

Life history traits influence in gonad composition of two sympatric species of flatfish

Juan Luis Gadea Alvarez^{1,2,*}, Martín Bessonart^{1,3}, Larisa Magnone¹, Florencia Féola³, María Salhi¹

¹ Laboratorio de Recursos Naturales, Facultad de Ciencias, Universidad de la República

(Iguá 4225 CP 11400 Montevideo, Uruguay)

² Departamento de Acuicultura, Dirección Nacional de Recursos Acuáticos (DINARA) - Ministerio de Ganadería Agricultura y Pesca (Constituyente 1497 CP 11200 Montevideo, Uruguay)

³ Estación Biológica, DINARA, Parque Nacional Cabo Polonio (Cabo Polonio CP 27202 Rocha, Uruguay)

*Corresponding author: juanluisgadea@gmail.com

ABSTRACT

Paralichthys orbignyanus and *Paralichthys patagonicus* are flatfish with different life history traits, having in common the condition of breeding in seawater. *Paralichthys patagonicus* remain their whole life in open seawater and *Paralichthys orbignyanus* are sometimes found in brackish water bodies. As marine and estuarine food webs have different fatty acid (FA) compositions, the aim of this study was to characterize the gonadal maturation of *P. orbignyanus* and *P. patagonicus* females through the analysis of lipid content and FA profile in order to understand to what extent life history traits are reflected in the ovarian composition. During gonadal maturation lipid content increased and FA profiles changed in both species, but the lipid increase was greater in *P. orbignyanus*. The N-3FA and n-3HUFA proportions increased in both species but were higher in *P. orbignyanus*. The differences between the lifestyles of these species were reflected in the ovarian FA profile mainly as a result of differences in their FA metabolism, causing a greater accumulation of n-3FA and n-3HUFA in *P. orbignyanus* than in *P. patagonicus*. The higher lipid accumulation in *P. orbignyanus*' ovaries could indicate that this species, feeding in brackish water bodies, has the possibility of storing more energy than *P. patagonicus*.

Descriptors: Fatty acid profile, Lipid content, Gonadal maturation.

RESUMO

Paralichthys patagonicus e *Paralichthys orbignyanus* apresentam diferentes atributos nas suas histórias de vida, embora ambas se reproduzam no mar. *Paralichthys patagonicus* permanece toda a vida em águas abertas, enquanto *P. orbignyanus* é comumente encontrado em corpos de água salobra. Considerando que redes alimentares marinhas e estuarinas têm diferente composição de ácidos graxos (FA), o objetivo deste estudo foi caracterizar a maturação gonadal de fêmeas de *P. orbignyanus* e *P. patagonicus* analisando o conteúdo lipídico e perfil FA, a fim de estabelecer se as diferenças nas histórias de vida são refletidas na composição do ovário. Durante a maturação gonadal, em ambas as espécies, houve aumento do teor lipídico e o perfil FA mudou, mas o incremento foi maior em *P. orbignyanus*. As proporções de N-3FA e n-3HUFA aumentaram em ambas as espécies, mas foram maiores em *P. orbignyanus*. As diferenças entre os estilos de vida destas espécies foram refletidas no perfil de FA dos ovários, principalmente como resultado das diferenças no metabolismo de FA, causando maior acúmulo de n-3FA e n-3HUFA em *P. orbignyanus*. O maior acúmulo de lipídios nos ovários de *P. orbignyanus* poderia indicar que esta espécie, ao se alimentar em águas salobras, tem a possibilidade de armazenar mais energia do que *P. patagonicus*.

Descritores: Perfil de ácidos graxos, Conteúdo lipídico, Maturação gonadal.

INTRODUCTION

In the Argentinian-Uruguayan *Common Fishery Zone* (ZCP), *Paralichthys patagonicus* (JORDAN, 1889) and *Paralichthys orbignyanus* (VALENCIENNES, 1839) are the flatfishes of greatest commercial importance (FABRÉ; DÍAZ DE ASTARLOA, 1996; DÍAZ DE ASTARLOA; MUNROE, 1998; DÍAZ DE ASTARLOA, 2002; HAIMOVICI; ARAÚJO, 2005; RICO, 2010). *P. orbignyanus* can be described as a marine/estuarine euryhaline teleost (SAMPAIO; BIANCHINI, 2002) which enters estuaries and is usually more abundant in shallow coastal waters (FABRÉ; DÍAZ DE ASTARLOA, 1996; DÍAZ DE ASTARLOA; MUNROE, 1998), coastal lagoons (SILVEIRA et al., 1995; RIVERA et al., 2001; NORBIS; GALLI, 2004) and in the mouths of rivers and streams (PLAVAN et al., 2010). This ability to live in estuarine environments together with its wide tolerance to environmental factors such as salinity and pH (WASIELESKY et al., 1997; SAMPAIO et al., 2001, 2002) and its high market price, make *P. orbignyanus* an interesting candidate for aquaculture (ROBALDO, 2003; BAMBILL et al., 2006; RADONIC et al., 2007; SAMPAIO et al., 2007; RADONIC; MACCHI, 2009; LANES et al., 2010).

The main difference between the lifestyles of *P. patagonicus* and *P. orbignyanus* seems to be that *P. patagonicus* remains all its life in open water, while *P. orbignyanus* usually lives in brackish coastal water bodies. However, both species depend on the marine environment for reproduction (SILVEIRA et al., 1995; ROBALDO, 2003; CERQUEIRA, 2005; SAMPAIO et al., 2007; CECCON et al., 2010; MILITELLI, 2011).

P. orbignyanus gametes and larvae can only survive and develop in seawater due to their anatomical and physiological characteristics. Therefore these fishes depend on seawater to reproduce although they can also live in brackish waters (CERQUEIRA, 2005; SAMPAIO et al., 2007).

Besides sharing the place where they breed, these species also share the breeding season. The spawning season of *P. orbignyanus* extends from October to April (SILVEIRA et al., 1995; ROBALDO, 2003), with a peak of reproductive activity between January and April (LANES et al., 2010), while that of *P. patagonicus* lasts from September to February with a peak of maximum reproductive activity in November (MACHI; DÍAZ DE ASTARLOA, 1996). During the rest of the year outside the breeding season it is usual to find *P. orbignyanus* feeding in brackish waters, coastal lagoons or rivers, whereas

P. patagonicus spends the resting phase feeding in the marine environment.

Fish, like all vertebrates studied thus far, require certain long chain polyunsaturated fatty acids (PUFA) for their normal development and reproduction (SARGENT et al., 1999a, 2002). Such PUFA are called essential fatty acids (EFA) and they include members of the n-3 and the n-6 series. In their biologically active form the EFA are docosahexaenoic acid (DHA, 22:6 n-3), eicosapentaenoic acid (EPA, 20:5 n-3) and arachidonic acid (ARA, 20:4 n-6), distinctively called highly unsaturated fatty acids (HUFA). Usually, terrestrial vertebrates and freshwater fishes are capable of producing DHA and EPA from linolenic acid (LNA, 18:3n-3) and ARA from linoleic acid (LA, 18:2n-6). In such species LNA and LA are dietary EFA. However, marine fishes cannot perform these conversions efficiently, therefore they must obtain DHA, EPA and ARA directly from the diet (SARGENT et al., 1999a, 1999b, 2002).

Marine and brackish water food webs have different fatty acid (FA) compositions and it is not clear whether marine fishes can meet their FA requirements in brackish water (ALFARO et al., 2006; RICHOUX; FRONEMAN, 2008; ARTS et al., 2009; KOUSSOROPLIS et al., 2011). Marine ecosystems are rich in PUFA, mainly HUFA of the n-3 series (n-3HUFA) such as DHA and EPA. These n-3HUFA are provided by organisms such as diatoms and flagellates which are at the base of marine food webs (ARTS et al., 2009). In estuarine ecosystems saturated FA (SAFA) are predominant over monounsaturated FA (MUFA) and PUFA (ALFARO et al., 2006; RICHOUX; FRONEMAN, 2008; KOUSSOROPLIS et al., 2011), therefore DHA and EPA are less abundant in these ecosystems than in seawater.

As marine and estuarine food webs have different FA composition, the aim of this study was to characterize the gonadal maturation of *P. orbignyanus* and *P. patagonicus* females through the analysis of lipid content and FA profile in order to understand to what extent life history traits are reflected in the ovarian composition.

MATERIAL AND METHODS

FISH SAMPLING AND DATA COLLECTION

P. orbignyanus and *P. patagonicus* adult females were collected at the beginning and at the end of the breeding seasons (spring and fall respectively) and during the resting phase (winter). Fishes caught in the full spawning

season (summer) were not included in this analysis due to the low number of samples obtained and the extreme variability observed in the ovarian composition.

P. orbignyanus females collected during fall and winter were obtained in mid-May and mid-July, respectively, from artisanal fisheries in “Laguna de Rocha”, a brackish water coastal lagoon semi-connected to the ocean in southeastern Uruguay. Females collected during spring were captured in the Rio de la Plata and the adjacent Atlantic coastal areas from November to December during a coastal survey of the National Committee for Aquatic Resources (DINARA, B/I “Aldebarán”). *P. patagonicus* females collected in the fall were captured in March-April during a DINARA coastal survey. Coastal industrial fisheries in Montevideo port gave us females collected during winter and spring in mid-August and late October, respectively. All *P. patagonicus* samples were captured in the Rio de la Plata and the adjacent Atlantic coastal areas. Flounder species were identified according to COUSSEAU; PERROTA, 2000.

Total length, total weight and gonad weight of each fish collected were measured. Samples of gonads were stored at -20° C for biochemical analysis. The bodily condition of individuals was estimated using Fulton’s condition factor (K) (RICKER, 1975), as follows:

$$K = 100 (TW/TL^3)$$

where K is Fulton’s condition factor, TW is total fish weight and TL is the total length.

BIOCHEMICAL ANALYSIS

Lipids were extracted with chloroform: methanol (2:1) following the method described by FOLCH et al. (1957) and quantified gravimetrically. FA methyl esters were obtained by transesterification with methanol in sulphuric acid (CHRISTIE, 1982), separated and quantified using gas chromatography. During lipid and FA analysis, samples were protected from oxidation by keeping them under nitrogen gas and using butylated hydroxy toluene (100 mg L⁻¹ of solvent). FA methyl esters were analysed using a gas chromatograph (Hewlett Packard 5890; Hewlett-Packard Company, Wilmington, DE, USA) equipped with a flame ionization detector and a Supelcowax fused silical capillary column (30 m 9 0.32 mm ID, Supelco, Bellefonte, PA, USA). Nitrogen was used as carrier gas. Samples were injected in split mode at 250° C. Column temperature was maintained at 180° C for 12 min, then increased to 212° C

at a rate of 2° C min⁻¹ and maintained at 212° C for 13 min. FA were identified by comparing retention times of methyl ester standards (Supelco) using a well characterized fish oil as a reference.

STATISTICAL ANALYSIS

Data were analysed statistically using a one-way analysis of variance (ANOVA) and Student’s t-test for means comparison (SOKAL; ROHLF, 2009). The significance level was set at $p < 0.05$.

RESULTS

OVARIAN COMPOSITION AND CONDITION FACTOR THROUGHOUT THE REPRODUCTIVE CYCLE

The evolution of ovarian composition and condition factor during the reproductive cycle is shown in Table 1. Total lipids was significantly lower in fall than in winter and spring for both species. Besides, *P. orbignyanus* showed a significantly higher amount of lipids in spring than in the other seasons. Moisture content was constant in ovaries of both species. The condition factor (K) in *P. patagonicus* was significantly higher in spring, whereas no significant differences between winter and fall were seen. In *P. orbignyanus* the K value remained constant throughout the three seasons (Table 1).

COMPOSITION OF OVARIES AND CONDITION FACTOR IN DIFFERENT SEASONS (COMPARISON BETWEEN SPECIES)

Total lipid content in the ovaries of *P. orbignyanus* was higher than in those of *P. patagonicus* in winter and spring, while no significant differences between species were found in the fall (Table 1). The moisture content was significantly different only in winter, resulting in a higher value in *P. orbignyanus*. The K value in winter and fall was significantly higher in *P. orbignyanus* than in *P. patagonicus*, while in spring no significant differences between species were seen (Table 1).

OVARY FATTY ACID PROFILE DURING THE REPRODUCTIVE CYCLE

The evolution of the ovarian FA profile throughout the reproductive cycle of *P. patagonicus* and *P. orbignyanus* is shown in Table 2. In all ovarian samples analysed 41 FA were identified, representing over 95% of all FA detected.

Table 1. Moisture evolution (%) and total lipids (% dry weight) of ovaries and condition factor (K) of *P. orbignyanus* and *P. patagonicus* throughout the reproductive cycle.

	<i>Paralichthys orbignyanus</i>			<i>Paralichthys patagonicus</i>		
	fall (n = 6)	winter (n = 11)	spring (n = 10)	fall (n = 12)	winter (6)	spring (n = 11)
Moisture	82.72 ± 2.75	83.95 ± 2.54A	83.27 ± 3.15	82.76 ± 1.67	81.22 ± 0.56B	81.94 ± 1.49
Total lipid	4.45 ± 1.21c	5.55 ± 0.62bA	13.29 ± 4.26aA	3.92 ± 0.34b	4.92 ± 0.35aB	5.05 ± 1.33aB
K	1.13 ± 0.04A	1.10 ± 0.07A	1.04 ± 0.22	1.01 ± 0.06bB	0.98 ± 0.10bB	1.06 ± 0.01a

Results are expressed as means ± SD; n = number of samples; within a row, means without a common superscript lowercase letter differed significantly between seasons for each species and values without a common superscript uppercase letter differed significantly between both species in the same season (ANOVA, $p < 0.05$).

PUFA were predominant in both species, followed by SAFA and finally by MUFA. The SAFA percentage was significantly higher in the fall for both species, showing significant differences between winter and spring in *P. orbignyanus*. Only in *P. orbignyanus* was the MUFA percentage significantly different between seasons, being higher in spring than in the other seasons. The predominant SAFA were 16:0 and 18:0, while the predominant MUFA were 18:1n-9 and 18:1n-7. The PUFA percentage remained constant in *P. orbignyanus*, while in *P. patagonicus* this value was significantly lower in the fall. In both species the predominant PUFA were DHA, ARA and EPA. N-3 FA, n-3 HUFA and DHA proportions were significantly lower in fall than in winter and spring for both species, while the opposite was observed for n-6 FA, n-6 HUFA and ARA. The EPA percentage was significantly lower in fall than in winter and spring in *P. patagonicus*, while in *P. orbignyanus* it remained constant throughout the reproductive cycle. In *P. patagonicus* the 18:2n-6 proportion was significantly higher in winter than in fall and spring, while in *P. orbignyanus* it remained constant throughout the reproductive cycle. In contrast, the 18:3n-3 percentage was significantly lower in winter than in fall and spring for both species. The 18:4n-3 proportion was higher in spring than in winter for both species, particularly for *P. orbignyanus*. The DHA/EPA ratio in *P. patagonicus* was lower in the fall than in winter and spring, and in *P. orbignyanus* no significant differences were found at any time during the reproductive cycle. Meanwhile, for both species DHA/ARA and EPA/ARA ratios were lower in fall than in winter and spring (Table 2).

OVARIAN FATTY ACID PROFILE IN DIFFERENT SEASONS (COMPARISON BETWEEN SPECIES)

A comparison of the ovarian FA profile between the two species is shown in Table 2. In spring, proportions of SAFA, MUFA and PUFA showed no significant

differences. In fall, the PUFA percentage was significantly higher in *P. orbignyanus*, while in *P. patagonicus* the MUFA percentage was significantly higher in fall and winter. In winter the SAFA proportion was significantly higher in *P. orbignyanus*. Except in winter, when no significant variations were found, the proportions of n-3 FA and n-3 HUFA were significantly higher in *P. orbignyanus* than in *P. patagonicus*. Differences in n-3 HUFA proportions in spring were mainly determined by variations in minor n-3 HUFA, such as 22:5n-3 and 20:4n-3, since no significant differences between species were found in EPA and DHA proportions in spring. The percentage of 18:3n-3 was significantly higher in *P. orbignyanus* in winter and in *P. patagonicus* in spring, while in fall no significant differences between both species were observed. Meanwhile proportions of n-6 FA and n-6 HUFA were significantly higher in *P. patagonicus* than in *P. orbignyanus* in the three seasons studied. However, the ARA percentage was higher in *P. patagonicus* in fall and spring, while in winter no significant differences between the two species were found. The percentage of 18:2n-6 was always significantly higher in *P. orbignyanus*. The DHA/EPA ratio only showed significant differences between the two species in winter, it being higher in *P. orbignyanus*. The EPA/ARA and DHA/ARA ratios were significantly higher in *P. orbignyanus* in the three seasons studied (Table 2).

DISCUSSION

It is well known that fishes experience different changes in their biochemical composition throughout their lifetime in response to diet variations, environmental factors and reproductive cycles (HUYNH et al., 2007; PÉREZ et al., 2007; HUANG et al., 2010; XU et al., 2010).

Important seasonal changes in gonadal biochemical composition were observed in both the sympatric flatfish species studied in this research. These changes consisted mainly in the lipid accumulation during the

Table 2. Fatty acid profile evolution during the reproductive cycle (% of total fatty acids) in the ovaries of *P. orbignyanus* and *P. patagonicus*.

Fatty acids	<i>Paralichthys orbignyanus</i>			<i>Paralichthys patagonicus</i>		
	fall (n = 6)	winter (n = 11)	spring (n = 10)	fall (n = 12)	winter (6)	spring (n = 11)
14:0	1.25 ± 0.32bB	1.30 ± 0.19b	2.03 ± 0.73aA	1.55 ± 0.25A	1.38 ± 0.35	1.49 ± 0.37B
15:0	0.49 ± 0.07bA	0.55 ± 0.04aA	0.52 ± 0.10ab	0.43 ± 0.04B	0.49 ± 0.07B	0.49 ± 0.13
16:0	20.74 ± 0.75bA	23.00 ± 1.13a	19.77 ± 3.22b	18.74 ± 0.93bB	21.90 ± 1.35a	19.81 ± 3.41ab
16:1*	4.07 ± 0.32b	3.59 ± 0.31cB	7.28 ± 1.62a	4.50 ± 0.95b	5.63 ± 1.25aA	6.19 ± 0.99a
16:2**	1.32 ± 0.14aB	0.89 ± 0.12bB	1.06 ± 0.40ab	1.75 ± 0.34aA	2.05 ± 0.64aA	1.14 ± 0.24b
18:0	7.95 ± 0.34aB	6.97 ± 0.48bA	4.70 ± 1.26cB	9.18 ± 0.65aA	5.87 ± 0.41bB	5.98 ± 0.82bA
18:1n-9	10.04 ± 0.62bB	12.35 ± 0.97a	13.70 ± 2.59a	15.21 ± 1.15aA	13.06 ± 1.54b	14.73 ± 2.09ab
18:1n-7	3.90 ± 0.24aA	3.89 ± 0.23aA	3.15 ± 0.33bB	2.98 ± 0.30bB	3.33 ± 0.11aB	3.55 ± 0.42aA
18:2n-6	0.93 ± 0.12A	1.03 ± 0.14A	1.01 ± 0.22A	0.54 ± 0.07bB	0.65 ± 0.10aB	0.62 ± 0.13abB
18:3n-3	0.81 ± 0.30a	0.27 ± 0.05cA	0.47 ± 0.15bB	0.86 ± 0.21a	0.13 ± 0.03bB	0.98 ± 0.23aA
18:4n-3	0.27 ± 0.05b	0.27 ± 0.10bA	0.99 ± 0.40aA	0.28 ± 0.03a	0.09 ± 0.03bB	0.28 ± 0.05aB
20:1n-9	0.75 ± 0.11	0.81 ± 0.08	1.35 ± 1.21	0.79 ± 0.09	0.83 ± 0.17	0.89 ± 0.19
20:1n-7	0.22 ± 0.05	0.20 ± 0.03B	0.26 ± 0.12	0.21 ± 0.04b	0.28 ± 0.05aA	0.28 ± 0.06a
20:2n-6	0.23 ± 0.06b	0.37 ± 0.04a	0.23 ± 0.08b	0.21 ± 0.03b	0.33 ± 0.05a	0.22 ± 0.04b
20:4n-6	7.81 ± 1.50aB	6.17 ± 0.64b	4.23 ± 1.26cB	10.35 ± 0.75aA	6.99 ± 1.32b	6.86 ± 1.64bA
20:4n-3	0.30 ± 0.12abA	0.31 ± 0.06bA	0.44 ± 0.18aA	0.18 ± 0.05B	0.19 ± 0.05B	0.19 ± 0.06B
20:5n-3	4.18 ± 1.07A	4.77 ± 0.64	5.18 ± 0.81	3.08 ± 0.59bB	4.97 ± 0.47a	4.46 ± 1.11a
22:1 +	0.46 ± 0.22abA	0.62 ± 0.08aA	0.33 ± 0.15b	0.27 ± 0.15bB	0.41 ± 0.08aB	0.36 ± 0.14ab
22:5n-3	3.29 ± 0.26bA	3.33 ± 0.36bB	3.93 ± 0.49aA	2.60 ± 0.47bB	4.22 ± 0.38aA	3.27 ± 0.78aB
22:6n-3	19.53 ± 1.41bA	23.46 ± 1.99aA	22.31 ± 2.43a	14.73 ± 1.63bB	20.26 ± 3.11aB	18.86 ± 4.76a
SAFA	34.65 ± 0.92a	33.05 ± 1.52bA	28.29 ± 2.75c	33.84 ± 1.33a	30.65 ± 1.46bB	30.16 ± 4.24b
MUFA	20.89 ± 0.72bB	21.93 ± 1.15bB	26.90 ± 4.11a	26.15 ± 1.96A	24.14 ± 2.82A	27.59 ± 3.71
PUFA	44.45 ± 0.85A	45.02 ± 2.57	44.81 ± 2.59	40.01 ± 2.07bB	45.21 ± 3.71a	42.24 ± 7.38ab
n-9	12.28 ± 0.90bB	13.72 ± 1.04a	15.91 ± 3.58a	17.48 ± 1.25aA	14.31 ± 1.61b	17.12 ± 2.15a
n-3	29.91 ± 2.39bA	32.69 ± 2.40a	33.75 ± 2.53aA	22.69 ± 1.88bB	30.09 ± 3.29a	28.81 ± 6.27aB
n-6	10.71 ± 1.83aB	10.11 ± 0.90a	6.72 ± 1.56bB	12.92 ± 0.84aA	11.45 ± 2.19ab	9.47 ± 2.07bA
n-3/n-6	2.88 ± 0.64bA	3.25 ± 0.37bA	5.27 ± 1.25aA	1.76 ± 0.16bB	2.72 ± 0.62aB	3.12 ± 0.74aB
n-3 HUFA	27.52 ± 2.58bA	32.12 ± 2.38a	32.02 ± 2.33aA	20.73 ± 1.93bB	29.80 ± 3.30a	26.95 ± 6.42aB
n-6 HUFA	9.55 ± 1.72aB	8.71 ± 0.88aB	5.48 ± 1.59bB	12.17 ± 0.85aA	10.47 ± 2.10bA	8.63 ± 2.10bA
DHA/EPA	4.93 ± 1.23	4.98 ± 0.69A	4.41 ± 0.88	4.91 ± 0.80a	4.08 ± 0.59bB	4.25 ± 0.46b
EPA/ARA	0.57 ± 0.22cA	0.78 ± 0.16b	1.33 ± 0.46aA	0.30 ± 0.06bB	0.73 ± 0.16a	0.68 ± 0.22aB
DHA/ARA	2.59 ± 0.59cA	3.83 ± 0.47bA	5.69 ± 1.67aA	1.43 ± 0.18bB	2.98 ± 0.66aB	2.86 ± 0.90aB

Results are expressed as means ± SD; n = number of samples; within a row, means without a common superscript lowercase letter differed significantly between seasons for each species and means without a common superscript uppercase letter differed significantly between species in the same season (ANOVA, $p < 0.05$). * includes n-9, n-7 and n-5 isomers; ** includes n-3 and n-4 isomers; + includes n-11, n-9 and n-7 isomers.

resting phase (from the end of one breeding season to the beginning of the new breeding season), together with important changes in FA profile throughout the reproductive cycle. The accumulation of lipid reserves in gonads in marine teleosts has been widely studied (TAKAMA et al., 1985; CHATZIFOTIS et al., 2004; GARRIDO et al., 2007; HUYNH et al., 2007). The lipid mobilization to the gonads supplies raw material, energy and EFA

necessary for the genesis and normal egg development (SARGENT et al., 2002).

FA composition of the ovaries of *P. orbignyanus* and *P. patagonicus* showed a typical marine profile, mainly characterized by PUFA predominance and high levels of n-3 FA and n-3 HUFA with significant amounts of DHA (SARGENT et al., 2002; ARTS et al., 2009). These characteristics prevailed throughout the year regardless of

the season even in *P. orbignyanus* females collected in a brackish coastal lagoon semi-connected to the ocean.

In both species PUFA was composed mostly of n-3HUFA. It is known that HUFA affect fishes' reproduction either directly or through their metabolites (SARGENT et al., 2002). As marine fishes are not able to synthesize their own HUFA efficiently they must obtain them from their diet (SARGENT et al., 1999a, 1999b, 2002). Lipid and FA composition of broodstock diet has been identified as one of the most important dietary factors determining reproductive success in fishes (IZQUIERDO et al., 2001). In some species, it has been observed that n-3HUFA content in broodstock diets increases fecundity, fertilization, egg quality and larval viability (FERNÁNDEZ-PALACIOS et al., 1995; FURUITA et al., 2000). Thus, taking into account the high levels of n-3HUFA observed in the ovaries of *P. patagonicus* and *P. orbignyanus*, high diet requirements of n-3HUFA could be expected.

Gonads of both species had particularly high levels of ARA, which exceeded the EPA proportion in almost all the specimens analyzed, mainly those captured during the resting phase. In other fish species studied the EPA/ARA ratio in gonads and eggs is greater than 1 (GARRIDO et al., 2007; HUYNH et al., 2007; HUANG et al., 2010; XU et al., 2010), even in other flatfishes such as *Solea senegalensis* (NORAMBUENA et al., 2012) and *Paralichthys adspersus* (WILSON, 2009). The selective retention of ARA has been observed in other estuarine fishes and it has been attributed to the environmental stress conditions to which fishes are exposed (KOUSSOROPLIS et al., 2011). There is evidence that ARA is important in generating an appropriate physiological response to environmental stress, such as changes in salinity (BELL; SARGENT, 2003).

Regarding bodily condition, the K values showed differences between the two species throughout the year. Such differences could be caused by their different lifestyles. According to the K values, *P. patagonicus* females were in better physical condition at the beginning of the spawning season than during the resting phase. On the other hand, the physical condition of *P. orbignyanus* females remained constant throughout the reproductive cycle. K values in *P. patagonicus* were significantly lower than in *P. orbignyanus* during the resting phase but they were similar for both species at the beginning of the spawning season. On the other hand, although the ovarian lipid content increased significantly in both species during gonadal maturation, it was significantly higher in *P. orbignyanus*.

We believe that during the cold seasons (fall and winter) there is less food available for flatfishes in the sea, which could be reflected in a decrease of K values and a reduced accumulation of lipids in *P. patagonicus*' gonads during the resting phase. *P. orbignyanus* would not be affected by the lower availability of food during the cold season because it can live in brackish water courses probably taking refuge during adverse months in estuarine environments where more food is available. On the other hand, another factor to be considered is that osmoregulation is an energy expensive process (TSENG; HWANG, 2008), therefore for a euryhaline fish such as *P. orbignyanus* it might be more economical to live in an isosmotic environment. The isosmotic point estimate for *P. orbignyanus* is 10.9‰ (SAMPAIO; BIANCHINI, 2002), the salinity likely to be found in a brackish coastal lagoon such as "Laguna de Rocha" (CONDE et al., 2000). However, it is also known that in *P. orbignyanus*, as in other species, growth hormone levels increase after suffering hyperosmotic stress, whereby the highest growth rates in *P. orbignyanus* would be obtained in seawater (MEIER et al., 2009). Despite this, SAMPAIO et al., 2001 observed no significant variations in the growth rates of *P. orbignyanus* cultured at salinities such as 30ppt and 11ppt. Thus, the combined effect of sparing energy with osmoregulation and getting more food by remaining in brackish water environments could allow *P. orbignyanus* to maintain a stable condition factor throughout the year, unlike *P. patagonicus* that lives in seawater all the year round. We need further research into lipid and FA composition of fish muscle in order to establish whether *P. orbignyanus* females were better fed than *P. patagonicus* during the recovery phase.

The ability of *P. orbignyanus* to maintain its condition factor stable and accumulate lipids in its tissues during cold seasons based mainly on an estuarine diet, does not explain the higher amount of n-3HUFA accumulated in their gonads than in those of *P. patagonicus*, which feed solely in seawater. Comparing FA profiles between the two species, we observed that the proportions of n-3 FA and n-3HUFA were higher in *P. orbignyanus*, while n-6 FA and the n-6HUFA proportions were higher in *P. patagonicus*, contrary to what we expected to find considering their different lifestyles. Assuming that the estuarine diet of *P. orbignyanus* is poorer in n-3HUFA than in the marine diet of *P. patagonicus*, the proportion of n-3HUFA found in *P. orbignyanus* equivalent to or even higher than that found in *P. patagonicus* could be explained by a selective accumulation in the ovaries of *P. orbignyanus* of specific

FA which appear in low concentration in their diet. This ability has been observed in other estuarine fishes of high trophic level, presenting more FA such as n-3HUFA than that found in their diet. Such selective retention of HUFA has been associated with a limited availability of this FA in the food web and also clearly shows an efficient ability to retain FA (KOUSSOROPLIS et al., 2011). *P. orbignyanus* is a high trophic level predator in coastal lagoons (RODRÍGUEZ-GRAÑA et al., 2008), so the accumulation of certain FA which are found in low proportion in their estuarine diet, such as n-3HUFA, is to be expected. Additionally we could consider the possibility of a greater ability of *P. orbignyanus* to elongate and desaturate LNA to DHA and EPA. This is in accordance with the higher proportion of 18:4n-3 and 20:4n-3 (the intermediate metabolites in the process to elongate and desaturate LNA to DHA EPA) (SARGENT et al., 2002) found in *P. orbignyanus* compared to that in *P. patagonicus*. This ability is shared with many vertebrates; it is not, however, common in marine fishes (SARGENT et al., 1999a, 1999b, 2002) although there is strong evidence suggesting that turbot (*Scophthalmus maximus*), another species of flatfish, has the ability to transform LNA and LA, respectively, into EPA and ARA (SARGENT et al., 2002). However, this is a hypothesis and further laboratory experiments are needed to establish whether *P. orbignyanus* is capable of selecting and retaining FA which are in low concentration in its diet or if they are capable to elongating and desaturating LNA efficiently. Furthermore, it was observed that the proportion of LA was greater in *P. orbignyanus* throughout its reproductive cycle, probably reflecting the FA composition of its estuarine diet. This FA is abundant in terrestrial/continental food webs such as freshwater streams and may also be abundant in estuaries (ALFARO et al., 2006; RICHOUX; FRONEMAN, 2008; KOUSSOROPLIS et al., 2011) being much rarer in the marine environment (SARGENT et al., 2002. ARTS et al., 2009).

In brief, differences between the lifestyles of these two species are reflected in the ovarian FA profile mainly as a result of differences in their FA metabolism, causing higher accumulation of n-3 FA and n-3 HUFA in *P. orbignyanus* than in the more oceanic *P. patagonicus* flatfishes.

The greater accumulation of lipids in the ovaries of *P. orbignyanus* could indicate that this species, feeding in brackish water bodies, is able to accumulate more energy than *P. patagonicus*, but to confirm this hypothesis further studies of muscle lipid and FA composition are needed.

ACKNOWLEDGEMENTS

We would like to thank the Dirección Nacional de Recursos Acuáticos (DINARA) for providing us with biological samples from the B/I Aldebarán coastal surveys. We are also grateful to the fishermen working at Rocha lagoon for allowing us access to the biological samples of *P. orbignyanus* during their work. We also thank the anonymous reviewers for their supportive comments which helped us in the preparation of this manuscript.

REFERENCES

- ALFARO, A. C.; THOMAS, F.; SERGENT, L.; DUXBURY, M. Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. *Estuar. Coast. Shelf Sci.*, v. 70, n. 1/2, p. 271-286, 2006.
- ARTS, M. T.; BRETT, M. T.; KAINZ, M. J. (Eds.). *Lipids in Aquatic Ecosystems*. New York: Springer, 2009. 377 p.
- BAMBILL, G. A.; OKA, M.; RADONIC, M.; LOPEZ, A. V.; MÜLLER, M. I.; BOCCANFUSO, J. J.; BIANCA, F. A. Broodstock management and induced spawning of flounder *Paralichthys orbignyanus* (Valenciennes, 1839) under a closed recirculated system. *Rev. Biol. Mar. Oceanogr.*, v. 41, n. 1, p. 45-55, 2006.
- BELL, J. G.; SARGENT, J. R. Arachidonic acid in aquaculture feeds: current status and future opportunities. *Aquaculture*, v. 218, n.1/4, p. 491-499, 2003.
- CERQUEIRA, V. R. Egg development of *Paralichthys orbignyanus* (Valenciennes, 1839). *Braz. Arch. Biol. Techn.*, v. 48, n. 3, p. 459-465, 2005.
- CHATZIFOTIS, S.; MUJE, P.; PAVLIDISA, M.; AGREN, J.; PAALAVUO, M.; MÖLSÄ, H. Evolution of tissue composition and serum metabolites during gonadal development in the common dentex (*Dentex dentex*). *Aquaculture*, v. 236, n. 1/4, p. 557-573, 2004.
- CHRISTIE, W. W. *Lipid analysis: isolation, separation, identification, and structural analysis of lipids*. Oxford: Pergamon Press, 1982. 207 p.
- CONDE, D.; AUBRIOT, L.; SOMMARUGA, R. Changes in UV penetration associated with marine intrusions and freshwater discharge in a shallow coastal lagoon of the southern Atlantic Ocean. *Mar. Ecol. Prog. Ser.*, v. 207, p. 19-31, 2000.
- COUSSEAU, M. B.; PERROTTA, R. B. *Peces Marinos de Argentina: biología, distribución y pesca*. Mar del Plata: INIDEP-SAGPyA, 2004. 167 p.
- DÍAZ DE ASTARLOA, J. M. A review of the flatfish fisheries of the south Atlantic Ocean. *Rev. Biol. Mar. Oceanogr.*, v. 37, n. 2, p. 113-125, 2002.
- DÍAZ DE ASTARLOA, J. M.; MUNROE, T. A. Systematics, distribution and ecology of commercially important paralichthyid flounders occurring in Argentinian-Uruguayan waters (*Paralichthys*, Paralichthyidae): an overview. *J. Sea Res.*, v. 39, n. 1/2, p. 1-9, 1998.

- FABRÉ, N. N.; DÍAZ DE ASTARLOA, J. M. Pleuronectiformes de importancia comercial del Atlántico sudoccidental, entre los 34°30' y 55° S. Distribución y consideraciones sobre su pesca. *Rev. Invest. Des. Pesq.*, v. 10, p. 45-55, 1996.
- FERNÁNDEZ-PALACIOS, H.; IZQUIERDO, M. S.; ROBAINA, L.; VALENCIA, A.; SALHI, M.; VERGARA, J. Effect of n-3 HUFA level in broodstock diets on egg quality of gilthead seabream (*Sparus aurata* L.). *Aquaculture*, v. 132, p. 325-337, 1995.
- FOLCH, J. M.; LEES, M.; SLOANE STANLEY, G. H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, v. 226, n. 1, p. 497-509, 1957.
- FURUITA, H.; TANAKA, H.; YAMAMOTO, T.; SHIRAIISHI, M.; TAKEUCHI, T. Effects of n-3 HUFA levels in broodstock diet on the reproductive performance and egg and larval quality of the Japanese flounder, *Paralichthys olivaceus*. *Aquaculture*, v. 187, n. 3/4, p. 387-398, 2000.
- GARRIDO, S.; ROSA, R.; BEN-HAMADOU, R.; CUNHA, M. E.; CHÍCHARO, M. A.; VAN DER LINGEN, C. D. Effect of maternal fat reserves on the fatty acid composition of sardine (*Sardina pilchardus*) oocytes. *Comp. Biochem. Phys. B.*, v. 148, n. 4, p. 398-409, 2007.
- HAIMOVICI, M.; ARAÚJO, J. N. *Paralichthys patagonicus* (Jordan, 1889). In: ROSSI, C. L. W.; CERGOLE, M. C.; ÁVILA-DA-SILVA, A. O. (Eds.). *Análise das Principais Pescarias Comerciais da Região Sudeste-Sul do Brasil: Dinâmica Populacional das Espécies em Exploração. Série Documentos Revizee-Score Sul*. São Paulo: IOUSP, 2005. p. 116-123.
- HUANG, X.; YIN, Y.; SHI, Z.; LI, W.; ZHOU, H.; LV, W. Lipid content and fatty acid composition in wild-caught silver pomfret (*Pampus argenteus*) broodstocks: Effects on gonad development. *Aquaculture*, v. 310, n. 1/2, p. 192-199, 2010.
- HUYNH, M. D.; KITTS, D. D.; HU, C.; TRITES, A. W. Comparison of fatty acid profiles of spawning and non-spawning Pacific herring, *Clupea harengus pallasi*. *Comp. Biochem. Phys. B.*, v. 146, n. 4, p. 504-511, 2007.
- IZQUIERDO, M. S.; FERNÁNDEZ-PALACIOS, H.; TACON, A. G. J. Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture*, v. 197, n. 1/4, p. 25-42, 2001.
- KOUSSOROPLIS, A. M.; BEC, A.; PERGA, M. E.; KOUTRAKIS, E.; BOURDIER, G.; DESVILETTES, C. Fatty acid transfer in the food web of a coastal Mediterranean lagoon: Evidence for high arachidonic acid retention in fish. *Estuar. Coast. Shelf Sci.*, v. 91, n. 3, p. 450-461, 2011.
- LANES, C. F. C.; OKAMOTO, M. H.; BIANCHINI, A.; MARINS, L. F.; SAMPAIO, L. A. Sperm quality of Brazilian flounder *Paralichthys orbignyanus* throughout the reproductive season. *Aquac. Res.*, v. 41, p. 199-207, 2010.
- MACHI, G. J.; DÍAZ DE ASTARLOA, J. M. Ciclo Reproductivo y Fecundidad del Lengüado *Paralichthys patagonicus* Jordan, en Jordan y Goss 1889. *Rev. Invest. Des. Pesq.*, v. 10, p. 73-83, 1996.
- MEIER, K. M.; FIGUEIREDO, M. A.; KAMIMURA, M. T.; LAURINO, J.; MAGGIONI, R.; PINTO, L. S.; DELLAGOSTIN, O. A.; TESSER, M. B.; SAMPAIO, L. A.; MARINS, L. F. Increased growth hormone (GH), growth hormone receptor (GHR), and insulin-like growth factor I (IGF-I) gene transcription after hyperosmotic stress in the Brazilian flounder *Paralichthys orbignyanus*. *Fish Physiol. Biochem.*, v. 35, p. 501-509, 2009.
- MILITELLI, M. I. *Paralichthys patagonicus* spawning areas and reproductive potential in the Bonaerense Coastal Zone, Argentina (34°-42°S). *Lat. Am. J. Aquat. Res.*, v. 39, n. 1, p. 131-137, 2011.
- NORAMBUENA, F.; ESTEVEZ, A.; BELL, G.; CARAZO, I.; DUNCAN, N. Proximate and fatty acid compositions in muscle, liver and gonads of wild versus cultured broodstock of Senegalese sole (*Solea senegalensis*). *Aquaculture*, v. 356-357, p. 176-185, 2012.
- NORBIS, W.; GALLI, O. Hábitos de alimentación del lengüado *Paralichthys orbignyanus* (Valenciennes, 1842) en una laguna costera somera del Atlántico Sur: Rocha, Uruguay. *Cienc. Mar.*, v. 30, n. 4, p. 619-626, 2004.
- PLAVAN, A. A.; PASSADORE, C.; GIMENEZ, L. Fish assemblage in a temperate estuary on the uruguay coast: seasonal variation and environmental influence. *Braz. J. Oceanogr.*, v. 58, n. 4, p. 299-314, 2010.
- PÉREZ, M. J.; RODRÍGUEZ, C.; CEJAS, J. R.; MARTÍN, M. V.; JEREZ, S.; LORENZO, A. Lipid and fatty acid content in wild white seabream (*Diplodus sargus*) broodstock at different stages of the reproductive cycle. *Comp. Biochem. Phys. B.*, v. 146, n. 2, p. 187-196, 2007.
- RADONIC, M.; MACCHI, G. J. Gonadal sex differentiation in cultured juvenile flounder, *Paralichthys orbignyanus* (Valenciennes, 1989). *J. World Aquac. Soc.*, v. 40, n. 1, p. 129-133, 2009.
- RADONIC, M.; MÜLLER, M. I.; LÓPEZ, A. V.; BAMBILL, G. A.; SPINEDI, M.; BOCCANFUSO, J. J. Improvement in flounder *Paralichthys orbignyanus* controlled spawning in Argentina. *Cienc. Mar.*, v. 33, n. 2, p. 187-196, 2007.
- RICO, M. R. Pesquería de lenguados en el ecosistema costero bonaerense al norte de 39° S. *Frete Marít.*, v. 21, p. 129-135, 2010.
- RICHOUX, N.; FRONEMAN, P. Trophic ecology of dominant zooplankton and macrofauna in a temperate, oligotrophic South African estuary: a fatty acid approach. *Mar. Ecol. Prog. Ser.*, v. 357, p. 121-137, 2008.
- RICKER, W. Computation and interpretation of biological statistics of fish populations. *Bull. Fish. Res. Board Can.*, v. 191, 1975. p. 382.
- RIVERA, A.; GARCÍA, S. B.; DÍAZ DE ASTARLOA, J. M. Feeding Ecology of Flatfish Juveniles (Pleuronectiformes) in Mar Chiquita Coastal Lagoon (Buenos Aires, Argentina). *Estuaries*, v. 24, n. 6A, p. 917-925, 2001.
- ROBALDO, R. B. Estudo comparativo da reprodução do linguado *Paralichthys orbignyanus* (Valenciennes, 1839) no ambiente e em cativeiro. PhD Thesis. Rio Grande: Fundação Universidade Federal do Rio Grande, 2003. 190 p.
- RODRÍGUEZ-GRAÑA, L.; CALLIARI, D.; CONDE, D.; SELLANES, J.; URRUTIA, R. Food web of a SW Atlantic shallow coastal lagoon: spatial environmental variability does not impose substantial changes in the trophic structure. *Mar. Ecol. Prog. Ser.*, v. 362, p. 69-83, 2008.
- SAMPAIO, L. A.; BIANCHINI, A. Salinity effects on osmoregulation and growth of the euryhaline flounder *Paralichthys orbignyanus*. *J. Exp. Mar. Biol. Ecol.*, v. 269, p. 187-196, 2002.
- SAMPAIO, L. A.; BIANCHINI, A.; CERQUEIRA, V. R. Growth of juvenile Brazilian flounder, *Paralichthys orbignyanus* cultured in different salinities. *J. Appl. Aquaculture.*, v. 11, p. 67-75, 2001.

- SAMPAIO, L. A.; FREITAS, L. S.; OKAMOTO, M. H.; LOUZADA, L. R.; RODRIGUES, R. V.; ROBALDO, R. B. Effects of salinity on Brazilian flounder *Paralichthys orbignyanus* from fertilization to juvenile settlement. *Aquaculture*, v. 262, n. 2/4, p. 340-346, 2007.
- SARGENT, J.; BELL, G.; McEVOY, L.; TOCHER, D.; ESTEVEZ, A. Recent developments in the essential fatty acid nutrition of fish. *Aquaculture*, v. 177, n. 1/4, p. 191-199, 1999a.
- SARGENT, J.; McEVOY, L.; ESTEVEZ, A.; BELL, G.; BELL, M.; HENDERSON, J.; TOCHER, D. Lipid nutrition of marine fish during early development: current status and future directions. *Aquaculture*, v. 179, p. 217-229, 1999b.
- SARGENT, J. R.; TOCHER, D. R.; BELL, J. G. The lipids. In: HALVER, J. E.; HARDY, R. W. (Eds.). *Fish Nutrition*. 3. ed. San Diego: Elsevier, 2002. p. 181-257.
- SILVEIRA, M. P. M.; COUSIN, J. C. B.; HAIMOVICI, M. Estrutura ovárica e testicular do linguado *Paralichthys orbignyanus* (Valenciennes, 1939). *Atlântica*, v. 17, p. 135-152, 1995.
- SOKAL, R. R.; ROLHF, F. J. *Introduction to Biostatistics*. 2. ed. New York: Dover Publications, 2009.
- TAKAMA, K.; LOVE, R. M.; SMITH, G. L. Selectivity in mobilization of stored fatty acids by maturing cod, *Gadus morrhua* L. *Comp. Biochem. Physiol.*, v. 80B, n. 4, p. 713-718, 1985.
- TSENG, Y. C.; HWANG, P. P. Some insights into energy metabolism for osmoregulation in fish. *Comp. Biochem. Physiol. C.*, v. 148, n. 4, p. 419-429, 2008.
- WASIELESKY, Jr., W.; BIANCHINI, A.; SANTOS, M. H. S.; POERSCH, L. Tolerance of juvenile flatfish *Paralichthys orbignyanus* to acid stress. *J. World Aquacult. Soc.*, v. 28, n. 2, p. 202-204, 1997.
- WILSON, R. Dietary effects of n-3 highly unsaturated fatty acid levels on egg and larval quality, and the fatty acid composition of the eggs of Chilean flounder *Paralichthys adspersus* broodstock. *Aquac. Res.*, v. 40, n. 12, p. 1400-1409, 2009.
- XU, J.; YAN, B.; TENG, Y.; LOU, G.; LU, Z. Analysis of nutrient composition and fatty acid profiles of Japanese sea bass *Lateolabrax japonicus* (Cuvier) reared in seawater and freshwater. *J. Food Comp. Anal.*, v. 23, n. 5, p. 401-405, 2010.