Ontogeny of the digestive tract in sharpsnout sea bream *Diplodus puntazzo* (Cetti, 1777)

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**Summary.** The ontogeny of the digestive tract was studied histologically and histochemically in sharpsnout sea bream *Diplodus puntazzo* from hatching (0 DAH, Days After Hatching) until day 57 (57 DAH). At hatching, the digestive tract appeared as a histologically undifferentiated straight tube lying dorsally to the yolk sac. When the mouth opened at 3 DAH, the digestive tract was differentiated into buccopharynx, oesophagus, incipient stomach and intestine. The pancreas, liver and gall bladder were also differentiated at this stage and both the bile and pancreatic duct had opened into the anterior intestine. Active feeding began in 50% of larvae at 4 DAH, although permanence of yolk reserves until 7 DAH suggests a period of both endogenous and exogenous feeding. Nutrient absorption was first visible from 5 DAH, as colourless supra- and infranuclear vacuoles in the anterior intestinal mucosa, suggesting a lipid content, as well as supranuclear, eosinophilic vacuoles, containing protein, in the posterior intestinal mucosa. Early caecal development could be detected from 10 DAH, whereas gastric glands appeared at 30 DAH, indicating the transition from larval to juvenile stage and the acquisition of an adult mode of digestion. Goblet cells appeared in the digestive tract of sharpsnout sea bream larvae shortly after first feeding. The mucus content of goblet cells varied with the digestive region and, in the buccal cavity and oesophagus, also with the developmental phase. This study provides knowledge for better husbandry practices in the aquaculture industry, as well as for the implementation of future nutritional studies.

**Key words:** Digestive system, *Diplodus puntazzo*, Larval development, Histology, Histochemistry

**Introduction**

The sharpsnout sea bream *Diplodus puntazzo*, Cetti 1777, represents an appreciated fishery resource occurring throughout the Mediterranean, the Black Sea and the eastern coasts of the Atlantic ocean, from the Bay of Biscay to Sierra Leone, the Canary Islands and Cape Verde. It is considered as one of the most interesting species for diversification in aquaculture due to its high market price, ease of reproduction in captivity (Faranda et al., 1985; Caggiano et al., 1993; Greco et al., 1993), high growth rate and food conversion efficiency (Hernández et al., 2001; Favaloro et al., 2002). Although the first rearing trials date back to more than 20 years (Divanach and Kentouri, 1982), massive production of this species is still limited because of hatchery problems during larval rearing, particularly at transition from endogenous to exogenous feeding and weaning to artificial diets. Such problems, some of which arise from a lack of knowledge concerning nutritional requirements and feeding sequences, result in low and unpredictable survival rates (ranging from 3 to 40%) (Marangos, 1995). Better results have recently been obtained using the mesocosm larval rearing system, which attained 54% survival (Papandroulakis et al., 2004).

A detailed understanding of the development of the digestive tract, particularly as far as ingestion, digestion and assimilation mechanisms are involved, will contribute to the improvement of larval survival and growth in different rearing conditions, through the development of feeding protocols that match the nutritional requirements and digestive ability of the larvae. In this context, a histological and histochemical description of the larval digestive system represents the first step towards the determination of the functional relationships between feeding and assimilation.

The organogenesis of the digestive system has been investigated in a number of commercially valuable
finfish species, including the halibut (Blaxter et al., 1983), European turbot (Cousin and Baudin Laurencin, 1985), cod (Kjørsvik et al., 1991), the Dover and Senegal sole (Boulhic and Gabaudan, 1992; Ribeiro et al., 1999), summer and yellowtail flounder (Bisbal and Bengston, 1995; Baglione et al., 1997), gilthead seabream (Sarasquete et al., 1995; Elbal et al., 2004), haddock (Hamlin et al., 2000), sea bass (García-Hernández et al., 2001), and red seabream (Gordon and Hecht, 2002), white seabream (Ortiz-Delgado et al., 2003), California halibut (Gisbert et al., 2004), common dentex (Santamaría et al., 2004), common pandora (Micale et al., 2006), shi drum (Zaiss et al., 2006), red porgy (Darias et al., 2007) and redbanded seabream (Sánchez-Amaya et al., 2007). These studies bear witness to a common ontogenetical process of the digestive system, even though considerable differences exist in the timing at which developmental events occur.

Different aspects of early life development of sharpsnout sea bream have been investigated in the last decade (Romano et al., 1999; Baglione et al., 2003; Sfakianakis et al., 2005; Korkut et al., 2006; Kouttouki et al., 2006; Saavedra et al., 2007; Suzer et al., 2007). However, the histological and histochemical changes that take place during gut ontogeny have never been reported. The purpose of this paper was to describe the morphogenesis of the digestive tract and its associated organs (liver, gall bladder and pancreas) in sharpsnout sea bream reared using the mesocosm technique (Papandoulakis et al., 2004), in order to provide the necessary knowledge for better husbandry practices in the aquaculture industry, as well as for the implementation of future nutritional studies.

Materials and methods

Larval rearing using the mesocosm technique

Larval rearing was carried out at the mesocosm hatchery of the Institute of Aquaculture, Hellenic Center for Marine Research (HCMR) (Crete, Greece). Two hundred thousand eggs obtained from a wild broodstock spawning naturally in tanks, were introduced into a 40 m³ semi-indoor tank initially filled with surface seawater (40 psu) and subsequently supplied with water from a shallow well (32 psu). The daily water renewal increased progressively from 20% to 400% at 42 days after hatching (DAH) and remained constant up to the end of the experimental trial (57 DAH). The tank was exposed to natural daylight, with additional light provided during the night (600 lux). During the experiment, oxygen saturation ranged between 90 and 95%. Water temperature decreased gradually from 22.3°C at the beginning of the experiment to 19.0±0.3°C at 7 DAH and remained constant thereafter. From 3 to 38 DAH, the culture was carried out in the presence of phytoplankton (Chlorella minutissima), which has been isolated from Heraklion bay since 1992 and mass produced in photo-bioreactors at HCMR (Hatzianthassios et al., 2001). The phytoplankton concentration which was added daily was 300-400x10^3 cells ml⁻¹ from 3 to 25 DAH, 200x10^3 cells ml⁻¹ from 25 to 30 DAH and 100-150x10^3 cells ml⁻¹ from 30 to 38 DAH, at which time phytoplankton use was discontinued. A surface skimmer was installed between 4 and 15 DAH to keep the surface free from oils, a prerequisite for successful swim-bladder inflation. From 4 to 30 DAH, larvae were fed on rotifers (Brachionus plicatilis) enriched with a commercial nutrient emulsion (DHA Protein Selco, INVE S.A., Belgium, containing 27% crude protein, 29% crude lipids and 5 mg/g Σω HUFAs [Highly Unsaturated Fatty Acids]), supplied twice daily to maintain a concentration of 2-3 rotifers ml⁻¹ in the tank. From 18 to 53 DAH, Artemia spp. nauplii enriched with a commercial nutrient emulsion (AI DHA Selco, INVE S.A., Belgium, containing 67% crude lipids and min. 200 mg/g Σω HUFAs) were provided continually in order to maintain a concentration of 0.05-0.35 nauplii ml⁻¹. Commercial feed crumbles of progressively larger size (R1 100; Proton 2/3, particle size 200-300 µm; NRD 3/5, particle size 300-500 µm; and NRD 5/8, particle size 500-800 µm, INVE S.A., Belgium) were provided continually from 29 DAH onwards, by means of electrical feeders. The biochemical composition of the commercial crumbles was 55-59% crude proteins, 14-16% crude lipids and 28-30 mg/g of Σω HUFAs, with a DHA/EPA (Docosahexaenoic Acid/Eicosapentaenoic Acid) ratio of 2.

Morphology, histology and histochemistry

A random sample of 30 larvae was collected daily from hatching (0 DAH) to 20 DAH, every second day from 22 to 30 DAH, and every third day from 33 to 57 DAH, and anaesthetized with clove oil (Mylonas et al., 2005). A sub-sample of 10 larvae was observed under a stereoscope equipped with a graded eyepiece for morphological and morphometrical evaluations. Total length (TL) was measured to the nearest 0.01 mm. Wet weight (WW) was measured at 20, 30, 42, 51 and 57 DAH, while mortalities were recorded after 30 DAH, when siphoning of the bottom of the tank was initiated. For the histological study, 10 larvae were fixed in Bouin’s solution, dehydrated in graded alcohols, embedded in a methacrylate resin (Technovit 7100®, Heraeus Kulzer, Germany) and cut in 3-5 µm-thick sagittal sections. The Haematoxylin/Eosin (HE) stain was used for general histological observations under a light microscope to describe the development of the digestive tract and accessory glands. The following histochemical reactions were performed on the remaining 10 larvae, either 4% formaldehyde- or Bouin’s fluid-fixed, and subsequently embedded in paraffin: Diastase/Periodic Acid Schiff (D/PAS) for glycogen (Bancroft et al., 1990); Alcian Blue 8GX pH 2.5/Periodic Acid Schiff (AB/PAS) for carboxylated (sulphated or not) and neutral glycoconjugates (Mowry, 1963); High Iron Diamine/Alcian Blue 8GX pH 2.5
Digestive tract ontogeny in D. puntazzo

(HID/AB) for sulphated and carboxylated, non-sulphated glycoconjugates (Spicer, 1965; Reid et al., 1989). Histochemical methods for general proteins (Bromophenol Blue, BB), as well as for proteins containing tryptophan (p-dimethylaminobenzaldehyde, DMAB), and proteins containing amino acids rich in disulfide and sulphhydryl linkages, such as cysteine (performic acid-Alcian Blue method, followed by eosin, PFA/AB/EO), were performed according to Pearse (1985) and Bancroft et al. (1990).

Results

Growth and morphology

The growth of sharpsnout sea bream larvae followed an exponential curve (Fig. 1). Survival was 68% at the end of the mesocosm rearing phase, and the final number

![Graph showing growth and feeding protocol](image)

**Fig. 1.** Feeding protocol and growth in size (mean ± S.D., n=10) of sharpsnout sea bream larvae from hatching (0 DAH) to 57 days (57 DAH).

**Table 1.** Major events during larval development of sharpsnout seabream Diplodus puntazzo.

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<th>Stage</th>
<th>DAH</th>
<th>TL (mm)</th>
<th>Feeding mode</th>
<th>Histo-morphological observations</th>
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DAH: Days After Hatching. Total length is expressed as mean±S.D. at the beginning of each stage. Numbers in brackets indicate the age in DAH.
of sharpsnout sea bream was 135,000 individuals. At 51 DAH, the mean (± SD) TL was 16.98±0.98, and the mean (± SD) WW was 540±135 mg.

At hatching (TL: 2.55±0.05 mm SD, standard deviation, n=10), the digestive tract appeared as a straight tube lying dorsally to the yolk sac. The anterior and posterior ends were closed. At 3 DAH (TL: 3.21±0.11 mm, n=10) the mouth was open, but no food could be identified in the digestive tract. At the same stage the eyes were pigmented and the pectoral fins were visible. At 4 DAH (TL: 3.25±0.07 mm, n=10) about 50% of the larvae were actively feeding and the yolk sac was almost completely resorbed. At the same stage the anus was open and the first chromatophores (4-6) appeared. At 8 DAH (TL: 3.74±0.20 mm, n=10) the oil drop was resorbed and the swim bladder was inflated in 100% of larvae. Notochord flexion began at 14 DAH (TL: 4.93±0.23 mm, n=10) in 30% of larvae and was completed in all fish by 18 DAH (TL: 5.85±0.25 mm, n=10). Anal and dorsal fins were completely formed by 33 DAH (TL: 8.69±1.03 mm, n=10).

Histology and histochemistry

The major ontogenetic events occurring during larval development of sharpsnout seabream are summarized in Table 1. The results of histochemical reactions showing the distribution of carbohydrates and proteins in the different gut regions throughout larval development are reported in Table 2.

At hatching the digestive tract appeared histologically undifferentiated (Fig. 2A). Within 2 days from hatching, sharpsnout seabream underwent rapid developmental changes, so that four segments could be distinguished in the digestive tract, namely the buccopharynx, oesophagus, presumptive stomach and intestine. At this stage, a conspicuous mass of strongly eosinophilic yolk globules was still visible in the resorbing yolk sac (Fig. 2B).

Buccopharynx

At mouth opening by 3 DAH, the oral cavity was lined by a simple squamous epithelium, while the pharynx was lined by a pseudostratified epithelium (Fig. 3A). The first goblet cells appeared at 5 DAH in the pharyngeal mucosa (Fig. 3B). These goblet cells were AB-positive with both the AB-PAS and HID-AB sequences, indicating the presence of carboxylated, non-sulphated glycoconjugates, but were negative to both PAS and protein reactions. The first buccopharyngeal taste buds appeared among epithelial cells at 11 DAH (Fig. 3C). Buccal goblet cells were visible from 24 DAH, especially in the ventral surface of the oral cavity (Fig. 3D), and were shown to contain carboxylated glycoconjugates (AB-positive) from 31 DAH. From 36 DAH they were HID-positive in the HID-AB sequence, showing the presence of sulphated glycoconjugates, in addition to carboxylated, non-sulphated ones (Fig. 3E). Taste buds and goblet cells became more numerous as larvae grew. Rudimentary pharyngeal teeth could be detected from 20 DAH as BB-positive (Fig. 3F) and

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<th>Table 2. Histochemical distribution of carbohydrates and protein in the digestive tract during larval development of sharpsnout seabream Diplodus puntazzo.</th>
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<tr>
<td><strong>Neutral glycoconjugates</strong></td>
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<td>Buccal cavity</td>
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<td>Goblet cells</td>
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<tr>
<td>Pharynx</td>
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<td>Goblet cells</td>
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<td>Oesophagus</td>
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<td>Epithelium</td>
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<td>Gastric glands</td>
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<td>Intestine</td>
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<td>Goblet cells</td>
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<td>Supranuclear inclusions</td>
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<td>(posterior intestine)</td>
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<td>Liver</td>
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<td>Pancreas</td>
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<td>Zymogen granules</td>
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Reaction intensity: 0, negative; 1, weak; 2, moderate; 3, strong. Timing of appearance of a positive reaction is reported in brackets as Days After Hatching [DAH].
erupted between 42 and 45 DAH, while mandibular and maxillary teeth, which were first visible at 30 DAH, had not yet erupted at 57 DAH.

**Oesophagus**

At 2 DAH the oesophagus could be distinguished as a short, narrow duct lined by a columnar, ciliated epithelium (Fig. 4A). Developing circular smooth muscle cells were visible in the oesophagus wall. At 5 DAH the inner mucosal surface of the oesophagus was expanded in several longitudinal folds. At the same stage, goblet cells appeared interspersed between epithelial cells. The oesophageal goblet cells were AB-positive from 7 DAH and AB- and HID-positive from 17 DAH (Fig. 4B), indicating the sequential appearance of carboxylated and sulphated glycoconjugates, respectively. On the other hand, these goblet cells contained no neutral glycoconjugates (PAS-negative) and were unreactive to protein reactions. The oesophageal goblet cells became more and more numerous throughout larval development, showing a strong positivity to protein reactions, particularly the PFA/AB/EO sequence for cysteine-rich proteins, from 20 DAH. A submucosa formed by loose connective tissue, a circular muscle layer (*tunica muscularis*) and a thin outer serosa completed the oesophagus wall.

**Stomach**

The presumptive stomach started to differentiate at 2 DAH as a dilated tract posterior to the oesophagus, lined by a simple cuboidal epithelium with basal nuclei (Fig. 4A). At 5-6 DAH a primordial pyloric sphincter developed, separating the future stomach from the anterior portion of the intestine. The stomach epithelium close to the sphincter, from which the pyloric portion of the stomach would develop, became columnar (Fig. 5A). At this stage, the stomach appeared elongated and its mucosal surface was expanded in mainly longitudinal folds. At 10 DAH, the intestinal mucosa immediately posterior to the pyloric sphincter showed pronounced evaginations, indicating the beginning of caecal development (Fig. 5B). By the same time, the cardiac portion of the stomach, lined by a simple cuboidal epithelium, could clearly be distinguished from the fundic and pyloric ones (Fig. 5B), both lined by a thicker columnar epithelium, which appeared PAS-positive in its...
Fig. 3. A. Detail of the squamous buccal (upper picture) and pseudostratified pharyngeal (lower picture) epithelia in a 3-day-old sharpsnout sea bream larva; HE. B. Detail of pharyngeal cavity in a 5-day-old larva showing a goblet cell (arrowhead); HE. C. Oral cavity in a 11-day-old larva, showing a taste bud (tb) on the tongue (t) surface; HE. D. Oral cavity in a 24-day-old larva, showing the presence of two goblet cells (arrowheads); HE. E. Oral cavity in a 36-day-old larva, showing HID-positive goblet cells containing acidic sulphated glycoconjugates (arrowheads); HID-AB. F. Pharyngeal epithelium in a 21-day-old larva, showing developing teeth (asterisk); BB. o: oesophagus; oc: oral cavity; ph: pharynx; arrowheads: goblet cells.
apical zone from 30 DAH. The gastric epithelium appeared devoid of goblet cells.

Gastric glands appeared at 30 DAH as strongly BB-positive mucosal acinar cell aggregates in the fundic region (Fig. 5C), exhibiting also a weak AB- and PAS-positivity. By this time, a *muscularis mucosae*, a submucosa and a muscle tunica could be clearly distinguished in the stomach, surrounded by an outer serous membrane. The musculature surrounding the pyloric sphincter was well developed. Gastric glands proliferated as larval development proceeded, but only in the fundic portion, so that a fully developed glandular stomach could be observed by 39 DAH (Fig. 5D).

**Intestine**

Prior to mouth opening (2 DAH) an anterior (prevalvular) intestine could be distinguished from a bending posterior (postvalvular) intestine, by a constriction indicating the future ileo-rectal valve (Fig. 6A). No histological difference was observed between the two segments, both of them showing a single epithelial layer composed of columnar cells with basal nuclei, with a strongly eosinophilic brush border containing neutral glycoconjugates (PAS-positive), glycogen and proteins, particularly those rich in tryptophan. Loop formation started in the anterior intestine by 3 DAH. At the same stage primordial mucosal folding began, which increased with larval growth and appeared well developed in both portions of the intestine by 28 DAH. The ileo-rectal valve appeared completely formed by 10 DAH (Fig. 5B).

Soon after the beginning of exogenous feeding (5 DAH), unstained, supra- and infranuclear vacuoles could be seen in some tracts of the anterior intestinal epithelium (Fig. 6B). Such vacuoles, which were negative to both protein and carbohydrate reactions, increased in number as the larvae grew (Fig. 6D,E). Another type of inclusion was apparent in the epithelial cells of the posterior intestine from 5 DAH, consisting of large, eosinophilic, BB-positive supranuclear vacuoles, containing protein (Fig. 6C). These vacuoles remained a constant feature of the posterior intestinal mucosa throughout larval development (Fig. 6F). Goblet cells appeared interspersed between enterocytes of both anterior and posterior intestine from 33 DAH, some of them containing neutral mucins and some acidic ones (Figs. 6D-E).

**Pancreas, liver and gall bladder**

At hatching, the accessory digestive organs were absent. At 2 DAH the incipient exocrine pancreas was visible as polyhedral, strongly basophilic cells with a round nucleus and a prominent nucleolus, lying dorsally to the yolk sac wall and ventrally to the developing gut. The endocrine pancreas could already be distinguished at this stage as an islet of irregularly shaped cells with a pale cytoplasm and a large, central nucleus containing granular chromatin, lying within the exocrine cells (Fig. 7A). By 3 DAH conspicuous eosinophilic zymogen granules appeared within the exocrine cells, at the apical portion of their cytoplasm, and the main pancreatic duct was visible, opening into the anterior intestine (Fig. 7E). At this precocious stage the exocrine pancreas was proportionally very large in comparison to the last stages of larval growth. The zymogen granules were positive to protein reactions (BB and DMAB) from 7 DAH, while

![Fig. 4. A. View of the anterior portion of the digestive tract in a 2-day-old sharpsnout sea bream larva, showing the oesophagus (o), lined by a columnar, ciliated epithelium, and the dilated, presumptive stomach (s), lined by a simple, cuboidal epithelium. Developing smooth muscle cells (arrow) of the oesophageal wall are visible; HE. B. Pharynx (ph) and oesophagus (o) in a 23-day-old larva, showing HID-positive goblet cells, containing acidic sulphated glycoconjugates, particularly abundant in the oesophagus; HID-AB. ys: yolk sac.](image)
Fig. 5. A. View of the digestive tract in a 6-day-old sharpsnout sea bream larva, showing the pyloric sphincter (ps), separating the stomach (s) from the anterior intestine (ai). Note how the cuboidal gastric epithelium becomes columnar in proximity of the sphincter; HE. B. General view of the digestive system in a 10-day-old larva. A developing caecal ridge (asterisk) is visible just after the pyloric sphincter (ps). The fundic portion of the stomach (f), lined by a columnar epithelium, may be distinguished from the cardiac portion (c), lined by a cuboidal epithelium; HE. C. Fundic portion of the stomach of a 30-day-old larva, showing two gastric glands (gg); HE. D. Fully developed glandular stomach in a 39-day-old larva, showing numerous gastric glands (gg); HE. ai: anterior intestine; f: fundic stomach; l: liver; o: oesophagus; p: pancreas; pi: posterior intestine; ps: pyloric sphincter; sb: swim bladder; v: ileo-rectal valve.
they were negative to AB-PAS.

The immature liver could be distinguished at 2 DAH as a chord-like structure, close to the pancreas, consisting of swollen, conspicuously vacuolized cells, with the nuclei pushed towards the periphery (Fig. 7B). At the beginning of exogenous feeding by 4 DAH, the
Fig. 7. A. Differentiation of the pancreas in a 2-day-old sharpsnout sea bream larva. The endocrine cells, with a pale cytoplasm and granular nuclear chromatin, are surrounded by the strongly basophilic exocrine cells, with a prominent nucleolus; HE. B. Immature liver (l) in a 2-day-old larva. The hepatocytes appear swollen, because of marked vacuolization; HE. C. Mature liver in a 4-day-old larva, showing moderately vacuolized hepatocytes; HE. D. Granulation and vacuolization of hepatic parenchyma in a 13-day-old larva; HE. E. Opening of the main pancreatic duct (white asterisk) and of the bile duct (black asterisk) into the anterior intestine (ai) in a 3-day-old larva; HE. F. Detail of the gall bladder in a 33-day-old larva, showing the thickened epithelium formed by cuboidal and cylindrical cells; HE. gb: gall bladder; i: intestine; l: liver; p: pancreas; ys: yolk sac.
mature hepatocytes appeared as polyhedral cells, tightly packed between sinusoids, often around a central vein, with a prominent, round nucleus and a granular, slightly vacuolar cytoplasm (Fig. 7C). The granules were shown to contain glycogen, as well as abundant protein, especially those rich in tryptophan, whereas the vacuoles appeared empty. Granulation and vacuolization of the cytoplasm increased as exogenous feeding proceeded (Fig. 7D).

The gall bladder, lined by a simple squamous epithelium, was visible between the pancreas and liver from 2 DAH (Fig. 2). At 3 DAH, the bile duct was already formed and opened into the anterior intestine (Fig. 7E). At 33 DAH the epithelium of the gall bladder appeared thickened, with cuboidal and cylindrical cells visible in the same section (Fig. 7F). A basal lamina separated the epithelium from the underlying layer of connective tissue, containing scattered smooth muscle cells.

**Discussion**

Larval development in sharpsnout seabream can be divided into three stages, based upon its feeding mode: an endotrophic stage from hatching to mouth opening (0-3 DAH), an endo-exotrophic stage (4-7 DAH) lasting until complete exhaustion of yolk reserves and a strictly exotrophic stage from 8 DAH onwards. Similarly to most marine fish species, sharpsnout seabream larvae possessed an undifferentiated digestive tract at hatching. Major developmental events took place between day 2 and 10, starting with regional gut differentiation into four histologically distinct segments (i.e. buccopharynx, oesophagus, incipient stomach and intestine), which occurred before mouth opening.

At mouth opening (3 DAH) the gall bladder, liver and pancreas were already differentiated and connected to the intestine by the bile and main pancreatic duct, respectively. A marked, colourless vacuolization of hepatocytes was visible before first feeding, suggesting accumulation of lipid from yolk and the oil globule by the primordial liver. Folding of intestinal mucosa, which would ensure more efficient digestion and absorption processes, was also apparent at mouth opening in *D. puntazzo*, as has also been reported in *D. sargus* (Ortiz-Delgado et al., 2003) and *P. auriga* (Sánchez-Amaya et al., 2007), and considerably earlier than in other Sparids studied so far (Santamaría et al., 2004; Micale et al., 2006; Darias et al., 2007). During this first phase, sharpsnout seabream larvae relied entirely on yolk reserves for their nutrition and showed a PAS-positive brush border of the intestinal epithelium, which could be related to the absorption of easily digested substances, such as disaccharides and short-chain fatty acids, similarly to what has been demonstrated in the gastric mucosa of the tilapia *Tilapia nilotica* (Osman and Cacetti, 1991). The oesophageal epithelium of *D. puntazzo* exhibited ciliated cells during this stage, which have also been described in the oesophagus of *D. dentex* (Santamaría et al., 2004) before starting exogenous feeding, and could be related to water transport and osmoregulation. Histological and histochemical observations suggest that the development of the digestive tract, involving the presence of functional liver, pancreas and gall bladder, enabled early sharpsnout seabream larvae to ingest, digest and assimilate the first exogenous food even before endogenous reserves were completely resorbed.

Twenty-four hours after mouth opening, rotifers could be detected in the digestive tract of 50% of the larvae, although yolk reserves were completely exhausted at 8 DAH, indicating a brief period of combined endogenous and exogenous feeding. In a previous study with sharpsnout sea bream (Faranda et al., 1985), yolk consumption occurred faster than in the present study. The different rearing protocols between the two studies may account for this discrepancy: in fact, the mesocosm rearing technique, employed in the present study, is known to provide more natural conditions compared to intensive rearing techniques, as well as a more stable prey population, resulting in better feeding and, hence, slower yolk consumption (Zaiss et al., 2006). The absence of a transitional starvation period between endogenous and exogenous feeding, such as that reported in the yellowfin porgy *Acanthopagrus latus* (Leu and Chou, 1996) and gilthead sea bream *Sparus aurata* (Sarasquete et al., 1995), which may be caused by a too low frequency distribution of prey or their inadequate size (Parra and Yúfera, 2000), is indicative of an adequate feeding protocol employed in the present study during early larval stages. Glycogen and protein accumulation in the liver could be demonstrated as soon as exogenous feeding began, as PAS- and BB-positive granules, respectively. On the other hand, the large, unstained vacuoles of probable lipid origin, were reduced at the beginning of the endo-exotrophic stage. It could be suggested that lipid from yolk reserves accumulated by the liver before mouth opening was almost exhausted at transition from endogenous to exogenous feeding, as has been reported in other species (Diaz et al., 2002).

Soon after the beginning of exogenous feeding (5 DAH), intestinal absorption of nutrients was apparent as supra- and infranuclear unstained vacuoles in the enterocytes of anterior intestine, as well as supranuclear BB-positive vacuoles in the enterocytes of posterior intestine. The former suggested lipid absorption, as has been reported in other fish larvae (Depiano et al., 1991; Bisbal and Bengston, 1995; Diaz et al., 1997; Ortiz-Delgado et al., 2003; Zaiss et al., 2006; Sánchez-Amaya et al., 2007). On the other hand, protein absorption vacuoles in the posterior intestine, which have been described in both larval and adult gastric and agastic fish (Krementz and Chapman, 1975; Albertini-Berhaut, 1987; Micale et al., 2005, 2006), are involved in the pinocytic uptake of proteins, which are subsequently digested intracellularly (Sire and Vernier, 1992). In some teleosts, they are present only until formation of the
gastric glands, suggesting a transient mechanism for protein digestion, related to the immaturity of the digestive tract (Tanaka, 1972; Luizi et al., 1999; Ortiz-Delgado et al., 2003; Elbal et al., 2004; Zaiss et al., 2006). Since no reduction in the number of protein vacuoles was apparent after a glandular stomach was formed in sharpsnout sea bass, similarly to what has been reported in the European sea bass (García Hernández et al., 2001), common pandora (Micale et al., 2006) and red porgy (Darias et al., 2007), it can be suggested that a combined intra- and extracellular protein digestion mechanism is active in sharpsnout sea bass.

The formation of the pyloric caeca has been considered in many species as the last major event during ontogeny of the digestive system in fish larvae, indicating the transition from a larval to a juvenile stage (Bisbal and Bengston, 1995; Hamlin et al., 2000; Chen et al., 2006; Micale et al., 2006). On the contrary, caecal development started precociously in sharpsnout sea bass (10 DAH), well before the formation of the gastric glands, as in the European sea bass (García Hernández et al., 2001). In the absence of functional gastric glands, the precocious development of pyloric caeca, in which several enzymatic activities have been demonstrated (Cousin et al., 1987), may enhance the digestive capability of larvae. Moreover, as the pyloric caeca have the same histological structure as the intestine, their early development increases the total available absorption area (Houssain and Dutta, 1998), ensuring a more efficient utilization of feed.

The formation of pepsin-producing gastric glands during larval development in fish is crucial for extracellular luminal digestion of complex protein. In most examined gastric species, a glandular stomach is fully developed several weeks after the start of exogenous feeding (Stroband and Kroon, 1981; Segner et al., 1994; Ribeiro et al., 1999). In sharpsnout sea bass, the first gastric glands were formed at the completion of the first month of life, similarly to what was observed in common pandora Pagellus erythrinus (Micale et al., 2006), and a fully developed glandular stomach could be observed by 39 DAH. This is in agreement with the sharp increase in pepsin-specific activity observed between 32 and 40 DAH in a recent study on digestive enzymes of larval sharpsnout sea bass (Şüzer et al., 2007). Gastric glands were detected more precociously in other Sparids, such as Diplodus sargus (Ortiz-Delgado et al., 2003), Dentex dentex (Santamaria et al., 2004), Pagrus pagrus (Darias et al., 2007) and Pagoauriga (Sánchez-Amaya et al., 2007), but considerably later in the gilthead sea bass Sparus aurata (Elbal et al., 2004). The location of gastric glands is species-specific and has been related to feeding habits (Ortiz-Delgado et al., 2003). In D. puntazzo, gastric glands occurred only in the fundic region of the stomach, as was also shown in the yellowtail flounder Pleuronectes ferruginea (Baglione et al., 1997), the summer flounder Paralichthys dentatus (Bisbal and Bengston, 1995), the turbot Scophthalmus maximus (Segner et al., 1994) and the Dover sole Solea solea (Veggetti et al., 1999). On the other hand, gastric glands were only formed in the cardiac region in D. sargus (Ortiz-Delgado et al., 2003), shi drum Umbrina cirrosa (Zaiss et al., 2006) and red porgy P. pagrus (Darias et al., 2007), whereas an entirely glandular stomach occurs in P. erythrinus (Micale et al., 2006). Neutral mucosubstances could be detected by the PAS reaction in the gastric epithelial cells at the same time as the gastric glands and could indicate the involvement of the former in mucosa protection from auto-digestion processes caused by hydrochloric acid and the enzymes secreted by the gastric glands, as in the milkfish Chanos chanos (Ferraris et al., 1987) and the gilthead sea bass (Elbal et al., 2004). On the other hand, neutral glycoconjugates have also been reported in the stomach epithelium of adult Mediterranean amberjack Seriola dumerillii (Grau et al., 1992) and larval white seabream Diplodus sargus (Ortiz-Delgado et al., 2003), and this fact has been related to the absorption of easily digestible substances, such as disaccharides and short-chain fatty acids. Neither proteins rich in tryptophan, which have been related to pepsinogen synthesis in the amberjack S. dumerillii (Grau et al., 1992), nor proteins rich in cysteine, were found in the gastric glands of D. puntazzo, whereas they do occur in the congener species D. sargus (Ortiz-Delgado et al., 2003). Similarly, cysteine- and tryptophan-rich proteins have been reported in the gastric glands of P. pagrus (Darias et al., 2007), but not in those of P. auriga (Sánchez-Amaya et al., 2007). This would suggest that the presence of these aminoacid residues in the gastric glands of fish is species-specific.

The first goblet cells in the digestive tract of sharpsnout sea bass were visible in the pharynx and oesophagus shortly after the first exogenous feeding, the buccal goblet cells appearing thereafter, while goblet cells in the intestine appeared finally at 33 DAH. Such a late appearance of intestinal goblet cells has been reported only in common pandora (Micale et al., 2006) and red porgy (Darias et al., 2007). The time at which mucus secreting cells are formed varies among species, although their sequence of appearance along the different segments of digestive tract (i.e. firstly, oesophagus, secondly, buccal cavity, and finally, intestine) is common to many examined species (García-Hernández et al., 2001; Ortiz-Delgado et al., 2003; Santamaria et al., 2004; Sánchez-Amaya et al., 2007). Glycoproteins produced by goblet cells may play an important lubricant role for the buccopharyngeal and oesophageal mucosa, which lack salivary glands. Mucosubstances may also play a role in protecting the digestive mucosa from bacterial and viral attacks (Gisbert et al., 2004), as well as in pre-gastric digestion (Baglione et al., 1997). The mucous content of goblet cells in D. puntazzo varied with the digestive region and, in some cases, with the developmental phase. Carboxylated glycoconjugates were detected in the

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goblet cells from all segments of the digestive tract, whereas neutral glycoconjugates were found only in the intestine, where their presence could be related to absorption processes. On the other hand, the presence of sulphated glycoconjugates in the buccal and oesophageal goblet cells could be related to an enhancement of mucus viscosity, possibly supporting a particle trapping function, as has been reported in the carp, *Cyprinus carpio* (Sibbing and Uribe, 1984). Sulphated glycoconjugates could be detected only after the appearance of the carboxylated ones, suggesting a progressive development of the mucus content, as reported in human salivary glands (Harrison et al., 1987). Oesophageal goblet cells contained proteins as well, especially those containing cysteine, similarly to what has been reported in *D. sargus* and *P. pagrus* (Ortiz-Delgado et al., 2003; Darias et al., 2007).

Taste buds appeared in the bucco-pharynx by 11 DAH, suggesting that sharpsnout seabream larvae are able to select the prey on an organoleptic basis well before weaning. This information does not agree with what has been reported in a previous study (Boglione et al., 2003) using scanning electron microscopy. The different methodology might account for this discrepancy, since observation of histological structures by light microscopy may reveal some details that may be missed by surface examination, such as allowed by SEM.

The histological and histochemical description of digestive system ontogeny in sharpsnout seabream reported in the present study complements previous information on larval digestive enzymes in the same species (Suzer et al., 2007), and represents a first step towards the determination of the functional relationships between feeding and assimilation in this marine species of great interest to the Mediterranean aquaculture industry. The absence of a completely functional stomach during the first month of life, during which dramatic developmental changes take place, which are crucial for rearing success, would be considered problematic in routinely intensive rearing techniques. The appropriateness of the mesocosm rearing system for sharpsnout sea bream larvae is witnessed by the high survival rate and body weight of the produced fry. In fact, in another study where larvae were reared with the green water technique, and the feeding regime also included rotifers, *A. salina* nauplii and enriched metanauplii, followed by artificial feed, the survival ranged between 18% and 22%, and the body weight at 60 DAH ranged between 0.15 and 0.18 g (Marangos, 1995), which is significantly less than in the present study. Also, in another study using the mesocosm rearing technique, albeit with an *Amphiodinium* spp. infection at 34 DAH, the final survival rate of sharpsnout sea bream at 50 DAH was 54%. TL was 19.6±0.9 mm and body weight was 107±32 mg (Papandroulakis et al., 2004). Therefore, the use of mesocosm rearing technology may be the key for the success of sharpsnout sea bream larval rearing in the present study. This is due to the relatively long-term self-sufficiency of live-food items in the mesocosm culture system, both from endogenous and exogenous origin (Papandroulakis et al., 2004). The variety of digestible food items provided by this rearing system is likely to have allowed sharpsnout sea bream larvae, possessing an immature and incomplete digestive system, to fulfil their dietary requirements.

**References**


embryonic development in three teleost species: *Dicentrarchus labrax*, *Sparus aurata* and *Stizostedion lucioperca*. Aquat. Living Resour. 15, 169-176.


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