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Shrinkage correction and length conversion equations for *Theragra chalcogramma*, *Mallotus villosus* and *Thaleichthys pacificus*

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Preservation of walleye pollock *Theragra chalcogramma,* capelin *Mallotus villosus* and eulachon *Thaleichthys pacificus* by freezing decreased fork length (L_F) up to 1·8, 5·6 and 2·7% and reduced mass by up to 8·4, 3·5 and 1·1%, respectively. Shrinkage of walleye pollock standard length (L_S) was greater for fish in 95% ethanol *v.* 5% formalin and for fish in 10% formalin *v.* frozen. Equations describing the shrinkage and loss in mass for these species are presented as well as conversions between different length measurements (L_S, L_F and total length, L_T) for fishes that were frozen. © 2005 The Fisheries Society of the British Isles (No claim to original US government works)

Key words: body mass loss; capelin; eulachon; length conversion; shrinkage; walleye pollock.

Scientific research frequently requires preservation of samples collected in the field, and generally, preservation results in body shrinkage and loss of mass (Treasurer, 1990; Armstrong & Stewart, 1997; Kristoffersen & Salvanes, 1998). Equations to correct for the effect of preservation on fish size are often needed in ecological and physiological studies to combine field and laboratory data (Kruse & Dalley, 1990; Fox, 1996; Porter *et al.*, 2001). Equations for conversion between preserved and fresh length and mass are not currently available in the literature for post-larval walleye pollock *Theragra chalcogramma* (Pallas), capelin *Mallotus villosus* (Müller) or eulachon *Thaleichthys pacificus* (Richardson). These fishes occupy an important mid-level trophic level in the food web of the Gulf of Alaska. The objective therefore was to examine the effect of preservation on fish size using different preservatives and to provide the necessary conversion equations for these species. Freezing was of particular interest because it is commonly used in studies examining fish diet and growth. The fin rays of frozen fishes, however, are easily damaged, making certain measurements of length difficult to obtain. Thus, in this study, conversion equations

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among different length measurements from thawed individuals are also included.

Data on fish length and body mass were obtained from samples collected in the western Gulf of Alaska from 1998 to 2003. Juvenile walleye pollock (age 0 to 2 years), juvenile capelin (age 0 to 1 year), and post-larval eulachon were collected using a small-mesh (3 mm mesh codend liner) midwater trawl (Wilson *et al.*, 1996). Fish were selected by size from the catch, placed on chilled trays, and processed within an hour of being landed on deck. Fresh fork length (L_F) was measured to the nearest mm, and fresh whole wet mass of blotted-dry individuals was measured to the nearest 0·1 g. Each fish was then individually tagged. All capelin, eulachon and most walleye pollock were frozen at -80° C before being transferred to a storage freezer at -20° C.

Preservative effects on walleye pollock standard length (L_S) were also determined to provide a means to standardize historical National Marine Fisheries Service (NMFS) data. Four preservation methods were used: 1) freezing, 2) 10% formalin (in sea water buffered with 4·0% sodium borate), 3) 5% formalin (in sea water buffered with 2·0% sodium borate) and 4) 95% ethanol. The L_S of walleye pollock were measured immediately before being preserved. Fish to be frozen were handled as described above, while the other fish were placed into jars containing one of the fixatives.

Capelin and eulachon samples were reanalysed in the laboratory after being frozen for 10 and 3 months, respectively, whereas walleye pollock samples were reanalysed within 1·5 to 27 months of being preserved. Frozen samples were thawed in sea water, and samples preserved in fixatives were rinsed with sea water. Following Ricker (1971), L_S, L_F and total lengths (L_T) of each preserved individual were measured to the nearest mm. Each fish was then blotted dry and weighed to the nearest 0·001 g. Wet mass was measured to a higher precision in the laboratory relative to in the field because of the more stable environment.

Preservation effects on fish length and mass were examined statistically using paired-sample *t*-tests. Least-squares linear regression was used to examine the effect of preservation time on shrinkage for frozen walleye pollock, which spanned the largest amount of time. The relationship between fresh and preserved measurements was described using least-squares linear regression. Examination of residual plots revealed no evidence of non-linearity, although heteroscedasticity was found when fresh mass was regressed on preserved mass of walleye pollock that had been frozen. This was a result of the large range of fish masses and was easily resolved by dividing the fish into a small ($L_S < 120$ mm) and large ($L_S > 120$ mm) group, distinguishing age 0 year and older fish based on their length frequency distributions in September (Brodeur & Wilson, 1996). The percentage change in length or body mass was calculated as: $100(\text{fresh size} - \text{preserved size})(\text{fresh size})^{-1}$, where fresh size was determined from the linear regression. The range of per cent shrinkage was determined by calculating the per cent change in length or mass for both the smallest and largest fishes used in each equation. For walleye pollock, statistical comparison among preservation methods (frozen, 95% ethanol, 5% formalin and 10% formalin) was accomplished using ANCOVA with preserved length as the covariate. Due to differences in the size ranges of the samples, preservative effect could only be tested between 5% formalin and 95% ethanol and between

freezing and 10% formalin. A lack of sufficient size overlap prohibited testing between other combinations of preservatives.

Geometric mean (GM) regression was used, following Ricker (1973), to calculate length conversions among L_S, L_F and L_T of thawed individuals. To examine whether these equations derived from preserved fishes could be applied to fresh fishes, a preservation effect on the L_F and L_S relationship was tested for walleye pollock using an ANCOVA with L_S as the covariate.

Freezing significantly affected individual length and body mass for all species (Table I). The mean pair-wise difference for each species (fresh minus thawed) indicated a loss in length and mass. On average, L_F decreased by 1·8, 4·2 and 2·4 mm and body mass decreased by 0·44, 0·12, and 0·26 g for juvenile walleye pollock, capelin and eulachon, respectively. When included in linear regression models of walleye pollock L_S, L_F and mass shrinkage, the number of days spent frozen did not have a significant effect on the regressions ($n = 243$, $P = 0{\cdot}444$, $n = 262$, $P = 0{\cdot}966$ and $n = 308$, $P = 0{\cdot}571$, respectively). This test was not applied to capelin and eulachon, because samples of each species were processed over a much shorter time period of <2 weeks.

Least-squares regression equations also reflected reductions in mass and length due to freezing fishes of the observed sizes (Table II). For each species, the slopes of the equations were greater than unity indicating that absolute loss due to freezing increased with fish size. For all species, L_F shrinkage did not exceed 5·6%, whereas mass loss was more variable, ranging from an increase of 3·5% to a loss of 8·4% for all species.

Preservation of walleye pollock samples in other preservatives also resulted in significant decreases in length and mass (Table I). Walleye pollock L_S decreased by an average of 2·0, 2·7, 0·4 and 1·1 mm when preserved by freezing, 10% formalin, 5% formalin and 95% formalin, respectively. Least-squares linear regressions describing walleye pollock length reduction in each preservative are

TABLE I. Paired-sample *t*-test results of decreases in standard length (L_S), fork length (L_F) and mass (M) of *Theragra chalcogramma*, *Mallotus villosus* and *Thaleichthys pacificus* due to different preservative types. Mean shrinkage was calculated as the average change in length or body mass (fresh minus preserved) of all individuals

Species	Preservative	Measurement	Mean shrinkage (mm, g)	S.D.	d.f.	P
Theragra chalcogramma	Freezing	L_F	1·838	1·818	261	<0·001
	Freezing	M	0·440	0·963	307	<0·001
	Freezing	L_S	2·016	1·203	242	<0·001
	95% ethanol	L_S	1·110	0·699	157	<0·001
	5% formalin	L_S	0·381	0·558	35	<0·001
	10% formalin	L_S	2·715	1·419	86	<0·001
	10% formalin	M	0·257	0·311	144	<0·001
Mallotus villosus	Freezing	L_F	4·244	1·725	81	<0·001
	Freezing	M	0·123	0·200	82	<0·001
Thaleichthys pacificus	Freezing	L_F	2·364	1·576	128	<0·001
	Freezing	M	0·261	0·459	129	<0·001

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TABLE II. Least-squares linear regression equations for shrinkage of *Theragra chalcogramma*, *Mallotus villosus* and *Thaleichthys pacificus* due to different preservative types. Fresh standard length (L_{S_0}), preserved standard length (L_{S_1}), fresh fork length (L_{F_0}) and thawed fork length (L_{F_1}) were measured in mm, and fresh mass (M_0) and thawed mass (M_1) were measured in g. The range of data are based on preserved measurements. Due to heteroscedasticity, two equations were used to describe loss of walleye pollock body mass after freezing. Per cent shrinkage ranges were calculated using the minimum and maximum sizes for each regression and are presented in that order

Species	Preservative	Conversion equation	n	r^2	Data range	Shrinkage (%)
Theragra chalcogramma	Freezing	$L_{F_0} = 1{\cdot}019L_{F_1} - 0{\cdot}141$	262	0·999	48–249 mm	1·6 to 1·8
	Freezing	$M_0 = 1{\cdot}052M_1 + 0{\cdot}017$	204	0·971	0·4–9·3 g	8·4 to 5·1
	Freezing	$M_0 = 1{\cdot}022M_1 - 0{\cdot}478$	104	0·999	22·2–134·6 g	0·0 to 1·8
	Freezing	$L_{S_0} = 1{\cdot}012L_{S_1} + 1{\cdot}119$	243	0·998	37–217 mm	4·1 to 1·7
	95% ethanol	$L_{S_0} = 1{\cdot}000L_{S_1} + 1{\cdot}088$	87	0·990	18–55 mm	5·7 to 1·9
	5% formalin	$L_{S_0} = 1{\cdot}012L_{S_1} - 0{\cdot}002$	36	0·996	21–60 mm	1·2
	10% formalin	$L_{S_0} = 1{\cdot}039L_{S_1} + 0{\cdot}141$	158	0·988	40–98 mm	4·1 to 3·9
	10% formalin	$M_0 = 1{\cdot}087M_1 + 0{\cdot}035$	145	0·968	0·5–7·8 g	13·4 to 8·4
Mallotus villosus	Freezing	$L_{F_0} = 1{\cdot}014L_{F_1} + 3{\cdot}041$	82	0·982	67–114 mm	5·6 to 3·9
	Freezing	$M_0 = 1{\cdot}040M_1 - 0{\cdot}031$	83	0·994	1·3–9·6 g	1·7 to 3·5
Thaleichthys pacificus	Freezing	$L_{F_0} = 1{\cdot}009L_{F_1} + 1{\cdot}147$	129	0·999	62–216 mm	2·7 to 1·4
	Freezing	$M_0 = 1{\cdot}012M_1 - 0{\cdot}053$	130	>0·999	1·2–78·5 g	−3·5 to 1·1

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presented in Table II. Fresh body mass was not measured for walleye pollock preserved in 5% formalin and 95% ethanol and as a result only mass loss for walleye pollock preserved in 10% formalin and by freezing could be calculated and included in Table II.

ANCOVA results between preservatives indicated significant differences in juvenile walleye pollock length reduction. Slopes of pollock L_S shrinkage were not significantly different between 5% formalin and 95% ethanol treatments (ANCOVA, d.f. = 119, $P = 0{\cdot}483$), whereas there was a significant difference between intercepts (d.f. = 120, $P < 0{\cdot}001$). Walleye pollock length reduction was on average 0·73 mm greater when preserved in 95% ethanol. To test for differences between the 10% formalin and freezing treatments, a sub-sample of the frozen walleye pollock was used, equalizing the length range of the two treatments (37–102 mm L_S). Although no significant difference between slopes was detected (d.f. = 385, $P = 0{\cdot}524$), the difference in the intercepts of the 10% formalin and freezing treatments was significant (d.f. = 386, $P < 0{\cdot}001$). Freezing caused less L_S shrinkage than 10% formalin did for walleye pollock by an average of 0·82 mm.

The GM regressions were used to characterize the relationships among three commonly used measures of length (L_S, L_F and L_T) for each species after being preserved by freezing (Table III). In examining the applicability of these equations for use on fresh fishes using ANCOVA, freezing was found to have a significant effect on the walleye pollock L_F and L_S conversion. The slopes of the fresh and thawed linear regressions were found to be marginally not significant (ANCOVA, d.f. = 396, $P = 0{\cdot}055$). Using a common-slope model, however there was a significant difference in the y-intercepts of the two linear regressions (d.f. = 397, $P < 0{\cdot}001$), with the difference between L_S and L_F being 0·66 mm greater on average for frozen walleye pollock.

Length and body mass measurements are vital to many studies of fish age, growth and condition, often requiring collection of samples in the field and then subsequent analyses in a laboratory setting. Due to preservation effects on fish size, measurements may need to be adjusted for changes in length and mass due to the preservation process using appropriate equations, particularly when combining different data sets. Although available for larval walleye pollock

TABLE III. Geometric-mean regressions of the relationship between standard (L_{S_l}), fork (L_{F_l}), and total (L_{T_l}) lengths of thawed *Theragra chalcogramma*, *Mallotus villosus* and *Thaleichthys pacificus* after being preserved by freezing

Species	GM regression	n	r^2	Size range
Theragra chalcogramma	$L_{F_l} = 1{\cdot}078L_{S_l} + 0{\cdot}397$	1690	$> 0{\cdot}999$	40–283 mm L_{S_l}
	$L_{T_l} = 1{\cdot}101L_{S_l} + 0{\cdot}080$	1709	$> 0{\cdot}999$	40–283 mm L_{S_l}
	$L_{T_l} = 1{\cdot}022L_{F_l} - 0{\cdot}330$	1688	$> 0{\cdot}999$	44–306 mm L_{F_l}
Mallotus villosus	$L_{F_l} = 1{\cdot}063L_{S_l} + 1{\cdot}192$	543	0·998	59–121 mm L_{S_l}
	$L_{T_l} = 1{\cdot}143L_{S_l} + 0{\cdot}896$	530	0·996	59–118 mm L_{S_l}
	$L_{T_l} = 1{\cdot}075L_{F_l} - 0{\cdot}449$	529	0·997	63–126 mm L_{F_l}
Thaleichthys pacificus	$L_{F_l} = 1{\cdot}076L_{S_l} + 1{\cdot}144$	557	0·999	58–199 mm L_{S_l}
	$L_{T_l} = 1{\cdot}160L_{S_l} + 1{\cdot}538$	469	0·999	58–199 mm L_{S_l}
	$L_{T_l} = 1{\cdot}078L_{F_l} + 0{\cdot}451$	465	0·999	62–216 mm L_{F_l}

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(Theilacker & Porter, 1995; Porter *et al.*, 2001) and larval capelin (Kruse & Dalley, 1990), the results presented here are the first shrinkage correction equations for post-larval walleye pollock, capelin and eulachon.

Although fish mass may increase due to preservation in formalin (Stobo, 1972; Billy, 1982), this is not generally the case with freezing. The gain of mass by small eulachon in this study may be due to absorption of water during the thawing process, when samples are immersed in salt water. Small eulachon excluded, the range of length and mass changes presented here is well within the range of reported findings in the literature, despite the complications in comparing different shrinkage studies. Not only do species of fishes react uniquely to preservation, but differences in fish size can also lead to varying degrees of shrinkage (Johnston & Mathias, 1993). Studies have suggested that the per cent of length and mass loss is inversely proportional to fish size, most likely a result of decreasing surface area to volume ratios (Hay, 1984). The type, concentration and salinity of preservative used to store fishes also has a significant effect on the length and mass changes of the samples (Fowler & Smith, 1983; Hay, 1984; Moku *et al.*, 2004).

With regard to effects of different preservatives, this study corroborates those of Fowler & Smith (1983), Moku *et al.* (2004) and Fox (1996) who showed that shrinkage was greater in ethanol than in formaldehyde for larvae of silver hake *Merluccius bilinearis* (Mitchill), myctophids and herring *Clupea harengus* L. respectively. While some research has shown that certain species of fishes decrease in length and mass significantly more by freezing than when in formalin, *e.g.* larval walleye *Stizostedion vitreum* (Mitchill) (Johnston & Mathias, 1993), and cisco *Coregonus artedii* Lesueur and yellow perch *Perca flavescens* (Mitchill) (Engel, 1974), this study indicates that juvenile walleye pollock shrinkage is greater in 10% formalin by an average of 0·82 mm. This information is useful in justifying freezing as an equally valid means of preserving walleye pollock samples to be used for studies concerning length and body mass. The advantage of this would be eliminating the need for the unnecessary handling of a hazardous chemical such as formaldehyde.

Studies of fishes preserved in formalin and ethanol have shown the amount of shrinkage stabilizes after an initial critical period of <1 month (Lockwood & Daly, 1975; Fox, 1996; Smith & Walker, 2003; Moku *et al.*, 2004). All of the samples preserved in formalin and ethanol in this study were measured after 1·5 months, and thus, it is assumed that preservation time did not greatly influence the results. Due to a lack of similar research on freezing, the effect of preservation time was examined in this study for frozen walleye pollock. The number of days spent frozen did not significantly affect the amount of walleye pollock shrinkage for the time period examined here. This study, however, was not designed to address this issue specifically, and more research should be conducted to directly examine the temporal effects of freezing.

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