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CHANGE OF LIPID AND FATTY ACID COMPOSITION DURING DEVELOPMENT OF *GALAXIAS MACULATUS* (*OSMERIFORMES: GALAXIIDAE*) EGGS AND LARVAE

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Introduction

It is recognized that environmental factors such as temperature, salinity, light, or available food type affect the lipid contents and fatty acid composition of aquatic organisms. For instance, successive changes in salinity during the migrations between fresh and saltwater in diadromous fish have been shown to cause significant changes in their metabolism (Sheridan, 1989), obviously affecting their biochemical composition mainly as a preparation or response to the osmoregulation process originated by the change of environment.

Since *Galaxias maculatus* can indiscriminately live in lacustrine and estuarine environments (freshwater and brackishwater, respectively), the aim of the present study was to compare the evolution of the fatty acid composition of eggs and larvae from estuarine, freshwater, or cultured populations in order to understand the effect of their broodstock origin.

Materials and methods

Sexually mature freshwater and estuarine broodstock were captured wild, and cultured broodstock were taken from F1 adults reared in captivity from wild parents. Eggs were obtained by artificial fertilization and incubated at 13±1°C and 0ppt for freshwater and cultured broodstock, and 10ppt for estuarine broodstock.

After fertilization, eggs from various females at 3-4 hours post-fertilization (1st cleavage), and embryons in the epibolic stage, embryons in the organogenesis stage, embryons in the ocular pigmentation stage, and embryos close to hatching were sampled. Once the larvae were hatched, they were kept in starvation in 2-l tanks at room temperature (12±1°C) at the same water salinity condition as in

incubation. Samples were then taken from newly hatched larvae, larvae with half of the yolk-sac reabsorbed, and larvae with the yolk-sac fully reabsorbed.

Each analysis was performed in duplicate from two samples from each pool and expressed as a percentage of dry weight. Total lipid was extracted from 0.1g of samples (eggs or larvae) and homogenizing in chloroform:methanol (2:1, v:v), according to Folch et al. (1957) and stored at -80°C prior to analysis. Fatty acid methyl esters were prepared from the extracted lipids according to Morrison and Smith (1964). Fatty acids were identified by separation in a gas chromatograph (Hewlett Packard 5890 series II Plus, Wilmington, USA) using a 30m × 0.25mm i.d. × 0.25μm capillary column HP-225 (Hewlett Packard, Wilmington, USA). 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, USA).

Results and discussion

The results indicate that the level of lipids in embryos and larvae from estuarine broodstock is lower than those from freshwater. This is probably a product of changes in salinity, not changes in diet (Table I).

The ratio n-3/n-6 polyunsaturated fatty acids (PUFA) increased considerably after hatching in larvae from estuarine fish, but not in those from freshwater or cultured conditions. The ratio of n-3/n-6 PUFA in eggs and larvae from estuarine fish corresponds to a marine pattern, while those from freshwater clearly correspond to a freshwater pattern. This agrees with Tocher et al. (1995), where changes in the composition of fatty acids resulted from a change in salinity rather than diet in juveniles of some salmonid species. In three cases, DHA was more abundant than EPA. It was also observed that both PUFAs were primarily conserved during embryogenesis and then consumed during larval development, independent of the original environment of the breeder (Table I).

Conclusion

Both environment and diet of broodstock fish affected lipid and fatty acid composition of *Galaxias maculatus* embryos and larvae as well as their 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 salinity. Further experiments are conducted to confirm this hypothesis.

Table I. Change of lipids, ratio n-3/n-6 PUFA , DHA (22:6n-3), and EPA (20:5n-3) during embryonic and larval development of *Galaxias maculatus*: eggs at 3-4 hours post-fertilization (A), embryons in the epibolic stage (B), embryons in the organogenesis stage (C), embryons in the ocular pigmentation stage (D) and embryo close to hatching (E), newly hatched larvae (F); larvae with half yolk-sac reabsorbed (G); and larvae with yolk-sac fully reabsorbed (H).

Populations	Fatty acids	A	B	C	D	E	F	G	H
Estuarine	% lipids	17.8±1.45	12.6±0.38	12.4±0.23	13.0±1.18	24.3±1.13	20.8±2.62	24.1±1.72	20.3±1.12
	n-3/n-6	7.3±0.46	5.6±0.33	4.4±0.08	6.4±0.33	4.7±0.72	16.7±1.31	15.1±0.24	11.0±0.86
	EPA	8.9±0.13	6.9±0.18	6.7±0.15	7.4±0.15	3.6±0.61	9.3±0.21	6.6±0.20	4.2±0.06
	DHA	19.5±0.62	15.7±0.3	15.2±0.91	17.9±0.54	10.7±1.97	24.3±0.8	21.7±1.72	14.2±0.43
Captivity	% lipids	26.4±0.37	22.9±0.97	19.3±1.95	12.6±0.33	18.5±0.09	29.8±1.58	28.9±0.50	25.6±1.83
	n-3/n-6	5.6±0.40	6.2±0.14	6.3±0.20	7.2±0.39	6.7±0.67	7.2±0.30	7.0±0.20	4.3±0.36
	EPA	4.9±0.06	4.7±0.03	4.6±0.10	5.1±0.16	5.2±0.01	5.0±0.51	5.0±0.06	2.1±0.01
	DHA	17.0±0.47	16.0±0.19	18.7±0.89	22.2±3.39	20.5±0.00	23.2±2.33	21.0±2.03	10.6±0.1
Freshwater	% lipids	24.4±1.49	21.8±0.58	24.9±0.00	26.3±1.07	24.2±2.97	23.1±0.03	24.7±2.24	27.1±1.57
	n-3/n-6	2.5±0.08	3.2±0.00	3.2±0.35	1.1±0.01	2.7±0.09	2.2±0.00	2.4±0.13	1.9±0.00
	EPA	4.6±0.06	5.8±0.01	5.3±0.45	2.2±0.12	4.1±0.29	3.2±0.00	2.2±0.16	1.4±0.04
	DHA	8.4±0.14	9.9±0.02	9.0±1.38	6.3±0.35	9.4±0.7	8.6±0.01	5.8±0.02	4.7±0.22

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