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Larval ontogeny of redbanded seabream *Pagrus auriga* Valenciennes, 1843 with special reference to the digestive system. A histological and histochemical approach

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Abstract

The larval ontogeny of redbanded seabream *Pagrus auriga* was studied histologically and histochemically from 0 until 30 days after hatching (DAH). According to the source of food and the structural changes in the digestive tract, larval development was divided into four stages: (1) lecitotrophic (0–2 DAH), (2) lecitoexotrophic (3–4 DAH), (3) exotrophic I (5–15 DAH), and (4) exotrophic II (16–30 DAH). During the first three stages, larvae underwent an intense organogenesis, this being particularly intense from stage 2 (mouth and anus opening) to early stage 4 (appearance of gastric glands). Subsequent development during stage 4 was characterized by the proliferation and growth of pre-existing structures. During stage 1, the mouth and the anus were closed and the digestive tract undifferentiated (straight tubular segment), as well as the majority of the organs (observed as undifferentiated cells groups or primordial structures). As resorption of endogenous reserves proceeded (3–4 DAH) larvae acquired initial absorptive and digestive equipments necessary for first feeding (enterocytes brush border, zymogen pancreatic granules, and ducts connecting accessory glands to the gut). During stage 2, the digestive tract started to differentiate and buccopharyngeal cavity, oesophagus, incipient stomach, and anterior, mid and posterior intestine were distinguished. During stages 2 and 3, prey capture became guaranteed (early development of jaw, fins, teeth, and taste buds) and the digestive and absorptive processes continued developing (appearance of the gut mucosa folds and protein supranuclear inclusions and lipid infranuclear vesicles in enterocytes). The endocrine elements (Langerhans islets and thyroid follicles) except corpuscles of Stannius appeared from 3 to 5 DAH (mouth opening and total yolk resorption). During stage 2 and early stage 3 (3–7 DAH), the circulatory and excretory systems became functional, with the compartmentalization of the heart and the development of renal corpuscles, tubules, and collecting ducts. The beginning of stage 4 was marked by the appearance of gastric glands (16 DAH), which subsequently proliferated in association with the increase in size of the accessory glands. Such event ensured the development of gastric digestion, which around 30 DAH became fully guaranteed (transition from larval to juvenile stage). During stage 4, gill filaments and lamellae proliferated, the heart completed its compartmentalization, the pronephric and mesonephric regions in the kidney developed, and endocrine elements

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proliferated. The structural information presented here constitutes an initial step towards the determination of the functional systemic capabilities of *P. auriga* larvae, and thus the physiological requirements needed for optimal welfare and growth.

Keywords: Sparidae; *Pagrus auriga*; Ontogeny; Larval development; Histology; Histochemistry

1. Introduction

The larval stage constitutes a critical period during fish life history, in which ontogeny causes important structural and functional changes in the body tissues, organs and systems. For this reason, both the success and progress of larviculture depend specially on an adequate knowledge of the development of such elements, in order to adjust culture conditions to the ontogenic status of the larvae. Among these organs and systems, the digestive system deserves special attention, given that a comprehensive knowledge of its morphofunctional characteristics at each developmental stage is essential to establish feeding protocols matching the larvae nutritional requirements, and thus ensuring the correct larval development and optimal growth in culture (Segner et al., 1993, 1994; Yúfera et al., 2000; Zambonino-Infante and Cahu, 2001). Moreover, having in mind that both the beginning of the exogenous feeding after yolksac resorption and weaning, i.e. the transition from live preys to compound diet feeding, are very important bottlenecks in fish larvae culture, the study of such developmental aspects is of particular importance to try to overcome these problems. In this context, morphological, histological and histochemical data represent an initial step towards the determination of the functional systemic capabilities of larvae, and thus the physiological requirements needed for optimal welfare and growth.

Several studies have described the ontogeny of the different organs and systems (mainly the digestive tract and its accessory glands; see previous paragraph) during larval development of diverse marine fish species. Some examples are turbot *Psetta maxima* (Segner et al., 1994; Padrós and Crespo, 1996), Atlantic cod *Gadus morhua* (Kjørsvik et al., 1991; Morrison, 1993), Japanese eel *Anguilla japonica* (Kurokawa et al., 2004), gilthead seabream *Sparus aurata* (Sarasquete et al., 1993, 1995; Elbal et al., 2004), European seabass *Dicentrarthus labrax* (García-Hernández et al., 2001), Senegalese sole *S. senegalensis* (Sarasquete et al., 1996, 2001; Ribeiro et al., 1999), white seabream *Diplodus sargus* (Ortiz-Delgado et al., 2003), common dentex *Dentex dentex* (Santamaría et al., 2004), common pandora *Pagellus erythrinus* (Micale et al., 2006) and common seabream *Pagrus pagrus* (Darias et al., in press). From these studies it can be concluded that the basic mechanisms of organogenesis are similar in all teleosts, even though there are considerable differences regarding the relative timing in the ontogeny, which in turn is affected by the general life history of each species and a number of abiotic and biotic factors, such as water temperature or food availability (reviewed by Falk-Petersen, 2005).

The success of *S. aurata* production in southern Europe aquaculture has stimulated the culture of other high-valuable Sparidae. One of these species is red-banded seabream *Pagrus auriga* which, as well as having good commercial value, exhibits both high fecundity and growth rates (Cárdenas et al., 2003; Prieto et al., 2003). Moreover, it inhabits southern and warm waters, including the Mediterranean Sea and the eastern Atlantic Ocean from Portugal to Angola (also found in Madeira and the Canary Islands) (Hureau, 1996), a quality which adequately fits the requirements of southern Europe aquaculture. Some studies have recently been published on *P. auriga* larval development focusing on growth under different feeding, light, and thermal regimes (Prieto et al., 2003; Lozano et al., 2004; Aguirre et al., 2006), on ontogeny of digestive enzymes (Moyano et al., 2005) and on skeletal development (Gavaia et al., 2005). However, to the authors' knowledge, an integrated study on the histological and histochemical aspects of the organogenesis during *P. auriga* larval development is not currently available in the literature. Therefore, the present study aims to describe the main histological and histochemical changes of the endogenous yolk reserves, digestive system and other organs (liver, pancreas, gills, gallbladder, spleen, kidney, heart) taking place in *P. auriga* larvae during the first month of life and to correlate them with physiological events.

2. Materials and methods

2.1. Larvae, rearing conditions and sampling

P. auriga larvae used in the present study were obtained by natural spawning from a captive wild broodstock held at CIFAP "El Toruño" aquaculture facilities (El Puerto de Santa María, Cádiz, Spain). Larval culture