	0.18	26.4	0.22	-2	2
Gluskie Cr.	13	5	28.0	0.36	26.5	0.27	25.9	0.23	6	7
Forfar Cr.	14	6	32.4	0.27	28.9	0.30	28.4	0.27	11	12
Forfar Cr.	5	6	31.0	1.41	29.3	0.66	29.5	0.85	6	5
Forfar Cr.	15	6	32.1	0.46	28.2	0.34	29.4	0.40	12	9
Gluskie Cr.	8	6	27.3	0.45	25.8	0.36	27.3	0.35	5	0
O'Ne-eil Cr.	14	6	33.1	0.38	30.3	0.31	30.3	0.31	8	8
Gluskie Cr.	11	7	43.4	0.53	40.9	0.61	40.4	0.55	6	7

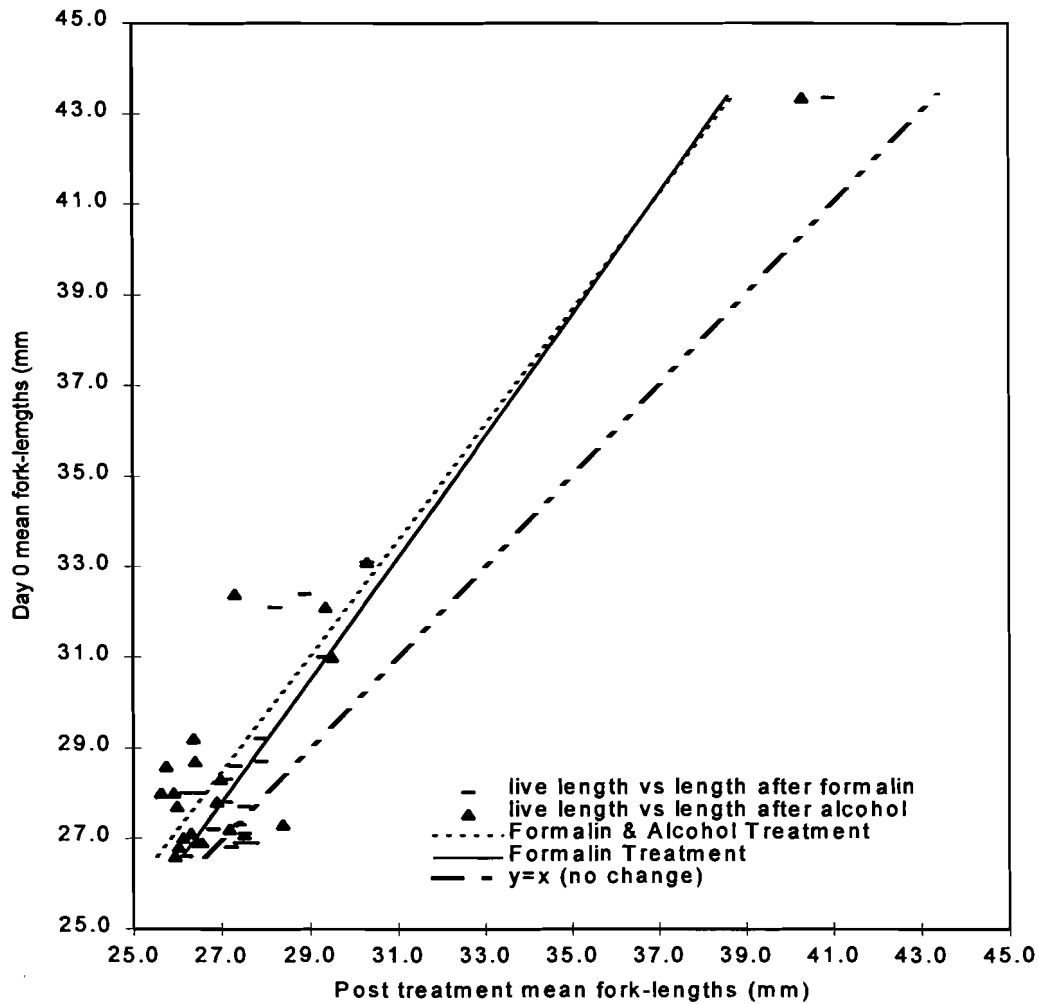


Figure 1. Weighted regressions of post treatment fork-length predicting live fork-length. The null model $Y=X$ was used to test the significance of the weighted regressions.