

## Length correction for early-juvenile Brazilian herring *Sardinella janeiro* (Eigenmann, 1894) after preservation in formalin, ethanol and freezing

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This work aims to quantify the variation in total length and body mass for the early-juvenile Brazilian herring *Sardinella janeiro* and to determine total length and body mass correction equation to allow fresh measures to be calculated from preserved ones. Fishes were randomly assigned to one of five preservation methods (freezing at -20° C, 2.5% and 5% formalin, 70% and 95% ethanol), and measured for total length (TL) and body mass (W) before preservation, and on days 5, 15, 30, and 60 after storage. Significant reductions in total length and body mass occurred during the first 5 days after preservation and continued to contract significantly at a lesser rate through 30 days in most methods. Exceptions were shown for body mass in freezing and 5% formalin, where the greatest losses occurred after 30 days of preservation. The degree of shrinkage for total length and body mass was very much dependent on fish size, with smaller specimens shrinking more than larger ones. The fresh total length and body mass can be back-calculated using equations that describe the relationship between fresh and preserved individuals after 60 days storage for all methods except for body mass in freezing.

Este trabalho objetivou quantificar a variação do comprimento total e massa corporal para pós-larvas de *Sardinella janeiro* e determinar as equações de correção para o cálculo do comprimento total e da massa corporal a partir de espécimes preservados. Os peixes foram submetidos aleatoriamente a cinco métodos de preservação (congelamento - 20° C, formalina 2,5% e 5%, álcool 70% e 95%), e medidos o comprimento total (CT) e pesada a massa corporal (P) antes da preservação, e 5, 15, 30 e 60 dias após a preservação. Foram observadas perdas significantes no comprimento total e na massa corporal durante os cinco primeiros dias após a preservação, prosseguindo em menor intensidade até o 30° dia na maioria dos métodos. Exceções foram observadas para a massa corporal em congelamento e formalina 5%, com perdas ocorrendo mesmo depois de 30 dias de preservação. O grau de perdas para comprimento total e massa corporal foi significativamente dependente do tamanho dos peixes, com os menores indivíduos sofrendo as maiores perdas. O comprimento total e massa corporal de indivíduos frescos podem ser retro-calculados usando equações que descrevem a relação entre indivíduos frescos e após 60 dias de preservação para a maioria dos métodos, exceto para a massa corporal em congelamento.

**Key words:** Shrinkage, Storage, Back-calculation, Initial length, Size conversion, Clupeidae.

### Introduction

Storage in formalin, ethanol or by freezing are frequently used to preserve fish for scientific purposes, and generally result in shrinkage of fish body mass and length (Buchheister & Wilson, 2005; Fey & Hare, 2005). The preserved individuals can be used to estimate population parameters such as size structure, growth rate, condition factor and length-body mass relationship, with these parameters being indicators of well being state of populations. Since these parameters are assigned from preserved species, such measures could not be reflecting the real species conditions, and the results of

the subsequent analyses being significantly biased (Fey, 1999; Porter *et al.*, 2001; Santos *et al.*, 2005). With the purpose of correcting such variation in specimens length and body mass which usually occur with the preservation, several workers have proposed a correction factor to estimate initial length and body mass by using appropriate equations (*e.g.* Buchheister & Wilson, 2005; Fey & Hare, 2005; Fey, 1999, 2002; Porter *et al.*, 2001).

The time of preservation, species age and the preservation method are the most important factors affecting shrinkage, generally losses of length and body mass. The highest losses occur in the first days of preservation, in the youngest

individuals (Fey, 1999, 2002; Radtke, 1989) and in specimens preserved in ethanol at concentrations near to absolute (Hjörleifsson & Klein-MacPhee, 1992). Loss in length can vary between 5% and 40% for larvae preserved in 70% and 95% ethanol, respectively (Fowler & Smith, 1983; Jennings, 1991; Radtke, 1989). The amount of shrinkage generally depends on the osmolarity of the preservative (Tucker & Chester, 1984), thus seawater formaldehyde solution will lead to a greater shrinkage than freshwater formaldehyde. Morkert & Bergstedt (1990) reported that length of larvae usually decrease about 4.3% after preservation in 10% formalin, varying according to concentration, salinity and the particular species. Despite of such effects, preservation in formalin is the most common for the ichthyoplankton (Smith & Richardson, 1977). However, because of decalcification of skeletal structure in formalin and ethanol that acts as a weakly acid, the freezing is the most recommended method for the preservation of larvae for age estimation from daily rings in otoliths (Santos *et al.*, 2005; Brothers, 1987).

Hjörleifsson & Klein-MacPhee (1992) recommend that if there is previous knowledge about a given species shrinkage variability, it is important to choose the preservation method. This procedure minimizes possible errors or even can increase the accuracy of the results, since such losses are species-specific. The most important use of shrinkage studies is to predict live size and body mass. The type of preservative used depends on the purpose of the study as well as the precision with which fresh length and body mass can be estimated from preserved length and body mass. Due to preservation effect on fish size, measurements may need to be adjusted for changes in length and mass using appropriate equation, particularly when combining different data sets. The present work aims to quantify the variation in length and body mass for the Brazilian herring *Sardinella janeiro* (Eigenmann, 1894) preserved in freezing, formalin and ethanol, and to indicate length and body mass correction equations to allow live measures to be calculated from preserved ones.

### Material and Methods

Data on fish length and body mass were obtained from samples collected in sandy beaches in Rio de Janeiro, Southeastern Brazil, from March to April 2006. Early-juvenile *S. janeiro* were collected using a small-mesh beach seine (12 x 2m; 4mm meshed cod-end liner). Immediately after to be collected, fishes were anesthetized by addition of a small amount of benzocaine and placed in plastic bags containing local seawater and ice to keep temperature low during transportation. Fresh total length (TL) was measured to the nearest 0.01mm with a caliper and fresh whole wet body mass (W) of blotted-dry individuals was measured to the nearest 0.001g using an electronic balance. Samples were divided in 5 similar size groups, corresponding to each preservation method, ranging from 15.07 to 28.11mm TL and 0.021 to 0.092 g. Each group of sample was stored by freezing at -20 °C, in 2.5% formalin (buffered with 2% sodium borate mixed with

seawater 32-35, pH 7.6), 5% formalin (buffered with 4% sodium borate mixed with seawater 32-35, pH 8.1), 70% ethanol and 95% ethanol.

Fishes were measured and weighed five times: before preservation, then at 5, 15, 30 and 60 days after preservation. For fishes storage in freezing, the body mass was measured only after thawing to avoid influence of possible ice incorporated in the tissues that could overestimate the species body mass. The percentage change in length or body mass was calculated as:  $100 \frac{(\text{fresh size} - \text{preserved size})}{(\text{fresh size})}$  or  $100 \frac{(\text{fresh body mass} - \text{preserved body mass})}{(\text{fresh body mass})}$ . ANOVA was used to compare individual's total length among the five groups before preservation and to compare shrinkage among the methods after 60 days preservation. A *t*-test for  $H_0$ : slope = 0 ( $p < 0.05$ ) was used to verify if there is difference in shrinkage throughout fish size. Equations describing the shrinkage in body mass and length are given based on least-square regression relationships between fresh and preserved body mass and length after 60 days. These equations were used for conversion between preserved and fresh body mass and length. To determine if a single correction factor is appropriate for a calculation of fresh length or body mass, a *t*-test (*t*-test analysis for  $H_0$ : slope = 1,  $p > 0.05$ ) was performed. If the *y*-intercept was significantly different from zero (*t*-test analysis for  $H_0$ : *y*-intercept = 0,  $p < 0.05$ ) it can be used as a single correction factor.

### Results

The means of the TL of *S. janeiro* were similar ( $F = 0.37$ ;  $d.f = 4$ ;  $p = 0.83$ ) among the five groups on day zero, indicating that the early-juvenile had been randomly sorted. The examined preservation methods influenced significantly on length and body mass of *S. janeiro* (ANOVA,  $p < 0.001$ ). Furthermore, losses in total length and body mass after 60 days showed significant differences among the preservation methods (Tukey HSD,  $p < 0.001$ ).

**Shrinkage with freezing.** The decrease in body length was initially rapid, and the majority of the length reduction occurred in the first 5 days (means = 1.31%; SE = 0.16). After 60 days the mean decrease in total length was  $1.91\% \pm 0.16$  SE (Fig. 1). The highest reductions in body mass were seen within the first 30 days ( $7.13\% \pm 0.54$  SE). The means body mass loss after 60 was  $7.18\% \pm 0.53$  SE (Fig. 2).

**Shrinkage with 2.5 % formalin.** The decrease in body length was highest in the first 5 days ( $1.78\% \pm 0.14$  SE), and length after 60 days of preservation was  $3.81\% \pm 0.12$  SE (Fig. 1). The highest reduction in body mass occurred during the first 5 days of preservation ( $2.71\% \pm 0.44$  SE) reaching  $6.47\% \pm 0.38$  SE after 30 days and  $6.56\% \pm 0.43$  SE after 60 days (Fig. 2).

**Shrinkage with 5% formalin.** The majority of the length reduction occurred during the first 5 days ( $1.96\% \pm 0.20$  SE). After 60 days of preservation the mean shrinkage was 4.83%

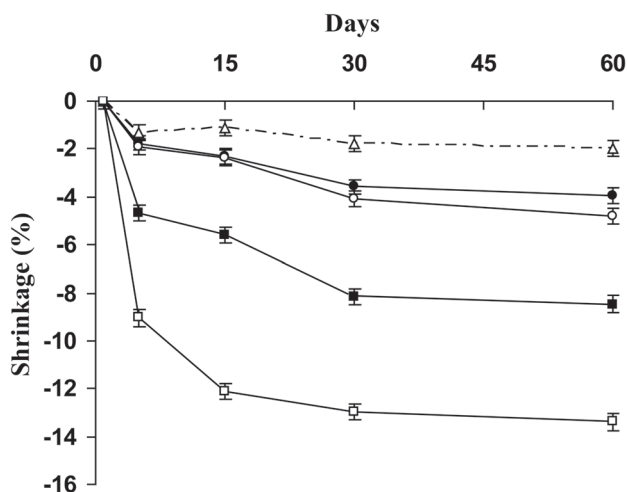
$\pm 0.17$  SE (Fig. 1). Body mass reduction was higher during the first 30 days of preservation ( $6.90\% \pm 0.34$  SE) (Fig. 2). After 60 days the body mass loss reached  $7.52\% \pm 0.36$  SE.

**Shrinkage with 70% ethanol.** Reduction in body length was higher in the first 5 days of preservation ( $4.64\% \pm 0.21$  SE), and after 60 days was  $8.68\% \pm 0.18$  SE (Fig. 1). The highest body mass reduction occurred during the first 5 days ( $7.53\% \pm 0.39$  SE) (Fig. 2). Mean body mass loss after 60 was  $10.60\% \pm 0.44$  SE.

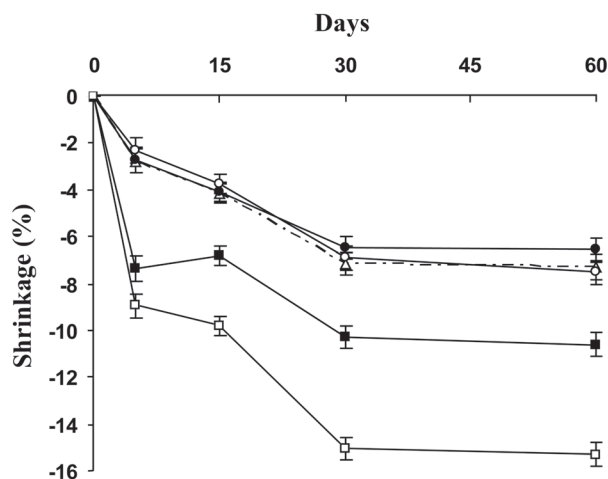
**Shrinkage with 95% ethanol.** The decrease in body length was initially rapid, and the majority of length reduction occurred in the first 5 days ( $9.23\% \pm 0.26$  SE). After 60 days of preservation loss in body length was  $13.49\% \pm 0.23$  SE (Fig. 1). The highest body mass reduction occurred mainly in the first 5 days of preservation ( $8.74\% \pm 0.35$  SE) (Fig. 2). Mean loss after 60 days was  $15.29\% \pm 0.34$  SE.

Losses in total length and body mass varied according to total length. The percentage deviation decreased with increasing body size; the smaller the individuals the higher losses were recorded for all preservation methods ( $t$ -test,  $H_0$ : slope = 0,  $p < 0.001$ ) (Fig. 3-4). The reduction in total length after 60 days of preservation, in decreasing order, was for fishes preserved in 95% ethanol, 70% ethanol, 5% formalin, 2.5% formalin and freezing (Tukey HSD,  $p < 0.005$ ). Loss of body mass was higher in specimens preserved in 95% ethanol, followed, in decreasing order, by 70% ethanol, 5% formalin, freezing and 2.5% formalin. Body mass loss did not differ significantly between freezing, 2.5% and 5% formalin (Tukey HSD,  $p = 0.40$ ).

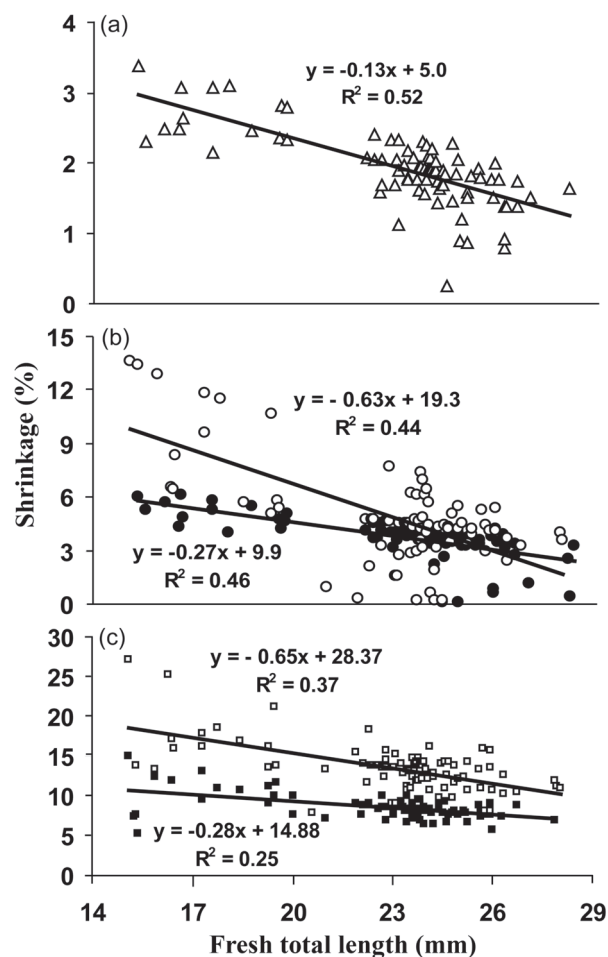
The relationships between fresh and preserved total length and body mass were described by a linear regression for all 5 preservatives (Table 1). All  $y$ -intercepts were highly significantly different from zero ( $H_0$ :  $y$ -intercept = 0;  $t$ -test of



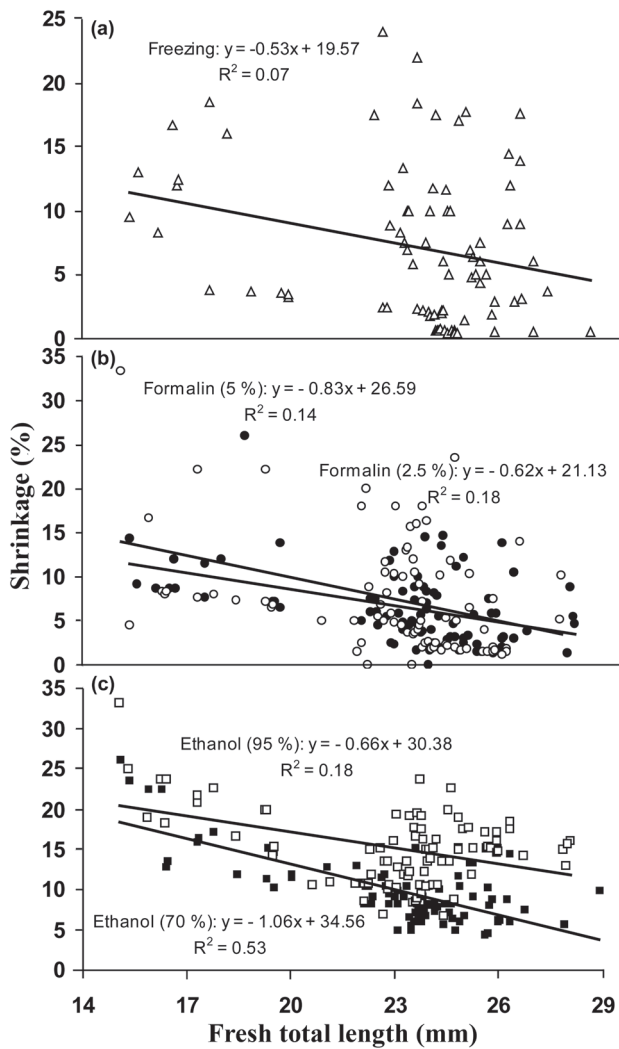
**Fig. 1.** Mean percentage shrinkage of total length for early-juvenile of *Sardinella janeiro* during storage in freezing (open triangle), 2.5% formalin (full circle), 5% formalin (open circle), 70% ethanol (full square) and 95% ethanol (open square).



**Fig. 2.** Mean percentage shrinkage of body mass for early-juvenile of *Sardinella janeiro* during storage in freezing (open triangle), 2.5% formalin (full circle), 5% formalin (open circle), 70% ethanol (full square) and 95% ethanol (open square).



**Fig. 3.** The relationship between fresh total length and relative (%) shrinkage in total length after 60 days of preservation for early-juvenile of *Sardinella janeiro*. (a) Freezing (open triangle), (b) 2.5% formalin (full circle), 5% formalin (open circle), (c) 70% ethanol (full square) and 95% ethanol (open square).



**Fig. 4.** The relationship between fresh total length and relative (%) shrinkage in body mass after 60 days of preservation for early-juvenile of *Sardinella janeiro*. (a) Freezing (open triangle), (b) 2.5% formalin (full circle), 5% formalin (open circle), (c) 70% ethanol (full square) and 95% ethanol (open square).

regression intercept,  $p < 0.001$ ) with exception of the freezing method for body mass ( $p = 0.053$ ). The slopes of the regressions of preserved total length at 60 days were not significantly different from one ( $H_0$ : slope = 1;  $t$ -test of slope,  $p > 0.05$ ) in 70% and 95% ethanol and of body mass in freezing and 2.5% and 5% formalin. Significant differences from one in slopes ( $H_0$ : slope = 1;  $t$ -test of slope,  $p < 0.036$ ) were detected for preserved length at 60 days in freezing, and 2.5% and 5% formalin, and of body mass in 70% and 95% ethanol. Therefore, a single correction  $y$ -intercept ( $y$ -intercept<sup>1</sup> 0 and slope = 1) can be used for conversion between preserved and fresh length in 70% and 95% ethanol and between preserved and fresh body mass in 2.5% and 5% formalin. The equation can be used for length correction for preserved length at 60 days in freezing, and 2.5% and 5% formalin, and of body mass in 70% and 95% ethanol. Correction factor or equations are not appropriate for correcting body mass storage in freezing.

## Discussion

Shrinkage of early-juvenile Brazilian herring *S. janeiro* varied among the preservation methods. The greatest shrinkage mean value of preserved length ( $13.49\% \pm 0.23$  SE) and body mass ( $15.29\% \pm 0.34$  SE) was in ethanol (mainly 95% ethanol) when compared with formalin or storage in freezing. Shrinkage varies because of the differing ionic strengths of these solutions (Parker, 1963; Hay, 1982; Tucker & Chester, 1984). Some studies report a smaller shrinkage in ethanol (e.g. Theilacker, 1980; Hjörleifsson & Klein-MacPhee, 1992; Porter *et al.*, 2001) when compared with formalin, but the majority has found a higher shrinkage in ethanol (e.g. Fowler & Smith, 1983; Kruse & Dalley, 1990; Fox, 1996; Kristoffersen & Salvanes, 1998; Moku *et al.*, 2004; Thorstad *et al.*, 2007). Ethanol has been found to distort larvae and cause greater variability in shrinkage estimates compared with formaldehyde preservation (Fox, 1996). Different processes cause body mass loss in formaldehyde and ethanol, respectively. Both formaldehyde and ethanol extract water from tissue. Formaldehyde also dissolves glycogen, glucose, some phospholipids and inorganic salts (Steedman, 1976),

**Table 1.** Least-squares linear regression equations for shrinkage of *Sardinella janeiro* for different preservation methods and  $t$ -test statistics ( $H_0$ : slope = 1 and  $y$ -intercept = 0). The ranges of data are based on preserved measurements.  $TL_p$  = preserved total length;  $W_p$  = preserved body mass.  $y$ -inter =  $y$ -intercept. ns = nonsignificant differences ( $p > 0.05$ ).

Preservation Method	Regression equation	n	R <sup>2</sup>	TL <sub>p</sub> and W <sub>p</sub> ranges	t-test Slope=1	p	t-test y-inter = 0	p
Freezing -20°C	TL <sub>fresh</sub> = 0.992TL <sub>p</sub> + 0.6152	82	0.999	14.32-27.01mm	2.26	0.035	8.04	<0.001
	W <sub>fresh</sub> = 1.0263W <sub>p</sub> + 0.0023							
Formalin 2.5%	TL <sub>fresh</sub> = 0.9797L <sub>p</sub> + 1.2928	85	0.994	14.05-26.94mm	2.36	0.019	6.76	<0.001
	W <sub>fresh</sub> = 1.005 W <sub>p</sub> + 0.0027							
Formalin 5%	TL <sub>fresh</sub> = 0.9226L <sub>p</sub> + 2.7632	84	0.982	14.22-26.67mm	5.27	<0.001	8.89	<0.001
	W <sub>fresh</sub> = 1.0003 W <sub>p</sub> + 0.0034							
Ethanol 70%	TL <sub>fresh</sub> = 1.0024L <sub>p</sub> + 1.9031	83	0.992	13.74-26.01mm	0.24	ns	6.35	<0.001
	W <sub>fresh</sub> = 1.0425 W <sub>p</sub> + 0.0025							
Ethanol 95%	TL <sub>fresh</sub> = 0.9812L <sub>p</sub> + 3.4276	85	0.970	13.02-25.05mm	0.99	ns	9.03	<0.001
	W <sub>fresh</sub> = 1.1281 W <sub>p</sub> + 0.0018							

while ethanol extracts much of the lipids present in specimens (Glauert, 1974). Thus, extraction of the lipids which are abundant in clupeids (e.g. *S. janeiro*) may account for the largest reduction in body mass.

The higher shrinkage in ethanol may partly be caused by a genuine shrinkage, and partly by higher rigidity of the fish after preservation compared with fish preserved on formaldehyde, making it harder to flatten fish (Kristoffersen & Salvanes, 1998). Thus, measures of the length of ethanol preserved fish may be underestimated when compared with formaldehyde preserved fishes. Therefore, the greatest losses of weight and length in ethanol in the present study may be caused by water and organic loss as well as by rigidity after preservation, which influences loss in body mass and length, respectively, when compared with specimens preserved in formalin and freezing.

In all preservation methods loss in length and body mass varied according to the time of preservation. Shrinkage was greatest soon after preservation, that is, mean shrinkage values in the first 5 days significantly exceeded zero ( $p < 0.001$ ) in most methods for both length and body mass. The only exception was for body mass in freezing and 5% formalin, where the greatest losses occurred after 30 days of preservation. According to Fey & Hare (2005) larvae of *Brevoortia tyrannus* (Latrobe, 1802) preserved in 95% ethanol shrinkage stabilizes within a few days. Larvae of *Cololabis saira* (Brevoort, 1856) preserved in 5% formalin have shown that the amount of shrinkage stabilizes after one month (Oozeki *et al.*, 1991). In general most studies agree that the length shrinkage stabilizes between a few days to one month (Fey, 1999; Fox, 1996; Smith & Walker, 2003; Moku *et al.*, 2004).

As the fish size increased, a decrease in shrinkage was observed in this study. This situation have been widely reported in the literature, such as for the clupeids *Clupea harengus* Linnaeus (Hay, 1982; Fey, 2002), *B. tyrannus* (Fey & Hare, 2005) and *Sprattus sprattus* Linnaeus (Fey, 1999) and other fish species such as *Pleuronectes americanus* (Walbaum, 1792) (Hjörleifsson & Klein-MacPhee, 1992), *Stizostedion vitreum* (Mitchill, 1818) (Johnson & Mathias, 1993), *Merluccius bilinearis* (Mitchill, 1814) (Fowler & Smith, 1983), *Gadus morhua* Linnaeus (Radtke & Waiwood, 1980; Radtke, 1989), and *Enchelyopus cimbrius* Linnaeus (Fey, 1999). This phenomenon is probably closely related to the decrease in the water content of larval tissues as length increases (Ehrlich, 1974). Additionally, it could be also likely a result of decreasing surface area in relation to volume ratios as reported by Hay (1984).

The size range is important to detect trends in shrinkage between small and large fishes. In this work, TL range from 15 to 28mm. This seems to be sufficient to detect changes in shrinkage between small and large fishes in all preservation methods. Thorstad *et al.* (2007) did not find correlation between body mass or length and mean shrinkage after preservation in ethanol, probably because of the more limited size range in juvenile European minnow *Phoxinus phoxinus* Linnaeus.

The length and body mass of preserved *S. janeiro* can be converted to fresh length and body mass through conversion equations. For this, a single correction may be applied to estimate fresh length and body mass from 60 days preserved specimens in 70% and 95% ethanol for length, and in 2.5% and 5% formalin for body mass. These findings are in accordance to Fey & Hare (2005) and Buchheister & Wilson (2005), who reported a single correction for length of *B. tyrannus*, *Theragra chalcogramma* (Pallas, 1814), *Mallotus villosus* (Müller, 1776) and *Thaleichthys pacificus* (Richardson, 1836) preserved in 95% ethanol. However, single correction can be applied only in situations with  $b$  (slope) = 1 and a ( $y$ -intercept) different from zero.

The complete equations for conversion between fresh and preserved individuals are valid when  $b \neq 1$  and the  $y$ -intercept  $\neq$  zero. This was the case for length in freezing, 2.5% and 5% formalin, and for body mass in 70% and 95% ethanol method. Additionally, linear regression provided good fits to plots of fresh lengths against stored lengths after stabilization. Hjörleifsson & Klein-MacPhee (1992), Fox (1996), Morkert & Bergstedt (1990) reported models to predict fresh lengths from preserved specimens of *P. americanus*, *C. harengus* and *Petromyzon marinus* Linnaeus. Furthermore, Smity & Walker (2003) proposed equations for both length and body mass conversion of *Cyprinus carpio* Linnaeus preserved in 70% and 95% ethanol. On the other hand, correction equation is not appropriate for *S. janeiro* body mass storage in freezing. These findings were also reported by Hjörleifsson & Klein-MacPhee (1992) for standard length of winter flounder *P. americanus* larvae frozen preserved. Predicting fresh body mass from frozen samples is impossible when high variability in shrinkage occurs.

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