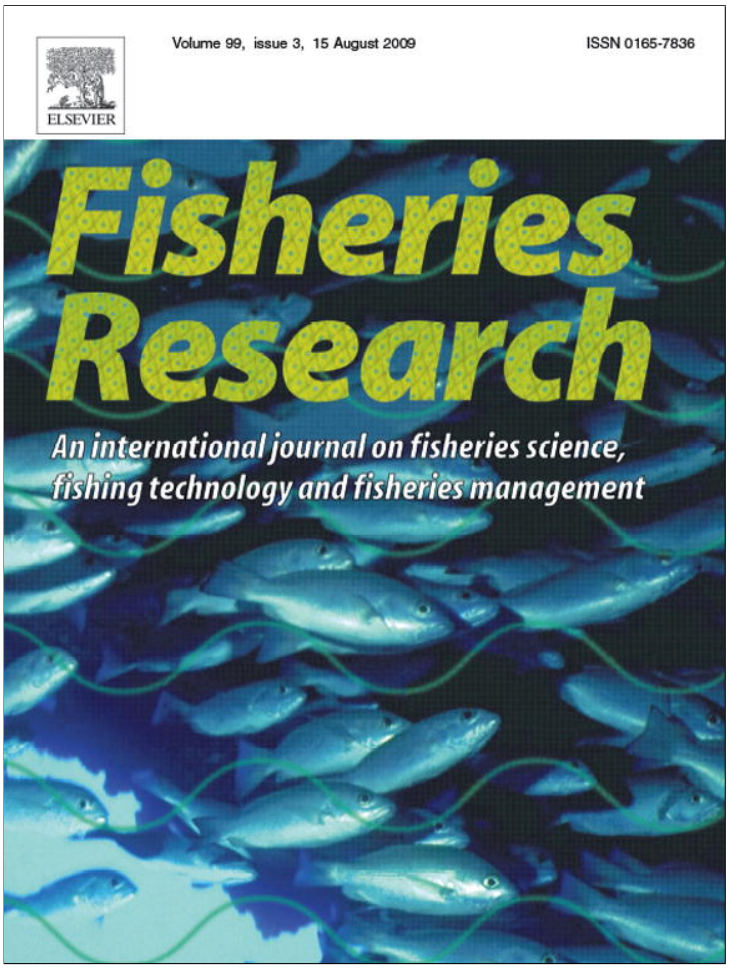

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Short communication

The effect of freezing on the length and weight measurements of ruffe (*Gymnocephalus cernuus*)

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ABSTRACT

The ruffe is a percid native to Europe and Asia that has invaded several new areas in the last several decades. For population studies ruffe are often frozen en masse after collection in the field and thawed at a later time for measurements of length and weight. I determined the effect of freezing and thawing on ruffe total length (TL) and weight (W) by making these measurements on freshly caught ruffe and ruffe that had been frozen and thawed. Frozen and thawed fish generally shrank by between 1.25% and 1.63% (95% CI) of their fresh TL and approximately 5–10% of their fresh W. Shrinkage in W increased as the length of time being frozen increased. The $\log_e(W)$– $\log_e(\text{TL})$ relationship differed between fresh and thawed ruffe. Relative underestimation of actual fresh W using thawed TL and a $\log_e(W)$– $\log_e(\text{TL})$ relationship constructed from thawed fish was 7.50%. All observed shrinkage amounts were substantially different than within-technician measurement variability. These results suggest that fresh specimens should be used if it is important to understand the true fresh measurements of ruffe. In the absence of being able to do this, (provided) corrections for errors due to thawing should be used.

1. Introduction

Length and weight measurements are important data used to understand the population and community dynamics of fish populations (Anderson and Neumann, 1996). For reasons of efficiency, the lengths and weights of many species of fish are recorded in the laboratory after the fish have been frozen for a period of time and then thawed. Freezing and thawing results in shrinkage of both the length and weight of many fish but the amount of shrinkage varies among species (Table 1). Thus, the freezing and thawing of fish may skew length and weight measurements, length–weight relationships, and associated measures such as relative condition, growth rates, population biomass, and production.

The ruffe (*Gymnocephalus cernuus*) is a percid native to Europe and Asia (Craig, 1987) that has been accidentally introduced into several large lakes around the world (Matthey, 1966; Pratt et al., 1992; Gunderson et al., 1998; Ogle, 1998; Winfield et al., 2002; Lorenzoni et al., 2007). In addition, ruffe are a major component of the fish community in many water bodies to which it is native (Duncan, 1990; Bronte et al., 1998; Winfield et al., 2007). Ruffe are often frozen en masse, thawed at a later time, and then measured and weighed in the laboratory. The effect of freezing on the length and weight measurements or the length–weight relationship of ruffe is unknown. My purpose here is to determine the effect, if any, of freezing on total length (TL) and weight (W) measurements and the length–weight relationship of ruffe.

2. Methods

Ruffe were collected from the St. Louis River Harbor between Duluth, MN and Superior, WI on 20 September 2007 and 12 May 2008. All captured ruffe were immediately placed on ice in a cooler and transported back to the laboratory. In the laboratory, within 8 h of being collected, individual ruffe were measured for TL (within 1 mm) and weighed (within 0.1 g). Ruffe were then individually labeled, placed en masse in a plastic bag, and frozen (at approximately −10 °C). After 75 ($n = 39$ ruffe), 148 ($n = 25$), or 301 ($n = 25$) days the ruffe were allowed to gradually thaw (approximately 6 h at approximately 23 °C) and were then measured again for TL and weighed. All fresh and thawed weight measurements were made after blotting excess moisture and mucus from the surface of the fish with a paper towel. Three ruffe in the 75 days frozen sample and two ruffe in the 148 days frozen sample were excluded from further analysis because of substantial damage to the caudal fins. Mean and variance of TL and variance of W were similar, but mean W differed slightly, among the three samples (Table 2).

For each fish, the percentage absolute change in TL and W due to freezing and thawing was calculated with $\%Y_{changed} = 100 \times |(Y_{thawed} - Y_{fresh})|/Y_{fresh}$ where Y generically represents TL or W. The effects of Y_{fresh} and the length of freezing time factor (*FRZNLEN*) on

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Table 1
Summary of literature review of percentage change amounts (negative means "shrinkage") for total length (TL) or weight (*W*). The range of specimens examined (TL in cm; *W* in g) and the results of tests for a length effect on the percentage change are also shown when known. Author codes are: (1) Ajah and Nunoo (2003), (2) Al-Hassan et al. (2000), (3) Buchheister and Wilson (2005), (4) DiStefano et al. (1994), (5) Engel (1974) and (6) Treasurer (1990).

Measure	Species	Fresh size	Percent change	Length effect	Author
TL	*Campostoma anomalum*	9.4 [a]	−2.88	–	(4)
TL	*Coregonus artedii*	<21	−3.7	No	(5)
TL	*Coregonus artedii*	≥21	−1.6	No	(5)
TL	*Mullus barbatus*	–	−2.36	–	(2)
TL	*Mullus surmeletus*	–	−4.01	–	(2)
TL	*Perca flavescens*	13.3–17.1	−0.7	No	(5)
W	*Campostoma anomalum*	7.76 [a]	−9.65	–	(4)
W	*Coregonus artedii*	<21	−2.3	No	(5)
W	*Coregonus artedii*	≥21	−1.6	No	(5)
W	*Esox lucius*	19–83	−2.55	–	(6)
W	*Mallotus villosus*	1.3–9.6	−2.6 [b]	–	(3)
W	*Perca flavescens*	13.3–17.1	−1.7	No	(5)
W	*Perca fluviatilis*	11–34	−2.65	–	(6)
W	*Sardinella aurita*	6.7–11.4	−14.2	–	(1)
W	*Thaleichthys pacificus*	1.2–78.5	+0.7 [b]	–	(3)
W	*Theragra chalcogramma*	22.2–134.6	−0.9 [a]	–	(3)
W	*Theragra chalcogramma*	0.4–9.3	−6.7 [b]	–	(3)

[a] Mean.
[b] Midpoint of provided range.

$\%Y_{changed}$ were assessed with a linear model that included both of these main effects and their interaction. The fresh measurements used in this model were "centered" (i.e., $Y^{*}_{fresh} = Y_{fresh} - \bar{Y}_{fresh}$) so that the model intercept could be interpreted as the mean $\%Y_{changed}$ at the mean value of Y_{fresh}. Post hoc multiple comparison tests for slopes used a false discovery rate correction (Benjamini and Hochberg, 1995; Storey, 2002; Verhoeven et al., 2005) whereas those for intercepts (in the absence of different slopes) used the Tukey HSD procedure.

I examined the effect of freezing (*FROZEN*; fresh or thawed) and *FRZNLEN* on the length–weight relationship with a linear model that included $\log_e(W)$ as the response variable and $\log_e$(TL), *FROZEN*, *FRZNLEN*, and all two- and three-way interactions between these variables as the explanatory variables. This model allows comparison of the slopes and intercepts for the six $\log_e(W)$–$\log_e$(TL) regression lines defined by the three length of freezing treatments and before or after being frozen in those freezing treatments. The significance of each model term was assessed with type II *F*-tests (Fox, 1997).

I assessed the relative impact of using TL from thawed fish and a $\log_e(W)$– $\log_e$(TL) relationship based on thawed fish to predict a perceived actual *W* by first creating a hypothetical group of fish with "fresh" TL at every mm over the range of observed TL in this study. I then simulated the actual fresh *W* for each of these fish with the fresh $\log_e(W)$– $\log_e$(TL) relationship, simulated the thawed TL of these fish by "shrinking" the TL by the observed constant shrinkage percentage (i.e., 1.44%), and predicted the perceived fresh *W* using the thawed TL values and the thawed $\log_e(W)$– $\log_e$(TL) relationship. To isolate the effect of using just thawed TL, I also predicted the perceived fresh *W* using the thawed TL value and the fresh $\log_e(W)$–$\log_e$(TL) relationship. The final relative impacts of using the thawed fish and the thawed relationship or just the thawed fish were measured by the mean percentage difference between the perceived and actual fresh *W*.

Within-technician variability in TL and *W* was assessed by the same technician recording these measurements a second time on the thawed fish from the 301 days frozen sample. The percentage absolute difference in measurements was calculated $\%Y_{diff} = 100 \times |(Y_2 - Y_1)|/Y_1$ where the subscripts represent the order of measurements.

All statistical analyses were computed with R Version 2.9.0 (R Development Core Team, 2009). All linear models were fit with lm(), type III ANOVA results were obtained with anova(), and type II ANOVA results were obtained with Anova() (from the "car" package). Comparisons of slopes with the false discovery rate were implemented with comp.slopes() and the Tukey HSD method for comparing intercepts was implemented with comp.intercepts() (from the "NCStats" package). All analyses used a 5% significance level, experimentwise error rate, or false discovery rate.

Table 2
Mean (and SD) of each measurement type for all three lengths of freezing. The ANOVA and Levene's test results for the comparison of means and variances across all lengths of freezing within each measurement type are shown. Different letters next to values indicate significant differences within that measurement type.

	Total length	Weight
75 days	98.1 (17.7)	10.96 (4.88)[a]
148 days	102.7 (19.8)	12.75 (6.44)[ab]
301 days	108.6 (16.8)	14.84 (5.79)[b]
ANOVA *p*	0.0889	0.0344
Levene's *p*	0.5172	0.3234

3. Results and discussion

The within-technician variability was between 0.28% and 0.68% (95% CI) for repeat TL and 0.78% and 1.89% for repeat *W* measurements. The mean repeat TL measurements by the same technician were not statistically different ($p \geq 0.4165$) indicating no bias towards shorter or longer measurements. However, the mean second *W* measurement by the same technician was significantly lower than the mean first *W* measurement ($p < 0.00005$). As these measurements were sequential in time this bias could be explained by further drying of the specimens despite our efforts to keep the fish moist. Nevertheless, comparing observed percentage weight change to this measurement "error" will provide a conservative comparison for determining weight change due to freezing and thawing.

Neither the length of freezing time nor the fresh TL had a significant effect on the percent change in TL due to freezing and thawing (Table 3). Thus, on average, the TL declined between 1.25% and 1.63% due to freezing. The relationship (i.e., slopes) between percentage change in *W* and fresh *W* did not differ among the three lengths of time frozen (Table 3). However, the percentage loss in *W* (i.e., intercepts) generally increased as the time frozen increased with a

Table 3
Intercepts and slopes from linear regression model fits to percent change on fresh measurements for each combination of measurement type and length of freezing and combined across all lengths of freezing (Common). *p*-Values for comparing slopes and intercepts (Comparison *p*) and for testing that the common slope or intercept is equal to zero (Common *p*) are shown. *p*-Values that should not be interpreted are left blank.

	Total length	Weight
Slopes		
75 days	0.002	0.148
148 days	−0.003	0.128
301 days	−0.024	0.104
Comparison *p*	0.1388	0.9609
Common	−1.444	0.061
Common *p*	0.3555	0.3067
Intercepts		
75 days	−1.50	−6.18[a]
148 days	−1.46	−7.48[ab]
301 days	−1.19	−9.43[b]
Comparison *p*	0.6092	0.0014
Common	−1.44	–
Common *p*	<0.00005	–

significant difference between the 75 and 301 days frozen groups. On average, the weights for fish frozen for 75 days declined between 5.02% and 7.83% and those frozen for 301 days declined between 8.13% and 10.27%.

The lack of an effect of length on percent shrinkage in ruffe is consistent with what has been found for other species (Table 1). However, the shrinkage percentage for TL appears to be low and the shrinkage percentage for *W* appears to be high when compared to other species (Table 1). This observation may be explained by ruffe being generally smaller then the other species. My results suggest, at least within approximately the first year of being frozen, that the percentage weight loss of ruffe increases with increasing amount of time frozen. I am unaware of any other study that has studied the effect of length of freezing on the amount of shrinkage.

The significant relationship (i.e., slopes) between $\log_e(W)$ and $\log_e(\mathrm{TL})$ ($p < 0.00005$) did not differ between ruffe when measured fresh or thawed ($p = 0.4061$), between the different lengths of freezing ($p = 0.7526$), or the interaction between these two main effects ($p = 0.9679$). However, the parallel $\log_e(W)$– $\log_e(\mathrm{TL})$ regression lines were statistically significantly offset between the freshly measured and the frozen and thawed ruffe ($p = 0.0082$), but not between lengths of freezing ($p = 0.7511$).

These observations about the $\log_e(W)$– $\log_e(\mathrm{TL})$ relationship can be explained geometrically (Fig. 1) by noting that the $\log_e(W)$–$\log_e(\mathrm{TL})$ relationship is positive and that a shrinkage in TL shifts a given fish to the "left" and a shrinkage in *W* shifts the fish "down." These two shrinkage amounts are constant percentages such that each fish on the $\log_e(W)$– $\log_e(\mathrm{TL})$ scale is shifted constant amounts. Because the shrinkage percentage for *W* is greater than that for TL the "downward" movement is greater then the "leftward" movement resulting in the thawed results ultimately being below the fresh results.

Estimates of *W* from TL taken on thawed ruffe using a $\log_e(W)$–$\log_e(\mathrm{TL})$ relationship constructed from thawed fish will underestimate the true fresh *W* by an average of 7.50%. If the $\log_e(W)$–$\log_e(\mathrm{TL})$ relationship constructed from fresh fish is used instead then the true fresh *W* is underestimated by an average of 4.10%. Thus, the error in predicting fresh *W* arises from using both a thawed TL and a $\log_e(W)$– $\log_e(\mathrm{TL})$ relationship constructed from thawed fish, despite the seemingly small difference between the $\log_e(W)$– $\log_e(\mathrm{TL})$ relationships for the fresh and thawed specimens (Fig. 1).

The bias in estimating *W* by using frozen and thawed specimens can be avoided by using fresh measured TL and a $\log_e(W)$– $\log_e(\mathrm{TL})$ relationship developed from fresh fish. However, if this is not possible, the *W* bias can be mitigated in one of two ways. First, fresh TL estimated from TL measured on thawed fish can be used with a $\log_e(W)$– $\log_e(\mathrm{TL})$ relationship developed from fresh fish. The fresh TL can estimated by dividing the thawed TL by 0.9856. Second, *W* predicted from thawed TL and a thawed $\log_e(W)$– $\log_e(\mathrm{TL})$ relationship can be converted to fresh *W*. This conversion can be computed by dividing the predicted *W* by 0.9250 (assumes an average 7.50% error).

My results show that the process of freezing ruffe en masse and later thawing them for the purpose of measuring length and weight imparts a statistical and practical bias in these measurements. Biases in length and weight measurements are on the order of three or six to seven times greater than measurement error for these measures, respectively. Weights predicted from thawed TL using thawed length–weight relationships are in error by approximately five times measurement error for the weight variable. Thus, if it is important to understand the true fresh measurements of ruffe then fresh specimens should be used. In the absence of being able to do this, corrections for errors due to thawing should be used.

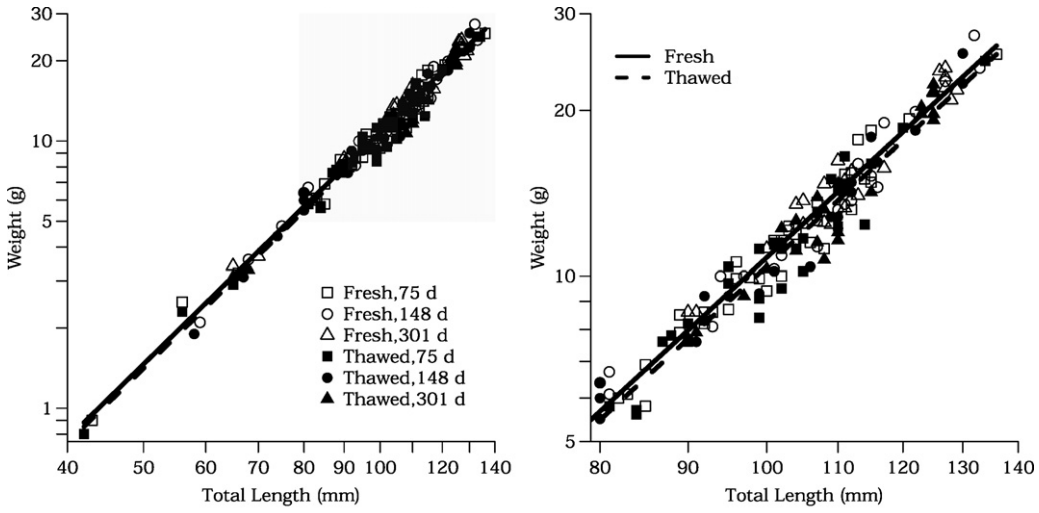

Fig. 1. Length–weight relationships, on the log–log scale, for each combination of measurements on fresh or thawed fish in the 75, 148, or 301 days frozen treatments. Significantly different regression lines for the fresh and thawed measurement groups are shown. To highlight the bulk of the data, the plot on the right is the shaded are of the plot on the left.

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