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The effect of lipid type on lipid digestion enzymes during larval development of the California halibut, *Paralichthys californicus*

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ABSTRACT

The possible use of alternative lipid sources as enrichment of live food during larval culture is of great interest. Evaluating the effect of lipid type on the activity of digestive enzymes and development in larvae of marine fish is crucial to improve the growth, survival and quality of cultured larvae. Thus, the objectives of this study were to characterize the bile-salt-dependent lipase (BSDL) and phospholipase A_2 (PLA_2) activities in the digestive tract of California halibut larvae and evaluated the effect of lipid type on growth, survival and lipid digestive enzymes during the larval period. Two types of lipids were evaluated as enrichments of live feed (rotifers and *Artemia*): Algamac-3000 a marine lipid source (MAR treatment) and flaxseed oil a terrestrial lipid source (FSO treatment). Additionally, at the end of experiment the quality of larvae from each dietary treatment were assessed by gradually increasing the water temperature from 18 °C to 32 °C. Growth in terms of DW, TL and survival were significantly higher in larvae fed live-prey enriched with MAR treatment, 24.24 ± 1.80 mg, 10.13 ± 0.42 mm and 21.23 ± 0.39% respectively, compared to those fed live-prey enriched with FSO treatment 9.36 ± 1.34 mg, 7.47 ± 0.75 mm and 3.72 ± 4.79%. Initiation and completion of metamorphosis were earlier for larvae fed the MAR treatment. BSDL and PLA_2 activity were detected before exogenous feeding commenced (3 dph). BSDL activity was similar in both treatments until 30 dph, when a significantly higher peak in total activity was observed in larvae fed live-prey enriched with the MAR treatment, while activity in larvae fed with FSO treatment peaked until 34 dph. Nonetheless, activity returned to comparable values in both treatments by 42 dph. No significant differences were found in PLA_2 activity between treatments. This indicates that there is a temporary negative effect of lipid type in BSDL activity, but not in the PLA_2. The larval quality tests revealed that larvae fed with FSO treatment had a higher stress index (29.92 ± 14.66) than those fed with the MAR treatment (1.88 ± 2.65). The FSO treatment did not provide essential fatty acids (LC-PUFAs n-3) required for adequate larval development and appropriate stress response and is not a suitable enrichment-lipid for live-prey in California halibut larval culture.

1. Introduction

The California halibut (*Paralichthys californicus*) is a flounder species with high potential for aquaculture in the Northwest of México and the Southwestern US, due to its adaptability to culture conditions, excellent market value and consumer preference (Haaker, 1975; Love and Brooks, 1990; Fischer et al., 1995). However, the development of culture techniques for this species has been limited in part due to the lack of knowledge, among other factors, of the digestive capacity, nutrient requirements and appropriate weaning protocols during the larval period.

The development and manufacturing of microdiets for marine fish larvae has only managed to partially replace the use of live food in the early stages of development in few species and this is species-specific (Cahu and Zambonino-Infante, 2001; Rønnestad et al., 2013). Consequently, it is essential to study the digestive capacity of new aquaculture species, through the characterization of digestive enzymes during ontogeny to assess the digestive capacity of the larvae and formulate highly digestible weaning microdiets (Conceição et al., 2010; Hamre et al., 2013).

An adequate supply of essential fatty acids (EFA) such as, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA) during the altricial stage is an indispensable factor for achieving suitable growth, survival and adequate larval development (Bell et al., 2003; Hamre et al., 2013). Other important lipids for the normal development of marine fish larvae are the phospholipids

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(Tocher et al., 2008; Cahu et al., 2009).

Traditionally, fish oil is the main source of PUFAs and LC-PUFAs for feeding marine fish larvae (Izquierdo and Koven, 2011). In recent years, the high demand for this resource has been limited due to increasingly higher costs and reduced availability of this raw material (Tacon and Metian, 2008; Turchini et al., 2009). This has generated interest in new alternative sources of PUFAs and LC-PUFAs for partial or total replacement of fish oil used in feeding marine fish (Turchini et al., 2009; Eryalçın et al., 2013). Within the land-based oils, the flaxseed oil (FSO) is a potential alternative for the replacement of fish oil due to relatively high PUFAs n-3 and n-6 content (NRC, 2011).

In teleost fish, the most important digestive lipase is the bile salt-dependent lipase (BSDL), produced by the pancreas, which hydrolyses triacylglycerols for subsequent absorption by the intestinal enterocytes (Gjellesvik, 1991; Izquierdo et al., 2000; Hoehne-Reitan et al., 2001). BSDL has been well studied in several species and characterized (Perez-Casanova et al., 2004; Faulk and Holt, 2009). However, phospholipase A2 (PLA_2), the enzyme responsible for catalyzing the hydrolysis of the ester link in position sn-2 of phospholipids (PL), has not been well studied in fish (Van den Bosch, 1982; Fujikawa et al., 2012) but has been reported in species such as sea bream *Pagrus major* (Iijima et al., 1997), turbot *Scophthalmus maximus* (Hoehne-Reitan et al., 2003), European sea bass *Dicentrarchus labrax* (Zambonino Infante and Cahu, 1999) and Atlantic cod *Gadus morhua* (Sæle et al., 2011).

Little is known with respect to the effect of the lipid type in the activity of digestive enzymes and development in marine fish larvae. Therefore, the objectives of this study were to evaluate the effect of two lipid sources (one terrestrial vs. of marine origin) on growth, survival and enzyme activity during the larval period, in order to increase our knowledge to formulate weaning diets and develop feeding strategies according to the digestive capacity of larvae.

2. Material and methods

2.1. Larval rearing and culture system

Fertilized eggs of California halibut were obtained from naturally spawning broodstock kept at the Marine Fish Culture Laboratory (MFCL) of CICESE, in Ensenada, Baja California, México. Once the eggs were collected, a sample was analyzed under a microscope to check the development and viability. The eggs were first incubated in 2000-L cylindro-conical tanks with gentle aeration. At 8 dph (once the quality of the spawn and larvae were assessed and determine appropriate for our experimental design) larvae were randomly distributed into six 200-L cylindrical fiberglass gray tanks (n = 3 per treatment) at density of 13 larvae L^{-1} in a closed recirculating seawater system equipped with biological and mechanical filters, heat pump and UV sterilized. Water quality was monitored daily before feeding to maintain the temperature at 18.0 ± 0.2 °C, dissolved oxygen at 6.8 ± 0.4 mg L^{-1}, salinity at 33.9 ± 0.3‰, 10% water exchange per day and water flow of 1.5 L min^{-1}. The ammonia, nitrites and nitrates were measured every three days using a commercial kit (Api Pharmaceutic Aquarium Kit) to keep values at < 1.0 N-NH3 mg L^{-1}. A photoperiod of 14 h light: 10 h dark and 72 lx of light intensity at water surface provided by overhead fluorescent lights.

2.2. Experimental design

Two treatments were evaluated in this study: 1) live feed enriched with an oil emulsion of marine origin (MAR), Algamac 3000TM (Bio-Marine Inc.,) rich in LC-PUFAs n-3, which is used as part of the normal protocol for larval rearing at the MFCL and can be considered as the control treatment; and 2) live feed enriched with an oil emulsion of terrestrial origin, flaxseed oil (FSO), Flaxseed oil omega-3 (Spectrum Organic Products) rich in n-3 PUFAs. To evaluate the effect of each type of lipid, the experimental phase began with 8 dph larvae, when it was possible to dissect the digestive tract of the larvae. The experimental period lasted 42 dph.

2.3. Experimental emulsions and live feed production and enrichment

Live feed was produce using the protocols describe in Zacarías-Soto et al. (2006). Live feed enriching was performed in 45-L vessels at a temperature of 28 °C, salinity 33‰ and constant aeration and light intensity. The marine oil treatment (MAR) consisted of enriching 300 *Artemia* mL^{-1} with 300 mg L^{-1} of Algamac 3000TM (Bio-Marine Inc.) while 200–500 rotifers mL^{-1} were enriched with 125 mg L^{-1} with the same product. Enrichment emulsion for the FSO treatment consisted of an emulsion of 5 mL FSO (Spectrum Organic Products), 0.5 g of soy lecithin as an emulsifier (Gelcaps, Banner Pharmacaps, Inc.) and 100 mL of distilled water. The emulsion was prepared by mixing all ingredients in a commercial blender for a period of 2 to 3 min. A sample of the emulsion was observed under a microscope to evaluate if the size of the micelles (10 to 30 μm) were properly formed and of proper size to be consumed by the live feed. Additionally, a test was made with an artificial colorant, red Sudan IV (Sigma Cat. S-8756), to confirm that the emulsion was properly ingested by the live feed. To enrich the live feed, a 0.8 mL of the prepared emulsion L^{-1} was added to 500 rotifers mL^{-1} and 100 to 200 *Artemia* mL^{-1}. Live feed was enrichment for 12 h before being fed to the larvae.

2.4. Larval feeding protocol

After enrichment and before feeding the larvae, the live feed was rinsed in a cleaning device for 15 min with UV disinfected seawater and for 5 min with freshwater to remove residues of emulsion and reduce the bacterial load in the live feed. Larval feeding was performed according to the protocol described by Zacarías-Soto et al. (2006) and Vizcaíno-Ochoa et al. (2010) with minor modifications. Briefly, larvae were fed starting at 3 dph with enriched rotifers with the initial prey concentration of 5 rotifers mL^{-1}, increasing to 7 rotifers mL^{-1} at 12 dph. Thereafter this density was maintained up to 16 dph. From 17 to 21 dph, larvae were co-fed with enriched *Artemia*. At this time rotifer supply was gradually decreased at a rate of 20% per day with the concurrent increase in the concentration of *Artemia*. After 21 dph, larvae were fed only with enriched *Artemia* at an initial density of 3–4 mL^{-1} increasing up to 7–8 mL^{-1} towards the end of the live feed experiment at 42 dph. The supply of live food was performed twice a day (8:00 and 14:00).

2.5. Larval performance

The total length (TL) of 30 randomly selected larvae from each tank was measured every two days from 8 to 18 dph and every four days until 42 dph. Once sampled, the larvae were measured from the mouth to the end of the notochord using a stereomicroscope Wild Heerbrug (MAX ERB Instrumental Company, USA). Larval dry weight (DW) was recorded using an analytical balance (XB 120A Precisa Instruments Ltd., Switzerland) at 8 and 42 dph after rinsing with distilled water to remove any salt crystals and placed in an oven at 60 °C for 24 h. All larvae were anesthetized with tricaine methanesulphonate (MS 222, 0.1 g L^{-1}, Argent Chemical Laboratories, Inc., Redmond, WA, U.S.A.) before measuring.

To characterize larval development before, during and after metamorphosis, growth in terms of TL was divided into 3 stages: 1) before metamorphosis ranging from 8 to 18 dph; 2) during metamorphosis from 18 to 34 dph and 3) after metamorphosis from 34 to 42 dph. In each stage, the specific growth rate (SGR) was calculated using the formula of Hardy and Barrows (2002):