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Combined effects of dietary HUFA level and temperature on sea bass (*Dicentrarchus labrax*) larvae development

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Abstract

The purpose of this study was to investigate the combined effect of the incorporation of vegetable products in diet and temperature on enzymatic pathways for high unsaturated fatty acids (HUFA) desaturation in sea bass larvae. Four replicated groups were fed a low (LH; 0.8% EPA + DHA) or a high (HH; 2.2% EPA + DHA) n-3 HUFA microparticulated diet from mouth opening, six days post-hatching and were reared at 16 or 22 °C. The four experimental conditions (LH16, HH16, LH22 and HH22) were tested for 45 days. At the end of the experiment, body weight, total length and biomass were affected by temperature ($P<0.001$), while biomass as well as fresh body weight was also influenced by diet ($P<0.05$ and $P<0.001$ respectively). This always lead to the same ranking of experimental conditions: HH22 > LH22 > HH16 > LH16. The larval skeletal development was more advanced in 22 °C-groups than in 16 °C-ones ($P<0.001$), while it was not affected by diet. Amylase and trypsin pancreatic secretions did not vary between d-25 and d-45, indicating that pancreatic maturation was achieved at d-25. Low temperature combined with low dietary HUFA delayed intestinal maturation ($P<0.001$), while low temperature combined with high HUFA diet allowed larvae compensating for the initial intestinal maturation retardation. Lipase gene expression was down-regulated in HH16 group at d-25 ($P<0.05$) and in the two 16 °C-groups at d-45 ($P<0.001$), while lipase enzymatic activity was similar in all groups. This suggested the presence of a post-transcriptional regulation of this gene. PPAR α and PPAR β were not affected neither by temperature, nor by diet, suggesting that lipid metabolism was not significantly affected by a lowering in dietary n-3 HUFA when isolipidic diets were used. A higher DHA content was found in larvae than in their diets (×2 for LH; ×1.5 for HH) but the DHA content in PL of d-45 LH larvae was lower than the initial one, which revealed a HUFA deficiency in this group. Delta 6-desaturase (Δ6D) gene expression was significantly up-regulated by HUFA deprived diet ($P<0.05$) whatever the temperature was. This was supported by the increase in 18:3n-6 in LH larvae ($P<0.001$), which indicated a desaturation from 18:2n-6 by the Δ6D. This study clearly showed that larvae were able to adapt to an n-3 deprived diet by a stimulation of enzymatic pathways for HUFA desaturation, and that this adaptation was not affected by temperature.
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Keywords: Aquaculture; Delta-6 desaturase; *Dicentrarchus labrax*; HUFA; Desaturation

1. Introduction

In marine fish, larval stage represents a transitional ontogenetic period of simultaneous growth and development, which causes substantial changes in structure, physiology and morphology, all of which modify the

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physiological and behavioural capabilities and subsequently the ability of the fish to deal with challenges to its survival (Fuiman, 1997). Larval development strongly depends on environmental parameters, such as temperatures, and on diet (Koumoundouros et al., 1999; Sargent et al., 1999). In particular, the importance of dietary n-3 high unsaturated fatty acids (HUFA, eicosapentaenoic EPA 20:5n-3, docosahexaenoic DHA 22:6n-3 and arachidonic ArA 20:4n-6 acids) influence on larvae has been demonstrated by several studies (Kanazawa, 1993; Koven et al., 2001) as they function as critical structural and physiological components of the cell membranes of most tissues and are essential for growth, development and survival (for review, see Sargent et al., 1999). A dietary deficiency in DHA in larvae of farmed marine teleosts has been correlated with poor growth, high mortality and susceptibility to stress and disease (Cahu et al., 2003; Robin and Peron, 2004).

In contrast to freshwater fish, marine fish require the presence of preformed HUFA in their diet as they have a low capacity to bioconvert 18 carbon atom fatty acids (linoleic 18:2n-6 and alpha-linolenic 18:3n-3) into HUFA with 20 or 22 carbon atoms (arachidonic 20:4n-6, EPA and DHA; Mourente and Tocher, 1994). The first step of this bioconversion pathway requires the presence of the delta 6-desaturase gene (Δ6D). This gene has been cloned in several freshwater species such as zebrafish (*Danio rerio* AF309556), common carp (*Cyprinus carpio* AF309557), rainbow trout (*Onchorhynchus mykiss*; Seiliez et al., 2001). Δ6D gene has also been cloned in two marine fish species: gilthead seabream and turbot (Seiliez et al., 2003; Zheng et al., 2004). In gilthead seabream, an enhanced expression of the gene was obtained by feeding juveniles a HUFA-free diet. Cho et al. (1999) and Seiliez et al. (2001) previously showed that dietary HUFA inhibits the Δ6D gene expression in mammals and in rainbow trout. The deficiency in Δ6D activity usually observed in marine fish can be related to the abundance of HUFA n-3 in marine food chain, which has induced an adaptation (Sargent et al., 1995) or a repression of the Δ6D activity (Olsen et al., 1990).

As long as fish oil and meals represent primarily ingredients of aquafeeds, larvae n-3 HUFA requirements are easily covered. However, the high increase in farmed fish production in addition to the stagnation or rarefaction of natural stocks leads to look at substitutes for fish products commonly used in aquaculture (Lodemel et al., 2001; Ringo et al., 2002). Incorporation of vegetable compounds in fish feeds constitutes at the present time the only solution in Europe, although it do not bring n-3 HUFA to cover marine fish requirement but PUFAs with 18 carbons (C18), which may disturb fish physiology (Parpoura and Alexis, 2001). So it should be interesting to obtain fish able to adapt their metabolism developing enzymatic pathways in order to bioconvert C18 fatty acids supplied by vegetable products into HUFA. However, in larval stages this capacity could be affected by environmental factors, specially by temperature, which is one of the greatest factors acting on fish ontogeny (Koumoundouros et al., 1999). Interaction between temperature and dietary n-3 HUFA has been investigated in European sea bass juveniles (Person-Le Ruyet et al., 2004) and showed that a 3-month deficiency in dietary n-3 HUFA did not drastically impair fish capacity to adapt to a high temperature (29 °C).

The aim of this study was to examine the effect of specific dietary n-3 HUFA combined with water temperature on the development of some metabolic functions, particularly on the enzymatic pathways for HUFA desaturation during sea bass (*Dicentrarchus labrax*) larval development. The expression of Δ6D in response to these experimental conditions was specially studied.

2. Materials and methods

2.1. Rearing conditions and experimental design

Three days post-hatching sea bass larvae were obtained from the commercial fish farm Aquanord (Gravelines, France) and experiments were conducted at the IFREMER-Brest. Larvae were dispatched in 20 conical fiberglass tanks (35 l; initial shocking density: 60 larvae l^{-1}, *i.e.* 2500 larvae $tank^{-1}$), and temperature was progressively increased from 14 °C to 16 °C within 2 days. After an acclimation period of 2 days, temperature was progressively increased to 22 °C in 8 tanks while other tanks remained at 16 °C. All groups were fed microparticulated diets from mouth opening at day 6 (d-6) to d-45. Larvae weighted 0.36±0.01 mg at d-6. Two isolipidic diets (Table 1) differenced by a low (LH) or high (HH) HUFA content were tested: 0.8 and 2.2% EPA+DHA on dry matter basis, respectively. The four experimental conditions were LH16, HH16, LH22 and HH22, with 6 tanks per conditions at 16 °C and 4 at 22 °C. Diets were automatically distributed in excess 18 h/24 h and the daily ration was progressively increased from 1 g per day per tank at d-6 to 10 g at d-45.

Tanks were supplied with running sea water (34.5‰) filtered through a sand filter, then passed successively through a tungsten heater and degassing column packed with plastic rings. The water renewing was progressively

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