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ABSTRACT

Gut development in *Perca fluviatilis*: a micro-anatomical study

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Introduction

In the lakes of Northern Italy, the survival of indigenous populations of European perch (*Perva fluviatilis* Linnaeus, 1758) is threatened by ecological competition exerted by several introduced exotic fish species. In 2006, our group activated a preliminary project for rearing perch larvae. The objective was the development of a good rearing protocol for perch larvae, with the aim of obtaining adult fish for (a) the repopulation of the natural environmental sites, and (b) for aquaculture purposes. The lack of knowledge about the organogenesis of the European perch larvae, mainly concerning gut development, was the first target of our rearing project. In this survey, we have studied the histological and histochemical features of the digestive system of *P. fluviatilis* during development, as well as the gut modifications involved during larval development. Moreover, immunohistochemical reactions have been used to study the changing events related to the regulation and appearance of secretory functions in the alimentary canal of larvae.

Materials and methods

In April 2006, three sticky strings of *P. fluviatilis* eggs laid on artificial substrates were collected from a reproduction site in the Lake of Varese (Italy). Eggs were maintained in

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controlled environments until larvae hatched in 140 degree/day. The European perch larvae were fed for a week with plankton from the sampled water site, then with Artemia-mix Sera (*Artemia salina* larvae) and with mosquito (*Culex spp.*) larvae. From the hatching day (0 dph) until day 57 (57 dph), every 3 days 5 larvae were killed with sodium methane sulphate, then fixed with 10% formaldehyde in saline phosphate buffer at 4°C, dehydrated and embedded in paraffin. Microtome sections (4–6 μm) of each larval age were used for histology (Hematoxylin-Eosin, HE) and the following histochemical reactions: Alcian Blue/ Periodic Acid Schiff pH 2.5 (AB/PAS) for glycoconjugates, and Bromophenol Blue (BB) for proteins. Other microtome sections were employed for immunohistochemistry, using the following rabbit polyclonal anti-sera: anti-pepsinogen II (USBiological), -serotonin (5-HT, Peninsula), -gastrin (Chemicon), -cholecystochinin-8 (-CCK-8, Peninsula), -neuropeptide Y (NPY, Peninsula), and -glucagon (from Med. Dr. J. M. Polak, London).

Results

In the *P. fluviatilis* embryo, the mouth had opened before larval hatching. In the larva at 3 dph, a complete and PAS-positive liver was present. At this time, the pancreas was developing, and mucous cells (AB- and PAS-positive) appeared in the oesophagus. An ileo-rectal valve separated a proximal from a distal intestinal tract. At 7 dph several mucous cells appeared in the growing intestinal canal, which were histochemically unreactive. Two days later (9 dph) in the proximal intestine, the enterocytes showed many eosinophil and BB-positive vacuoles. From the 11th dph to the 14th dph the stomach appeared, with a progressively enlarging lumen and thickening muscle wall. In the larvae at 17 dph, the intestinal mucous cells contained acid and neutral and glycoconjugates, and after 3 days (20 dph) the pyloric caeca appeared. The stomach was fully developed at 26 dph, when it showed oxyntopeptic cells, which were immunoreactive to the anti-pepsinogen serum.

In the alimentary canal of the newly hatched larvae, several endocrine cells (EC) immunoreactive (IR) to the anti-5-HT serum were observed. During larval development, numerous anti-5-HT-IR EC appeared in the stomach and in the distal tract of the intestine. In larvae at 20 dph, several anti-5-HT-IR neurons were observed in the myenteric plexus of the alimentary canal. In larvae at 14 dph, anti-gastrin- and anti-CCK-8-IR EC appeared and increased in number in the proximal tract of the intestine, localized at the base of the intestinal folds. At 20 dph, larvae showed several NPY-IR EC in the intestinal canal. Numerous anti-glucagon IR EC were observed in the pancreas endocrine islets. On the other hand, no anti-glucagon IR EC were found in the whole alimentary canal.

Discussions

In the European perch larvae, alimentary materials were present in the gut lumen from 3 dph, and yolk sac absorption was complete at 6 dph (Falk-Petersen 2005). In larvae at 3 dph, mouth and anus were opened and the alimentary canal was an undifferentiated tube. At this time, mucous cells containing acid glicoconjugates at the epithelial surface, and neutral glycoconjugates, namely immature elements, at the epithelial base appeared in the oesophagus (Elbal and Agulleiro 1986). In the *P. fluviatilis* larvae, the occurrence of mucous cells in the intestinal canal was detected at 7 dph. Before gastric differentiation, the European perch larvae were able to digest carbohydrates and lipids, but probably not proteins (Santamaria et al. 2004). The stomach appeared at 9 dph and become fully developed at 26 dph, when oxyntopeptic cells showed immunoreactivity to the anti-

pepsinogen serum. This agrees with results of Cuvier-Péres and Kestemont (2002) who detected pepsinogen in homogenate from the alimentary canal of *P. fluviatilis* larvae at 29 dph.

The gut neuroendocrine system is formed by several endocrine cells scattered in the gut epithelium, and by the intramural neurons, which co-operate to regulate the local physiological activity of the digestive apparatus. In the early larval stages, the intestinal epithelium showed several endocrine cells containing and secreting 5-HT. From the 20th dph, serotoninergic neurons appeared in the myenteric plexus of the gut. In *P. fluviatilis* larvae, 5-HT it the first neuroendocrine factor to regulate epithelial secretion and muscle activity in the gut. (Lee et al. 2004). At 20 dph, several endocrine cells containing a NPY-like molecule were observed in the intestinal epithelium of the larvae. NPY is a peptide involved in the control of feed uptake in many vertebrates (Volkoff et al. 2005). In the proximal intestine of the larvae at 14 dph, numerous endocrine cells CCK-8- and gastrin-IR were observed, and the two immunoreactivities were probably co-localized (Bosi et al. 2004). CCK-8 is a strong anorexigenic factor which is responsible for gallbladder contraction and bile secretion (Volkoff et al. 2005). In younger *P. fluviatilis* larvae, the pancreas, but not the alimentary canal, showed the occurrence of endocrine elements IR to glucagon anti-serum.

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