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Lipid and fatty acid composition during embryo and larval development of puye *Galaxias maculatus* Jenyns, 1842, obtained from estuarine, freshwater and cultured populations

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Galaxias maculatus eggs and larvae obtained from broodfish captured either in an estuarine or a freshwater environment, as well as from cultured broodstock were analysed to compare their lipid and fatty acid profiles. Results showed a lower lipid content in embryos and larvae from estuarine populations than those from fresh water, denoting the influence of environmental conditions. The n-3:n-6 ratio was higher in eggs from estuarine and cultured populations, being in the range of marine fishes, whereas for eggs from freshwater fish was lower and typical of freshwater fishes. The polyunsaturated fatty acids (PUFA), particularly docosahexaenoic acid (22:6n-3) and eicosapentaenoic acid (20:5n-3), were higher in eggs and larvae of broodstock coming from culture or estuarine environments than in those from fresh water. Moreover, these fatty acids markedly increased after hatching in larvae coming from estuarine populations, suggesting the effect of the environment on fatty acid profiles to physiologically prepare the larvae to adapt to higher salinity conditions. Linoleic acid (18:2n-6) content was higher in fresh water fish and its reduction during embryo and larval development was accompanied by a significant increase of arachidonic acid (20:4n-6), which was not observed in embryos or larvae from broodstock fish from estuary or aquaculture origin. Both environment and diet of broodstock fish affected lipid and fatty acid composition of *G. maculatus* embryo and larvae as well as their changes during development.

Key words: eggs; fatty acids; *Galaxias maculatus*; larvae; lipids; puye.

INTRODUCTION

Galaxias maculatus Jenyns, 1842, commonly called 'puye' in Chile, is a cold water fish found in the southern hemisphere. It is one of the most attractive species to be used in the diversification of Chilean aquaculture due to the high

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market value of its 'cristalino' juvenile. This specie is a normally catadroumous, stream dwelling species, but it also has a number of viable, landlocked populations, both populations markedly separated (De Silva *et al.*, 1998).

Several attempts have been made to develop the rearing techniques for this species (Mitchell, 1989; Dantagnan *et al.*, 1995; Dantagnan, 2003), although their nutritional requirements are as yet unknown. Lipids are among the most important nutritional factors affecting growth and survival in early life stages (Watanabe, 1982; Izquierdo *et al.*, 2000), hence the importance of determining lipid and fatty acid requirements of new species. Since the fish egg should contain all the nutrients required for the normal development of embryo and endogenous feeding larvae, the study of the evolution of fatty acid composition of eggs and larvae would contribute to determining their first feeding requirements (Izquierdo, 1996).

It is also recognized that environmental factors such as temperature (Farkas *et al.*, 1980; Henderson, 1996; Olsen *et al.*, 1999), salinity (Borlongan & Benitez, 1992; Tocher *et al.*, 1994), light (Ota & Yamada, 1971) or the type of food available (Watanabe, 1982) exert an effect on the lipid contents and fatty acid composition of aquatic organisms. Accordingly, seasonal or geographic changes also have a significant effect (Olsen & Skjervold, 1995; Jobling *et al.*, 1998; Zenebe *et al.*, 1998) on biochemical composition. These environmental changes primarily affect metabolic processes (Sheridan, 1989), causing accumulations which may vary in the different organs of aquatic organisms (Farkas *et al.*, 1980; Sheridan, 1989; Borlongan & Benitez, 1992). For instance, successive changes in salinity during migrations between fresh and salt water in diadromic fishes have been shown to cause significant changes in their metabolism (Sheridan, 1989), which affects their biochemical composition. This is mainly in preparation or response to osmoregulation due to changes in the environment. In some cases, there is some evidence to indicate that these changes can even occur before the fishes change environment, as a 'pre-adaptive' response (Tocher *et al.*, 1994). In this respect, it is known that salinity can primarily have an effect on the polyunsaturated fatty acid (PUFA) contents in fishes, given that the ratios of n-3:n-6 PUFA are different in freshwater and marine fishes (Daikoku *et al.*, 1982; Borlongan & Benitez, 1992). Other authors have shown that the increase of n-3 PUFA, mainly docosahexaenoic acid (22:6n-3), when organisms switch to a marine environment play a dominant role in osmoregulation (Sampekalo *et al.*, 1992; Tocher *et al.*, 1995).

Since, *G. maculatus* can live in different environments with varying salinity, the aim of the present study was to compare the changes of the fatty acid composition of eggs and larvae from broodfish coming from estuarine, freshwater or cultured populations.

MATERIALS AND METHODS

BROODSTOCK COLLECTION

Freshwater populations: broodstock fish were captured by net in Lake Riñihue, near Temuco (IX Region of Chile; 39°48′ S; 72°22′ W), in March, 1998, at a sexually mature stage, when the average range in water temperature was 10–14° C and salinity 0.

Estuary populations: broodstock fish were captured by net near Hornopirén, (X Region of Chile; 42°00 S; 73°00 W) in March, 1998, when the average range in water temperature was 11–15° C and in salinity was 10–15. Cultured population: broodstock used to obtain eggs and larvae were the first generation of adults reared in culture from wild parents captured near Hornopirén. Larval rearing of this first generation was conducted in salt water with salinity 10 and then kept in fresh water until sexual maturity. They were fed a commercial fish food used for salmonid farming. The water temperature at the time of spawning ranged between 10 and 13° C.

In each case *c*. 60 broodstock fish were used, of which 40 were female and 20 were male. Each female produces between 500 and 1000 eggs. Eggs were obtained from each of the three types of broodstock by stripping immediately after capture and fertilized '*in vitro*'. Afterwards they were incubated at 13 ± 1° C (mean ± S.D.) and at salinity of 0 if they were from freshwater and cultured broodstock or 10 if they were from estuarine broodstock.

SAMPLE COLLECTION

Lipid composition during embryo development: after fertilization, eggs from various females and embryo samples were taken at each stage of development until hatching of the larvae, specifically, eggs at 3–4 h post-fertilization (pf) (first cleavage), embryos in the epibolic stage (day 2 pf), embryos in the organogenesis stage (day 7 pf), embryos in the ocular pigmentation stage (day 10 pf) and embryo close to hatching (day 30 pf) according to Benzie (1968).

Lipid composition of larvae during yolk sac re-absorption: once the larvae were hatched they were kept in starvation in 2 l tanks at room temperature (12 ± 1° C, mean ± S.D.) at the same water salinity condition in which the eggs were incubated. The following samples were taken: newly hatched larvae (day 31 pf); larvae at 4 days post-hatching (ph) (half yolk sac reabsorbed) and larvae at 6 days ph (yolk sac fully reabsorbed). The sample were rinsed in distilled water and blotted on filter paper and were frozen at −80° C until analysis.

CHEMICAL ANALYSIS

Each analysis was performed in duplicate from two samples from each pool and expressed as a percentage of dry mass. Total lipid was extracted from 0·1 g of samples (eggs or larvae) and homogenized in chloroform/methanol (2:1, v/v), in accordance with the method proposed by Folch *et al.* (1957) and stored at −80° C prior to analysis. Fatty acid methyl esters were prepared from the extracted lipids according to the method proposed by Morrison & Smith (1964). Fatty acids were identified by separation in a gas chromatograph (Hewlett Packard 5890 series II Plus, Wilmington, NC, U.S.A.) using a 30 m × 0·25 mm internal diameter × 0·25 μm capillary column HP-225 (Hewlett Packard). Nitrogen was used as a carrier gas. Fatty acids were identified by comparison to a well characterized standard such as GLC 462 (Nu-Chek Prep, Elysian, WA, U.S.A.).

STATISTICAL ANALYSIS

To compare the different contents of fatty acids in the different developmental stages within and between populations, a two-way ANOVA was carried out. *A posteriori*, a Tukey test was done on average pairs. All analysis was done using the statistics programme STATISTIX version 7.0 (analytical software, Tallahassee, FL, U.S.A.).

RESULTS

The total lipids in the estuarine embryos varied significantly ($P < 0{\cdot}05$) between 12·4% in the organogenesis stage and 24·3% close to hatching. The

lipids in the embryos from captive fish varied between 12·6% in the ocular pigmentation stage and 29·3% in the newly hatched larvae. The lipid content in the freshwater fish embryos did not vary significantly ($P > 0{\cdot}05$) during embryo and larval development, remaining between 21·81 and 27·17%. Lipids were significantly more abundant ($P < 0{\cdot}05$) in the majority of the embryo development stages in the freshwater fish embryos than in those from estuary fish (Fig. 1).

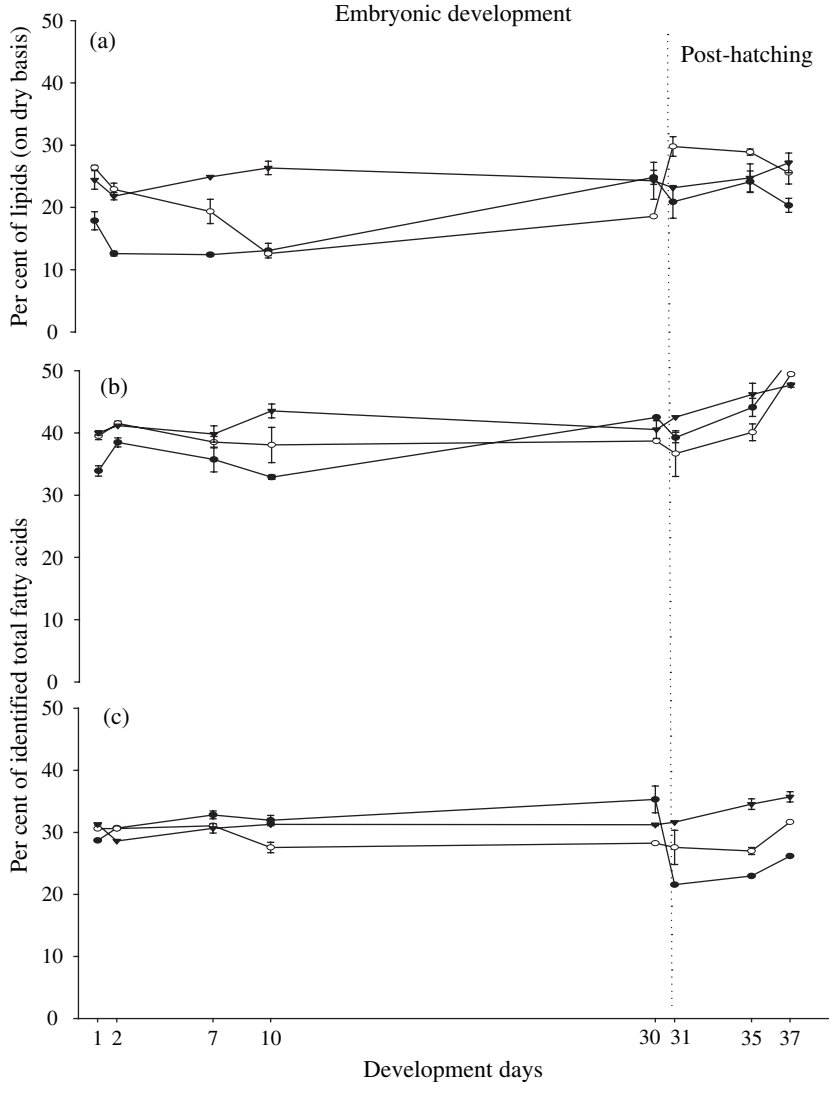

FIG. 1. Changes of (a) total lipid, (b) saturated fatty acids (SAFA) and (c) monounsaturated fatty acids (MUFA) contents during the embryonic and larval development of *Galaxias maculatus* of estuarine (–●–), cultured (–○–) and freshwater (–▼–) origin. Day 1 = recently fertilized eggs; day 2 = epibolic embryos; day 7 = embryos in the organogenesis stage; day 10 = embryos with ocular pigmentation; day 30 = embryos close to hatching; days 31, 35 and 37 = time since fertilization. Values are means ± S.D.

Saturated fatty acids (SAFA) (Fig. 1) during embryogenesis in the three populations represented 30–40% of the total fatty acids with no significant variation ($P > 0{\cdot}05$). After hatching, in all three cases, the SAFA increased significantly ($P < 0{\cdot}05$) as a proportion of the total fatty acids, particularly at the end of the absorption stage of the vitelinic sac. The predominant SAFA was palmitic (16:0), followed by stearic (18:0) and lauric (14:0) acids (Table I). Monounsaturated fatty acids (MUFA), on the other hand, were significantly reduced after hatching in the embryos from estuary fish, but not in the captive or freshwater embryos (Fig. 2). In all three cases the most abundant MUFA was oleic acid (18:1n-9), followed by palmitoleic acid (16:1n-7) (Table I). The quantity of PUFA did not vary significantly ($P > 0{\cdot}05$) during embryo development in all three populations (Fig. 2). A significant decrease ($P < 0{\cdot}05$) in the PUFA in all three populations was found after hatching, principally in the final stage of yolk sac absorption.

The ratio of n-3:n-6 PUFA varied between 5·63 and 7·23 in embryos from cultured broodstock, between 4·47 and 7·30 in those from the estuary environment and between 1·16 and 3·27 in those from fresh water (Fig. 2). This ratio increased after hatching ($P < 0{\cdot}05$) in larvae from estuarine fish to 16·07 but not in those from freshwater or culture conditions (Fig. 3). This ratio was always significantly lowest ($P < 0{\cdot}05$) in eggs and larvae from freshwater broodstock. The more abundant fatty acids of the n-3 series were 22:6n-3 and eicosapentaenoic acid (20:5n-3) in all cases, followed by docosapentaenoic (22:5n-3) and linolenic (18:3n-3) acids in the estuary and culture populations, and 18:3n-3 and 22:5n-3 acids in the freshwater sample. There was a significant ($P < 0{\cdot}05$) higher proportion of 22:6n-3 and 20:5n-3 in larvae and embryos from estuary fish than in those from the freshwater environment during almost the whole development of the embryo (Fig. 3). Both fatty acids were significantly reduced ($P < 0{\cdot}05$) in all three cases after hatching, principally in the final stage of yolk sac absorption (Fig. 3). In the freshwater population there was a significant increase in these fatty acids ($P < 0{\cdot}05$) until the organogenesis stage and then a significant decrease ($P < 0{\cdot}05$) until the yolk sac was fully absorbed. Linoleic acid (18:2n-6) and arachidonic acid (20:4n-6) were the most abundant PUFA of the n-6 series. Both were significantly more abundant ($P < 0{\cdot}05$) in embryos and larvae from freshwater fish than in those from estuary and captive fish. The 20:4n-6 increased significantly ($P < 0{\cdot}05$) in embryos from freshwater fish from the beginning of embryo development to the ocular pigmentation stage, while the level of 18:2n-6 diminished significantly ($P < 0{\cdot}05$) in parallel, until the organogenesis stage.

DISCUSSION

Total lipid contents of *G. maculatus* were within the expected range for cold water fishes with a long incubation period (Sargent, 1995). Comparing the lipid contents in embryos from the different environments, it was found that lipids in the embryos from freshwater fish were within the typical range for fishes in this type of environment, however, lipid contents were lower in the estuary embryos suggesting a pattern corresponding to marine environments rather than freshwater ones (Sargent, 1995) and denoting the influence of environmental

TABLE I. Mean ± S.D. fatty acid composition (percentage of identified total fatty acids) and total lipid content of fertilized eggs and larva immediately after hatching of *Galaxias maculations* according to origin

Fatty acids	Estuarine		Fresh water		Captive	
	Eggs	Larvae	Eggs	Larvae	Eggs	Larvae
C12:0	0·00 ± 0·00	0·00 ± 0·00	0·80 ± 0·01	0·66 ± 0·00	0·00 ± 0·00	0·00 ± 0·00
C14:0	3·05 ± 0·12	3·71 ± 0·26	4·98 ± 0·01	3·60 ± 0·00	6·75 ± 0·13	4·22 ± 0·42
C16:0	24·77 ± 1·07	30·44 ± 0·56	26·99 ± 0·26	29·21 ± 0·00	29·06 ± 0·46	26·21 ± 2·62
C18:0	6·09 ± 0·12	5·11 ± 0·01	1·97 ± 0·00	6·73 ± 0·00	3·71 ± 0·00	5·87 ± 0·59
C20:0	0·00 ± 0·00	0·00 ± 0·00	4·55 ± 0·00	1·28 ± 0·00	0·00 ± 0·00	0·00 ± 0·00
C22:0	0·00 ± 0·00	0·00 ± 0·00	1·00 ± 0·00	0·62 ± 0·00	0·00 ± 0·00	0·38 ± 0·38
C24:0	0·00 ± 0·00	0·00 ± 0·00	0·00 ± 0·00	0·60 ± 0·00	0·00 ± 0·00	0·00 ± 0·00
C14:1	0·12 ± 0·01	0·00 ± 0·00	0·19 ± 0·01	0·00 ± 0·00	0·00 ± 0·00	0·00 ± 0·00
C16:1n-7	8·00 ± 0·12	8·28 ± 0·21	11·14 ± 0·01	11·92 ± 0·00	8·69 ± 0·14	6·52 ± 0·65
C18:1n-9	14·40 ± 0·18	9·48 ± 0·09	13·16 ± 0·03	12·80 ± 0·00	16·67 ± 0·03	15·64 ± 1·56
C18:1n-7	3·64 ± 0·20	2·73 ± 0·01	5·86 ± 0·02	4·56 ± 0·00	3·28 ± 0·00	2·89 ± 0·29
C20:1n-9	1·36 ± 0·25	0·20 ± 0·04	0·51 ± 0·04	0·67 ± 0·00	0·92 ± 0·08	1·17 ± 0·12
C22:1n-9	0·00 ± 0·00	0·00 ± 0·00	0·00 ± 0·00	0·56 ± 0·00	0·00 ± 0·00	0·18 ± 0·02
C24:1	0·70 ± 0·18	0·87 ± 0·00	0·44 ± 0·05	1·14 ± 0·00	1·05 ± 0·05	1·19 ± 0·12
C18:2n-6	2·87 ± 0·15	1·12 ± 0·01	6·65 ± 0·012	4·29 ± 0·00	2·54 ± 0·04	2·12 ± 0·21
C18:3n-6	0·36 ± 0·02	0·31 ± 0·03	0·20 ± 0·00	0·60 ± 0·00	0·40 ± 0·00	0·30 ± 0·03
C18:3n-3	1·14 ± 0·02	0·57 ± 0·04	2·89 ± 0·03	3·43 ± 0·00	0·71 ± 0·00	0·61 ± 0·06
C20:2n-6	0·08 ± 0·01	0·00 ± 0·00	0·47 ± 0·01	0·02 ± 0·00	0·00 ± 0·00	0·12 ± 0·01
C20:3n-3	1·18 ± 0·00	0·14 ± 0·02	2·67 ± 0·02	1·13 ± 0·00	0·00 ± 0·00	0·46 ± 0·05
C20:3n-6	0·12 ± 0·01	0·00 ± 0·00	0·33 ± 0·01	0·63 ± 0·00	0·24 ± 0·00	0·00 ± 0·00
C20:4n-6	1·14 ± 0·06	0·69 ± 0·10	0·30 ± 0·01	1·94 ± 0·00	0·96 ± 0·01	1·40 ± 0·14
C20:5n-3	8·95 ± 0·13	9·31 ± 0·21	4·67 ± 0·06	3·27 ± 0·00	4·96 ± 0·06	5·07 ± 0·51
C22:2n-6	0·00 ± 0·00	0·00 ± 0·00	0·11 ± 0·05	0·00 ± 0·00	0·00 ± 0·00	0·00 ± 0·00
C22:4n-6	0·00 ± 0·00	0·19 ± 0·02	0·18 ± 0·01	0·43 ± 0·00	0·37 ± 0·02	0·36 ± 0·04
C22:5n-3	2·48 ± 0·12	2·56 ± 0·04	1·60 ± 0·28	1·32 ± 0·00	2·68 ± 0·04	2·03 ± 0·20
C22:6n-3	19·57 ± 0·62	24·30 ± 0·80	8·41 ± 0·14	8·61 ± 0·00	17·00 ± 0·46	23·23 ± 2·33
Total						
SAFA	33·91 ± 0·83	39·26 ± 0·83	40·22 ± 0·36	42·70 ± 0·00	39·5 ± 0·59	36·68 ± 3·67
MUFA	28·22 ± 0·06	21·56 ± 0·07	31·30 ± 0·10	31·64 ± 0·00	30·61 ± 0·04	27·60 ± 2·76
PUFA	37·87 ± 0·39	39·18 ± 0·90	28·48 ± 0·49	25·66 ± 0·00	29·87 ± 0·63	35·73 ± 3·57
n-3 PUFA	33·29 ± 0·60	36·88 ± 1·03	20·23 ± 0·54	17·76 ± 0·00	25·36 ± 0·56	31·42 ± 3·14
n-6 PUFA	4·58 ± 0·21	2·30 ± 0·12	8·25 ± 0·06	7·91 ± 0·00	4·51 ± 0·06	4·31 ± 0·43
n-3/n-6	7·26 ± 0·46	16·03 ± 1·31	2·54 ± 0·08	2·25 ± 0·00	5·62 ± 0·04	7·29 ± 0·03
EPA:DHA	0·43 ± 0·02	0·38 ± 0·00	0·55 ± 0·000	0·38 ± 0·00	0·29 ± 0·00	0·21 ± 0·00
Total lipid (% dry mass)	17·6 ± 1·45	20·9 ± 2·62	24·43 ± 1·49	23·15 ± 0·00	26·4 ± 0·37	29·80 ± 1·58

SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, decosahexaenoric acid.

conditions such as salinity and temperature. Indeed, after maturing in a freshwater environment, broodfish from these populations migrate to spawn in the estuary, an environment with higher salinity, where they reduce their food intake and even stop feeding restricting the influence of the diet in egg composition (McDowall, 1987). This lower lipid content in eggs from estuary broodfish may be related to the adaptation process to higher salinity and other special conditions such as tides and currents during migration to the estuary,

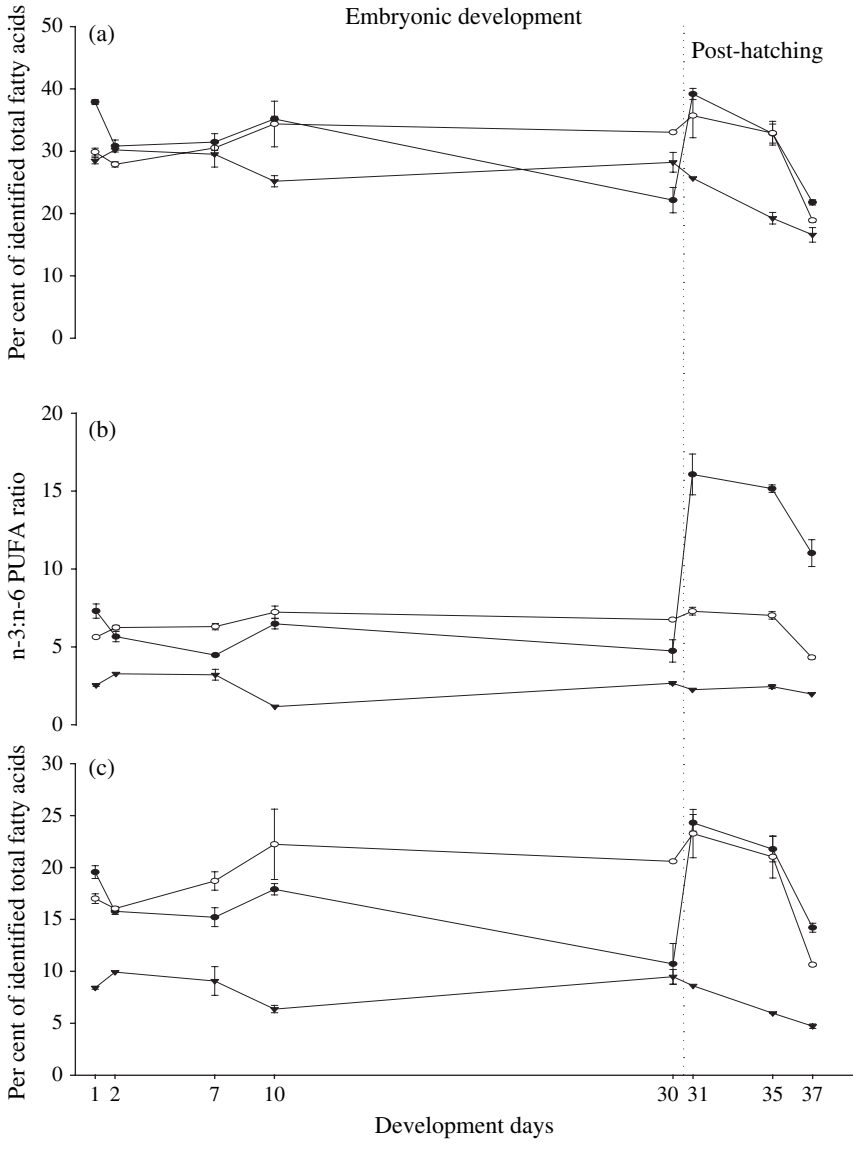

FIG. 2. Changes of (a) polyunsaturated fatty acids (PUFA), (b) ratio of n-3:n-6 PUFA and (c) 22:6n-3 during embryonic and larval development of *Galascias maculatus* of estuarine (–●–), cultured (–○–) and freshwater (–▼–) origin. Day 1 = recently fertilized eggs; day 2 = epibolic embryos; day 7 = embryos in the organogenesis stage; day 10 = embryos with ocular pigmentation; day 30 = embryos close to hatching; days 31, 35 and 37 = time since fertilization. Values are means ± S.D.

which requires an expenditure of energy. This, which is associated with reduced food intake, may contribute to reduce the lipid content in the embryos. Eggs of cultured fish on the contrary showed a higher lipid content reflecting previous feeding. This content tended to decrease during embryo development until the values matched those found in embryos coming from estuarine fish, both being

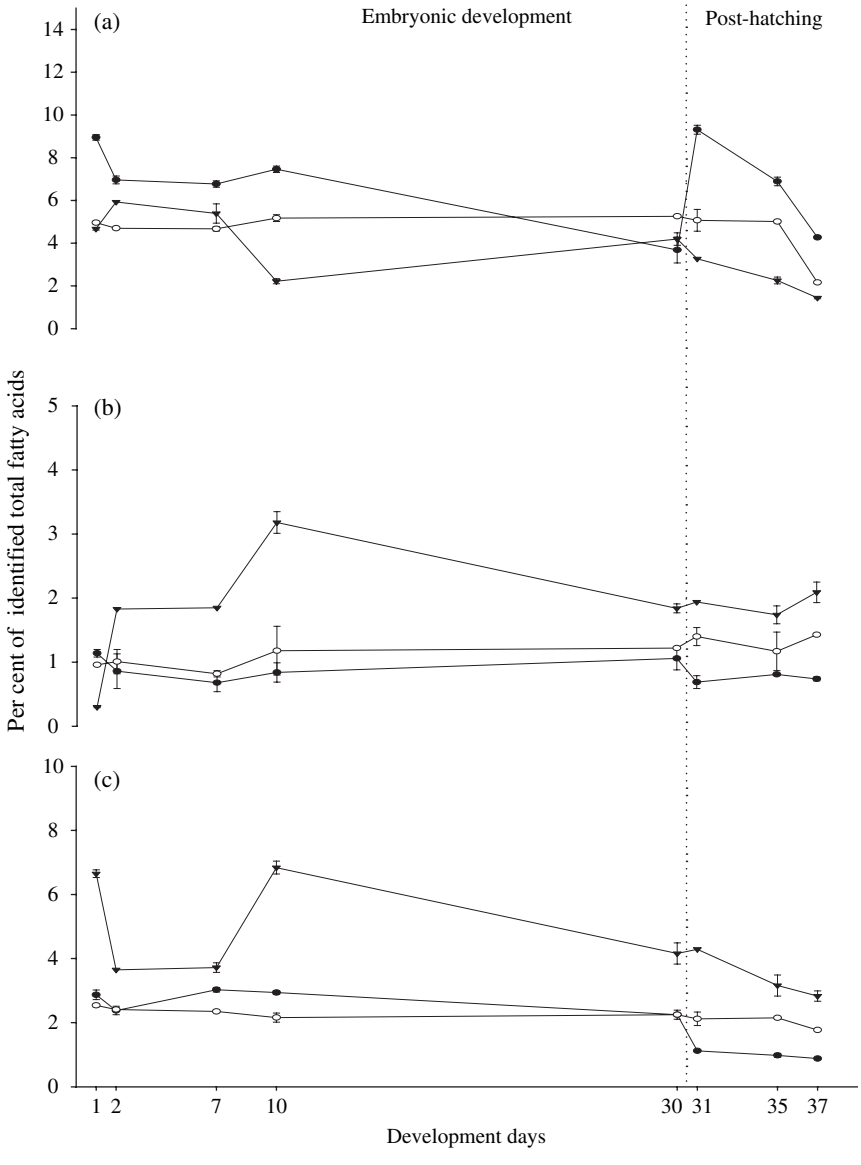

FIG. 3. Changes of (a) 20:5n-3, (b) 18:2n-6 and (c) 20:4n-6 during embryo and larval development of *Galaxias maculatus* of estuarine (–●–), cultured (–○–) and freshwater (–▼–) origin. Day 1 = recently fertilized eggs; day 2 = epibolic embryos; day 7 = embryos in the organogenesis stage; day 10 = embryos with ocular pigmentation; day 30 = embryos close to hatching; days 31, 35 and 37 = time since fertilization. Values are mean ± S.D.

incubated at the same salinity. Total lipid content in embryo and larvae during development may vary both in quality and quantity within the same species (Fraser *et al.*, 1988; Mourente & Vazquez, 1996) depending on environmental conditions (Wirth & Steffens, 1996), physiological events or energy demands (Sargent, 1995).

The quantity of SAFA did not vary during embryo development in fish from any of the three environments, nor was there any difference between embryos from the different environments. During both embryo development and yolk sac absorption, consistent with findings for the majority of species, the most abundant saturated fatty acid was 16:0, which is a main component of phospholipids, principally phosphatidylcholine and phosphatidylethanolamine (Tocher *et al.*, 1985; Mourente & Vazquez, 1996) and hence very important in membrane formation during embryogenesis. This pattern of conservation of saturated fatty acids during embryogenesis is typical of cold water fishes with long incubation periods (>20 days), such as Atlantic salmon *Salmo salar* L. (Cowey *et al.*, 1985), herring *Clupea harengus* L. (Tocher *et al.*, 1985) and cod *Gadus morhua* L. (Fraser *et al.*, 1988), and differs to the reduction found in warm water fishes with shorter incubation periods, *e.g.* red drum *Sciaenops ocelatus* (L.) (Vetter *et al.*, 1983), red seabream *Pagrus major* Temminck & Schlegel (Tandler *et al.*, 1989) and turbot *Scophthalmus maximus* (L.) (Rainuzzo, 1993).

The decrease in monounsaturated fatty acids during yolk sac absorption in the larvae from estuarine fish could be related with their importance as energy source, with 18:1n-9 and 16:1n-7 acids being the preferred substrates for catabolism (Izquierdo, 1996).

The PUFA in *G. maculatus* represented >30% of the total fatty acids in most cases, and their levels were maintained during embryogenesis denoting their importance in tissue and organs formation during embryogenesis. They were, however, reduced in the larvae during the yolk sack absorption stage suggesting their use as an important source of energy for the larva which is a very active swimmer (Dantagnan *et al.*, 1995) with a high energy requirement but a small yolk sac. This is in agreement with the β-oxidation of fatty acids from triacylglicerides found in during the yolk sac absorption in other fish species larvae (Rainuzzo, 1993). This pattern of PUFA utilization found in *G. maculatus* has also been observed in halibut *Hippoglossus hippoglossus* (L.) (Falk-Petersen *et al.*, 1989) and plaice *Pleuronectes platessa* L. (Rainuzzo *et al.*, 1993) and agrees well with the description given by Rainuzzo *et al.* (1992) for species with a high lipid content.

Increased PUFA contents in just hatched-out larvae from estuarine populations were related with the increase in n-3/n-6, 22:6n-3 and 20:5n-3, denoting again the importance of these fatty acids in higher salinity environments in comparison with fresh water. For instance, the ratio n-3:n-6 PUFA can differ considerably between freshwater and marine environments. The n-6 series tends to predominate in fresh water while the n-3 series appears to predominate in saltwater environments (Cowey & Sargent, 1972; Jobling, 1994). This ratio was higher in eggs from estuarine and cultured populations, being in the range of marine fishes (4·7–14·4; De Silva *et al.*, 1998), whereas for eggs from freshwater populations was lower and typical from freshwater fishes (0·5–3·8) (De Silva *et al.*, 1998). Hence, despite a previous freshwater nutritional influence of estuarine populations the n-3:n-6 ratios showed a clear marine pattern. This is in agreement with the marked effect of salinity over diet in the fatty acid composition of juveniles from certain salmonid species (Tocher *et al.*, 1995). Nevertheless the effect of broodstock diet on fatty acid composition of the eggs

could be observed in both, wild and cultured freshwater populations. Thus, embryos from cultured broodstock, which have been kept in fresh water for their entire life cycle but given diets based on fish meal and oil showed a n-3:n-6 ratio close to embryos from estuarine fish, whereas the embryos from freshwater broodstock which fed in a freshwater environment, showed a clear freshwater pattern in the ratio n-3:n-6 PUFA. These results, as well as those in other species (Sargent *et al.*, 1997; Czesny *et al.*, 2000), suggested the important effect of the broodstock diet in the fatty acid composition of embryos when there is no change in salinity or environmental conditions.

PUFA n-3, particularly 22:6n-3 and 20:5n-3, were higher in eggs and larvae of broodstock of *G. maculatus* coming from culture or estuarine environments than in those coming from fresh water. Moreover, these fatty acids markedly increased after hatching in larvae coming from estuarine populations, reaching the levels of cultured ones, both types of eggs being incubated at 10° C. These results suggest a predominant effect of the environment on fatty acid profiles to physiologically prepare the larvae to adapt to higher salinity conditions, directly affecting electrolyte transport across membranes and indirectly affecting osmoregulation through certain derivatives such as prostaglandins. These fatty acids were conserved during embryogenesis and larval development, denoting their importance and were used only at the end of the experimental period when starved larvae were exhausted. At this last period, 20:5n-3 decrease was more pronounced than that of 22:6n-3, showing a preferential consumption of 20:5n-3 despite it being less abundant. The 18:2n-6 acid content was higher in freshwater fish and its reduction during embryo and larval development was accompanied by a significant increase of 20:4n-6. This was not observed in embryos or larvae of broodstock fish from estuary or aquaculture origin, suggesting certain elongation and desaturation of 18:2n-6 to form 20:4n-6 only in fish coming from the freshwater environment.

In conclusion, both environment and diet of broodstock fish affected lipid and fatty acid composition of *G. maculatus* embryos and larvae as well as their changes during development, suggesting that differences in the requirements of first feeding fish may be predicted for larvae coming from different environments or reared in different water salinities.

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