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Short communication

Fatty acid composition of wild *Odontesthes bonariensis* (Valenciennes 1835) larvae: implications on lipid metabolism and trophic relationships

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Summary

The fatty acid composition of the pejerrey *Odontesthes bonariensis* larvae was studied to elucidate potential dietary relationships. Their principal fatty acids were characteristic for membrane lipids of aquatic organisms. The fatty acid composition varied little throughout seasons, with high proportions of 22 : 6(n-3) (27% of total fatty acids), which is biosynthesized *de novo* from dietary precursor fatty acids and/or accumulated from the diet. Other major fatty acids were 16 : 0 and 18 : 0. The diatom-typical 16 : 1(n-7) and other dietary fatty acids (zooplankton and microplankton) are not reflected in the larvae, thus limiting the use of fatty acids as trophic markers for food web relationships of atherinopsids.

Introduction

Fatty acid markers have been widely used to understand trophic relationships because they are transferred conservatively through the food web and indicate the assimilated diet. The fatty acid profile of a particular fish larvae species defines not only its diet but also its capacity of lipid biosynthesis, conversion and accumulation (Wiegand, 1996; Işik et al., 1999; Izquierdo et al., 2008). The polyunsaturated fatty acids 22 : 6(n-3), 20 : 5(n-3) and 20 : 4 (n-6) are considered essential because they play structural-functional roles in the membranes and are precursors of hormones. There is strong evidence in aquaculture that essential fatty acids sustain fish growth, development and reproduction (Sargent et al., 1995; Bogut et al., 2002; Köse and Yildiz, 2013). Despite the ecological and commercial importance of these fatty acids, little work has been carried out on native South American fishes.

The pejerrey *Odontesthes bonariensis* (Valenciennes 1835) is probably the most important native fish from Argentinian inland waters, and has also been introduced into several other countries. This typical zooplanktivorous fish is highly appreciated for its commercial value but is vulnerable to climate change due to the eutrophy and hydrological instability of its habitat and thermolabile sex determination (Kopprio et al., 2010). Although *O. bonariensis* is described as a freshwater species, its maximum size and weight is reached at intermediate salinities. The aim of this work was to understand the origin and modification of fatty acids from dietary sources in wild *O. bonariensis* larvae. Due to its zooplanktivorous feeding behaviour, we hypothesise that there is a fatty acid composition of the larvae that indicates the composition of their food items.

Materials and methods

Lake Chasicó is a circa 65 km^2 endorheic water body located in the Argentinian Pampa (38°37′S, 63°05′W). Limnological characteristics, fish larvae and plankton dynamics and location of sampling stations were described by Kopprio et al. (2010). Briefly, Lake Chasicó is at present eutrophic with clear waters and a moderate salinity (~20). Three sampling stations were selected: (i) Chapalcó Village (CV); (ii) the fishing spot "El Embudo" (EE); and (iii) the plant nursery "El Vivero" (EV), near the mouth of Chasicó River. Horizontal towing with a 500 μm mesh size Bongo net was used to catch *O. bonariensis* larvae monthly from October 2007 (early spring) to May 2008 (late autumn). However, larvae were only sporadically encountered, resulting in a total set of 13 samples throughout the campaign. Zooplankton were sampled by vertical net towing (200 μm mesh size) and transferred in 500 ml filtered lake water (GF/F Whatman). Microplankton were fractionated first with a net of 200 μm and then with a net of 20 μm mesh size and finally suspended in 500 ml filtered lake water. For lipid analysis, about 10–20 fish larvae (from 6 to 15 mm total length) were preserved in dichloromethane-methanol (2 : 1 by vol.) under nitrogen atmosphere at −30°C. Two aliquots of 100 ml from the 500 ml containing live plankton were filtered through GF/F filters and preserved as described above.

Lipids of the larvae of each sampling station and month (n = 13) and the filters containing plankton were extracted basically after Folch et al. (1957). The total lipid extract was transesterificated under nitrogen atmosphere with 3% concentrated sulphuric acid in methanol for 4 h at 80°C. The resulting fatty acid methyl esters were analysed by gas-liquid chromatography (Hewlett Packard 6890 GC) on a 30-m wall-coated capillary column (inner diameter 0.25 mm, film

thickness 0.25 μm; liquid phase DB-FFAP) according to Kattner and Fricke (1986). Fatty acids were quantified with 19 : 0 and 23 : 0 fatty acid methyl esters and identified with standard mixtures. Further confirmation was carried out by mass spectrometry.

From the percentages of the 46 fatty acids detected, those with ≥1% (mass % of total fatty acids) were considered. Differences of each fatty acid among groups were tested with the non-parametric Kruskal–Wallis one-way analysis of variance. Samples were ordinated by Principal Component Analysis (PCA) in PRIMER v5 to visualize their relationship to the fatty acids.

Results

Significant differences in fatty acid compositions were found among larvae of *O. bonariensis*, zooplankton and microplankton (Table 1). Larvae contained principally 22 : 6(n-3), 16 : 0 and 18 : 0. The fatty acids 16 : 0 and 22 : 6(n-3) were also important in zooplankton as well as 16 : 1(n-7). Microplankton presented the highest levels of 16 : 0, 16 : 1(n-7) and 20 : 5(n-3). The highest proportions of 20 : 4(n-3), 18 : 4(n-3) and 18 : 3(n-3) were observed in zooplankton. For the larvae, the 22 : 6(n-3)/20 : 5(n-3) ratio was about six and 12 times higher than that of zooplankton and microplankton, respectively.

In the PCA ordination, the larvae were arranged homogeneously, while the plankton groups had a higher heterogeneity (Fig. 1). The first axis explains the 76% and the second axis the 9% variability. Figure 1 summarizes the relations of the fatty acids with the principal components. The 22 : 6(n-3) (0.78) and 16 : 1(n-7) (−0.49) were fatty acids of higher coefficients in the linear combination of variables with the first component. This axis separated clearly the larvae at positive values from plankton principally by these two fatty acids. The larvae were also

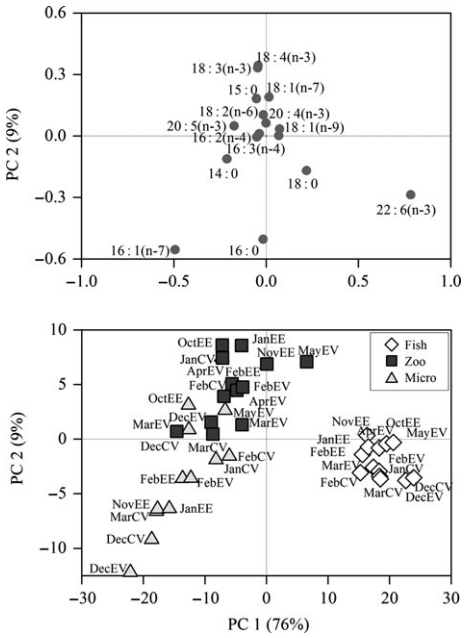

Fig. 1. Summary of the fatty acids with the principal components. The 22:6(n-3) (0.78) and 16:1(n-7) (−0.49) were the fatty acids of higher coefficients in the linear combination of variables with the first component. This axis separated clearly the larvae at positive values from plankton principally by these two fatty acids

negatively associated with the second component mainly due to their percentages of 16 : 0.

Fish larvae, located at the positive extreme of the first component, were collected in May at Station EV and in December at Stations CV and EV, being characterized by the highest individual percentages of 22 : 6(n-3) (~ 35%). Moreover, the larvae at Station EV in May had the maximum levels of 20 : 5(n-3) (9%). The maximum of 18 : 4(n-3) (~5%) and 20 : 4(n-3) (~2%) in the larvae lipids were found

Table 1
Composition of the main fatty acids (mean ± standard deviation of mass % of total fatty acids, n = 13) in larvae of *Odontesthes bonariensis*, zooplankton and microplankton collected from October 2007 to May 2008 at the three sampling sites. Data for zooplankton and microplankton were only considered when larvae were found. Chi square (X^2) and significance (P) after Kruskal–Wallis one-way analysis of the variance

Fatty acids	*O. bonariensis*	Zooplankton	Microplankton	$X^2_{2,\ 36}$	P
Saturated					
14 : 0	1.2 ± 0.2	6.7 ± 1.4	7.7 ± 3.4	21.7	<0.001
15 : 0	1.3 ± 0.5	3.9 ± 0.9	2.8 ± 2.1	19.2	<0.001
16 : 0	18.9 ± 2.4	17.6 ± 1.7	23.9 ± 3.6	20.2	<0.001
18 : 0	10.1 ± 2.5	3.5 ± 0.7	3.9 ± 2.0	24.5	<0.001
Monounsaturated					
16 : 1(n-7)	4.5 ± 0.8	14.5 ± 4.6	20.1 ± 6.9	27.5	<0.001
18 : 1(n-9)	4.8 ± 1.1	3.6 ± 0.9	3.6 ± 1.5	16.6	<0.001
18 : 1(n-7)	3.5 ± 0.6	5.8 ± 0.9	3.1 ± 1.5	24.0	<0.001
Polyunsaturated					
16 : 2(n-4)	0.3 ± 0.3	1.5 ± 0.5	1.6 ± 0.6	24.7	<0.001
16 : 3(n-4)	0.4 ± 0.2	0.9 ± 0.5	2.8 ± 2.3	20.2	<0.001
18 : 2(n-6)	1.8 ± 0.8	2.7 ± 0.8	2.4 ± 2.2	8.9	0.012
18 : 3(n-3)	1.2 ± 0.6	5.4 ± 2.1	2.4 ± 1.3	23.4	<0.001
18 : 4(n-3)	0.7 ± 0.7	4.8 ± 2.5	2.0 ± 1.3	21.4	<0.001
20 : 4(n-6)	3.2 ± 1.2	2.1 ± 0.5	1.2 ± 0.6	22.9	<0.001
20 : 4(n-3)	0.6 ± 0.5	1.2 ± 0.7	0.9 ± 1.0	3.6	0.165
20 : 5(n-3)	4.4 ± 1.5	9.8 ± 2.2	11.0 ± 2.2	24.5	<0.001
22 : 6(n-3)	26.8 ± 3.4	11.9 ± 4.5	5.7 ± 2.3	31.4	<0.001
Ratio					
22 : 6(n-3)/ 20 : 5(n-3)	6.7 ± 1.9	1.2 ± 0.3	0.6 ± 0.2	32.8	<0.001

in October at Station EE. The samples of late summer (January at Station CV and February at Station CV and EV) were ordinated to the left of the group. These larvae had the highest proportions of 16 : 0 (~ 25%) and 18 : 1(n-9) (~7%) and the lowest of the 22 : 6(n-3) (~ 28%). Furthermore, the maximum of 18 : 0 (~17%) was observed in larvae from February at Station EV.

The zooplankton samples from May at Station EV and November at Station EE were more similar to the larvae of *O. bonariensis* mainly because of the higher percentages of 22 : 6(n-3) (23 and 17%, respectively). In October at Station EE, zooplankton had the highest percentage of 18 : 4(n-3) (~10%) of all fractions. Other samples of zooplankton more related to the microplankton were characterized by lower proportions of the main polyunsaturated fatty acids and higher proportions of saturated and monounsaturated fatty acids.

Discussion

The fatty acids of the larvae of *O. bonariensis* were typical for membrane fatty acids of aquatic fishes, with little variation throughout locations and months. The 22 : 6(n-3) fatty acid together with 16 : 0 and also often with 20 : 5(n-3) are fundamental components of membranes in aquatic organisms (Sargent et al., 1995). Owing to its essential membrane functions 22 : 6(n-3) is crucial for the optimal development of larvae (Petursdottir et al., 2008; Tocher, 2010). As reviewed by Parrish (2009), polyunsaturated fatty acids are important in teleosts for normal somatic growth, survival, neuronal development, pigmentation and reproduction. They are generally ingested and accumulated from the zooplankton and microplankton diet, although some freshwater fishes are able to synthesize polyunsaturates from dietary precursors such as 18 : 2(n-6) and 18 : 3(n-3) (Bell and Tocher, 2009; Tocher, 2010).

Fatty acids serve as trophic markers; however, the planktivorous larvae of *O. bonariensis* scarcely reflected the main fatty acids from microplankton and zooplankton. The most abundant fatty acid in the diet, the diatom marker 16 : 1(n-7), is only of minor importance in the fish larvae and therefore probably used for energetic requirements. Also the other diatom marker and essential fatty acid 20 : 5(n-3), being a major component in microplankton and zooplankton, was less abundant in the fish larvae. The 18 : 0, which was about three times higher in the fish larvae than in the plankton fractions, is probably *de novo* synthesized. It supposes a higher carnivory of fish larvae.

The small monthly changes in the fatty acid composition of the fish larvae reflect a minor dependence of the fatty acid composition of fish larvae on the plankton fatty acids. In larvae from autumn, higher proportions of 22 : 6(n-3) coincided with higher levels in zooplankton and microplankton. During autumn, the higher abundance of dinoflagellates reflected in higher proportions of 22 : 6(n-3) and 18 : 4(n-3) in the plankton fractions, did not follow the trend of 22 : 6(n-3) in the larvae. The similar composition of the yolk sac of the larvae may contribute also to the relative uniform composition of fatty acids. However, the yolk sac is more than six times shorter than the total body length in *O. bonariensis* larvae immediately after hatching, and the survival of these larvae is associated with their skills to immediately access live food (Chalde et al., 2014).

The fatty acid composition of the *O. bonariensis* larvae is barely suitable to reveal predator-prey relationships as presented for the pelagic marine environment. The same was reported for the Mexican silverside *Chirostoma estor* Jordan 1879; this species also does not reflect the fatty acid composition of its diet (Palacios et al., 2007). The fatty acid compositions of the eggs, embryos, and the *C. estor* larvae were very similar to that of the *O. bonariensis* larvae. Both species belong to the family Atherinopsidae and display an elevated 22 : 6(n-3)/20 : 5(n-3) ratio, which may indicate chain extension. Both seem to be able to elongate and desaturate the dietary 18 : 3(n-3), 18 : 4(n-3), 20 : 4(n-3) and 20 : 5(n-3) fatty acids to 22 : 6(n-3). However, these fatty acids together with other fatty acids may be selectively accumulated or catabolized to sustain growth and to meet larval energetic requirements. This would limit or even exclude their value as markers. The capacity of *C. estor* for endogenous biosynthesis of long-chain polyunsaturated fatty acid was evidenced with ^{14}C labelled precursors, a pathway essentially active in saline conditions (Fonseca-Madrigal et al., 2012). The intermediate salinity of Lake Chasicó may also support this pathway in *O. bonariensis*.

We conclude that *O. bonariensis* has the capacity of chain elongation, especially of the dietary fatty acids with 18 and 20 carbon atoms of the (n-3) family, and may be stimulated by the intermediate salinity of Lake Chasicó. Laboratory studies involving isotopic markers, diets enriched and non-enriched with essential fatty acids as well as under saline and freshwater conditions are needed to demonstrate this capacity. In aquaculture, the addition of essential fatty acids with the diet is important for the successful growth of *O. bonariensis*. The source and fate of (n-3) and (n-6) fatty acid trophic markers have to be carefully considered in food webs involving atherinopsids.

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