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Organogenesis of digestive system, visual system and other structures in Atlantic bluefin tuna (*Thunnus thynnus*) larvae reared with copepods in mesocosm system

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ABSTRACT

This study examines the anatomical development of Atlantic bluefin tuna during the first month of life at histological level with emphasis in the gut development and those organs required to establish the hunting behaviour characteristic of this species. The tuna larvae have been reared in mesocosm with copepods (*Acartia* and *Trigriopus* sp.) as primary live prey during the first two weeks. The larvae hatched with 3.4 mm average TL and exhibited a very fast growth reaching between 4 and 5 cm at the end of the first month. The yolk reserves were consumed rapidly in two days. As an altricial species the gut and most of the sensory organs were not developed at hatching. The transformation of the gut from an undifferentiated canal at hatching up to a complex and segmented juvenile-like digestive tract occurred in three weeks. At hatching, the digestive tract appeared as a straight tube dorsal to the yolk-sac. The opening of both mouth and anus occurred at 2 days post-hatching (dph). From 4 dph onwards, intestinal loops started to be visible and the primordial future stomach started to develop. Intestinal brush border and increased folds in length were detected in the intestine from 4 dph. Acidophilic protein supranuclear inclusions within the hindgut enterocytes and the first signs of lipid absorption within enterocytes of the anterior intestine were evident from 6 dph. First gastric glands were detected at 11 dph, and at 17 dph the stomach was practically developed with the apparition of the first pyloric caeca. Newly hatched Atlantic bluefin tuna larvae had unpigmented eyes. The eyes were pigmented and functional at the opening of the mouth (between 2 and 3 dph), and rods appeared at 17 dph. Nevertheless twin cones were not observed during the first month. The bluefin tuna larvae quickly acquired a degree of development that allows for efficient predation and digestion of more complex feeds. In fact, the digestive system, sensory and visual structures, thyroid gland, swim bladder, and the kidney and heart differentiated very early during the larval ontogeny of this species, and they were well developed and practically fully functional around 17–18 dph under our rearing conditions. At 21 dph with the change in the allometry of mouth size the post-larvae exhibited most of the juvenile anatomical characteristics.

1. Introduction

The Atlantic bluefin Tuna (*Thunnus thynnus*) is one of the most commercially valuable marine fish species. This scombrid is distributed in the Atlantic Ocean and Mediterranean Sea (Fromentin and Powers, 2005; MacKenzie and Mariani, 2012; Rodríguez-Roda, 1964). Farming of this species based in captures for on-growing and fattening in cages had progressed during the last decade becoming a relevant industry in Mediterranean countries (Aguado-Giménez and García-García, 2005; Mylonas et al., 2010; Ottolenghi, 2008; Tićina et al., 2007). The maintenance of captive animals in cages for long periods is allowing significant advances in the knowledge of key processes to achieve the full domestication and farming of this species. Particularly important are the studies on reproduction, control of gametogenesis, spawning hormonal induction and obtaining of fertilised eggs (Corriero et al., 2007; De Metrio et al., 2010; Gordoa, 2010; Gordoa et al., 2009; Mylonas et al., 2007, 2010; Sarasquete et al., 2002). The full control of the production cycle is a necessary step for establishing a stable industrial farming of Atlantic bluefin tuna as for advancing in its sustainability (Mylonas et al., 2010). Nevertheless, the completion of the life cycle is not yet accomplished. Specifically, the larviculture of this species is still far from being satisfactory but there have been relevant steps using eggs obtained from natural or induced spawning (De la Gándara et al., 2011; Katavić et al., 2011). Thus, basic developmental and growth characteristics during the larval stage have been established (Besseau et al., 2011; Cataudella et al., 2011; Covès et al., 2011; De Metrio et al., 2010; Papandroulakis et al., 2011). Although with some variations, current rearing methodologies are based in general on mesocosm rearing system using rotifers, *Artemia* and fish yolk sac larvae as live feeds during the first weeks.

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This progress in the larval rearing of the Atlantic bluefin tuna is in part benefiting from the advances obtained in the sibling species Pacific bluefin tuna *Thunnus orientalis* (Masuma et al., 2011; Miyashita et al., 2001; Sawada et al., 2005; Takebe et al., 2012), in which the completion of the life cycle has been achieved (Sawada et al., 2005). The growth results of this Pacific tuna species improved progressively during the last decade with the application of better rearing systems and more appropriate feeding protocols (Nakagawa et al., 2011; Sawada, 2011). Larval feeding and weaning onto inert diets are crucial aspects that require priority treatment when larvae of a new species are growing in the rearing facilities.

All these studies in larvae and early juveniles as well as the age estimation from otoliths in natural populations of different tuna and other scombrid species revealed a very fast larval growth (Brothers et al., 1983; Jenkins and Davis, 1990; Lang et al., 1994) and a piscivory feeding behaviour with high voracity from early stages (Catalán et al., 2011; Hunter and Kimbrell, 1980; Sabate et al., 2010; Young and Davis, 1990). Such characteristics are concordant with precocity in the development of the digestive tract and those structures required for an efficient hunting and for a sustained and speedy swimming (e.g., visual functionality, fins, and swim bladder).

A good knowledge of the main events occurring at ontogenetic level, and the characteristics of the development during the larval phase is essential to advance in the design of more appropriate feeds and feeding protocols during this stage. Despite that most fish species exhibits a general pattern of somatic, sensory or endocrine development, temporal variability is an essential characteristic in the ontogeny among teleosts. These inter-specific ontogenic variations are related to transitional and definitive feeding habits when the juvenile stage is attained, and affect to the presence/absence and relative size of some structures as well as to the temporal sequence of histophysiological events. In addition, a high developmental plasticity has been observed in the larval stage in relation to environmental circumstances including dietary conditions (Pittman et al., 2013). In fact, rearing conditions (water physical and chemical characteristics, larval density, prey type, etc.) strongly affect growth, survival and larval performance.

In this study we have examined the anatomical and functional developments of Atlantic bluefin tuna during the first month of life at histological level with emphasis in the digestive system development and those organs and tissues required to establish the hunting behaviour characteristic of this species. With this aim the tuna larvae have been reared under mesocosm system with copepods as primary food source in contrast to the previous tested rearing systems.

2. Material and methods

Naturally fertilised eggs obtained from a captive broodstock were collected from cages placed in the Northern Mediterranean coast of Spain. Three egg batches were used in this study. Eggs were transferred to the hatchery facilities of FUTUNA Blue and incubated in 100 l tanks at a temperature of 23 °C and a salinity of 37 g l^{-1}. Larvae were reared in 25 m^3 tanks with water recirculation system at a temperature of 23–24 °C, a salinity of 37 g l^{-1} and a light/dark daily cycle of 16/8 h. Oxygen saturation in water ranged between 85 and 110%. The initial density was 4 larvae l^{-1}. Exogenous feeding started at 2 days post-hatching (dph). Larvae were fed on rotifers (*Brachionus plicatilis*) enriched with Ori-green (Skretting) during the first two days of feeding and on copepods from 3 to 18 dph. Copepods were cultured on mesocosm system and consisted in a mixture of *Acartia* sp. (95%) and *Trigriopus* sp. (5%). Rotifers and copepods were supplied twice a day to maintain a minimum density of 10 prey ml^{-1} in the rearing tanks. Weaning onto commercial diet (Skretting Tuna Starter; 300–500 of particle diameter) started at 16 dph. Microalgae *Tetraselmis chuii* was also provided from first feeding up to 15 dph.

Larvae from different spawnings were sampled intermittently during the first month of life. All sampled larvae were anaesthetised with ethyl-4-aminobenzoate. Specifically the larvae were sampled at 0, 1, 2, 3, 4, 6, 11, 17, 18, 25, 27 and 28. The day of hatching is considered as 0 dph. Total length (TL), yolk length and width and diameter of oil globule were measured in 10 to 15 larvae per sample during the yolk sac stage. TL was measured in 6 to 10 larvae per sample from 3 to 28 dph. Points with a sample size lower than 6 larvae were not considered to calculate the mean TL. Mouth size was estimated by measuring the lower jaw from the labial commissure to tip of the mouth. To construct the jaw length/TL ratio in relation to age, additional measurements were taken in dead juveniles between 30 and 38 dph that were not representative to be considered for the population growth. Yolk volume (V_y) was calculated from the formula of a prolate spheroid: $V_y = (\pi/6) \cdot (\text{yolk length}) \cdot (\text{yolk height})^2$. Oil globule volume was calculated from the diameter assuming a spherical form.

For the histological analysis 5 to 10 larvae per sample were fixed in 4% v/v buffered formaldehyde (pH 7.2) and embedded in paraffin blocks. Sagittal and transversal histological sections of whole specimens of 5–7 μm thickness were stained with haematoxylin–eosin (H–E) and haematoxylin–VOF (VOF: light green–orange G–acid fuchsin) according to Sarasquete and Gutiérrez (2005). Neutral lipids were visualised as empty vacuoles, because they dissolved during the paraffin procedure. PAS–diastase-PAS (glycogen and neutral glycoproteins) and Bromophenol Blue –BPB– (proteins) techniques were performed, according to the previous studies in fish species (Sarasquete et al., 2002) and monograph by Pearse (1985).

3. Results

3.1. Larval growth

The increase in total length during the first month is shown in Fig. 1. The larvae hatched with 3.4 mm average TL and exhibited a very fast growth reaching between 4 and 5 cm at the end of the first month. The yolk reserves were consumed rapidly (Fig. 2). This fast yolk consumption allowed the larvae to reach 3.8 mm at the opening of the mouth at the second day post-hatching. By this time the yolk was completely exhausted though the oil globule still lasted one more day to be consumed. The size of the jaws increased progressively faster than the total body length from the opening of the mouth up to 21 dph, and then the jaw length/TL ratio decreased (Fig. 3).

3.2. Organogenesis of digestive and visual systems and other structures

Main ontogenetic events occurring during the first month of Atlantic bluefin tuna larval life are summarised in Tables 1 and 2. The present tabular overview of organogenesis in *T. thynnus* provides valuable

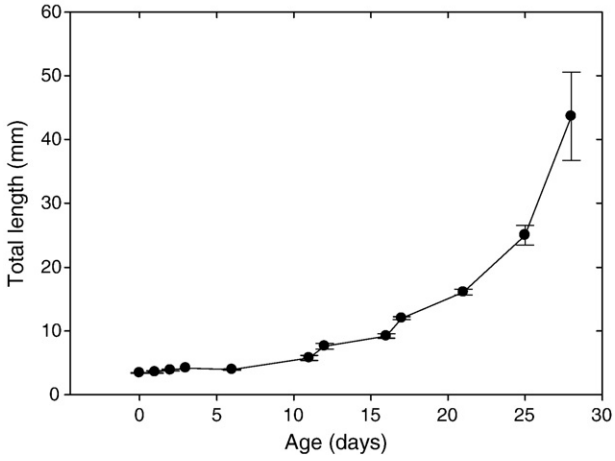

Fig. 1. Growth in total length of reared Atlantic bluefin tuna larvae during the first month of life.