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Ontogeny of the digestive system of meagre *Argyrosomus regius* reared in a mesocosm, and quantitative changes of lipids in the liver from hatching to juvenile

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ABSTRACT

Histological development of the digestive system was studied in association with feeding preferences in meagre (*Argyrosomus regius*) from hatching to juvenile (44 days after hatching, dah), using a mesocosm system. In addition, the liver lipid content was evaluated using histological methods (area covered with lipid vacuoles, ACLV%). The ontogeny of the digestive system was completed by 19 dah (or 444 degree days) with the gastric glands appearing at 15 dah (361 degree days), the pyloric ceaca at 17 dah (404 degree days) and the Y-shaped stomach formed at 19 dah (444 degree days). The rearing period was characterized first by relatively slow growth until tail flexion (~15 dah) and fast growth thereafter (mean ± SD total length of 45.14 ± 4.00 mm at 44 dah). When the feeding protocol included exclusively rotifers, mean ACLV remained low (2.39 ± 0.34%) while feeding on *Artemia nauplii* and copepods increased liver ACLV to 47.18 ± 6.56% at 20 dah. Changes in the feeding protocol were reflected in feeding preferences (stomach content), and variations of liver lipid content and the occurrence of vacuoles in the intestine. During transition from live prey to artificial feed (~28 dah), ACLV decreased significantly – indicating a malnutrition period – concomitant with a delay in the acceptance of artificial feed of 8 days. Thereafter, consumption of artificial feed resulted in an increased ACLV to 56.3 ± 7.6% at 36 dah. The results indicate that during early development meagre is a fast growing species, developing rapidly the structures and basic organs of the digestive system required to overcome successfully the critical stages of larval rearing. The study also shows that histological evaluation of liver lipid content using the ACLV may be a valuable tool in commercial aquaculture to improve larval rearing protocols, and production efficiency.

1. Introduction

Meagre (*Argyrosomus regius*) is a scienid species distributed in the Mediterranean Sea, Black Sea and the Atlantic coast of Europe, with maximum reported weight and total length of 103 kg and 182 cm, respectively (Chao, 1986; Quéro and Vayne, 1987). This species inhabits coastal ecosystems near the continental shelf, and can be found in lagoons or river Deltas (Griffiths and Heemstra, 1995) where it spawns in the spring. Flesh quality is considered exceptional and highly nutritious, and the species name *regius* (i.e., royal) was given because of its highly esteemed flesh quality (Poli et al., 2003).

Meagre has been considered a good species for the diversification of fin-fish aquaculture in the Mediterranean cage-culture industry (Quèmèner, 2002). Growth rates of > 1 kg year^{-1} have been reported (Monfort, 2010), which is many-times more than the growth of currently cultured species such as the gilthead seabream (*Sparus aurata*) and the European sea bass (*Dicentrarchus labrax*). In addition, meagre mature at a large size > 4 kg (Schuchardt et al., 2007), which is larger than the harvest size, thus avoiding problems of reduced growth associated with maturation. Although meagre fail to undergo oocyte maturation spontaneously in captivity, recently developed hormonal induction methods have proven effective in controlling spawning (Duncan et al., 2012; Fernández-Palacios et al., 2009a; Mylonas et al., 2011) and producing eggs of sufficient quality and quantity for commercial hatchery production. Larvae and juveniles have been reared with similar facilities and methodologies to other marine fishes using rotifers, *Artemia nauplii* and inert feeds (Estévez et al., 2007; Fernández-Palacios et al., 2007, 2009b; Hernández -Cruz et al., 2007; Roo et al., 2007, 2009, 2010; Vallés and Estévez, 2009). Therefore, the introduction of meagre in commercial aquaculture in the Mediterranean region may contribute positively to the further growth of the industry. Meagre was first produced in 1997 in France, and once juveniles became available outside France, European production grew from 103 mtn in 2003 to 2377 mtn in 2010 (FAO, 2012).

To optimize larval rearing technology, in order to achieve better production results (i.e., higher survival, less variable growth, reduced cannibalism, lower skeletal deformities, better welfare and reduced cost), it is essential to study the ontogeny of the digestive system in relation to the rearing method and employed feeding protocol. The

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most critical rearing period is during the early developmental stages, especially during the transition from endogenous to exogenous feeding (Watanabe and Kiron, 1994), and during weaning and the transition from live to artificial feeds (Garcia-Ortega et al., 2003; Hamlin et al., 2000). A number of genetically programmed, species-specific morphological and functional changes are observed in the digestive system of fish at the early developmental stages until metamorphosis to juvenile (Zambonino-Infante et al., 2008). However, the ontogeny of the digestive system exhibits significant plasticity, with temperature (Kamler, 2002) and larval rearing protocol (Papadakis et al., 2009) being the most influential environmental factors affecting the appearance and development of the various structures of the digestive system.

Histological examination of the timing of ontogenetic development of the digestive tract and accessory glands is considered the most accurate method for the evaluation of proper function and, thus, nutritional status of fish larvae (Hamlin et al., 2000). For example, hepatocyte morphology and vacuole formation in the cytoplasm are related to the nutritional status of the larvae (Chen et al., 2007; Gisbert et al., 2004a; O'Connell and Paloma, 1981). Understanding the changes of the digestive system occurring during ontogeny under specific rearing conditions is essential and may provide indicators of the nutritional status of the fish during early stages (Chen et al., 2007; Gisber et al., 2008; Treviño et al., 2011). These indicators, then may aid in the identification of periods of malnutrition of the reared organism and provide information for the optimization of commercial rearing technologies and feeding protocols (Zambonino-Infante et al., 2008). Although the ontogeny of the digestive system is basically similar among teleost fishes, the existence of significant species-specific differences related to the timing of appearance and complete functionality of the various structures or organs, makes it necessary to study each potential species in order to develop optimal rearing protocols (Conceição et al., 2007; Gisber et al., 2008; Zambonino-Infante et al., 2008). Such information is not yet available for the meagre.

The aim of the present study was to describe the ontogeny of the digestive system in meagre from hatching to metamorphosis using the mesocosm rearing system, and relate changes occurring in lipid deposition in the liver with the feeding protocol during rearing of meagre using the mesocosm method. The use of the mesocosm is advantageous in fin-fish larval rearing of species with little information of their nutritional requirements (Divanach and Kentouri, 2000; Koumoundouros et al., 2004) as this rearing system is more similar to the natural conditions. It is expected that the obtained information will aid in the improvement of other more intensive commercial larval rearing protocols for this species.

2. Materials and methods

Larval rearing was performed at the facilities of the Institute of Marine Biology, Biotechnology and Aquaculture of the Hellenic Centre for Marine Research (HCMR), Iraklion, Crete, Greece. Rearing was based on the mesocosm technology (Divanach, 1985; Divanach and Kentouri, 2000; Kentouri, 1985) as described below.

2.1. Mesocosm larval rearing and sampling

One hundred thousand meagre eggs were stocked in one 40-m^3 tank. Unfiltered water was pumped initially directly from the sea (salinity of 38 psu) and then was renewed with well-water (salinity of 32 psu). Daily renewal of water increased gradually from 20% first day after hatching (dah) to 400% at the end of the rearing period of 48 d. During daytime, the tank was exposed to ambient light and during the night artificial light (600 lx) was used. Oxygen saturation ranged between 90% and 95% and temperature between 19 and 23 °C from the start to the end of the rearing period. Surface skimmers were used between 4 and 15 dah to remove the surface oil and assist in swim bladder inflation. From 2 to 27 dah, larvae were provided with daily additions of microalgae *Chlorella minutissima*. Rotifers (*Brachionus plicatilis*), enriched previously (DHA Protein Selco, INVE S.A., Belgium) for 6 h in enrichment tanks (200–250 ind ml^{-1}) with aeration, were added daily from 3 to 8 dah at a concentration in the rearing tank of 2–3 ind ml^{-1}. Enriched (A1 DHA Selco, INVE S.A., Belgium) *A. nauplii* (Artemia) also remained in an enrichment tank (280 nauplii ml^{-1}) for 6 h with high aeration, and were then offered to the larvae from 7 to 30 dah at a starting concentration of 0.05 at 7 dah, increasing to 0.35 nauplii ml^{-1} at the beginning of the weaning stage.

Artificial feeds were added progressively from 15 to 44 dah. The feed size was adjusted gradually according to fish size (R1 100 and Proton 2/3 grain size 200–300 μm, NRD 3/5 grain size 300–500 μm, NRD 5/8 grain size 500–800 μm, INVE S.A., Belgium). Naturally produced copepods – mainly of the families Harpacticoidae and Tisbidae – appeared in the tank between 18 and 23 dah at water column concentrations ranging from 0.01 to 0.08 individuals ml^{-1}. The overall copepod concentration may have been underestimated since the majority of them were attached to the tank walls. At 48 dah fish were counted and transferred to on-growing tanks.

Random samples of larvae ($n = 10$) were taken at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 13, 14, 15, 17, 19, 20, 23, 26, 28, 30, 33, 36, 40 and 44 dah (day 0 was the day of hatching). Sampling was performed 2 h after feeding. Fish were measured in total length, examined with the aid of stereoscope for external morphology and were preserved for histology in buffered 4F:1G, containing 4% formaldehyde: 1% gluteraldehyde for at least 24 h (McDowell and Trump, 1976).

2.2. Histological analysis

Before embedding in methacrylate resin (Technovit 7100®, Heraeus Kulzer, Germany) larvae were dehydrated in gradually increased ethanol solutions (70–96%). Serial sections of 3 μm were obtained with a microtome (Reichert Jung, Biocut 2035, Germany). Sections were stained with Methylene Blue (Sigma, Germany)/Azure II (Sigma, Germany)/Basic Fuchsin (Polysciences, USA) according to (Bennett et al., 1976).

In order to describe the ontogeny of the digestive system and accessory glands all the sections were examined using compound microscope. Stomach content was examined using microphotographs taken from histological sections from 4 larvae of each daily sampling, at ×20 and ×40 magnification.

The ontogeny results were presented in relation to the age of the fish in dah as well as in temperature degree-days after hatching. The latter is estimated by the summation of the mean daily temperatures during rearing and allows for the influence of temperature to be factored in the rates of growth and development processes in poikilothermic animals.

2.3. Area covered with lipids vacuoles in the liver (ACLV %)

Four additional larvae were sampled at 6, 8, 11, 13, 15, 17, 20, 23, 26, 28, 30, 33, 36, 40 and 44 dah and processed histologically for the estimation of liver lipid content according to the methodology of Papadakis et al. (2009). Prior to 6 dah it was not possible to measure ACLV, due to the very small size of the liver. For each larva, 6 microphotographs were obtained at ×100 magnification from sections obtained from different areas of the liver. A total number of 384 photographs were analyzed. Photographs were converted to gray scale in order to convert the area occupied with lipid vacuoles in white, and the total area covered with lipid vacuoles was calculated using an image analysis software (Image J, NIH, USA). Other tissues that could be confused by the software as lipid vacuoles (e.g., blood capillaries) were manually excluded from the analysis. Measurement of the lipid vacuole-covered area was performed automatically following manual delineation. The results are presented as the percentage of the total area of the hepatic tissue of the photograph (without other non-hepatic elements) covered with lipid vacuoles.