Aquaculture Reports 17 (2020) 100377

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Lipid content and fatty acid dynamics of female muscle, oocytes and larvae of *Prochilodus argenteus* (Spix & Agassiz, 1829)

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ARTICLE INFO

Keywords:
Reproduction
Docosahexaenoic acid
Eicosapentaenoic acid
Arachidonic acid
Fish

ABSTRACT

This work aimed to determine fatty acid dynamics and lipid composition of female muscle, oocytes, newly hatched larvae (NHL) and first feeding larvae (FFL) of *Prochilodus argenteus*. Oocytes and NHL had the highest percentages of total lipids. Neutral lipids (NL) and polar lipids (PL) remained constant from oocytes through the larval stages ($P < 0.05$). Among NL, C18:2n 6 had its highest percentage in muscle ($P < 0.05$) and C18:3n 3 in muscle and oocytes. The highest percentages of arachidonic acid (AA) were in muscle and FFL. The highest percentages of n-9 and n-6 series were in muscle. The highest values of n-3HUFA were in the larval stages while the highest of n-6HUFA were in muscle and FFL. Percentage of ΣSFA in LN was highest in oocytes, NHL and FFL ($P < 0.05$) while ΣMUFA was highest in muscle, oocytes and NHL. The highest percentages of ΣPUFA were in muscle and FFL. For PL, however, C18:2n 6 and AA were highest in muscle ($P < 0.05$) while eicosapentaenoic acid was highest in muscle and oocytes. Percentage of ΣSFA in LP was highest in NHL and lowest in FFL and muscle. The highest percentage of ΣMUFA was in oocytes, NHL and FFL ($P < 0.05$). The highest percentages for series n-9 were in oocytes and FFL ($P < 0.05$), while the highest percentages for series n-6 were in muscle and oocytes. The lowest percentage for n-6HUFA was in NHL. The n-3 fatty acids were incorporated into the yolk from body reserves, which were stored prior to vitellogenesis and mobilized to the oocytes. The increase in docosahexaenoic acid reflects the ability of the species to lengthen and desaturate C18:3n 3 yielding n-3HUFA, thus demonstrating the importance of this fatty acid during early development.

1. Introduction

Lipids are the main energy source for, and provide essential fatty acids required by, a number of biological functions related to energy reserves, structural components for cell membrane formation (Copeman et al., 2002; Tocher, 2003) and immune response (Hamre et al., 2013; Tocher, 2010; Gizem et al., 2017). Lipids and fatty acids (FAs) are the most important maternal components affecting egg quality in fishes (Sargent et al., 1999a, b; Sargent et al., 2002).

Polar lipids (PLs) are important membrane lipids because they form the bilayer surface of cells (Wiegand, 1996). On the other hand, neutral lipids (NLs), including triacylglycerol (TAG) and wax esters-steryl esters (WE-SE), provide most of the energy consumed by developing embryos (Falk-Petersen et al., 1982; Phleger et al., 1997).

The concentration of fatty acids present in oocytes and fish larvae varies during initial development (Cruzado et al., 2013; Yeganeh, 2014) since they participate in anabolism, catabolism and the formation of structures and organs (Wiegand, 1996; Sargent et al., 1999b; Finn and Fyhn, 2010). Some studies have also related embryonic development and hatching success to essential polyunsaturated fatty acid (PUFA) content, namely docosahexaenoic acid (DHA; 22:6 n-3), eicosapentaenoic acid (EPA; 20:5 n-3) and arachidonic acid (ARA; 20:4 n-6), which

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https://doi.org/10.1016/j.aqrep.2020.100377
Received 4 September 2019; Received in revised form 7 May 2020; Accepted 15 May 2020
Available online 20 May 2020

are essential building blocks of cell membranes and are contained in storage lipids (March, 1993; Marteinsdottir and Begg, 2002). Furthermore, EPA and ARA are also precursors of eicosanoids, a group of highly biologically active hormones (Howard and Stanley, 1999).

In spite of its significance to studies on the nutrition of reproducing fish and larvae at the initial phase of feeding, there remains little knowledge about fatty acid dynamics during early development of many Neootropical fish species.

Prochilodus argenteus, popularly known as curimatã-pacu, is an endemic fish of the São Francisco River in Brazil and can reach body weights of up to 15 kg (Sato et al., 1996; Reis et al., 2003; Sato and Godinho, 2003). The species is iliophagous, consuming fine-particulate sludge deposited on river beds or in nurseries, and thus is important for nutrient cycling (Flecker, 1996; Godinho and Godinho, 2003). It is also rheophilic, has total spawning and rapid embryonic development, and does not perform parental care (Sato et al., 2003). It reproduces during the rainy season, from December to March, which coincides with seasonal floods, high temperatures and long photoperiods (Sato et al., 1996). The species responds positively to hormonal induction of artificial reproduction (Sato et al., 1996; Arantes et al., 2011) and possesses good quality meat with a pleasant taste, and thus has positive consumer acceptability.

Thus, this work aimed to understand the fatty acid dynamics and lipid composition of female broodstock muscle, oocytes and larvae of *P. argenteus*.

2. Materials and methods

All experimental procedures were evaluated and approved by the Ethics Commission on Animal Use of the Federal University of Minas Gerais and were in accordance with protocol number 240/2015/(CEUA/UFMG).

Wild male and female broodstock of *Prochilodus argenteus* were kept in captivity in a pond of 200 m^2 at stocking density of 1 fish/5 m^2 for one year at "Companhia de Desenvolvimento dos Vales do São Francisco e do Parnaíba (CODEVASF)", Três Marias, Brazil. Fish were fed *ad libitum* once a day with a commercial extruded diet as pellets with a diameter of 6 mm (Table 1). Bromatological analysis of the diet was performed at the Laboratory of Animal Nutrition of the Federal University of Minas Gerais according to the methods described in The Official Methods of Analysis of AOAC (2016). Fatty acid analyses are presented below.

2.1. Spawning and sampling procedure

To obtain oocytes and larvae for analysis, six males (average weight 2.6 ± 0.3 kg) and three females (average weight 1.760 ± 0.28 kg) were selected and subjected to artificial reproduction. The selected females possessed swollen abdomens and red genital papillae. Males were selected based on semen release. The females received two hormone doses of crude carp pituitary extract (CPE; 0.5 and 5 mg/kg), with twelve hours between doses. The males received a single dose (2.5 mg CPE/kg) at the same time that the females received their second dose (Ihering, 1935; Zaniboni-Filho and Weingartner, 2007).

Gametes were obtained at 234 degree-hours (average water temperature 26 °C) after hormone application by dry extrusion of the three females and six males separately. A 7 g sample of oocytes, or 17.500 oocytes (0.81 ± 0.04 mm, 0.4 ± 0.01 mg), from each female was frozen at −80 °C for future analysis. The remaining oocytes obtained from each female were fertilized with semen from two males. After homogenization of oocytes with semen, the eggs were hydrated using water at the same temperature as the incubator water. The fertilized eggs were transferred to a conic fiberglass incubator (250 L), with constant water renewal and a flow rate of 50 L/min at 26.0 ± 1.0 °C

At 20 h post-fertilization, after chorion rupture, 4 g, or 8.000, newly hatched larvae (NHL; 3.22 ± 0.04 mm, 0.5 ± 0.01 mg) were collected from each spawning. At 45 h post-fertilization, with opening of larvae mouths, 4 g, or 4,000, first-feeding larvae were collected (FFL; 5.99 ± 0.31 mm, 1.0 ± 0.01 mg). Oocytes and larvae were immediately frozen and kept at −80 °C until analysis.

The breeder females were euthanized with eugenol anesthetic at a

Table 1
Bromatological and fatty acid composition of the commercial diet offered to *Prochilodus argenteus* broodstock, based on dry matter.

Composition analyzed	Commercial diet
Protein (%)	32.8
Dry matter (%)	92.36
Mineral Matter (%)	10.55
Lipid (%PH)	7.17
Lipid (%PS)	7.77
Moisture (%)	7.69
Fatty acids	(%) of total FA
C16:0	21.96
C18:0	14.00
Σ SFA	40.99
C16:1n 7	2.68
C16:1n 9	0.02
C18:1n 9	29.81
C18:1n 7	1.53
Σ MUFA	35.33
C18:2n 6	20.39
C18:3n 3	1.31
C20:4n 6 (AA)	0.24
C20:5n 3(EPA)	0.05
C22:5n 3	0.00
C22:5n 6	0.02
C22:6n 3(DHA)	0.17
Σ PUFA	23.67
n-9	30.30
n-6	21.03
n-3	2.02
n-3HUFA	0.65
n-6HUFA	0.52
n-3/n-6	0.10
DHA/EPA	3.15
EPA/AA	0.23
DHA/AA	0.71

Commercial diet - Basic composition: soybean meal, Corn, wheat bran, feather flour, animal fat, meat meal, soybean oil, bicalcic phosphate. Mineral/Vitamin premix: Vit. A, Vit. D3, Vit. E, Vit. K3, Vit. B1, Vit. B2, Vit. B6, Vit. B12, Vit. C, Calcium Pantothenate, Niacin, Folic Acid, Choline Chloride, Biotin, Copper Sulphate, Iron Sulphate, Manganese Sulphate, Cobalt Carbonate, Calcium Iodate, Sodium Selenite, Zinc Sulphate, BHA, Ethoxyquin, Acid Citric, Inositol, Silicon Dioxide, mineral oil.Vit. A (min) 1000000.00 IU, Vit. D3 (min) 250000.00 IU, Vit. E (min) 12500.00 IU, Vit. K3 (min) 1250.00 mg, Vit. B1 (min) 1875.00 mg, Vit. B2 (min) 1875.00 mg, Vit. B6 (min) 1250.00 mg, Vit. B12 (min) 2500.00 mcg, Vit. 300 mg, Niacin (min) 10.00 g, Folic Acid (min) 625.00 mg, Biotin (min) 62.50 mg, Choline (min) 50.00 g, Copper (min), Iron (min) 6250.00 mg, Manganese (min) 1875.00 mg, Cobalt (min) 12.50 mg, Iodine (min) 62.50 mg, Zinc (min) 6250.00 mg, Selenium (min) 12.50 mg, Inositol (min) 12.50 g. Possible substitutes: Rice bran, soybean cone, proteinosis, soy lecithin,blood meal, calcined bone meal,refined fish oil.
Palmitic (C16:0), stearic (C18:0), palmitoleic acid (C16:1n 7), cis-7 hexadecenoic (C16:1n 9), oleic (C18:1n 9), vaccenic (C18:1n 7), linoleic C18:2n 6), linolenic (C18:3n 3), arachidonic (C20:4n 6) (AA), eicosapentaenoic (C20:5n 3) (EPA), docosapentaenoic (C22:5n 3), docosapentaenoic (22:5n 6), and docosahexaenoic (C22:6n 3) (DHA) acids, and saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), and highly unsaturated fatty acid (HUFA). Composition HUFA (C20:4n 6 (AA); C20:5n 3 (EPA); C22:6n 3 (DHA)).

concentration of 285 mg/L, according to the CETEA 396/2012 protocol, and samples of dorsal muscle (10 g) were collected from each, which were frozen and kept at −80 °C until analysis.

2.2. Lipid and fatty acid analysis

Lipid and fatty acid analyses were performed at the Natural Resources Laboratory - Institute of Ecology and Environmental Sciences, Montevideo, Uruguay. All muscle, oocyte and larval samples were lyophilized, followed by lipid extraction performed in duplicate.

Samples were analyzed for total, polar and neutral lipid content, and fatty acid profiles of neutral lipids and polar lipids were determined. Moisture content was determined by drying samples in an oven at 110 °C until constant weight.

Total lipid content was extracted using chloroform:methanol (2:1) following the method described by Folch et al. (1957), and quantified gravimetrically. Polar and neutral lipids were separated by adsorption chromatography using silica cartridges (Sep-Pack, Waters S.A., Milford, MA, USA - Sample extraction products). Fatty acid methyl esters (FAMEs) were obtained using transesterification with methanol in sulfuric acid, and then separated and quantified using gas chromatography (Hewlett Packard 5890) (Christie, 1982).

Nonadecanoic acid (19: 0) was used as an internal standard to evaluate the efficiency of the technique used in the laboratory. Samples were protected from oxidation during lipid and fatty acid analyses by maintaining them under a nitrogen atmosphere and using butylated hydroxyl toluene (100 mg/L of solvent). Fatty acid methyl esters were analyzed using a gas chromatograph (Hewlett Packard 5890; Hewlett-Packard Company, Wilmington, DE, USA) equipped with a flame ionization detector and a Supelcowax fused silica capillary column (30 m 9 0.32 mm ID, Supelco, Bellefonte, PA, USA) using nitrogen as a carrier. Samples were injected in split mode at 250 °C. Column temperature was maintained at 180 °C for 12 min, increased to 212 °C at a rate of 2 °C/min, and then maintained at 212 °C for 13 min. Fatty acids were identified by comparing the retention times of methyl ester standards (Supelco) and by reference to a well-characterized fish oil.

2.3. Light microscopy

Samples of oocytes, NHL and FFL were fixed in Bouin's fluid for 24 h at room temperature. The tissues were then dehydrated and embedded in paraffin. Transverse histological sections of 5-μm thickness were mounted on glass slides and stained with Sudan black (SB).

2.4. Statistical analysis

Statistical analysis was performed using Statistical Analysis System (SAS) version 9.0. Data were submitted to Cramér-von Mises normality test and Levene's test for homoscedasticity. Logarithmic transformation (natural logarithm) was applied to non-normal data. Data were tested by one-way ANOVA and compared using Tukey's test at 5% probability.

3. Results

Table 2 shows the percentages of total, neutral and polar lipids in muscle, oocytes, newly hatched larvae (NHL) and first feeding larvae (FFL) of *P. argenteus*. Oocytes and NHL had the highest percentages for total lipids ($P < 0.05$). Muscle had the highest percentage for neutral lipids ($P < 0.05$), with lower percentages for oocytes, NHL and FFL. In contrast, muscle had the lowest percentage for polar lipids ($P < 0.05$), with higher percentages in oocytes, NHL and FFL ($P < 0.05$).

The composition of neutral lipid (LN) fatty acids is presented in Table 3. Among the saturated fatty acids (SFA), C16:0 had its highest percentages in oocytes and NHL and FFL, and its lowest in muscle ($P < 0.05$). In contrast, C18:0 had its highest percentages in muscle and FFL ($P < 0.05$).

For monounsaturated fatty acids (MUFA) of the neutral lipid fraction, the highest percentages of C16:1n 7 were found in oocytes, followed by NHL with intermediate percentages and FFL and muscle with the lowest ($P < 0.05$) (Table 3). The highest percentages for the fatty acid C18:1n 9 were found in muscle and oocytes, while the highest percentages for C18:1n 7 were found in oocytes and NHL and FFL ($P < 0.05$). The fatty acid C16:1n 9 was not detected in NHL and FFL (Table 3).

For polyunsaturated fatty acids (PUFA) of the neutral lipid fraction, the highest percentages of C18:2n 6 were found in muscle, with lower percentages in oocytes, NHL and FFL ($P < 0.05$). The highest percentages of the fatty acid C18:3n 3 were found in muscle and oocytes, with lower percentages in NHL and FFL (Table 3).

The highest percentages of C20:4n 6 (AA) were found in muscle and FFL, intermediate percentages in NHL and the lowest in oocytes ($P < 0.05$). The highest percentage of C22:5n 3 was found in muscle, while the highest percentages of C22:6n 3 (DHA) were in NHL and FFL ($P < 0.05$). Both C20:5n 3 (EPA) and C22:5n 6 did not exhibit significant differences among life stages ($P > 0.05$) (Table 3).

The highest percentages of ΣSFA were found in oocytes and during embryonic development ($P < 0.05$). The highest percentages of ΣMUFA were found in muscle, oocytes and NHL ($P < 0.05$), while the highest percentages for ΣPUFA were in muscle and FFL, with intermediate percentages in NHL and lower percentages in oocytes ($P < 0.05$) (Table 3).

Fatty acids of series n-3, and the ratios n-3/n-6, DHA/EPA and EPA/AA, did not exhibit significant differences ($P > 0.05$) (Table 3). The highest percentages of n-9 and n-6 series were in muscle ($P < 0.05$), while the highest percentages of n-3HUFA were in NHL and FFL. The highest percentages of n-6HUFA were in muscle and FFL, with intermediate percentages in NHL and lower percentages in oocytes ($P < 0.05$). The highest percentages for the ratio DHA/AA were found in NHL and FFL ($P < 0.05$).

The fatty acids of the polar lipid fraction (PL) are presented in Table 4. Among the SFA, C16:0 had its highest percentages in oocytes and NHL and is lowest in FFL ($P < 0.05$). The SFA C18:0 had its highest percentage in oocytes ($P < 0.05$). For MUFA in the lipid fraction, the fatty acids C18:1n 9 and C18:1n 7 had their highest percentages in oocytes and FFL (Table 4); the lowest percentage of C18:1n 7 was in NHL ($P < 0.05$). The fatty acids C16:1n 7 and C16:1n 9 did not exhibit significant differences ($P > 0.05$), and C16:1n 9 was not detected in FFL (Table 4).

Among the PUFAs of the polar lipid fraction, the fatty acids C18:2n 6 and C22:5n 6 had their highest percentages in muscle, with the lower

Table 2
Total lipid content (% of wet weight) and percentage of neutral and polar lipid in female broodstock muscle, oocyte, newly hatched larvae (NHL) and first feeding larvae (FFL) of *Prochilodus argenteus*.

	Muscle	Oocyte	NHL	FFL	C.V (%)	P *value*
Total lipid	10.80 ± 1.79^{c}	25.61 ± 1.05^{a}	26.61 ± 1.80^{a}	19.90 ± 0.84^{b}	8.65	.0001
Neutral lipid	82.36 ± 1.46^{a}	55.22 ± 1.03^{b}	50.50 ± 1.26^{b}	62.22 ± 1.39^{b}	9.81	.0001
Polar lipid	17.64 ± 1.46^{b}	44.78 ± 1.03^{a}	49.50 ± 1.25^{a}	37.78 ± 1.39^{a}	9.62	.0001

Different letters on the same line represent significant differences according to Tukey's test ($P < 0.05$). Means (± standard deviation).

Table 3
Fatty acid composition of neutral lipid (% of total FA) in female broodstock muscle, oocyte, newly hatched larvae (NHL) and first feeding larvae (FFL).

	Muscle	Oocyte	NHL	FFL	P *value*
Fatty acids					
C16:0	26.29 ± 1.10^{b}	36.36 ± 3.45^{a}	37.48 ± 1.91^{a}	36.70 ± 1.26^{a}	.0001
C18:0	8.63 ± 0.46^{a}	4.42 ± 0.42^{b}	5.76 ± 0.27^{b}	9.71 ± 0.70^{a}	.0001
Σ SFA	30.19 ± 1.32^{b}	41.21 ± 2.95^{a}	44.06 ± 2.12^{a}	44.46 ± 1.54^{a}	.0001
C16:1n 7	7.63 ± 0.35^{b}	11.76 ± 1.96^{a}	10.36 ± 1.63^{ab}	6.53 ± 0.87^{b}	.0001
C16:1n 9	0.10 ± 0.06	0.07 ± 0.005	–	–	–
C18:1n 9	20.19 ± 1.22^{a}	18.10 ± 3.75^{a}	15.06 ± 3.14^{b}	12.49 ± 1.14^{b}	.0001
C18:1n 7	4.22 ± 0.06^{b}	6.35 ± 0.17^{a}	6.94 ± 0.36^{a}	5.70 ± 0.81^{a}	.0001
Σ MUFA	38.08 ± 1.00^{a}	40.99 ± 2.84^{a}	36.93 ± 3.33^{a}	29.13 ± 2.18^{b}	.0001
C18:2n 6	10.09 ± 0.80^{a}	3.89 ± 0.49^{b}	3.71 ± 0.29^{b}	2.89 ± 0.17^{b}	.0001
C18:3n 3	1.64 ± 0.23^{a}	1.26 ± 0.23^{a}	0.54 ± 0.04^{b}	0.38 ± 0.07^{b}	.0001
C20:4n 6 (AA)	1.21 ± 0.11^{a}	0.26 ± 0.01^{c}	0.64 ± 0.03^{b}	1.11 ± 0.16^{a}	.0001
C20:5n 3 (EPA)	0.17 ± 0.01	0.05 ± 0.005	0.61 ± 0.21	0.55 ± 0.42	.0085
C22:5n 3	0.88 ± 0.03^{a}	0.24 ± 0.08^{b}	0.46 ± 0.18^{b}	0.60 ± 0.13^{b}	.0001
C22:5n 6	0.50 ± 0.06	0.16 ± 0.10	0.28 ± 0.007	0.15 ± 0.22	.0020
C22:6n 3 (DHA)	1.38 ± 0.12^{b}	0.53 ± 0.17^{b}	2.08 ± 0.89^{a}	3.12 ± 0.92^{a}	.0001
Σ PUFA	22.69 ± 0.96^{a}	12.78 ± 0.48^{c}	13.35 ± 1.54^{bc}	18.34 ± 3.64^{a}	.0001
n-9	23.37 ± 1.45^{a}	18.51 ± 3.78^{b}	15.30 ± 3.23^{b}	12.79 ± 1.04^{b}	.0001
n-6	14.33 ± 0.88^{a}	6.14 ± 0.77^{b}	6.72 ± 0.13^{b}	6.82 ± 0.49^{b}	.0001
n-3	5.55 ± 0.20	2.97 ± 0.25	4.23 ± 1.51	5.11 ± 1.62	.0038
n-3HUFA	3.31 ± 0.12^{b}	1.24 ± 0.28^{b}	3.66 ± 1.45^{a}	4.70 ± 1.63^{a}	.0001
n-6HUFA	2.95 ± 0.05^{a}	1.23 ± 0.20^{c}	2.08 ± 0.18^{b}	2.80 ± 0.45^{a}	.0001
n-3/n-6	0.39 ± 0.02	0.49 ± 0.10	0.63 ± 0.24	0.74 ± 0.21	.0048
DHA/EPA	8.11 ± 1.16	10.12 ± 3.75	3.42 ± 0.03	5.67 ± 4.77	.0018
EPA/AA	0.14 ± 0.005	0.20 ± 0.01	0.94 ± 0.36	0.47 ± 0.33	.0172
DHA/AA	1.15 ± 0.20^{b}	2.05 ± 0.76^{b}	3.25 ± 1.21^{a}	2.80 ± 0.54^{a}	.0001

Values followed by different letters on the same line were significantly different (P < 0.05) by Tukey's test. Means (± standard deviation).
Palmitic (C16:0), stearic (C18:0), palmitoleic acid (C16:1n 7), cis-7 hexadecenoic (C16:1n 9), oleic (C18:1n 9), vaccenic (C18:1n 7), linoleic C18:2n 6), linolenic (C18:3n 3), arachidonic (C20:4n 6) (AA), eicosapentaenoic (C20:5n 3) (EPA), docosapentaenoic (C22:5n 3), docosapentaenoic (22:5n 6), docosahexaenoic (C22:6n 3) (DHA), saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA), highly unsaturated fatty acid (HUFA). Composition HUFA (C20:4n 6 (AA); C20:5n 3 (EPA); C22:6n 3 (DHA)).

Table 4
Fatty acid composition of polar lipid (% total FA) in female broodstock muscle, oocyte, newly hatched larvae (NHL) and first feeding larvae (FFL).

	Muscle	Oocyte	NHL	FFL	P *value*
Fatty acids					
C16:0	18.71 ± 2.03^{ab}	21.24 ± 1.58^{a}	25.56 ± 9.17^{a}	15.86 ± 0.51^{b}	.0001
C18:0	9.09 ± 0.59^{b}	12.25 ± 0.39^{a}	8.94 ± 2.23^{b}	8.08 ± 0.20^{b}	.0001
Σ SFA	34.17 ± 1.67^{c}	40.22 ± 1.02^{b}	43.25 ± 5.20^{a}	38.35 ± 3.04^{c}	.0001
C16:1n 7	1.43 ± 0.12	2.13 ± 0.71	4.62 ± 3.92	2.39 ± 0.09	.0090
C16:1n 9	0.49 ± 0.05	0.04 ± 0.01	0.19 ± 0.01	–	–
C18:1n 9	5.93 ± 0.78^{b}	10.89 ± 0.86^{a}	5.01 ± 1.04^{b}	9.76 ± 0.57^{a}	.0001
C18:1n 7	2.99 ± 0.25^{b}	4.53 ± 0.45^{a}	0.21 ± 0.01^{c}	4.31 ± 0.47^{a}	.0001
Σ MUFA	13.46 ± 148^{b}	20.18 ± 0.75^{a}	22.23 ± 7.98^{a}	18.60 ± 1.11^{a}	.0001
C18:2n 6	8.92 ± 1.06^{a}	5.56 ± 0.78^{b}	4.60 ± 0.66^{b}	4.49 ± 0.22^{b}	.0001
C18:3n 3	0.71 ± 0.08	0.43 ± 0.07	0.44 ± 0.01	0.37 ± 0.01	.0180
C20:4n 6 (AA)	9.45 ± 0.20^{a}	5.03 ± 0.57^{b}	3.65 ± 2.51^{b}	6.51 ± 0.77^{ab}	.0001
C20:5n 3 (EPA)	4.39 ± 0.71^{a}	3.22 ± 0.51^{a}	2.34 ± 1.55^{b}	2.84 ± 0.91^{b}	.0001
C22:5n 3	2.76 ± 0.08	2.44 ± 0.18	1.96 ± 1.07	2.30 ± 0.74	.0420
C22:5n 6	4.46 ± 0.16^{a}	1.65 ± 0.23^{b}	1.34 ± 0.76^{b}	1.94 ± 0.21^{b}	.0001
C22:6n 3 (DHA)	9.94 ± 0.63	13.22 ± 0.96	9.35 ± 5.96	14.37 ± 4.45	.0001
Σ PUFA	50.65 ± 1.92	40.53 ± 1.19	34.97 ± 17.11	43.78 ± 2.00	.0350
n-9	7.76 ± 0.98^{b}	11.22 ± 0.75^{a}	5.42 ± 0.75^{c}	9.92 ± 0.54^{a}	.0001
n-6	28.93 ± 1.98^{a}	19.68 ± 2.88^{a}	15.06 ± 6.13^{b}	18.29 ± 1.29^{b}	.0001
n-3	18.86 ± 1.25	20.29 ± 1.53	14.93 ± 8.75	21.22 ± 6.41	.0950
n-3HUFA	18.09 ± 1.38	19.77 ± 1.59	14.45 ± 8.73	20.80 ± 6.59	.0400
n-6HUFA	17.30 ± 0.61^{a}	11.48 ± 1.75^{a}	8.55 ± 5.05^{b}	12.86 ± 1.30^{a}	.0001
n-3/n-6	0.66 ± 0.07	1.03 ± 0.25	0.99 ± 0.30	1.16 ± 0.40	.0321
DHA/EPA	2.27 ± 0.23^{c}	4.11 ± 0.44^{b}	4.02 ± 0.33^{b}	5.06 ± 0.22^{a}	.0001
EPA/AA	0.46 ± 0.07	0.64 ± 0.18	0.64 ± 0.18	0.43 ± 0.10	.0021
DHA/AA	1.05 ± 0.05^{b}	2.63 ± 0.50^{a}	2.56 ± 0.57^{a}	2.20 ± 0.52^{a}	.0001

Values followed by different letters on the same line were significantly different (P < .05) by Tukey's test. Means (± standard deviation).
Palmitic (C16:0), stearic (C18:0), palmitoleic acid (C16:1n 7), cis-7 hexadecenoic (C16:1n 9), oleic (C18:1n 9), vaccenic (C18:1n 7), linoleic C18:2n 6), linoleic (C18:3n 3), arachidonic (C20:4n 6) (AA), eicosapentaenoic (C20:5n 3) (EPA), docosapentaenoic (C22:5n 3), docosapentaenoic (22:5n 6), docosahexaenoic (C22:6n 3) (DHA), saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA), highly unsaturated fatty acid (HUFA). Composition HUFA (C20:4n 6 (AA); C20:5n 3 (EPA); C22:6n 3 (DHA)).

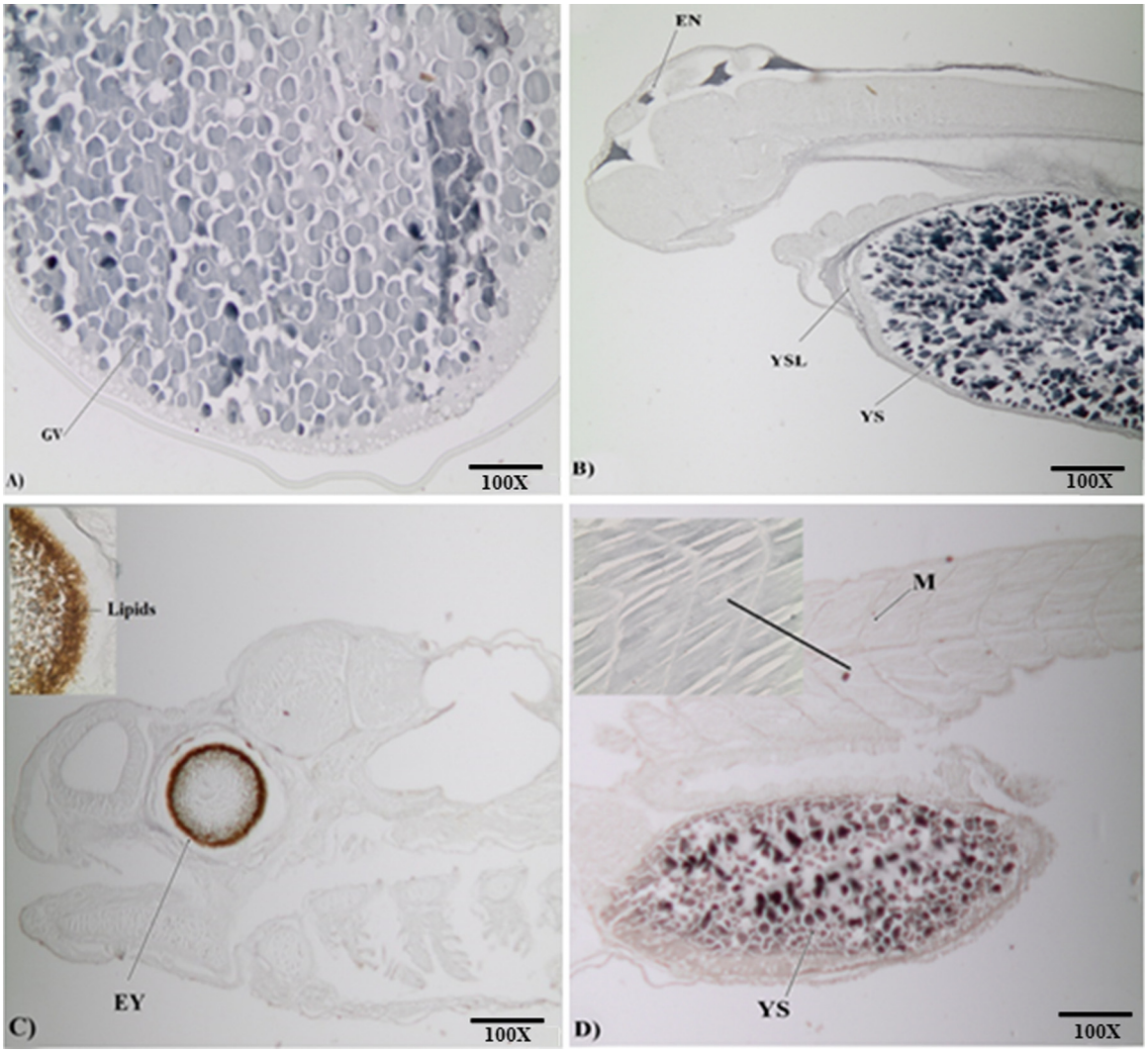

Fig. 1. Photomicrography of oocytes, newly hatched larvae (NHL) and first feeding larvae (FFL) of *Prochilodus argenteus*. A - vitellogenic oocytes stained with Sudan black showing the presence of lipids in yolk granules (GV); B - encephalon (EN), yolk sac (YS) and yolk syncytial layer (YSL), all stained with Sudan black. C and D – first feeding larvae (FFL) with yolk sac (YS), eyes (EY) and myomeres (M), stained with Sudan black.

percentages in oocytes and during embryonic development ($P < 0.05$) (Table 4). The highest percentage of fatty acid C20:4n 6 (AA) was in muscle, with an intermediated percentage in FFL and lowest in oocytes and NHL ($P < 0.05$). The percentages of C20:5n 3 (EPA) were highest in muscle and oocytes, with the percentages being lowest NHL and FFL ($P < 0.05$) (Table 4). No significant differences were found for the fatty acids C18:3n 3, C22: 5n 3 and C22:6n 3 (DHA) ($P > 0.05$).

The highest percentages of ΣSFA were in NHL, while the lowest were in FFL and muscle. The highest percentages of ΣMUFA were in oocytes, NHL and FFL with a lower percentage in muscle ($P < 0.05$). No significant differences ($P > 0.05$) were exhibited for ΣPUFA, fatty acids of the n-3 series, n-3HUFA and the ratios n-3/n-6 and EPA/AA (Table 4).

The highest percentages for the n-9 series were in oocytes and FFL, with a lower percentage in NHL ($P < 0.05$). The highest percentages for the n-6 series were in muscle and oocytes, with lower percentages during embryonic development ($P < 0.05$). The lowest percentage for the n-6HUFA series was in NHL ($P < 0.05$) (Table 4). The highest percentage for the ratio DHA/EPA was in FFL, with intermediate percentages in oocytes and NHL, and lowest in muscle. The highest percentages for the ratio DHA/AA were in oocytes, NHL and FFL ($P < 0.05$).

Histochemistry with Sudan black staining identified lipids in yolk globules (Fig. 1A). The early formation of the brain and the syncytial layer of the vitellogenic sac were observed in NHL (Fig. 1B). The presence of a reduced yolk sac and lipids in the composition of the eye, as well as the formation of myomeres in muscle tissue, were observed in FFL (25 h after hatching) (Fig. 1C and D).

4. Discussion

Lipids have important relationships with multiple functions during embryonic and larval development, such as serving as a source of metabolic energy and for the formation of organs and tissues (Sargent et al., 1999a, b; Izquierdo et al., 2000; Tocher et al., 2003). Histochemisty revealed lipids in the yolk of oocytes and larvae, however, the yolk sac reduced throughout embryonic development and during the formation of the eyes, retinal pigmentation and the brain. These observations have been reported previously for other species of fish, such as *Engraulis mordax* at the opening of the mouth (Lasker et al., 1970) and *Prochilodus costatus* and *P. argenteus* (Santos et al., 2016).

Lipids present in the yolk sac during initial development are used as an energy source for embryo development (Heming and Buddington, 1988; Wiegand, 1996). This was verified in the present study by the highest percentages of total lipids being recorded in oocytes and NHL, and lower percentages in FFL. A reduction of total lipids was also found

for *Carassius auratus* (Wiegand, 1996), *Maccullochella macquariensis* and *M. peelii peelii* (Gunasekera et al., 1999), indicating catabolism of this nutrient for energy production during larval development.

However, the percentage of polar and neutral lipids in oocytes, NHL and FFL remained constant, unlike total lipids. Polar lipids are the main components of biological membranes (Contreras-Guzmán, 2002), and are indispensable to many functions, including growth, resistance to stress (Nelson and Cox, 2005) and increased survival during larval and juvenile periods (Azarm et al., 2013). Neutral lipids, on the other hand, are catabolized to provide metabolic energy for larval development (Lovell, 1998), in addition to providing essential fatty acids that act on membrane synthesis and serve as important metabolic modulators and chemical signalers (Gurr and Harwood, 1991). This suggests that the studied species may be storing fatty acids for the beginning phase of exogenous feeding, when there is a need to search for food (Watanabe and Kiron, 1994).

MUFAs may also be needed as a source of energy throughout embryonic and larval development (Tulli and Tibaldi, 1997; Abi-Ayad et al., 2000; Dantagnan et al., 2007). These fatty acids play an important role in signaling the hypothalamus about the nutritional state of the animal, thus provoking integral and compensatory responses in stages of larval or adult development (Lam et al., 2005). Among MUFAs of the neutral lipid fraction, C16:1n 7 had its highest percentage in oocytes, after which it decreased throughout embryonic development. This reduction shows that this fatty acid is being catabolized as a source of energy or is being mobilized to form other fatty acids with longer chains. Similar reports were observed for the marine species *Scophthalmus maximus* (Mourente et al., 1991) and the freshwater species *C. auratus* (Wiegand, 1996) and *B. orthotaenia* (Martins et al., 2017).

The fatty acid C18:1n 9 was highest in female muscle and oocytes, indicating the transfer of nutrients from mother to oocytes, which may be a reflection of the high percentages of this fatty acid in the diet. These results elucidate the importance of maternal nutrition in transferring lipids and fatty acids (Terova et al., 1998).

In the PL fraction, C18:1n 9 and C18:1n 7 had their lowest percentages in NHL. There is a great demand for energy as substrates for rupturing the chorion during hatching (Wiegand, 1996), as verified in *Salminus hilarii* (Araújo et al., 2012). These fatty acids are also important in membrane fluidity (Wiegand, 1996) and in the formation of most organs and the digestive tract (Mello et al., 2009). They are also important for brain development, as described for *S. maximus* (Mourente et al., 1991).

Among the polyunsaturated fatty acids, C18:2n 6 in the NL and PL fractions had its highest percentages in the muscle of females, whereas C18:3n 3 of the NL fraction was highest in muscle and oocytes. These results show that these fatty acids are also incorporated into the yolk from the dietary source provided during vitellogenesis and are mobilized to oocytes (Wiegand, 1996). This finding underscores the importance of the composition of these fatty acids in the diet offered to broodstock. The reduction of C18:3n 3 in NHL and FFL, and the increase in the percentage of DHA in the respective phases, evidences potential bioconversion of fatty acids in this species through the enzymatic activity of desaturases and elongases (Ling et al., 2006; Jaya-Ram et al., 2008; Tocher, 2010), as observed in *Maccullochella peelii peelii* (Turchini et al., 2006), another freshwater species.

Some PUFAs deserve special attention, including DHA of the NL fraction, which had higher values in NHL and FFL. This is the most important fatty acid in the n-3 series and performs structural functions in cell membranes besides having several other important functions in fish, such as maintaining the structure and functional integrity of cells (Sargent et al., 1999b; Furuita et al., 2003), including the formation of neural tissues (Wiegand, 1996), brain and retina (Sargent, 1995; Wiegand, 1996). In addition, these tissues represent large proportions of the body mass of an embryo or larvae (Sargent, 1995), which is reflected in high requirements for DHA to ensure adequate visual and neural development of larvae, as histochemistry revealed by the presence of lipids in the eyes and in the formation of brain tissues in *P. argenteus* by histochemistry.

In the NL and PL fractions, greater percentages of AA were observed in the muscle of females and FFL. This fatty acid is related to growth, survival and resistance to stress through the production of eicosanoids (Bessonart et al., 1999; Bell and Sargent, 2003; Tocher, 2010). Considering eicosanoids produced from 20:4n 6 that are biologically active in fish, PUFAs (n-6) are essential nutrients for fish (Sargent, 1995). At reproduction, the fatty acid AA is mobilized, metabolized and utilized as a messenger by gonadotropin releasing hormone (GnRH) (Van Der Kraak, 1997).

The EPA of the PL fraction decreased throughout embryonic development. This fatty acid is responsible for maintaining the fluidity and permeability of biological membranes (Sargent et al., 2002), and larvae are extremely dependent on this property at this stage. According to Kamler (2008), larvae perform many of their biological functions through the fluidity and permeability of membranes and the chorion.

The sum of SFA of the NL fraction was highest in oocytes, NHL and FFL. These results show that these fatty acids were not used as an energy source. However, the SFA summation of the PL fraction was lower in FFL due to the higher energetic demand for the formation of new tissues (Sargent, 1995). On the other hand, the MUFAs of the NL fraction remained constant in female muscle, oocyte and NHL and reduced in FFL. This reduction may be related to energy consumption for swimming and searching for food. On the other hand, MUFAs of the PL fraction were stored from the oocyte phase to FFL, showing their necessity for later phases.

The sum of PUFAs of the NL fraction exhibited higher percentages in female muscle and FFL. These fatty acids are rich in n-3 series and are expected to be conserved for the formation of new tissues in embryos and larvae (Wiegand, 1996).

Oocytes, NHL and FFL possessed low percentages of n-9 and n-6 in the NL fraction, which may be indicative of the consumption of these classes for metabolic demand. However, the PL fatty acid n-9 was lower in NHL, and n-6 reduced during embryonic development. It is possible that this class of PUFAs was used for the formation of tissues and organs, and for the rupture of the chorion. The increase of n-3HUFA and n-6HUFA of the NL fraction in FFL suggests their necessity for larvae of *P. argenteus*, as was also observed with *B. orthotaenia* (Martins et al., 2017). However, the n-6HUFA of the PL fraction in *P. argenteus* decreased during embryonic and larval development. This reduction is due to these fatty acids acting as constituents of the structural phospholipids of cell membranes, in the tissue formation (neural tissue and neuromuscular junctions) (Sargent et al., 2002; Tocher, 2010).

5. Conclusion

The results of the present study show the transfer of nutrients from the matrix to the oocytes. The increase in DHA suggests there is enzymatic activity of desaturases and elongases to obtain n-3HUFA in this species. Understanding the chemical composition and changes in fatty acid composition in muscle, oocytes, NHL and FFL of *P. argenteus* can lead to better nutritional management of larvae at first exogenous feeding, as well as that of broodstock.

Author statement

Edenilce de Fátima Ferreira Martins: The conception and design of the study; and acquisition of data; analysis and interpretation of data; drafting the article or revising it critically for important intellectual content; final approval of the version to be submitted.

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Ethics committee

The fish husbandry and experimental protocols conducted in this study were approved by the Ethics Commission on Animal Use of the Federal University of Minas Gerais and were in accordance with Protocol 240/2015/ (CEUA/UFMG).

Software and data repository resources

None of the data were deposited in an official repository.

Declaration of Competing Interest

Authors and co-authors of this study declare that they have no conflicts of interest in the publication of this study.

Acknowledgements

The present research was funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-Brazil), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-Brazil), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG-Brazil). LUZ, R.K. received a research grant from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq No. 308547/2018-7).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.aqrep.2020.100377.

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