

Correction of the weight and length for juveniles *Atherinella brasiliensis* (Actinopterygii: Atherinopsidae) after fixation in formalin and preservation in ethanol

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ABSTRACT. The present study compared losses of weight and length in specimens of *Atherinella brasiliensis* (Quoy & Gaimard, 1825) after fixation in formalin 10% during ten days, and posterior preservation in ethanol 70% for 45 days, in two solvents: freshwater and marine water. Additionally, correction factors were proposed to calculate the correction for weight and length from preserved specimens. The specimens were weighted and measured after collection (fresh), and with 10, 20, 27, 34, 41 and 55 days of fixation plus preservation. The largest losses in weight occurred during the first 20 days ($10.35 \pm 0.31\%$ – freshwater; $11.29 \pm 0.44\%$ – marine water), continuing with lesser intensity up to the 27th day in freshwater dilution, and up to the 34th day in marine water dilution. Weight losses stabilized by the 34th day for both freshwater ($11.77 \pm 0.33\%$) and marine water ($13.62 \pm 0.41\%$). The largest losses in the total length for the two methods were also observed in the first 20 days ($7.42 \pm 0.27\%$ – freshwater; $9.76 \pm 0.26\%$ – marine water), stabilizing after the 27th day in freshwater ($8.60 \pm 0.26\%$) and 20th day in marine water ($9.76 \pm 0.26\%$). The losses of weight and total length were significantly dependent on fish size, with smaller individuals suffering the most significant losses. Complete regression equations were proposed for the retro-calculation of fresh weight and total length from the preserved specimens for freshwater ($W_{\text{fresh}} = 1.0536W_p + 0.0416$; $TL_{\text{fresh}} = 0.9588TL_p + 5.8437$) and marine water ($W_{\text{fresh}} = 1.0868W_p + 0.0451$; $TL_{\text{fresh}} = 0.8621TL_p + 10.425$).

KEY WORDS. Back-calculation; initial length; shrinkage; size conversion.

Fixation of fish specimens in formalin 10% and posterior preservation in ethanol 70% is a widely used process in ichthyological sampling, due to the usually high number of specimens collected, and the time required to transport and analyze them. Despite its usefulness in keeping specimens preserved for future analysis, this method can alter the weight and length of the subjects (ARMSTRONG & STEWART 1997, KRISTOFFERSEN & SALVANES 1998, SANTOS *et al.* 2009).

The duration of preservation, age of the specimen when collected, and the preservation method employed are factors determining alterations in total weight and length. Overall, the most profound alterations are recorded during the first days after fixation, in small juvenile individuals (RADTKE 1989, FEY 1999, 2002) and in specimens preserved in ethanol in concentration near absolute (HJÖRLEIFSSON & KLEIN-MACPHEE 1992). Larvae preserved in ethanol 70% to 95% can lose from 5% to 40% of their total length (FOWLER & SMITH 1983, RADTKE 1989, JENNINGS 1991). According to TUCKER & CHESTER (1984), larval shortening is a function of the osmolarity of the liquid used for preserva-

tion. For example, formaldehyde diluted in marine water can lead to more significant shortening when compared with freshwater. According to MORKERT & BERGSTEDT (1990), larval length is frequently shortened by about 10% after fixation in formalin. This value tends to vary as a function of concentration, salinity and species.

It is common practice to estimate important population parameters such as size structure, growth rate, condition factor and length/weight relationship from preserved specimens. Variables obtained from the latter can be misleading (PORTER *et al.* 2001, SANTOS *et al.* 2005). In attempting to correct variations in weight and length that result from preservation techniques, several studies have suggested a correction factor to estimate the initial weight and total length of specimens using appropriate equations (BUCHHEISTER & WILSON 2005, FEY & HARE 2005, SANTOS *et al.* 2009). However, the calculations of weight and total length losses proposed in the literature are generally limited to the effects of either fixation (in formalin) or preservation (in ethanol). Such studies do not consider the combined

effects fixation followed by preservation techniques, which usually follow one another in the preparation of biological samples. The present study aims to quantify and compare the loss in weight and length in specimens of *Atherinella brasiliensis* (Quoy & Gaimard, 1825) fixed in formalin 10% and posteriorly transferred to ethanol 70% in two dilution means: freshwater and marine water. Additionally, we propose correction factors for retro-calculation of these measurements from preserved specimens.

MATERIAL AND METHODS

Information on weight and total length of *A. brasiliensis* juveniles was obtained from 312 specimens collected in the mangrove of the Biological and Archeological Reserve of Guaratiba (23°01'-23°03'S, 44°34'-44°37'W), state of Rio de Janeiro (southeastern Brazil). Specimens were collected in fall, 2009 with beach seines (12 x 2.5 m; mesh size 8 mm between opposite knots). Immediately after collection, specimens were conditioned in plastic bags immersed in ice boxes and transferred to the laboratory, where each individual was weighed with a precision of 0.0001 g and measured with a precision of 0.01 mm (fresh measurement). In all treatments each specimen was wrapped in towel paper to dry the body surface before measurements.

After being measured, fresh specimens were separated in two groups of similar size structure, and allocated to different water solutions (freshwater versus marine water). One group was fixed in formalin 10% (formaldehyde 38%, PA, diluted in freshwater), buffered with Borax 4% and pH 7.6. The other group was fixed in formalin 10% (formaldehyde 38%, PA, diluted in marine water) buffered with Borax 4% and pH 7.4. After 10 days in the fixation solution, both groups were transferred to ethanol 70% (ethanol 99.3° diluted in freshwater or in marine water).

Individuals were weighed and measured again 10, 20, 27, 34, 41 and 55 days after the first measurements. The weight and total length losses were determined for comparisons between fresh specimens and preserved specimens, according to the following equation: Percentage of losses (shrinkage%) = ((fresh-preserved)/fresh) x 100). Repeated measures ANOVA and TUKEY HSD test ($p < 0.05$) were used to analyze the significance of percent losses in weight and total length between day intervals over 55 days of preservation, and the t-test ($p < 0.05$) was used to compare the two dilution methods. For each preservation method a linear regression was used to describe the relationship between the two measurements (fresh and preserved) for the weight and the total length and a t-test ($p < 0.05$) was used to ascertain significant differences between the linear coefficient (y-intercept) from 0, and the angular coefficient (slope) from 1. It was used to determine the use of simple correction factor (y-intercept $\neq 0$; slope = 1) or the complete correction factor (y-intercept $\neq 0$, slope $\neq 1$) to estimate the initial weight and the total length of specimens.

RESULTS

The mean initial total length of *A. brasiliensis* juveniles was similar for the two groups submitted to the two different methods of preservation (t-test = 0.20, $p = 0.84$), indicating that they had been randomly selected, therefore allowing posterior comparisons. The results of the two methods of preservation (freshwater versus marine water) differed significantly after 55 days, for both weight and total length (t-test = 4.24, $p < 0.001$).

Dilution in freshwater

Significant differences were found in losses in weight and total length among the preservation times according to repeated measures ANOVA.

The most pronounced weight losses occurred during the first 20 days ($10.35 \pm 0.305\%$). There were no significant weight losses between 27 and 55 days (Tukey HSD, $p > 0.05$), after specimens had lost $12.35 \pm 0.48\%$ of their weight (Fig. 1). Weight remained stable after 27 days.

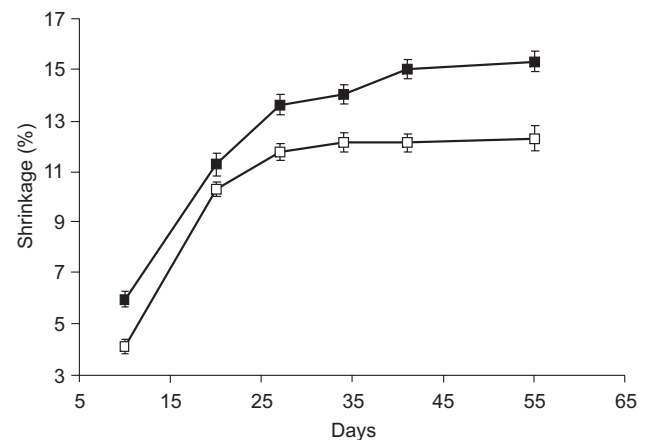


Figure 1. Shrinkage percentage in weight for *A. brasiliensis* juveniles in different dilutions: marine water (■) and freshwater (□).

The most pronounced decreases in total length were also observed in the first 20 days ($7.42 \pm 0.19\%$). No significant decreases in total length were observed after 27 days, an indication that length remained stable thereafter (Tukey HSD, $p > 0.05$); the mean loss after 55 days was $9.45 \pm 0.29\%$ (Fig. 2).

Dilution in marine water

The most pronounced reduction in weight occurred during the first 20 days ($11.29 \pm 0.44\%$). After 27 days no significant losses were observed in weight, indicating weight stabilization (Tukey HSD, $p > 0.05$) with cumulative mean losses in weight after 55 days being $14.91 \pm 0.42\%$ (Fig. 1).

The most pronounced decrease in total length was also observed in the first 20 days ($9.76 \pm 0.26\%$). No significant

losses in total length were observed after 20 days, indicating total length stabilizations (Tukey HSD, $p > 0.05$). Mean losses in total length after 55 days were $10.61 \pm 0.43\%$ (Fig. 2).

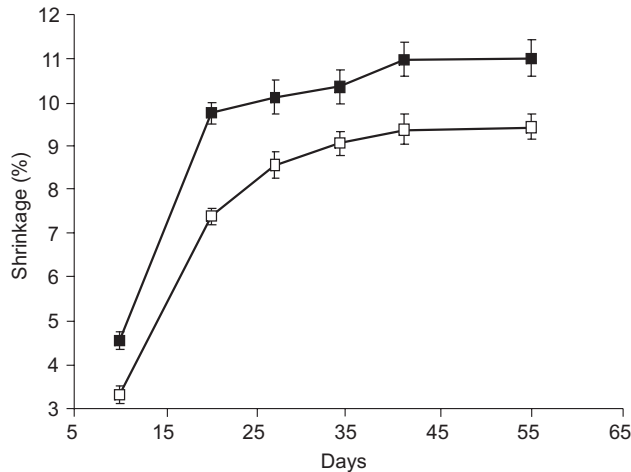


Figure 2. Shrinkage percentage in total length for *A. brasiliensis* juveniles in different dilutions: marine water (■) and freshwater (□).

Losses in weight and length varied according to the total length of specimens: smaller specimens had the most significant losses in the two preservation methods (t-test, H_0 : slope = 0, $p < 0.05$). The test of slope-homogeneity indicated that the fastest losses in length occurred in specimens diluted in marine water (Tukey HSD, $p > 0.0001$), while the losses in weight did not differ between the two preservation methods (Figs 3 and 4).

All linear coefficients (y-intercepts) resulting from regressions between fresh weight versus preserved weight, and fresh total length versus preserved total length were significantly different from zero (H_0 : y-intercept = 0; t-test, $p < 0.001$) in the two methods investigated (Figs 5 and 6).

Slopes (b) resulting from regression of fresh weight versus preserved weight and of fresh total length versus preserved total length for 55 days were significantly different from 1 (H_0 : slope = 1; t-test, $p < 0.05$); therefore the correction of weight and length should be performed by using the complete correction factor (Tab. I).

DISCUSSION

In our results, weight and total length losses in preserved specimens were associated with the preservation method. More precisely, the treatment using marine water accounted for the largest percentage losses overall. According to TUCKER & CHESTER (1984), alterations in body dimensions generally depend on the osmolarity of the preservative. Consequently, specimens kept in formaldehyde or ethanol diluted in marine water tend to undergo more marked alterations (loss in weight and length) when compared with specimens kept in formaldehyde or ethanol diluted in freshwater. MORKERT & BERGSTEDT (1990) recorded that lamprey larvae fixed in formalin 10% shrink according to formalin concentration and salinity. Our findings corroborate these results.

Ethanol has the capacity to extract water and lipids from tissues, resulting in weight losses (STEEDMAN 1976). Another factor that can account for differences in how much specimens decrease in length is the rigidity of specimens preserved in ethanol when compared with those fixed in formalin (KRISTOFFERSEN & SALVANES 1998), which makes measurements more difficult and leads to errors. Overall losses in specimens preserved in ethanol in the present study may be caused by losses *per se*, originated by organic losses, and also by comparatively higher rigidity of specimens after preservation.

Losses in weight and length by both methods have changed with time of preservation. According to FEY & HARE (2005) the length of juveniles of *Brevoortia tyrannus* (Latrobe, 1802) preserved in ethanol 95% stabilized within a few days. By contrast, the length of *Cololabis saira* (Brevoort, 1856) larvae preserved in formalin 5% stabilized after one month (OOZEKI *et al.* 1991). For several fish species, the length stabilized within a few days to one month (FEY 1999, SMITH & WALKER 2003, MOKU *et al.* 2004). Our findings indicate that juveniles of *A. brasiliensis* lose most weight and length up to 20 days into preservation.

The losses in total length recorded for *A. brasiliensis* were more pronounced in smaller specimens. This finding corroborates results obtained for other species (HJÖRLEIFSSON & KLEIN-MACPHEE 1992, FEY 2002, FEY & HARE 2005) such as *Pseudopleuronectes americanus* (Walbaum, 1792), *Clupea harengus* Linnaeus, 1758, *Osmerus eperlanus* (Linnaeus, 1758), and *B. tyrannus*. According to FEY (1999), alterations in larvae, post-

Table I. Correction equations to estimate the weight and total length for juvenile *A. brasiliensis*.

Preservation method	Regression equation	n	R ²	Total length and weight	t-test Slope = 1	p	t-test y-intercept = 0	p
Freshwater	TL _{fresh} = 0.9588TLP + 5.8437	156	0.98	26.72-64.44 mm	3.63	<0.05	7.52	<0.0001
	W _{fresh} = 1.0536WP + 0.0416			0.99	0.13-2.22 g	6.65	<0.05	5.28
Marine water	TL _{fresh} = 0.8621TLP + 10.425	156	0.96	27.01-63.89 mm	8.56	<0.05	11.88	<0.0001
	W _{fresh} = 1.0868WP + 0.0451			0.98	0.14-2.12 g	7.64	<0.05	4.70

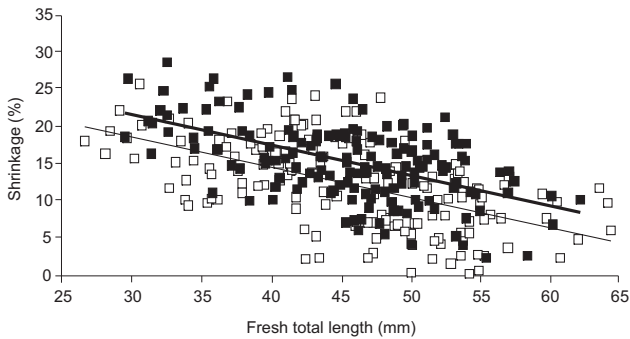


Figure 3. Shrinkage percentage in weight in relation to total length for *A. brasiliensis*: freshwater (□) ($y = -0.40x + 30.84$, $R^2 = 0.31$), marine water (■) ($y = -0.41x + 33.98$, $R^2 = 0.29$).

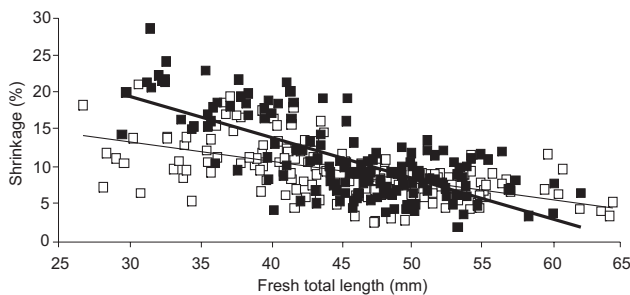


Figure 4. Shrinkage percentage for total length in relation to total length for *A. brasiliensis*: freshwater (□) ($y = -0.25x + 21.06$, $R^2 = 0.33$), marine water (■) ($y = -0.55x + 36.19$, $R^2 = 0.51$).

larvae and juveniles are a serious problem in ecological and biological studies of fish species, which can lead to errors in growth ratio estimation. Because preservation is inevitably associated with losses in weight and length correction factors are necessary to estimate initial weight and length using appropriate equations (BUCHHEISTER & WILSON 2005).

The weight and length of preserved *A. brasiliensis* can be converted to fresh weight and length by conversion equations. Conversion factors using complete equations are necessary to correct the weight and length of specimens in the present study. Our findings are corroborated by SANTOS *et al.* (2009) who proposed correction factor using complete equations for *Sardinella janieiro* (Eigenmann, 1894).

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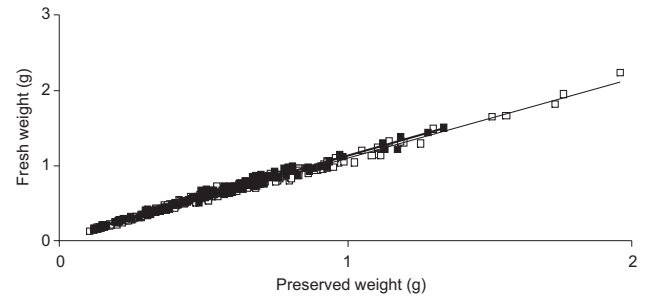


Figure 5. Relationship between fresh weight and preserved weight for *A. brasiliensis*: freshwater (□) ($y = 1.0536x + 0.0416$, $R^2 = 0.99$); marine water (■) ($y = 1.0868x + 0.0451$, $R^2 = 0.98$).

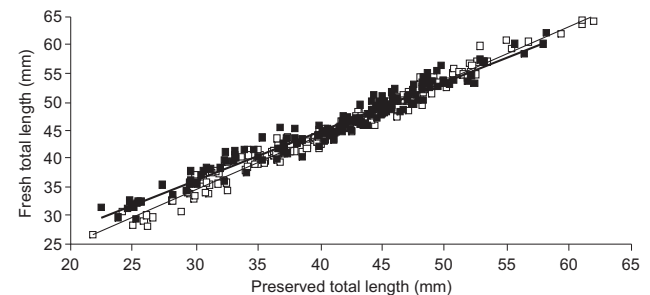


Figure 6. Relationship between fresh total length and preserved total length for *A. brasiliensis*: freshwater (□) ($y = 0.9588x + 5.8437$, $R^2 = 0.981$); marine water (■) ($y = 0.8621x + 10.425$, $R^2 = 0.96$).

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