Comparative Ontogeny of the Digestive Tract in Sharpsnout Sea Bream *Diplodus puntazzo* Cetti and Common Pandora *Pagellus erythrinus* L.

Valeria Micale[*,1] and Ugo Muglia[2]

[1]*National Research Council, Institute for Marine Coastal Environment (CNR-IAMC), Messina, Italy*

[2]*Department of Morphology, Biochemistry, Physiology and Animal Production, Faculty of Veterinary Medicine, University of Messina, Italy*

Abstract: The ontogeny of the alimentary tract and its associated structures (liver, pancreas, gall bladder) was compared between sharpsnout sea bream *Diplodus puntazzo* and *Pagellus erythrinus* and some differences were highlighted. The first goblet cells appeared earlier in sharpsnout seabream (5-7 DAH) and later in common pandora (9-11 DAH), as well as taste buds which appeared at 11 DAH in sharpsnout seabream and at 17 DAH in common pandora. On the contrary, stomach segmentation in a cardiac, fundic and pyloric portions was more precocious in common pandora than in sharpsnout seabream, although gastric glands were formed almost simultaneously. Early caecal development was much more precocious in sharpsnout seabream (10 DAH) than in common pandora (22 DAH). At 3 DAH in sharpsnout seabream and later (10 DAH) in common pandora, primordial mucosal folding of intestine began, which appeared well developed in the anterior portion, by 22 DAH in common pandora and by 28 DAH in sharpsnout seabream. The functional meanings of these aspects were discussed comparatively.

Keywords: Digestive system, *Pagellus erythrinus*, *Diplodus puntazzo*, ontogeny.

INTRODUCTION

The organogenesis of the digestive system has been investigated in a number of commercially valuable finfish species, including the halibut *Hippoglossus hippoglossus* L. [1], European turbot *Scophthalmus maximus* L. [2], cod *Gadus morhua* L. [3], the Dover and Senegal soles *Solea solea* L. and *Solea senegalensis* Kaup [4, 5], gilthead seabream *Sparus aurata* L. [6, 7], haddock *Melanogrammus aeglefinnus* L. [8], clownfish *Amphiprion percula* [9], white seabream *Diplodus sargus* [10] and California halibut *Paralichthys californicus* [11]. The sharpsnout sea bream *Diplodus puntazzo* (Cetti 1777) and the common pandora *Pagellus erythrinus* (L. 1758) are important fishery resources, occurring throughout the Mediterranean basin [12]. In addition, both species are considered as promising candidates for diversification in aquaculture due to their high market price, the possibility of reproduction in captivity, high growth rate and food conversion efficiency [13-23]. Massive production of these species is still limited because of hatchery problems with high mortalities occurring during larval rearing [24] when intensive larviculture techniques are used, particularly at transition from endogenous to exogenous feeding and weaning to artificial diets. Such problems, mainly arising from a lack of knowledge concerning nutriational requirements and feeding sequences, often result in low and unpredictable survival rates (ranging from 3 to 40%) [25]. Larval performances improve under mesocosm rearing, resulting in better larval quality and survival. The mesocosm rearing technique, which attained 54% of larval survival [26], is known to provide more natural conditions compared

to intensive rearing techniques, as well as a more stable prey population, resulting in a better feeding and, hence, slower yolk consumption [27].

Recently, details of the development of the digestive tract of the common pandora and the sharpsnout sea bream have been reported [28, 29]. These data contributed to clarify the process of assimilation through the development of feeding protocols matching the nutritional requirements and digestive ability of larvae, therefore leading to the improvement of larval survival in different rearing conditions. In consideration of the same environmental characteristics, the purpose of present paper is to compare the morphogenetic and histochemical data, reported from the above cited studies, to better elucidate the similarity of these two species.

FEEDINGS PROTOCOLS

The absence of a transitional starvation period between endogenous and exogenous feeding, in the species considered, such as that reported in the yellowfin porgy [30] and gilthead sea bream [6], which may be caused by a too low frequency distribution of prey or their inadequate size [31], is indicative of an adequate feeding protocol during early larval stages.

Pagellus erythrinus

Larvae of *P. erythrinus* were obtained by natural spawning from a broodstock adapted to captivity at the COISPA experimental aquaculture station (Bari, Italy). Floating eggs were incubated at 18.5-20°C, with moderate aeration in 1.5 m^3 tanks (density: 140×l^{-1}). The salinity of the inlet water was 34 and the pH 7.7. During larval rearing (initial density: 110 larvae×l^{-1}), the water turnover was 1.5 volumes×day^{-1}, the temperature was the same as during incubation and

*Address correspondence to this author at the Consiglio Nazionale delle Ricerche, Istituto per l'Ambiente Marino Costiero, Spianata S. Raineri 86, 98122 Messina, Italy; Tel: +39090669003; Fax: +39090669007; E-mail: valeria.micale@iamc.cnr.it

natural photoperiod (July-August in the Central Mediterranean) was applied. From the hatching day till the *Artemia* feeding stage, cultured microalgae *Isochrysis galbana* and *Tetraselmis suecica* were supplied as water conditioner to the rearing units, according to the pseudo-green method [32]. Starting from the 3rd day after hatching (DAH), rotifers (*Brachionus plicatilis*; small strain: 129 μm lorica length) enriched with the commercial emulsion DHA SELCO twice every 6 hours were also added. Average concentration of rotifers in the rearing tanks was 10 individuals ml^{-1}. The supply of *Artemia* nauplii enriched with DHA SELCO started on 28 DAH. Formulated feed was introduced in the diet from 35 DAH onwards. Any change of food was applied gradually with an overlapping phase. The use of microalgae seem to be essential during the first stage of common pandora larval rearing [24] as well as of other marine larvae (e.g. *S. aurata*, *P. pagrus*). Indeed, the positive effect of microalgae was highlighted by previous studies [e.g.33], suggesting that phytoplankton may act as a diet component and/or stimulating factor of digestive enzyme synthesis. Survival at the end of metamorphosis was 6%.

Diplodus puntazzo

Larval rearing using the mesocosm technique was carried out at the facilities of the Institute of Aquaculture, Hellenic Center for Marine Research in Crete, Greece. Eggs were obtained in October 2005 from natural spawning and incubated, at an initial density of 2.5 eggs per litre, in a 40 m^3 semi-indoor tank initially filled with surface sea-water (salinity: 40) and subsequently supplied with water from a shallow well (salinity: 32). The daily water renewal increased progressively from 5% to 200% at 35 DAH. Aeration was provided by wooden diffusers, guaranteeing a 90-95% oxygen saturation. Water temperature started from 21.5 °C and decreased to 19.0 ± 0.5 °C since 7 DAH. From 3 to 38 DAH, microalgae (*Chlorella minutissima*) were added twice daily (250-400 x 10^3 cells ml^{-1}). From 4 to 30 DAH, larvae

were fed on rotifers enriched with a commercial emulsion (DHA Protein Selco, INVE S.A., Belgium), supplied twice daily (3-4 rotifers ml^{-1}). From 18 to 53 DAH, *Artemia* spp. nauplii enriched with a commercial emulsion (A1 DAH Selco, INVE S.A., Belgium) were provided continuously to a concentration of 0.2 nauplii ml^{-1}. Formulated feed of progressively larger size was provided continuously from 29 DAH onwards, by means of electrical feeders.

COMPARATIVE EXTERNAL MORPHOLOGY

Growth of common pandora and sharpsnout sea bream larvae followed an exponential curve (Fig. **1**).

At hatching (TL, total length: 2.55 ± 0.12 mm, *n* = 40 and 2.55 ± 0.05 mm, *n* = 10 for common pandora and sharpsnout seabream, respectively), the alimentary canal appeared as a straight tube lying dorsally to the yolk sac. The anterior and posterior ends were closed. The anus opened at 2 DAH (TL: 3.05 ± 0.15 mm, *n* = 40) and 4 DAH (TL: 3.25 ± 0.07 mm, *n* = 10), respectively. At 3 DAH (TL: 3.18 ± 0.10 mm, *n* = 40) the mouth opened in common pandora and exogenous feeding began (few rotifer mastaxes as well as microalgae could be identified); on the other hand, in sharpsnout seabream the mouth was open at 3 DAH (TL: 3.21 ± 0.11 mm, *n* = 10), but no food could be identified in the digestive tract until 4 DAH, when about 50% of the larvae were actively feeding. In both species, the yolk sac was almost completely reabsorbed and the eyes were pigmented by 4 DAH (TL: 3.20 ± 0.11 mm, *n* = 40; TL: 3.25 ± 0.07 mm, n = 10, for common pandora and sharpsnout seabream respectively), while the oil droplet was reabsorbed at 6 DAH (TL: 3.34 ± 0.12 mm, *n* = 40) and 8 DAH (TL: 3.74 ± 0.20 mm, *n* = 10), respectively. The swim bladder was inflated at 8 DAH (TL: 3.74 ± 0.20 mm, *n* = 10) in 100% sharpsnout seabream larvae, while it started to be inflated at 9 DAH in common pandora.
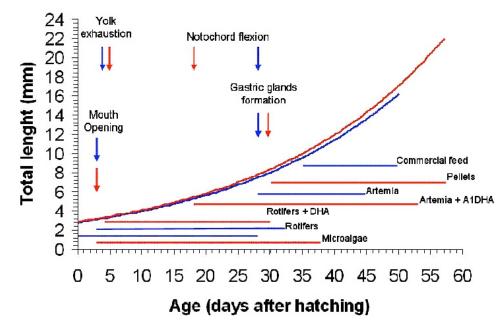

Fig. (1). Feeding protocol and growth in size of sharpsnout sea bream larvae from hatching to 57 days and common pandora larvae from hatching to 50 days. The main events of larval development schedule are also shown. Red: sharpsnout sea bream. Blue: common pandora.

COMPARATIVE DEVELOPMENT OF DIGESTIVE TRACT

The major ontogenetic events occurring during larval development of sharpsnout seabream and common pandora are summarized in Table **1**.

At hatching the alimentary canal appeared as a histo-logically undifferentiated straight tube lying dorsally to the yolk sac. Rapid developmental changes lead to the different-tiation of the digestive tract into four segments (i.e., bucco-pharinx, oesophagus, presumptive stomach and intestine) within 3 DAH in common pandora and 2 DAH in sharpsnout seabream. At this stage, strongly eosinophilic yolk globules were still visible in the resorbing yolk sac.

Buccopharynx

At mouth opening by 3 DAH in both species, the oral cavity was lined by a simple squamous epithelium, while the pharynx was lined by a pseudostratified epithelium. The first goblet cells appeared, in the pharyngeal mucosa, at 5-7 DAH in sharpsnout seabream and at 9-11 DAH in common pandora, more precociously than in mouth, at 24 and 17

DAH respectively. Taste buds appeared at 11 DAH in sharpsnout seabream, earlier than in common pandora (17 DAH). Taste buds and goblet cells became more numerous as larvae grew. Mandibular and maxillar teeth erupt respectively at 57 DAH in sharpsnout seabream and 30 DAH in common pandora while pharyngeal teethed erupt respectively at 42- 45 DAH and 36 DAH.

Oesophagus

In both species, at mouth opening (3 DAH) the bucco-pharynx, communicated with the anterior intestine through a short oesophagus with a rather narrow lumen. Two different regions could be distinguished in the oesophagus, based upon the histological features of their mucosa: an anterior region lined by a pseudostratified, (columnar in sharpsnout seabream) ciliated, epithelium, and a dilated posterior region lined by a simple, cuboidal epithelium, from which the future stomach will originate. Circular smooth muscle fibers were visible in the oesophagus wall. At 5 DAH in sharpsnout seabream goblet cells appeared scattered within the mucosal surface of the anterior oesophagus, which was expanded in several longitudinal folds. On the other hand, both goblet

Table 1.   Major Events During Larval Development of Sharpsnout Sea Bream and Common Pandora.
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

Stage	DAH	TL (mm)	Feeding Mode	Histo-Morphological Observations
Endotrophic	0-3 0-2	2.55±0.05 2.55±0.12	Endogenous	Segmentation of gut into buccopharynx, oesophagus, stomach anlage, anterior and posterior intestine [2] (3). Differentiation of liver [2] (2-3), gall bladder [2] (3), exocrine and endocrine pancreas [2] (3). Mouth opening [3] (3). Intestinal looping [3] (4) and early mucosal folding [3] (10). Opening of biliary and pancreatic ducts into anterior intestine [3] (4). Eyes pigmented [3] (4).
Endo-exotrophic	4-7 3-5	3.25±0.07 3.18±0.10	Endogenous and exogenous	Anus opening [4] (2). Protein and glycogen in the liver [4] PAS positive granules and colourness PAS negative vacuoles (11). Yolk sac resorption [4] (4) Protein [5] (2) and probable lipid [5] (2) vacuolization in posterior and anterior intestine, respectively. Goblet cells in pharynx and oesofagus [5] (11). Mucosal folding of the oesophagus [5] (20).
Exotrophic	8-57 6-50	3.74±0.20 3.34±0.12	Exogenous	Oil drop resorption [8] (6). Inflation of swim bladder [8] (9). Ileo-rectal valve fully developed [10] (6). Early caecal development [10] (22). Bucco-pharyngeal taste buds [11] (17). Notochord flexion [18] (28). Goblet cells in the buccal cavity [24] (17). Stomach segmentation [10] (16). Formation of gastric glands [30] (28). Anal and dorsal fins fully developed [33] (30). Goblet cells in the intestine [33] (33). Eruption of mandibular and maxillar teeth [57] (30). Eruption of pharyngeal teeth [42-45] (36).

Red: sharpsnout sea bream; Blue: common pandora.

cells and mucosal folding appeared later in pandora (at 11 and 20 DAH, respectively). A submucosa formed by loose connective tissue, a circular muscle layer (*tunica muscularis*) and a thin outer serosa completed the oesophagus wall in both species.

Stomach

The presumptive stomach, lined by a simple cuboidal epithelium with basal nuclei, started to differentiate as a dilatation of the posterior oesophagus in sharpsnout seabream at 2 DAH and in common pandora at 3 DAH. At 6 DAH (5-6 DAH in sharpsnout seabream) a primordial pyloric sphincter developed, separating the future stomach from the anterior portion of intestine.

In common pandora at 16 DAH the stomach exhibited a pouched shape and its epithelium began to differentiate, so that cardiac, fundic and pyloric portions could be distinguished, the former lined by a simple cuboidal epithelium, the latter two by a thicker columnar epithelium. An apical brush border of microvilli lined the gastric epithelium, which was devoid of goblet cells. At this stage epithelial folds separating the cardiac from the fundic portion of the stomach began to appear. Gastric glands appeared at 28 DAH, as submucosal acinar cell aggregates, concentrated mainly in the fundic region. Simultaneously, the intestinal mucosa immediately posterior to the pyloric sphincter showed pronounced evaginations, indicating the beginning of caecal development. 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 sphyncter was well developed. The number of gastric glands increased significantly as larval development proceeded, so that they appeared largely diffused throughout the stomach by 41 DAH (39 DAH in sharpsnout seabream).

In sharpsnout seabream, at 5-6 DAH, the stomach epithelium close to the sphincter, from which the pyloric portion of the stomach will develop, became columnar. 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. 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, both lined by a thicker columnar epithelium. The gastric epithelium appeared devoid of goblet cells. Gastric glands appeared at 30 DAH. 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, in the fundic portion of the stomach, as in common pandora.

Intestine

Prior to mouth opening (2 DAH) in sharpsnout seabream and later, at the time of mouth opening (3 DAH) in common pandora, the future ileo-rectal valve began to develop and clearly separated the anterior (prevalvular) intestine from the bending posterior (postvalvular) intestine. The ileo-rectal valve appeared completely formed in common pandora at 6 DAH, more precociously than in sharpsnout seabream (10 DAH). No histological difference was observed between the two intestinal segments, both of them showing a single epithelial layer composed of columnar cells with basal nuclei. With the beginning of exogenous feeding (3 DAH), in common pandora and in sharpsnout seabream, the lumen of the anterior intestine became dilated and loop formation (3-4 DAH) started in the anterior intestine. At 3 DAH in sharpsnout seabream and later (10 DAH), in common pandora, primordial mucosal folding began, which appeared well developed in the anterior intestine, in common pandora by 22 DAH and by 28 DAH in sharpsnout seabream. Goblet cells appeared interspersed between enterocytes of both anterior and posterior intestine from 33 DAH, more numerous in sharpsnout seabream than in common pandora.

In both the species, at 48 DAH mucosal folding of the anterior intestine appeared very prominent, whereas the posterior intestinal mucosa showed few, short folds.

Liver, Pancreas and Gall Bladder

At hatching, the accessory digestive organs were absent. At 2 DAH, the rudimentary liver and pancreas appeared in both species as a cord-like structure, lying between the intestine and the yolk sac wall close to the pancreas. The liver was constituted by irregularly shaped, basophilic cells with a round nucleus and a prominent nucleolus in common pandora, while it consisted of swollen, conspicuously vacuolized cells, with the nuclei pushed towards the periphery in sharpsnout seabream. Liver vacuolization was greatly reduced at the beginning of exogenous feeding by 4 DAH, and the mature hepatocytes appeared, as in common pandora (5 DAH), as polyhedral cells, tightly packed between sinusoids, often around a central vein, with a prominent, round nucleus and a granular, slightly vacuolar cytoplasm. From 11 DAH, in common pandora, cytoplasmic vacuoles started to appear within hepatocytes and they progressively increased with larval growth. In both species granulation and vacuolization of the cytoplasm due to synthesis and storage of nutrients increased as exogenous feeding proceeded.

At 3 DAH (2 DAH in sharpsnout seabream) the incipient pancreas could be detected lying dorsally to the yolk sac wall and ventrally to the developing gut. It was formed by exocrine polyhedral cells with round basal nuclei and a prominent nucleolus, containing dense eosinophilic zymogen granules at the apical portion of cytoplasm towards the lumen. At 2 DAH in sharpsnout seabream but later (4 DAH) in common pandora the endocrine pancreas could already be distinguished as islets of irregularly shaped cells with a pale cytoplasm and a large, central nucleus containing granular chromatin, lying within the exocrine cells. At these precocious stage, for both species, the exocrine pancreas was proportionally very large in comparison with the last stages of larval growth. By 3 DAH in sharpsnout seabream 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. Later, in common pandora, at 4 DAH pancreatic ducts were formed and the opening of the main

pancreatic duct into the anterior intestine, just after the pyloric sphyncter, could be clearly seen.

The gall bladder was visible from 2 DAH in sharpsnout seabream and from 3 DAH in common pandora, lying between the exocrine pancreas, liver and yolk sac wall. It was lined by a squamous epithelium, which appeared flattened and thin when the bladder was distended. On the contrary the opening of the bile duct into the anterior intestine, just after the pyloric sphincter, was visible at 3 DAH in sharpsnout seabream and at 4 DAH in common pandora. At 33 DAH in sharpsnout seabream and later at 36 DAH in common pandora, the epithelium of the gall bladder appeared thickened, with cuboidal and cylindrical cells visible in the same section. A basal lamina of connective tissue containing scattered smooth muscle cells underlay the epithelium.

COMPARATIVE MORPHO-FUNCTIONAL FEATURES

Similarly to most marine fish, in the two considered species, larval development can be divided into three stages, based upon feeding mode: an endotrophic stage (endogenous feeding) from hatching to mouth opening; an endo-exotrophic stage (mixed endogenous/exogenous feeding – first feeding) lasting until complete exhaustion of yolk reserves and a strictly exotrophic stage (exclusive exogenous feeding).

The differentiation of the digestive tract into four morphologically distinct regions namely the buccopharynx, oesophagus, presumptive stomach, lined by a simple cuboidal epithelium common to the two species considered, and intestine was established, within 3 DAH, coinciding with time of first feeding in common pandora and at 2DAH, at the end of endogenous feeding, in sharpsnout seabream. In both species first feeding occurred before complete resorption of yolk. A strictly exotrophic stage followed from 6 DAH in common pandora and 8 DAH in sharpsnout seabream. In this latter, the first gastric glands were formed at the accomplishment of the first month of life, as in common pandora, and a fully glandular stomach could be observed approximately ten days later. However, a sharp increase in pepsin-specific activity was delayed of about one week in sharpsnout seabream [34], compared to common pandora [35]. Gastric glands were detected more precociously, in other Sparids, such as *Diplodus sargus* [10], *Dentex dentex* [36], *Pagrus pagrus* [37] and *Pagrus auriga* [38], but considerably later in the gilthead seabream *Sparus aurata* [7].

The location of gastric glands is species-specific and has been related to feeding habits [10]. In *D. puntazzo*, gastric glands occurred only in the fundic region of the stomach, as was also shown in the yellowtail flounder *Pleuronectes ferruginea* [39], the summer flounder *Paralichthys dentatus* [40], the turbot *Scophthalmus maximus* [41] and the Dover sole *Solea solea* [42]. On the other hand, gastric glands were only found in the cardiac region in *D. sargus* [10], shi drum *Umbrina cirrosa* [28] and red porgy *P. pagrus* [37], whereas an entirely glandular stomach occurs in common pandora. 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* [43] and the gilthead sea bream [7]. On the other hand, neutral glycol-conjugates have also been reported in the stomach epithetlium of adult Mediterranean amberjack *Seriola dumerilii* [44] and larval white seabream *Diplodus sargus* [10], and this fact has been related to the absorption of easily digestible substances, such as disaccharides and shortchain fatty acids. Neither proteins rich in tryptophan,which have been related to pepsinogen synthesis in the amberjack *S. dumerilii* [44], nor proteins rich in cysteine, were found in the gastric glands of sharpsnout seabream, whereas they do occur in the congener species *D. sargus* [10]. Similarly, cysteine- and tryptophan-rich proteins have been reported in the gastric glands of *P. pagrus* [37], but not in those of *P. auriga* [45]. This would suggest that the presence of these aminoacid residues in the gastric glands of fish is species-specific.

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 [8, 29, 30, 40, 46]. On the contrary, caecal development started precociously in *D.puntazzo* (10 DAH) and much later in *P. erythrynus* (22 DAH). In both species, however, caecal development preceded the formation of gastric glands, as in *D. labrax* [47]. In the absence of functional gastric glands, the pre-cocious development of pyloric caeca, in which several enzymatic activities have been demonstrated [48], 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 [49], ensuring a more efficient utilization of feed. Intestinal coiling began at mouth opening in both species, as in *D. sargus* [10] and *P. auriga* [45], and considerably earlier than in other Sparids studied so far [36, 37]. Folding of intestinal mucosa, which would ensure more efficient digestion and absorption processes, started much earlier in sharpsnout seabream (3 DAH) than in common pandora (10 DAH). The presence of unstained vacuoles in the anterior intestine mucosa after the beginning of exogenous feeding (4-5 DAH in the two species considered), has been described in other species as an indicator of luminal absorption and storage of lipid [6, 10, 28, 40, 45, 50-52] during the larval phase, due to poor lipid metabolism, which improves with larval growth. Simultaneously with the beginning of exogenous feeding, lipid absorption was visible in the enterocytes of the anterior intestine, as small, supra- and infranuclear unstained vesicles. With the beginning of exogenous feeding (3-4 DAH), small supra- and infranuclear vacuoles could be seen in some tracts of the anterior intestinal epithelium. Such vacuoles, which were negative to both protein and carbohydrates reactions, thus suggesting lipid absorption, increased in number as the larvae grew. The small vesicles observed in the anterior intestinal epithelium, from 4 DAH onwards suggest that enterocytes are able to process and transfer the fat contents of ingested food from a very early developmental stage, unlike what happens in other freshwater and marine larvae, which exhibit large lipid droplets, suggesting a temporary storage of excess fatty acids [28-30, 52]. Protein absorption was visible shortly after first

feeding, as supranuclear Bromophenol blue-positive vacuoles occurring in the enterocytes of posterior intestine. These vacuoles, which have been described in the posterior intestine of both larval and adult gastric and agastric fish [53, 54], are involved in the pinocytotic uptake of proteins, which are subsequently digested intracellularly [55]. 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 [7, 10, 28, 56, 57]. Since no reduction in the number of protein vacuoles was observed after a glandular stomach was formed, similarly to what has been reported in the European sea bass [47] it can be suggested that a combined intra- and extracellular protein digestion mechanism is active in the two species considered.

Goblet cells appeared in the digestive tract after the first exogenous feeding, and their number increased with larval development. 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 [10, 36, 45, 47]. Glycoproteins produced by goblet cells may play an important lubricant role of 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 [11], as well as in pre-gastric digestion [39]. The mucous content of goblet cells in sharpsnout seabream varied with the digestive region and, in some cases, with the developmental phase. Variability in staining within a given goblet cell has been attributed to a temporal sequence in the mucus biosynthesis [58], from an early developmental stage during which the cell produces mainly PAS-positive neutral glycoproteins, through an intermediate stage during which carboxylation of the glycoproteins occur, until a final stage, when sulphated groups are conjugated to the glycoproteins. In sharpsnout seabream, pharyngeal goblet cells secreting acid sialomucins appeared at 5 DAH and their mucous content did not vary in the later stages. Acid mucins were also abundant in oesophageal goblet cells after 7 DAH, but these cells contained sulphated mucins ten days later as well as cystein-rich proteins thirteen days later. On the other hand, the goblet cells of buccal cavity, which were first visible at 24 DAH, were shown to contain neutral mucins at 31 DAH and sulphated mucins from 36 DAH. Finally, two types of goblet cells appeared in the intestinal epithelium coinciding with formation of gastric glands, one containing neutral mucins and the other one containing acid mucins. These data suggest that the variable staining of digestive goblet cells in *D. puntazzo* may be related to a progressive development of the mucus content in both the buccal cavity and oesophagus, but not in the pharynx and intestine.

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. Eosinophilic zymogen granules, where the precursors of pancreatic enzymes are accumulated, could be detected within the exocrine pancreatic cells, suggesting their genetically programmed, rather than dietary-induced, differentiation [5, 11, 59].

In common pandora the colourless, PAS-negative liver vacuoles, which increased progressively during larval development, are indicative of lipid storage, whereas in sharpsnout seabream, the large, unstained vacuoles of probable lipid origin, were reduced at the beginning of the endo-exotrophic stage. It could be suggested that, in the last species, 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 [52].

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