LIPID BODY COMPOSITION OF BLACK CATFISH, *Rhamdia quelen* (SILURIFORMES, HEPTAPTERIDAE), OF TWO POPULATIONS ADAPTED TO DIFFERENT ENVIRONMENTAL CONDITIONS

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ABSTRACT

The muscle lipid composition in fishes is affected by intrinsic (metabolic characteristics, migratory habits, age) and/or extrinsic factors (habitat, temperature, salinity). In particular, the fatty acids composition and the n-3:n-6 ratio in the different tissues are affected by salinity. In the present study, wild individuals of black catfish, *Rhamdia quelen,* were captured in two places exhibiting different environmental characteristics: a freshwater river (SG) and a shallow coastal lagoon (LR) with significant variations in salinity. Muscle lipid composition of individuals of the two populations was determined and compared. The highest values of total lipid were recorded in SG, reflecting, probably, the high food availability in the summer, period in which the fishes were captured. In the present study, the value of the n-3:n-6 ratio was approximately 2, characterizing a freshwater fish. However, when the two populations were analyzed, the value of the n-3:n-6 ratio in SG was significant lower than that registered in LR. This may be associated to ambient conditions and/or differences in the diets. While the specimens were collected from two different sites, during different seasons and under different environmental conditions, the ratios of the muscle essential fatty acids remained constant. Further studies should be held focusing these ratios under extreme environmental conditions in order to confirm these findings and, above all, to contribute to optimal diet formulation.

Key words: fatty acids; fish; black catfish; *Rhamdia quelen*; salinity; DHA; EPA

COMPOSIÇÃO LIPÍDICA DE JUNDIÁ, *Rhamdia quelen* (SILURIFORMES, HEPTAPTERIDAE), DE DUAS POPULAÇÕES ADAPTADAS A DIFERENTES CONDIÇÕES AMBIENTAIS

RESUMO

A composição lipídica do músculo de peixes é afetada por fatores intrínsecos (características metabólicas, hábitos migratórios, idade) e/ou extrínsecos (hábitat, temperatura, salinidade). Em particular, a composição em ácidos graxos e a relação n3:n6 em diferentes tecidos são afetadas pela salinidade. No presente estudo, indivíduos selvagens de jundiá, *Rhamdia quelen*, foram capturados em dois lugares com condições ambientais diferentes: um rio de água doce (SG) e uma lagoa costeira com significativas variações de salinidade. A composição em ácidos graxos do músculo de indivíduos das duas populações foi determinada e comparada. Os maiores valores foram registrados em SG, refletindo, possivelmente, a alta disponibilidade de alimento no verão, estação em que os indivíduos foram capturados. Neste estudo, o valor da relação n-3:n-6 esteve próximo de 2, caracterizando um peixe de água doce. Porém, quando comparadas as duas populações, observa-se que em indivíduos de SG o valor da relação n-3:n-6 é inferior ao registrado naqueles de LR. Este fato pode estar associado a condições ambientais e/ou dietas diferentes. Embora os exemplares tenham sido coletados em lugares, estações e condições ambientais diferentes, as relações entre ácidos graxos essenciais do músculo permaneceram constantes. Outros estudos que focalizem estas relações em condições ambientais extremas são necessários para confirmar estes dados e aportar informações para uma adequada formulação de dietas.

Palavras-chave: ácidos graxos; peixes; jundiá; *Rhamdia quelen*; salinidade; DHA; EPA

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INTRODUCTION

The black catfish, *Rhamdia quelen*, is a Siluriformes with an extensive geographical distribution, from the Central Region of Argentina up to southern Mexico (SILFVERGRIP, 1996). This fish species inhabits freshwater rivers and coastal lagoons with estuaries. It is omnivorous with carnivorous tendencies (RINGUELET *et al.*, 1967), and its diet is mainly composed by fish, insects, mollusks and crustaceans, employing a generalist approach (GARCÍA, 1995). This is a rustic species, presenting easiness to handle, excellent feed efficiency and rapid growth, even in the winter months, making it a good candidate for aquaculture production (LUCHINI, 1990; FRACALOSSI *et al.*, 2002). During the period from 1990 to 1999 there has been a continuous growth in the cultivation of this species in Brazil (FAO, 2000), particularly in the southern region (ULIANA *et al.*, 2001; MEYER and FRACALOSSI, 2004).

The body composition of fish, in particular the muscle lipid composition, is the result of its feed (STEFFENS, 1987; JUSTI *et al.*, 2003). However, the fatty acid composition may be affected by intrinsic factors, such as those related to metabolism (ROBIN *et al.*, 2003), migratory habits (SHERIDAN *et al.*, 1985; DE SILVA *et al.*, 1997), age or stage of development (SOIVIO *et al.*, 1989), or extrinsic factors: habitat (DE SILVA *et al.*, 1998), temperature (TAKEUCHI and WATANABE, 1982), and salinity (BORLONGAN and BENITEZ, 1992). The composition in fatty acids, essentially the polyunsaturated fatty acids (PUFAs), and the ratio between the series n-3: n-6 in the different tissues are affected by salinity. This ratio is more elevated in marine fish than in freshwater one (ACKMAN, 1998). Moreover, the recent work of HALILOGLU *et al.* (2004) with rainbow trout (*Oncorhynchus mykiss*), that fed the same diet but was exposed to different salinities, showed variation in the content of fatty acids in muscle and adipose tissues. This may be due to the structural and permeability roles played by fatty acids of the series n-3 in the membranes.

Thus, studies pointing out the lipid body composition of native species adapted to different environmental conditions represent an important contribution to the understanding of nutrient requirements for this species.

The aim of the present study is the comparison of the lipid composition in muscle tissue of black catfish of two populations adapted to distinctive habitats, with different salinity levels.

MATERIAL AND METHODS

Specimens of black catfish, *Rhamdia quelen,* (n=14) were captured in two places exhibiting different environmental characteristics: Negro River, in the San Gregorio zone (Departamento de Tacuarembó, Uruguay), and Laguna de Rocha (Departamento de Rocha, Uruguay) (Figure 1). The first place is a freshwater river, and the second one, a shallow coastal lagoon with an intermittent confluence with the Atlantic Ocean through a sand dam. This produces significant variations in salinity (range: 0.0 to 30.0 ppm.) throughout the year (CONDE *et al.*, 1999; AUBRIOT *et al.*, 2004). The fishes were captured with stationary nets in December 2002 from Negro River (SG) and in April 2003 from Laguna de Rocha (LR).

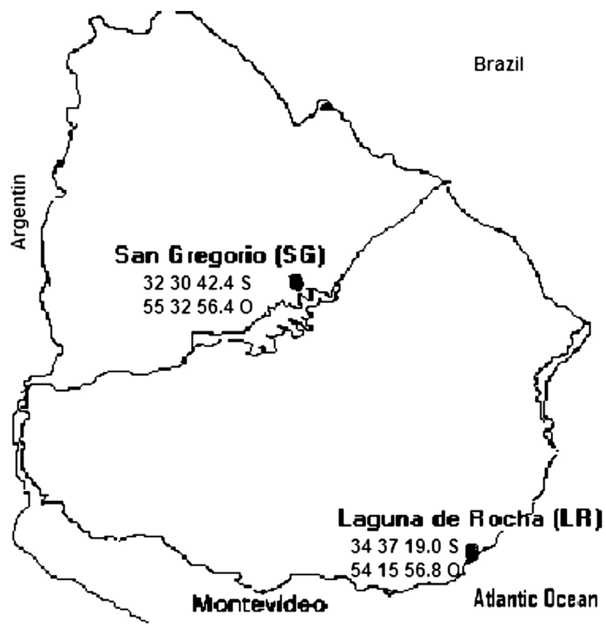

Figure 1. Map of Uruguay showing the two points of capture of black catfish, *Rhamdia quelen*: Negro River (SG), a freshwater river in the San Gregorio zone, and Laguna de Rocha (LR), a shallow estuarine lagoon

The collected fish specimens were weighed, measured and sexed when gonadal maturity was necessary. Muscle samples, representative of the edible portion, were obtained by cutting a steak from the right dorsal region. These samples were frozen at –20°C and lyophilized to facilitate handling. The muscle total lipid was determined by extraction with chloroform and methanol (2:1, v v^{-1}), according to the method described by FOLCH *et al.* (1957). Fatty acid methyl esters (FAMEs) were prepared through acid-catalyzed transesterification utilizing sulphuric acid in methanol, in accordance with the method of CHRISTIE (1982). FAMEs were separated and quantified by gas chromatography in a Hewllet Packard model 5890. Hydrogen was used as a carrier gas and temperature programming was from 180 °C (12 min) to 212 °C at 2 °C min^{-1}, with the temperature maintained during seven minutes. For the identification and quantification of the individual FAMEs, known standard and internal standard (C19:0, nonadecanoic acid) were used respectively.

The comparison of weight and length between the two populations was performed by a ***t*** test, while the comparison among the mean values of the different fatty acids and the ratios: n-3 : n-6, docosahexaenoic acid (DHA) : eicosapentaenoic acid (EPA), DHA : arachidonic acid (AA), EPA : AA were determined by a one-way ANOVA method. Differences between means were determined by Tukey´s test with a 5% significance level (STEEL and TORRIE, 1980).

RESULTS

In the present study, 14 individuals of black catfish, *Rhamdia quelen,* were utilized: seven individuals from SG (four females and three males) captured in December, and seven individuals from LR (four females and three males) captured in April. No differences were recorded between the length means (SG: 37.9±3.9 cm and LR: 40.1+4.9 cm; p=0.2959) and weight means (SG: 668.1±159.6 g and LR: 780.9+214 g; p=0.2993) for the specimens of the two populations.

The values for total lipids, expressed in dry weight (d.w.), were: 114±85.5 mg g^{-1} for SG and 41.5±6.8 mg g^{-1} for LR; these values expressed as percentage of wet weight were: 7.2±5.7% for SG and 3.3±0.48% for LR. The total lipids values in the specimens from the population of Negro River were significantly higher then those presented by the individuals from the population of Laguna de Rocha ($p < 0.1$). A total of forty-eight fatty acids were identified in the muscle tissue of individuals of the two populations of black catfish. Palmitic acid (C16:0) and oleic acid (C18:1n-9) were the most abundant fatty acids in the two studied populations, followed by C16:1n-7 in SG and C22:6n-3 in LR. Within the HUFAs group, the recorded values were: 7.4% DHA; 3.6% AA; and 2.3% EPA, for individuals of SG, and 10.2% DHA; 5.4% AA; and 3.3% EPA, for individuals of LR (Table 1).

Table 1. Fatty acids profile (expressed as percentage of recognized fatty acids) and ratios between fatty acids in the dorsal muscle of wild black catfish, *R. quelen*, captured in Negro River (SG) and in Laguna de Rocha (LR). The values are mean±std of seven individuals from each ambient.

Fatty acid	*SG*	*LR*
16:0	20.08 ± 1.16	21.75 ± 1.59 *
16:1 n-7	11.14 ± 2.82	5.63 ± 1.52 *
16:4 n-3	0.22 ± 0.18	0.58 ± 0.07 *
18:0	6.29 ± 1.10	9.31 ± 0.72 *
18:1 n-9	14.75 ± 3.45	12.60 ± 1.9 n.s.
18:2 n-6	3.38 ± 0.72	2.93 ± 0.75 n.s.
18:3 n-9 ± n-6	0.39 ± 0.08	0.19 ± 0.02 *
18:3 n-3	2.26 ± 0.43	1.50 ± 0.26 *
18:4 n-3	1.39 ± 0.63	3.07 ± 0.40 *
20:2 n-6	0.59 ± 0.07	0.21 ± 0.03 *
20:3 n-9	0.12 ± 0.08	0.70 ± 0.08 *
20:4 n-6 (AA)	3.57 ± 1.12	5.38 ± 0.91 *
20:3 n-3	0.42 ± 0.07	0.38 ± 0.07 n.s.
20:5 n-3 (EPA)	2.30 ± 0.57	3.26 ± 0.33 *
22:3 n-6	0.26 ± 0.07	0.15 ± 0.03 *
22:5 n-3	3.33 ± 0.64	3.28 ± 0.63 n.s.
22:6 n-3 (DHA)	7.39 ± 2.10	10.18 ± 2.17 *
Sat	33.91 ± 2.89	39.9 ± 1.90 *
MUFAs	36.07 ± 6.23	25.1 ± 2.60 *
n-3	18.02 ± 3.15	23.2 ± 2.80 *
n-6	9.04 ± 0.80	9.0 ± 1.30 n.s.
n-9	17.70 ± 3.40	15.0 ± 2.00 *
n-3:n-6	2.01 ± 0.33	2.6 ± 0.40 *
n-3 PUFAs	17.81 ± 3.03	5.9 ± 0.90 n.s.
n-6 PUFAs	9.04 ± 0.80	22.5 ± 2.9 n.s.
n-3 HUFAs	14.15 ± 2.64	9.0 ± 1.3 n.s.
n-6 HUFAs	5.27 ± 1.15	2.6 ± 0.2 n.s.
DHA:EPA	3.47 ± 1.57	3.1 ± 0.5 n.s.
DHA:AA	2.08 ± 0.30	1.9 ± 0.2 n.s.
EPA:AA	0.69 ± 0.26	0.62 ± 0.12 n.s.

Values with asterisk are significantly different at $p < 0.05$ according to ANOVA. n.s.= no significant
Sat=Saturated; MUFAs=Monounsaturated Fatty Acids; PUFAs=Polyunsaturated Fatty Acids; HUFAs=Highly Unsaturated Fatty Acids

Significant differences in HUFAs were observed, being the highest values registered in LR. Ratios C20:3n-9 : AA and C20:3n-9 : DHA were, respectively, 0.03 and 0.02 for specimens of SG, and respectively 0.13 and 0.07 for specimens of LR. The ratio n-3 : n-6 differed in the two populations ($p < 0.05$). However, the ratios of HUFAs (DHA : EPA, DHA : AA, EPA : AA) showed no significant differences in both the populations. The values for these ratios were respectively 3.47; 2.08; and 0.69 for SG and 3.11; 1.89; and 0.62 for LR (Table 1). The existence of a correlation between the fatty acid 16:1n-7 and the sum of PUFAs was recorded within each population: y= -0.84+36.24 (r=0.72) for SG and y= -0.35+16.6 (r=0.92) for LR.

DISCUSSION

The lipid content in many species of Siluriformes is usually low compared to that of other fish species (FACONNEAU and LAROCHE, 1996). In the present study, the values of total lipids in dry weight basis (114$\pm$85.5 mg g^{-1} d.w. for SG and 41.5$\pm$6.8 mg g^{-1} d.w. for LR) and wet weight basis (7.3% w.w. for SG and 3.3% w.w. for LR) are similar to those of other species of the same order: *Ictalurus punctatus,* 7.4% w.w. (CLEMENT and LOVELL, 1994); *Ictalurus nebulosus,* 2.7% w.w. (HENDERSON and TOCHER, 1987); *Clarias gariepinus,* 2.2% w.w. (NG *et al.*, 2003); *Heteropneustes fossilis,* 0.86% w.w. (HENDERSON and TOCHER, 1987); and *Silurus glanis,* 3% w.w. (MANTHEY *et al.*, 1988). The high variability in the muscle lipid content of fish is a well-documented fact (ZENEBE *et al.*, 1998). This is due to several factors: diet (JUSTI *et al.*, 2003), temperature (TAKEUCHI and WATANABE, 1982), salinity (BORLONGAN and BENITEZ, 1992), metabolic features (ROBIN *et al.*, 2003), age (SOIVIO *et al.*, 1989) and/or genetics. Within the order Siluriformes, various studies confirm this variability: *Clarias gariepinus,* with a variation of 18.7 to 90.8 mg g^{-1} d.w. (ZENEBE *et al.*, 1998), and *Ictalurus punctatus,* in which the amount of total lipids oscillates between 0.5% and 3% w.w. (CHANMUGAN *et al.*, 1986). In the present study, a high level of variability in lipid content in the muscle tissue of *Rhamdia quelen* was observed. The highest values were recorded in specimens of the SG population. This may be due to the high food availability in the summer, period in which the fish were captured. An increase in the diversity of diet of the black catfish in the hot months was pointed out by GARCIA (1995), studying a freshwater habitat located near the site of the current study.

As in other species of catfish, the main fatty acids recorded in this work were C16:0 and C18:1n-9, important sources of energy (SHIRAI *et al.*, 2002; TOCHER, 2003). Elevated concentrations of the fatty acid C20:3n-9 in the muscle is a signal of deficiency in the fish diet (WATANABE, 1982). Thus, it has been proposed that the ratios C20:3n-9:AA and C20:3n-9:DHA could serve as indicators of essential fatty acid deficiency for carp, with the ratio between these two fatty acids never exceeding 0.4 and 0.6 respectively (CASTELL *et al.*, 1972; WATANABE, 1975). The ratios C20:3n-9:AA and C20:3n9:DHA detected in the populations of the two studied sites were markedly low, suggesting that the animals exhibited no deficiency in essential fatty acids in the moment of capture.

The significantly lower values of DHA observed in the muscle of fish from SG when compared with the values registered in the fish from LR ($p < 0.05$) can be explained by the possible accumulation of DHA in the gonads, once the capture in SG was held during the species reproductive period (LUCHINI, 1990). According to JEONG *et al.* (2002), an accumulation of DHA in the gonad can be observed in the reproductive period of fish, fundamentally in the male, in which this fatty acid plays an important role in the structure of spermatozoon membrane. However, the higher content of EPA and AA in the animals of LR might also be explained by the need to adapt to a more stressful environment with pronounced changes in salinity. This observation was also made by HALILOGLU *et al.* (2004), examining the muscle tissue of rainbow trout cultivated in different levels of salinity. The mentioned fatty acids are precursors of eicosanoids (MUSTAFA and SRIVASTAVA, 1989), having an important function in the osmoregulation (JABS *et al.*, 1989; SARGENT *et al.*, 1999). Other feasible explanation is that the animals of SG were fattier than those of LR, leading different proportions of triacylglycerols (TAG) and phospholipids (PL) in the total lipids of fish from the two populations. In general, fattier fish showed a higher proportion of TAG, and these showed a lower proportion of HUFAs when compared to PL (ACKMAN, 1998). More studies separating the polar (PL) and neutral (TAG) fraction must be developed to light up this point.

The n-3:n-6 ratio in marine fish normally varies from 4.7 to 14.4, while in freshwater fish it oscillates between 0.5 and 3.8 (STEFFENS, 1997; DE SILVA *et al.,* 1998). In the present study, this ratio was approximately 2, characterizing a freshwater fish. Our findings agree with the values of the ratio n-3: n-6 observed for the wild African catfish (*C. gariepinus*), in which such values were 2.5±1.1 (ZENEBE *et al.,* 1998). However, when the two populations were compared, LR individuals showed a significant superior n-3:n-6 ratio and n-3 content in the muscle. This fact may be associated to two factors: ambient condition (salinity) and/or differences in the diets. In this way, HALILOGLU *et al.* (2004) demonstrated in rainbow trout the significant effects of the salinity in the n-3:n-6 ratio in adipose tissue and liver but not in gonad and muscle.

On other hand, in the present work an inverse relation between the 16:1n-7 content and the PUFAs content in muscle were registered in both fish populations. The same relationship was recorded for aquatic invertebrates, mainly insects (BELL *et al.,* 1994; GHIONI *et al.,* 1996), which are part of the catfish food chain. In this way, 23% of the diet of adult black catfish in Negro River are composed by insects (GARCÍA, 1995). This may possibly reflect the important role played by the diet in the determination of muscle lipid content. Unfortunately, there is not enough information about diet composition of the specimens captured in the Laguna de Rocha. Future studies correlating the lipid content profile in the muscle with the feeding habits of the species *Rhamdia quelen* within different environments are essential for determining the importance of the diet to this characteristic.

The metabolic interactions which exist among the PUFAs and the need of different tissue for each essential fatty acid: DHA, EPA, and AA, suggest that in order to evaluate these requirements not only the absolute values of each fatty acid in the tissue but also the ratio between them must be considered (SARGENT *et al.,* 1999). In the present study, while the specimens were collected from two different sites, during different seasons and under different environmental conditions, the ratios of the essential fatty acids remained constant in the muscle tissue. The ratio EPA:AA in the diet is important for determining the action of the eicosanoids (SARGENT *et al.,* 1999). Further studies should be held focusing the EPA:AA ratio under extreme environmental conditions, in order to confirm these findings and, above all, to contribute to optimal diet formulation, maximizing the production of the black catfish, *Rhamdia quelen*.

CONCLUSION

In general trend, the values of total lipids and ratio n-3:n-6 obtained in this study for the black catfish, *Rhamdia quelen*, are typical of freshwater fishes and similar to those encountered in species of the same family. However, in the LR population, adapted to salinity changes, a higher n-3:n-6 ratio and n-3 content were registered, probably due to different food habits or different environmental condition, like observed in others studies. While the specimens were collected in different sites and seasons and under different environmental conditions, the HUFAs ratios remained unchanged in the muscle tissue. This fact may be associated with the action of the eicosanoids and their important functions. Further studies should be held focusing in the HUFAs ratio under extreme environmental conditions in order to confirm these findings and, above all, to contribute to optimal diet formulation, maximizing the production of this species.

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