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Research Article

Feeding ω-3 PUFA enriched rotifers to *Galaxias maculatus* (Jenyns, 1842) larvae reared at different salinity conditions: effects on growth parameters, survival and fatty acids profile

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ABSTRACT. Despite the well known importance of ω-3 polyunsaturated fatty acids (PUFA) in marine and freshwater fish larvae, there are few studies on how essential fatty acid requirements and composition on whole body can be altered by changes in water salinity. The present study aimed to determine the effect of salinity on ω-3 PUFA requirements, larval growth survival and fatty acid composition of *Galaxias maculatus* larvae cultured at two different salinities (0 and 15 g L^{-1}) for 20 days while fed rotifers containing two different levels of ω-3 PUFA (1.87 and 3.16%). The results denoted a marked difference in ω-3 PUFA requirements and in the pattern of fatty acid deposition in the whole body of larvae reared at different salinities, depending of ω-3 PUFA in diets. Thus, to improve growth and survival larvae of *G. maculatus* reared at 0 g L^{-1} require higher levels of ω-3 PUFA, principally 18:3 ω-3. Larvae reared at salinities of 15 g L^{-1} require low levels of ω-3 PUFA for optimal survival, especially 18:3 ω-3. Eicosapentaenoic acid and docosahexaenoic acid content in the whole body of larvae was also affected by water salinity.

Keywords: larval nutrition, whitebait, *Galaxias maculatus*, fatty acid requirement.

Alimentación de larvas de *Galaxias maculatus* (Jenyns, 1842) con rotíferos enriquecidos con ω-3 PUFA cultivadas a diferentes condiciones de salinidad: efectos sobre el crecimiento, sobrevivencia y perfil de ácidos grasos

RESUMEN. A pesar que es bien conocida la importancia de los ácidos grasos poli-insaturados (PUFA), principalmente los de la serie ω-3, en larvas de peces marinos y de agua dulce, existen escasos estudios sobre cómo los requerimientos de ácidos grasos esenciales y de la composición en el cuerpo del pez pueden ser alterados por los cambios en la salinidad del agua. El presente estudio tuvo como objetivo determinar el efecto de la salinidad sobre los requerimientos de lo PUFA ω-3 y en la composición de ácidos grasos en larvas de *Galaxias maculatus* cultivadas durante 20 días posteclosión. Para esto se evaluaron dos niveles de salinidad (0 y 15 g L^{-1}) y dos concentraciones de PUFA ω-3 (1,87 y 3,16%), utilizando rotíferos enriquecidos. Los resultados muestran una marcada diferencia en los requerimientos de PUFA ω-3 y en el patrón de deposición de ácidos grasos en las larvas cultivadas a diferentes salinidades en función de la dieta. Así, para mejorar el crecimiento y la sobrevivencia en larvas de *G. maculatus* cultivdas a 0 g L^{-1} se requiere mayores niveles de PUFA ω-3, principalmente 18:3 ω-3 mientras que larvas cultivadas a salinidades de 15 g L^{-1} requieren menores niveles PUFA ω-3, especialmente 18:3 ω-3. El nivel de los ácidos eicosapentaenoico (EPA) y docosahexaenoico (DHA) contenidos en el cuerpo de las larvas se vieron afectados por la salinidad del agua.

Palabras clave: nutrición de larvas, puye, *Galaxias maculatus*, requerimientos de ácidos grasos.

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INTRODUCTION

Galaxias maculatus (Jenyns, 1848) is a small-size fish with circumpolar distribution around Antarctic, known in Chile and Argentina as puye or pueyen, and in New Zealand and Australia as whitebait (Mardones *et al.,* 2008). This fish lives in different salinity environments such as rivers, lakes and estuaries. Two types of populations have been described: anadromous, that spends their entire life cycle in freshwater and

diadromous that spawn in brackish water, where larvae hatch out, migrate to the sea before returning to freshwater as post larvae, and were remain for most of its adult life (McDowall, 1987).

This species has been studied and commercially exploited in Chile, Argentina and New Zealand due to the high economic value of its transparent eel-like juveniles in European, Mexican and New Zealand markets (Mardones *et al.,* 2008). However, due to wild population overfishing, annual catches have declined during the past few years and *G. maculatus* current conservation status is ranked as vulnerable due to the overexploitation of fisheries and predation by the introduced salmon species (Encina-Montoya *et al*., 2011). As result demand needs to be met by a sustained production through the development of breeding technologies. As a result of the high market value and commercial interest for this species, studies on the production and rearing of this fish have been developed in southern Chile (Barile *et al*., 2003; Mardones *et al*., 2008), but their nutritional requirements are not well known.

One of the most important nutrients during initial stages of any fish species are the essential fatty acids (EFA), especially highly unsaturated fatty acids (HUFA) (Watanabe, 1982; Watanabe & Kiron, 1994; Izquierdo, 1996). Several studies have focused on fatty acids requirements for larvae of different marine fish species and the findings indicate that HUFA dietary supplementation either to live preys or formulated diets resulted in positive effects on survival and growth parameters (Izquierdo *et al.,* 1989; Watanabe & Kiron, 1994; Sargent *et al.,* 1999; Izquierdo, 2005). Furthermore, the requirements for essential HUFA in fish may be affected by various environmental and geographical factors (Olsen & Skjervold, 1995; Zenebe *et al.,* 1998). Environmental conditions such as temperature (Farkas *et al.,* 1980; Olsen *et al.,* 1999; Vagner *et al.,* 2007), salinity (Borlongan & Benitez, 1992; Haliloglu *et al.,* 2004; Dantagnan *et al.,* 2010; Xu *et al.,* 2010) and light (Ota & Yamada, 1971) have been reported to affect the lipid content and fatty acids profile on fish tissues. Among the factors mentioned above, water salinity plays an important role regarding tissue lipid composition in diadromous fish (Sampekalo *et al.,* 1992). Changes between freshwater and seawater ecosystems affect the fish metabolism, modifying the fatty acids composition of cellular membranes, mainly ω-3 HUFA (Daikoku *et al.,* 1982; Sheridan, 1989). These modifications play an important role in osmoregulation (Sampekalo *et al.,* 1992; Tocher *et al.,* 1995) and its occurrence seem to be determined more by variations in salinity than by the diet composition (Haliloglu *et al.,* 2004; Dantagnan *et al.,* 2007).

Thus, it is important to determine the ω-3 and ω-6 PUFA dietary requirements under different salinities (Izquierdo, 2005). The determination of appropriate ω-3 PUFA in diets for fish larvae has been extensively studied in many marine and freshwater species (Satoh *et al.,* 1989; Izquierdo *et al*., 1990; Dickey-Collas & Geffen 1992; Geurden *et al*., 1995), whereas studies in diadromic species such as *G. maculatu* are still scarce. Thus, the present study aimed to determine the effect of salinity on the optimum dietary ω-3 PUFA for survival and growth the larval stage and effect on and body biochemical composition.

MATERIALS AND METHODS

Experiment design and fish rearing conditions

The feeding trial was designed as a 2×2 factorial with two levels of ω-3 PUFA in the experimental emulsions (1.87 and 3.16%) used to enrich life prey (Rotifers) and two different salinity conditions as the other main factor (0 and 15 g L^{-1}). Experimental treatments were evaluated in triplicate.

Galaxias maculatus larvae were obtained from a total of 60 wild specimens (40 females and 20 males) captured in the Reloncaví Estuary near Hornopirén, Chile. Fish were transported to the hatchery of the Catholic University of Temuco where they were adapted to experimental conditions that simulated the estuary environment. The eggs were obtained by stripping and fertilized *in vitro*. Subsequently, the fertilized eggs were incubated at 13 ± 1ºC and a salinity level of 0 g L^{-1}. Newly hatched larvae, with an initial length of 6.00 ± 0.26 mm, were randomly distributed into 12 cylindrical polyurethane tanks (5 L), at an initial density of 60 larvae per liter.

Tanks were supplied with filtered water (5 µm) at different salinities, being totally renewed every three days after total absorption of the yolk sac (day 7). Water temperature was 15 ± 1ºC and a photoperiod of 24 h with natural light in the hatchery was kept during the whole experimental period. Larval culture was kept closed flux, with partial renewal of water every three days. No microalgae were added to the rearing tanks to have a better control of the rotifers fatty acid profile.

Experimental emulsions were manufactured with 6% vitamin premix, 4% soybean lecithin and 90% of the oils blend formulated with ROPUFA 25 ω-6, ROPUFA 30 ω-3, ROPUFA 30-EPA (Roche Ltda., Santiago, Chile), EPA 28 (Nippai Company Ltd., Tokyo, Japan), olive oil and corn oil. Rotifers were

grown at 22ºC and fed with commercial yeast (*Saccharomyces cerevisiae*) before being enriched. Rotifers were enriched using 0.05 g emulsion L^{-1} per 180-200 rotifers mL^{-1} at 20-22ºC during 18 h, washed with clean filtered seawater and then fed to the larvae. The fatty acid composition of rotifers is shown in Table 1. Fish larvae were fed during 20 days post-hatching at a rate of 5 rotifers mL^{-1} and gradually increased to 15 rotifers m L^{-1} by day 20.

Sampling and calculations

Growth was measured by using the length-specific growth rate (SGR). This variable was calculated by the following equation: SGR = ln [final TL – ln (initial TL)] * 100/days), where TL is total body length. Survival rate was determined by the difference between the numbers of larvae at the beginning and at the end of the experiment. After twenty days of feeding period, samples of all larvae and rotifers were filtered, freshwater washed and stored at -80ºC for posterior biochemical analysis.

Chemical analyses of body composition

Water content was determined according to the methods of AOAC (1995). Lipids were extracted with a mix of chloroform and methanol (2:1) (Folch *et al*., 1957) and neutral and polar fractions were separated by absorption chromatography on silica cartridges (Sep-pack, Waters, Milford, MA) (Juaneda & Roquelin, 1985). Methyl esters from fatty acids obtained from lipids were prepared following the method proposed by Morrison & Smith (1964). Fatty acids were separated by gas-liquid chromatography (Hewlett Packard 5890 series II Plus, Wilmington, USA) using a 30 x 0.25 mm id x 0.25 µm capillary column HP-225 (Hewlett Packard, Wilmington, USA). Nitrogen was used as carrier gas. Fatty acids were identified by comparison to a well characterized standard such as GLC 462 (Nu-Chek Prep, Elysian, USA). The fatty acids from the whole body larvae were expressed as dry basis percentage.

Statistical analyses

Data was analyzed by a two-way variance analysis. Significant differences among treatment means were determined using Tukey's test, with critical limits being set at $P < 0.05$. Arcsine square root transformations of percentage data were conducted to achieve homogeneity of variance. All the statistical analyses were performed using the software Statistica 10 (StatSoft, Tulsa, USA).

RESULTS

After 20 days of feeding trial, survival of *G. maculatus* larvae was affected by water salinity and the interactions between water salinity and diet ($P < 0.05$) (Table 2). Fish reared at 0 g L^{-1} showed a significant higher survival rate ($P < 0.05$) when rotifers ω-3 PUFA content was increased from 1.87 to 3.16 % (Fig. 1). In contrast, fish reared at salinity of 15 g L^{-1} showed a significant decrease in survival rate ($P < 0.05$) when the rotifers content of ω-3 PUFA increased from 1.87 to 3.16% (Fig. 1). Growth of *G. maculatus* larvae was affected by water salinity, diet and the interactions between water salinity and diet ($P < 0.05$) (Table 2). At 0 g L^{-1} salinity the lowest SGR was recorded in the larvae fed rotifers containing 1.87% ω-3 PUFA and the highest SGR in those fed

Table 1. Fatty acids composition and total lipid contents (percentage of dry weight) of rotifer enriched with the experimental lipid emulsions.

Fatty acid	Treatments	
	1	2
C12:0	0.14±0.01	0.11±0.01
C14:0	0.43±0.03	0.83±0.09
C16:0	3.15±0.66	3.79±0.22
C18:0	2.12±0.67	2.31±0.08
C20:0	0.10±0.02	0.79±0.08
C22:0	0.25±0.01	0.18±0.04
C24:0	0.14±0.01	0.07±0.01
C14: 1	0.05±0.00	0.00±0.00
C16: 1	2.46±0.42	5.45±0.38
C18: 1ω-9	9.76±0.72	6.70±0.23
C18: 1ω-7	0.91±0.16	1.33±0.06
C20: 1ω-9	0.86±0.10	0.91±0.04
C22: 1ω-9	0.18±0.02	0.20±0.01
C24: 1	0.07±0.01	0.08±0.01
C18: 2ω-6	3.36±0.21	2.38±0.10
C18: 3ω-6	0.03±0.00	0.74±0.07
C18: 3ω-3	0.21±0.01[a]	0.91±0.04[b]
C20: 2ω-6	0.06±0.01	0.00±0.00
C20: 3ω-3	0.04±0.00	0.46±0.08
C20: 3ω-6	0.03±0.00	0.15±0.01
C20: 4ω-6	0.12±0.01	0.06±0.00
C20: 5ω-3	0.98±0.02	1.18±0.04
C22: 2ω-6	0.01±0.01	0.00±0.00
C22: 4ω-6	0.00±0.00	0.13±0.00
C22: 5ω-3	0.12±0.01	0.11±0.01
C22: 6ω-3	0.52±0.01	0.51±0.03
Σ ω-6 PUFA	3.61±0.25	3.46±0.19
Σ ω-3 PUFA	1.87±0.04[a]	3.16±0.19[b]
ω-3/ω-6	0.52±0.04[a]	0.91±0.01[b]
EPA/DHA	1.88±0.03	2.32±0.05
Total lipid (%)	26.10±0.34	26.26±2.39

Values represent means ± SD (n = 6)
Values with different superscript letter indicate significant difference ($P > 0.05$).
Dry basis

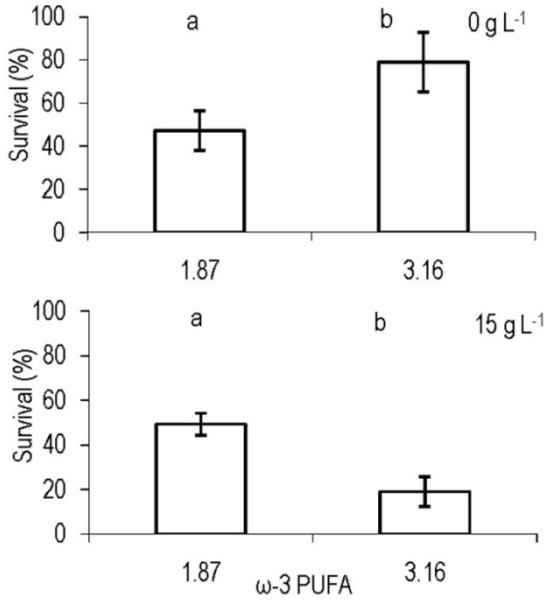

Figure 1. Survival of *Galaxias maculatus* larvae reared at different salinities and two ω-3 PUFA dietary levels after 20 days post-hatching (Values are means ± 2 SD).

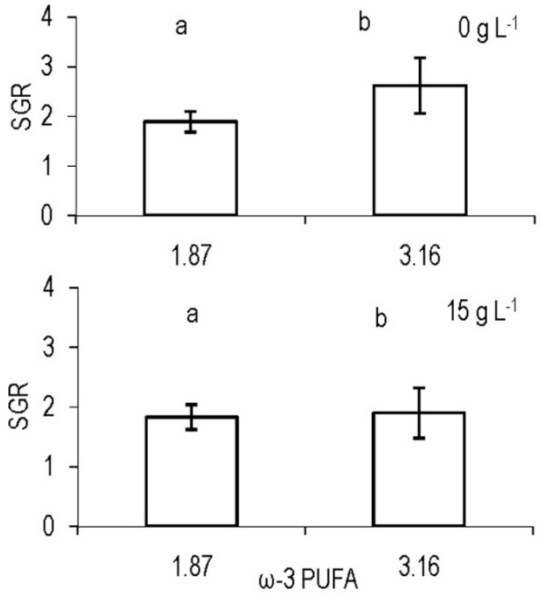

Figure 2. Specific Growth Ratio (SGR) of *Galaxias maculatus* larvae reared at different salinities and two ω-3 PUFA dietary levels after 20 days (Values are means ± 2 SD).

rotifers with 3.16% of ω-3 PUFA (Fig. 2). However, at 15 g L^{-1} salinity growth rates were low and there was not a marked effect of ω-3 PUFA (Fig. 2) ($P > 0.05$).

Lipid profile analysis of larvae showed that the most abundant monounsaturated fatty acids were 16:1, 18:1 ω-9 and 18:1 ω-7 (Tables 3 and 4). These were mainly accumulated in neutral fractions, regardless water salinity and fatty acid level in rotifers. Larvae reared at 0 and 15 g L^{-1} and fed high ω-3 PUFA enriched rotifers contained high ω-3 PUFA accumulated in the neutral fraction as opposed to larvae fed with low ω-3 PUFA content rotifers, in which, ω-3 PUFA were accumulated in the polar fraction, especially DHA (Tables 3 and 4).

DISCUSSION

The results from the present study showed that there were both independent and synergistic significant ($P < 0.05$) effects of salinity and dietary ω-3 PUFA on survival and growth in *G. maculatus* larvae. EPA and DHA content in both diets are relatively close, but the best larval survival and growth at 0 g L^{-1} were obtained when ω-3 PUFA dietary levels, mostly 18:3 ω-3, were high. When *G. maculatus* larvae are reared in freshwater and fed high 18:3 ω-3 content, they are able to accumulate higher EPA and DHA levels in the neutral fraction, contributing in this way to cover the requirements of these fatty acids and improving the growth and survival rates. Thus, 18:3 ω-3 is efficient as EFA for *Galaxias maculatus* reared in freshwater, considering that freshwater fishes are able to convert 18:3 ω-3 into 20:5 ω-3 and 22:6 ω-3 (Sargent *et al.,* 1995).

When larvae were reared at 15 g L^{-1} and fed with rotifers with a highest content of ω-*3* PUFA, mainly 18:3*ω-3*, growth did not improve. These results suggest that fatty acids requirements at 15 g L^{-1} are not met by 18:3ω-*3* increment to improve growth and higher level DHA is necessary to be incorporated in the diet. This agrees with our previous results (Dantagnan *et al*., 2010) and other studies with marine fish larvae for which DHA requirement is highly relevant and it is necessary to be incorporated in the diet to improve growth (Watanabe, 1993; Gaspasin & Duray, 2001; Bransden *et al*., 2005; Lund *et al*., 2007; Vizcaino-Ochoa *et al*., 2010). High survivals were found in larvae fed with low dietary ω-3 PUFA reared at 15 g L^{-1}. Furthermore, this group of larvae presented a high DHA level on polar fraction from whole body lipids. DHA is an essential fatty acid for fish larvae on brackish environments because it has an important role on membranes permeability and

Table 2. ANOVA table showing the effects of salinity and diets on total survival and growth. In bold are indicate the significant values. ($P < 0.05$).

	Total survival				Specific Growth Ratio (SGR)			
	df	MS	F	*P*	df	MS	F	*P*
Salinity	1	0.26	29.59	**0.00**	1	8.15	39.82	**0.00**
Diet	1	0.00	0.02	0.88	1	8.32	40.68	**0.00**
Salinity x diet	1	0.30	33.52	**0.00**	1	5.80	28.34	**0.00**
Error	7	0.009			214	0.20		

Table 3. Fatty acids composition (porcentaje en base seca) from neutral and polar fractions in larvae fed with the experimental diets and reared at salinity of 0 g L^{-1}. N: Neutral, P: Polar.

Fatty acids (%)*	Treatment 1			Treatment 2		
	N	*P*	Ratio N/P	N	*P*	Ratio N/P
C12:0	0.00	0.00	-	0.12	0.00	-
C14:0	0.58	0.18	3.22	0.80	0.22	3.63
C16:0	5.64	2.31	2.44	5.28	2.53	2.09
C18:0	6.85	2.83	2.42	4.06	1.15	3.53
C20:0	0.14	0.06	2.33	0.04	0.00	-
C22:0	0.37	0.12	3.08	0.06	0.00	-
C24:0	0.36	0.14	2.57	0.12	0.09	1.33
C14: 1	0.12	0.02	6.00	0.00	0.00	-
C16: 1	1.17	0.37	3.16	2.22	1.15	1.91
C18: 1ω-9	4.01	1.21	3.31	3.25	2.15	1.51
C18: 1ω-7	0.72	0.19	3.78	0.98	0.54	1.81
C20: 1ω-9	0.47	0.10	4.70	0.36	0.09	4.00
C22: 1ω-9	0.19	0.01	19.00	0.10	0.01	10.00
C24: 1	0.22	0.05	4.40	0.18	0.06	3.00
C18: 2ω-6	1.19	0.42	2.83	1.54	0.20	7.70
C18: 3ω-6	0.19	0.04	4.75	0.42	0.04	10.05
C18: 3ω-3	0.27	0.05	5.40	0.11	0.03	3.66
C20: 2ω-6	0.02	0.02	1.00	0.00	0.00	-
C20: 3ω-3	0.10	0.10	1.00	0.09	0.06	1.50
C20: 3ω-6	0.04	0.01	4.00	0.11	0.00	-
C20: 4ω-6	0.03	0.04	0.75	0.20	0.12	1.66
C20: 5ω-3	0.08	0.08	1.00	0.53	0.45	1.17
C22: 2ω-6	0.05	0.00	-	0.00	0.00	-
C22: 4ω-6	0.02	0.00	-	0.03	0.00	-
C22: 5ω-3	0.01	0.02	0.50	0.13	0.09	1.44
C22: 6ω-3	0.12	0.32	0.37	0.77	0.48	1.45
Σ ω-6 PUFA	1.55	0.53	2.92	2.30	0.36	6.38
Σ ω-3 PUFA	0.59	0.57	1.03	1.62	1.11	1.47
ω-3/ω-6	0.38	1.07	0.35	0.71	3.11	0.22
EPA/DHA	0.67	0.25	2.69	0.68	0.95	0.71

osmoregulation (Sampekalo *et al.,* 1992). Thus, several studies have suggested that DHA accumulation patterns in polar lipids are linked to survival (Mourente & Tocher, 1992, 1993; Koven *et al.,* 1993). According to Rodríguez *et al*. (1997) low DHA incorporation in polar lipids usually results in low survival rates on marine fish larvae.

In summary, ω-3 PUFA dietary changes, principally 18:3 ω-3, can determine important alterations in the accumulation pattern of PUFA in larvae reared in

Table 4. Fatty acids composition (percentage of dry weight) from neutral and polar fractions in larvae fed with the experimental diets and reared at salinity of 15 g L^{-1}. N: Neutral, P: Polar.

Fatty acids (%)	Treatment 1			Treatment 2		
	N	P	Ratio N/P	N	P	Ratio N/P
C12:0	0.05	0.00	-	0.24	0.00	-
C14:0	0.16	0.10	1.60	0.25	0.04	6.25
C16:0	1.69	1,52	1.11	0.11	0.75	0.14
C18:0	1.80	1.16	1.55	4.29	0.31	1.38
C20:0	0.06	0.01	6.00	0.09	0.04	2.25
C22:0	0.10	0.04	2.50	0.39	0.20	1.95
C24:0	0.13	0.05	2.60	0.32	0.01	3.20
C14: 1	0.03	0.00	-	0.72	0.00	-
C16: 1	0.72	0.43	1.67	0.17	0.05	3.40
C18: 1ω-9	1.80	1.48	1.21	2.89	0.57	5.07
C18: 1ω-7	0.38	0.24	1.58	0.72	0.15	4.80
C20: 1ω-9	0.22	0.07	3.14	0.28	0.03	9.33
C22: 1ω-9	0.18	0.08	2.25	0.36	0.10	3.60
C24: 1	0.06	0.01	6.00	0.09	0.02	4.50
C18: 2ω-6	0.50	0.45	1.11	0.18	0.26	0.69
C18: 3ω-6	0.02	0.01	2.00	1.64	0.04	0.11
C18: 3ω-3	0.06	0.03	2.00	0.18	0.02	9.00
C20: 2ω-6	0.01	0.02	0.50	0.04	0.00	--
C20: 3ω-3	0.05	0.03	1.66	0.31	0.00	-
C20: 3w-6	0.01	0.02	0.50	0.05	0.02	2.50
C20: 4ω-6	0.03	0.07	0.42	0.09	0.06	1.50
C20: 5ω-3	0.11	0.24	0.45	0.42	0.15	2.80
C22: 2ω-6	0.02	0.00	-	0.00	0.00	-
C22: 4ω-6	0.07	0.02	3.50	0.34	0.10	3.40
C22: 5ω-3	0.02	0.05	0.40	0.08	0.04	2.00
C22: 6ω-3	0.09	0.50	0.18	0.22	0.27	0.81
Σ ω 6 PUFA	0.65	0.58	1.12	2.33	0.38	6.00
Σ ω 3 PUFA	0.34	0.84	0.40	1.19	0.48	2.47
ω-3/ω6–	0.51	1.45	0.35	0.51	1.28	0.40
EPA/DHA	1.17	0.48	2.43	1.92	0.00	-

freshwater and brackish water, affecting the larval survival and growth. This is probably the result of 18:3 ω-3 bioconversion in freshwater. In seawater, DHA is mostly accumulated on the polar fraction and does not depend on the level of 18:3 ω-3 in diets. Thus, EPA and DHA deficiencies can be covered by 18:3 ω-3 in freshwater, while in seawater dietary DHA incorporation is required. This may have important repercussions during the migration periods of anadromic or catadromic fish and may affect the rearing performance of this species when cultured under diferent salinities.

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REFERENCES

Association of Official Agricultural Chemists (AOAC). 1995. Official methods of analysis of the Association of Analytical Chemist. AOAC International, Washington, 1085 pp.

Barile J., A. Borquez, P. Dantagnan, A. Mardones, J. Quevedo, I. Salgado, I. Valdebenito & R. Vega. 2003. Antecedentes para el cultivo del puye *Galaxias maculatus* (Pisces: Galaxiidae). Editorial Gráfica-casur, Temuco, 144 pp.

Borlongan, I. & L. Benitez. 1992. Lipid and fatty acid composition of milkfish (*Chanos chanos* Forsskål) grown in freshwater and seawater. Aquaculture, 104: 79-89.

Bransden, M., S. Battaglene, D. Morehead, G. Dunstan & P. Nichols. 2005. Effect of dietary 22:ω-3 on growth, survival and tissue fatty acid profile of striped trumpeter (*Latris lineata*) larvae fed enriched *Artemia*. Aquaculture, 243: 331-344.

Daikoku, T., I. Yano & M. Masui. 1982. Lipid and fatty acid composition and their changes in the different organs and tissue of guppy, *Poecilia reticulata* on sea water adaption. Comp. Biochem. Phys., 73a: 167-174.

Dantagnan, H., A. Borquez, I. Valdebenito, I. Salgado, E. Serrano & M.S. Izquierdo. 2007. Lipid and fatty acid composition along embryo and larval development of *Galaxias maculatus* obtained from estuarine, fresh water and cultured populations. J. Fish Biol., 70: 770-781.

Dantagnan, P., A. Borquez, A. Hernández & M.S. Izquierdo. 2010. Effect of EPA/DHA ratios on the growth and survival of *Galaxias maculatus* (Jenyns, 1842) larvae reared under different salinity regimes. Aquacult. Res., 41: e239-e244.

Dickey-Collas, M. & A.J. Geffen. 1992. Importance of the fatty acids 20:5ω-3 and 22:6ω-3 in the diet of place (*Pleuronectes platessa*) larvae. Mar. Biol., 113: 463-468.

Encina-Montoya, F., R. Vega, A. Mardones, T. Rueda & A. Tello. 2011. Characterization of whitebait (*Galaxias maculatus*) respiratory rates to optimize intensive culture carrying capacities. Aquacult. Res., 42: 835-843.

Farkas, T., I. Csegeri, F. Majoros & J. Olah. 1980. Metabolism of fatty acids in fish. III. Combined effect of environmental temperature and diet on formation and deposition of fatty acids in the carp, *Cyprinus carpio* Linnaeus, 1758. Aquaculture, 20: 29-40.

Folch, J., M. Lees & G.H.S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissue. J. Biol. Chem., 226: 497-509.

Gaspasin, R.S. & M.N. Duray. 2001. Effects of DHA-enriched live food on growth, survival and incidence of opercular deformities in milkfish (*Chanos chanos*). Aquaculture, 193: 49-63.

Geurden, I., J. Radünz-Neto & P. Bergot. 1995. Essentiality of dietary phospholipids for carp (*Cyprinus carpio* L.) larvae. Aquaculture, 131: 303-314.

Haliloglu, H.I., A. Bayr, A.N. Sirkecioglu, N.M. Aras & M. Atamanalp. 2004. Comparison of fatty acid composition in some tissues of rainbow trout (*Onchorhynchus mykiss*) living in seawater and freshwater. Food Chem., 86: 55-49.

Izquierdo, M.S., T. Watanabe, T. Takeuchi, T. Arakawa & C. Kitajima. 1989. Requirement of larval red sea bream *Pagrus major* for essential fatty acids. Nippon Suisan Gakk., 55: 859-867.

Izquierdo, M.S., T. Watanabe, T. Takeuchi, T. Arakawa & C. Kitajima. 1990. Optimal EFA levels in *Artemia* to meet the EFA requirements of red seabream (*Pagrus major*). In: M. Takeda & T. Watanabe (eds.). The current status of fish nutrition in aquaculture. Tokyo University Fisheries, Tokyo, pp. 221-232.

Izquierdo, M.S. 1996. Essential fatty acid requirements of cultured marine fish larvae. Aquacult. Nutr., 2: 183-191.

Izquierdo, M.S. 2005. Essential fatty acid requirements in Mediterranean fish species. Cah. Options Médit., 63: 91-102.

Juaneda, P. & G. Roqueline. 1985. Rapid and convenient separation of phospholipids from heart using silica cartridges. Lipids, 20: 40-41.

Koven, W.M., A. Tandler, D. Sklan & G.W. Kissil. 1993. The association of eicosapentaenoic and docosahexaenoic acids in the main phospholipds of different-age-*Sparus aurata* larvae with growth. Aquaculture, 116: 71-82.

Lund, I., S.J. Steenfeldt & B.W. Hansen. 2007. Effect of dietary arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid on survival growth and pigmentation in larvae of common sole (*Solea solea* L.). Aquaculture, 273: 532-544.

Mardones, A., R. Vega & F. Encina. 2008. Cultivation of whitebait (*Galaxias maculatus*) in Chile. Aquacult. Res., 39: 731-737.

McDowall, R. 1987. Evolution and importance of diadromy: the occurrence and distribution of diadromy among fishes. Am. Fish. S. S., 1: 1-13.

Morrison, W. & L. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron-fluoride methanol. J. Lipids Res., 5: 600-608.

Mourente, G. & D.R. Tocher. 1992. Effects of weaning onto a pelleted diet on docosahexaenoic acid (22:6ω-3) levels in brain of developing turbot (*Scopthalmus maximus* L.). Aquaculture, 105: 363-377.

Mourente, G. & D.R. Tocher. 1993. Incorporation and metabolism of ^{14}C-labelled polyunsaturated fatty acids in juvenile gilthead sea bream *Sparus aurata* in vivo. Fish Physiol. Biochem., 10: 443-453.

Olsen, Y. & H. Skjervold. 1995. Variation in content of ω-3 fatty acids in farmed Atlantic salmon, with special emphasis on effects of non-dietary factors. Aquacult. Int., 3: 22-35.

Olsen, R.E., E. Lovaas & O. Lie. 1999. The influence of temperature, dietary polyunsaturated fatty acids, α-tocopherol and spermine on fatty acid composition and indices of oxidative stress in juvenile Arctic char, *Salvelinus alpinus* L. Fish Physiol. Biochem., 20: 13-29.

Ota, T. & M. Yamada. 1971. Lipids of masu salmon *Oncorhynchus masou.* I. Variations of the lipid content and fatty acid composition of juvenile masu

salmon during the period of smolt-transformation, and the influence of light upon those variations. Bull. Fac. Fish. Hokkaido Univ. 22: 151-158.

Rodríguez, C., J. Perez, M. Diaz, M.S. Izquierdo, H. Fernández-Palacios & A. Lorenzo. 1997. Influence of the EPA/DHA ratio in rotifers on gilthead seabream (*Sparus aurata*) larval development. Aquaculture, 150: 77-89.

Sampekalo, J., T. Takeuchi & T. Watanabe. 1992. Comparison of gill lipids between freshwater fish. J. Tokyo Univ. Fish., 79: 71-76.

Sargent, J., J.G. Bell, M.V. Bell, R.J. Henderson & D.R. Tocher. 1995. Requirement criteria for essential fatty acids. J. Appl. Ichthyol., 11: 183-198.

Sargent, J., L. Mc Evoy, A. Estevez, G. Bell, M. Bell, J. Henderson & D. Tocher. 1999. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture, 179: 217-229.

Satoh, S., W.R. Poe & R.P. Wilson. 1989. Effect of dietary ω-3 fatty acids on weight gain and liver polar lipid fatty acid composition of fingerling channel catfish. J. Nutr., 120: 23-28.

Sheridan, M. 1989. Alterationsin lipid metabolism accompanying smoltification and seawater adaptation of salmonid fish. Aquaculture, 82: 191-203.

Tocher, D.R., J.D. Castell, J.R. Dick & J. Sargent. 1995. Effects of salinity on the fatty acid composition of total lipid and individual glycerophospholipid classes of Atlantic salmon (*Salmo salar*) and turbot (*Scopthalmus maximus*) cells in culture. Fish. Physiol. Biochem., 14: 125-137.

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Vagner, M., J.H. Robin, J.L. Zambonino-Infante & J. Person-Le Ruyet. 2007. Combined effects of dietary HUFA level and temperature on sea bass (*Dicentrarchus labrax*) larvae development. Aquaculture, 266: 179-190.

Vizcaino-Ochoa, V., J.P. Lazo, B. Barón-Sevilla & M. Drawbridge. 2010. The effect of dietary docosa-hexaenoic acid (DHA) on growth, survival and pigmentation of California halibut *Paralichthys californicus* larvae (Ayres, 1810). Aquaculture, 302: 228-234.

Watanabe, T. 1982. Lipid nutrition in fish. Comp. Biochem. Physiol., 73: 3-15.

Watanabe, T. 1993. Importance of dosahexanoic acid in marine larval fish. J. World Aquacult. Soc., 24: 152-161.

Watanabe, T. & V. Kiron. 1994. Prospects in larval fish dietetics. Aquaculture, 124: 223-251.

Xu, J., B. Yan, Y. Teng, G. Lou & Z. Lu. 2010. Analysis of nutrient composition and fatty acid profiles of Japanese sea bass *Laterolabrax japonicus* (Cuvier) reared in seawater and freshwater. J. Food Compos. Anal., 23: 401-405.

Zenebe, T., G. Ahlgrent & M. Bomberg. 1998. Fatty acid content of some freshwater fish of commercial importance from tropical lakes in the Ethiopian Rift Valley. J. Fish Biol., 53: 987-1005.