Effect of preservatives on caloric density in the muscles of *Hoplias* aff. *malabaricus* (Bloch, 1794) (Osteichthyes, Erythrinidae)

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ABSTRACT. The preservation of samples for caloric values determination in bioenergetic studies of fish has been presented literature with a variety of methods. To identify the effect of preservatives over the results obtained with different preservation techniques, adult individuals of *Hoplias* aff. *malabaricus* (Osteichthyes, Erythrinidae) were collected in Paraná river floodplain. From each fish, five samples were extracted from its dorsal muscle and submitted each sample for a different treatment: ice (-10ºC), formaldehyde (4%), alcohol (70%), liquid nitrogen (-180ºC) and a control sample immediately oven dried (60ºC). After 30 days, the treatments were oven dried (60ºC) and caloric values of each sample were determined in a caloric bomb. Significant differences among the treatments were found (ANOVA: $n = 206$; $F = 50.28$; $p < 0.001$). Some evaluations of the effects of each treatment were presented for further comparisons with the results published in literature.

Key words: bioenergetic, calorimetry, *Hoplias* aff. *malabaricus*, preservatives.

RESUMO. Efeito dos conservantes sobre a densidade calórica em músculos de *Hoplias* aff. *malabaricus* (Bloch, 1794) (Osteichthyes, Erythrinidae). A conservação de amostras para a determinação dos valores calóricos em estudos de bioenergética de peixes tem se apresentado, na literatura, com uma variedade de métodos. Com o intuito de identificar o efeito de conservantes sobre os resultados obtidos com as diferentes técnicas de conservação, foram coletados exemplares adultos de *Hoplias* aff. *malabaricus* (Osteichthyes, Erythrinidae) na planície de inundação do rio Paraná. De cada exemplar, foram retiradas 5 amostras da musculatura, as quais foram submetidas aos seguintes tratamentos: formol (4%), álcool (70%), congelamento (-10ºC), nitrogênio líquido (-180ºC) e estufa à 60ºC (amostra controle). Depois de 30 dias, todas as amostras foram secas em estufa à 60ºC e posteriormente procedeu-se à determinação calórica de cada uma das amostras em bomba calorimétrica. Detectaram-se diferenças significativas entre os tratamentos (ANOVA: $N=206$; $F=50,28$; $p<0,001$). Para comparações com os resultados constatados na literatura, são apresentadas estimativas dos efeitos de cada tratamento.

Palavras-chave: bioenergética, calorimetria, *Hoplias* aff. *malabaricus*, conservantes.

Introduction

Many direct and indirect methods may be used to determine the energetic content of organic matter. The energy lost as heat by animals is determined through calorimeter, which is a general technique to measure heat flow between two bodies (Cho *et al.,* 1982). Calorimeter measures directly the quantity of heat released in the process of combustion of the matter (Doria and Andrian, 1997). The conservation of samples for later analysis of caloric values in studies of fish bioenergetics has been presented in the literature with a variety of preservatives: formaldehyde (Madon and Culver, 1993; Abitia-Cardenas *et al*., 1997); liquid nitrogen (Doria and Andrian, 1997), ice (Nishiyama, 1970; Kitchell *et al.*, 1977; Saldaña and Vernables, 1983; Rand *et al*., 1994; Hartman and Brandt, 1995; Bryan *et al*., 1996; Hop *et al*., 1997; Berg *et al*., 1998) and freeze-dried to constant mass (Mattingly and McLure, 1982). Many studies have been published without mentioning such preservatives used above or even other sort of preservatives, including alcohol, previously to the determination of calorimeters. The fact that differences exist in caloric results of different treatments might impair comparisons.

The effects of different preservatives on caloric contents in muscle samples of fish submitted to

different preservatives were investigated and these results were compared with those presented in published literature.

Material e methods

Forty-two adult individuals of *Hoplias* aff. *malabaricus* (Bloch, 1794) were collected in Baía River in May (high water period), and on October 1999 (low water period) in the lakes of Paraná River, near the municipality of Porto Rico-PR, Brazil. Standard length (Ls) in cm of each individual was taken and five samples of muscle were extracted closed to dorsal fin from the same fish. They underwent different preservation treatments before combustion by calorimetric bomb: alcohol (70%), formaldehyde (4%), liquid nitrogen (-180ºC), ice (-10ºC) and a control sample. This later sample was immediately dried at 60ºC before of any modifications associated of decompositions of the muscle tissues. After a 30-day period, samples from different preservatives were also oven dried in the same conditions like control sample (60ºC) and their caloric density determined at random in a Parr Model 1261 oxygen bomb calorimeter. Bonferroni correction was applied for multiple testing.

Results and discussion

The range of the standard length was 20.5cm to 35.5cm while total weight was between 174.7g and 567.2g.

The lowest caloric values belonged to samples conditioned in ice and liquid nitrogen, as well as that control samples oven dried (Figure 1). These results are associated with the power of evaporation during the process of dehydration. Formaldehyde forms crystals in the tissue cells cytoplasm. These crystals promote increase in caloric. In the case of alcohol, the large range is due to the different levels of evaporation during the dehydration. It is necessary to be more careful when obtaining the weight constant during the dehydration. Thus, samples in formaldehyde had the highest means, whereas samples in alcohol the highest confidence intervals. Samples in nitrogen, alcohol and formaldehyde were respectively enriched in 43.12, 165.15 and 292.14 cal/g dry wt in contrast to control samples. Only samples preserved in ice had their caloric values devoid of 50.06 cal. g/dry wt when compared to control samples. Significant differences between treatments were found (one-way ANOVA: N=206, F=50.28, p<0.001).

The matrix of pair wise comparison probabilities is shown in Table 1. Control samples were similar to values recorded in the case of freezing and liquid nitrogen samples.

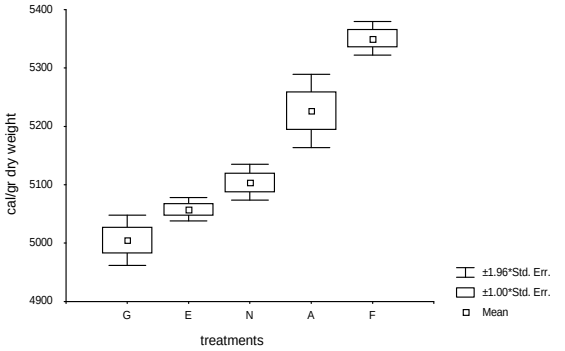

Figure 1. Means, standard error and confidence interval of caloric values (cal/gr dry weight) of muscles of adult *Hoplias malabaricus* submitted to different preservatives (G = frozen; E = control sample; N = nitrogen liquid; A = alcohol; F = formaldehyde)

Table 1. Matrix of pair-wise comparison probabilities of Scheffe's Test (A = alcohol; E = control samples; F = formaldehyde; G = frozen; N = nitrogen liquid) (⋆ significant 5%)

	A	E	F	G
A	1.000			
E	<0.001⋆	1.000		
F	<0.001⋆	<0.001⋆	1.000	
G	<0.001⋆	0.606	<0.001⋆	1.000
N	<0.001⋆	0.622	<0.001⋆	0.037

Researchers perceived seasonal variations in body composition and energy contents associated with a decrease in food availability in the environment, reproductive conditions, sex, age and ontogeny (Strange and Pelton, 1987; Doria and Andrian, 1997). In this research individuals were collected during two distinct periods (at high and low water). Periods indicate cyclic occurrence of food availability in the environment and species' s reproductive conditions. No significant differences were found between periods (ANOVA: N=206, F<0.01, P=0.982) and caloric values of treatments were seasonally independent from fat accumulated. When the caloric densities of samples were analysed according to size of individuals, they showed the same trends as those of the different preservation methods (Figure 2). Samples in alcohol and formaldehyde had the highest caloric values (ANCOVA: N=200, F=47.73, p<0.001). Length did not interfere on the caloric values (ANCOVA: N=200, F=0.133, p=0.716).

Protein (12.4-20.2%) and ash (1.0-5.2%) contents varied less than fat contents (0.2-28.8%) for the 21 species of fish of commercial fishery from the Amazon floodplain (Junk, 1976). In their studies on

fresh and sea water fish Economidis *et al.* (1981) found many similarities in the caloric and ash contents of species from both environments. Interval suggested by authors for fresh water species preserved in ice (5069±119 cal/g dry wt) coincides with values found during the present research in *Hoplias* aff. *malabaricus*. In this way, the results deduced from this species may be used with great reliability for other species of fish. However, such suppositions may not be valid for juveniles or even for small size fish, once their body composition is determined by metabolic rate. Further and stricter analyses are necessary before predictions may be accepted with any reliability.

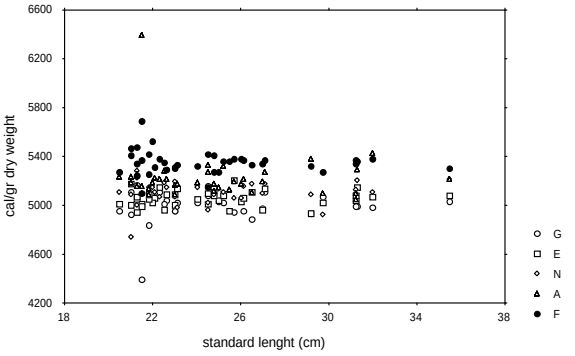

Figure 2. Relationship between caloric values (cal/gr dry wt) of muscle of *Hoplias malabaricus* submitted to different preservatives and standard length (G = frozen; E = control samples; N = nitrogen liquid; A = alcohol; F = formaldehyde)

Alcohol and formaldehyde cause an intense dehydration in biological material. Simultaneously they prevent protein destruction and decomposition of tissues by microbes' action. Therefore, the caloric increase caused by these preservatives may be dependent not only on chemical composition but also on water concentration in the organism, thus presenting an inherent caloric value. There is a general relationship between changes in energy density and modifications in water contents among taxa (Hartman and Brandt, 1995), water percentage in the individual is a good indicator of relative contents of lipids and actual energy. Lowest water percentages imply greater relative content of lipids and higher energy content in fish (Jonsson *et al*., 1997). Lipids seem to be readily depleted from the carcass stores, which, in turn, imply mobilisation to support energy requirements (Jorgensen *et al*., 1997). Thus, equations suggested for inter conversions may present angular (b) and intercept (a) coefficients dependent on interspecies variations of water concentration in the body. Table 2 shows correlation results between treatments. Only alcohol x formaldehyde, alcohol x ice and formaldehyde x ice treatments have inter convertible results ($p<0.05$), although their correlation coefficient was less than 0.74.

Table 2. Parameters of the linear equation between treatments with significant probabilities ($p<0.05$) (A = alcohol; F = formaldehyde; G = frozen; A = intercept, B= angular coefficient; R = Pearson's coefficient of linear correlation)

Treatment	A	B	p-level	R
G x A	11.340×10^3	-1.220	<0.001	0,74
G x F	4.069×10^3	0.253	0.014	0,40

There is an immediate application for researchers working on models and those using fishery management. Improvements have to be continually sought after so that the working of the ecosystem and the role of organisms in the energy flow can be constantly refined.

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