

## Histology of the developing digestive system and the effect of food deprivation in larval green sturgeon (*Acipenser medirostris*)

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### Abstract

The histological development of the digestive tract in hatchery-reared green sturgeon (*Acipenser medirostris*) larvae and the effects of food deprivation on the digestive system organization were studied from hatching until 31 days post-hatching (dph). At hatching, the larval digestive system consisted of two rudiments: a large endodermal yolk sac and a primordial hind-gut. During the endogenous feeding phase, the wall of the yolk sac differentiated into the stomach (glandular and non-glandular regions) and the anterior and intermediate intestine, while the hind-gut primordium differentiated into the spiral valve and rectum. At the onset of exogenous feeding (15 dph at 16 °C), the organization and cytoarchitecture of the digestive system in green sturgeon larvae was generally similar to those of juveniles and adults. Larvae deprived of food exhibited a progressive deterioration, with subtle pathological changes observed after 5-d starvation: shrinkage of digestive epithelia, tissue degeneration, and necrosis were observed at 10–15 d of starvation (30 dph). No changes were observed in the mucous secretion of different regions of the digestive tract of food-deprived larvae. The histological analysis of the larval digestive system may be used to evaluate the nutritional condition of larval green sturgeon in their nursery habitats in spawning rivers, which are affected by dams and flow diversions.

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### Résumé

**Histologie du système digestif en développement et l'effet de privation de nourriture chez les larves de l'esturgeon (*Acipenser medirostris*).** Le développement histologique du tractus digestif chez les larves d'esturgeon vert (*Acipenser medirostris*) élevées en éclosion et la privation de nourriture sur l'organisation du système digestif ont été étudiés de l'éclosion jusqu'à 31 jours après l'éclosion (j). À l'éclosion, le système digestif larvaire est caractérisé par un épais endoderme du sac vitellin et un intestin postérieur primaire. Durant la phase de nutrition endogène, la paroi du sac vitellin se différencie en estomac (régions glandulaire et non glandulaire) et en intestin antérieur et intermédiaire, tandis que l'intestin postérieur primaire se différencie en valve spiralee et en rectum. Au début de la phase de nutrition exogène (15 j à 16 °C), l'organisation et la structure cellulaire du système digestif chez les larves sont généralement similaire à celles des juvéniles et des adultes. Des larves privées de nourriture présentent une détérioration progressive, avec de légers changements pathologiques observés après 5 j de jeûne : retrécissement de l'épithélium digestif, dégénération du tissu et nécrose ont été observés à 10–15 j de jeûne (30 j après éclosion). Aucun changement n'a été observé dans la sécrétion des muqueuses des différentes régions du tractus digestif des larves privées de nourriture. L'analyse histologique du système digestif larvaire peut être utilisée pour évaluer les conditions nutritionnelles des larves de cet esturgeon présentes dans les fleuves, où s'effectue la reproduction, affectés de barrages ou soumis aux aléas des courants de marée.

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**Keywords:** *Acipenser medirostris*; Larvae; Digestive system; Histology; Starvation

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## 1. Introduction

Green sturgeon is the least studied species among North American acipenserids, and is considered a rare or vulnerable species in the United States and Canada (Birnstein, 1993) and an endangered species in Russia (Artyukhin and Andronov, 1990). In North America, the only known spawning populations of green sturgeon are in the Klamath, Rogue and Sacramento Rivers, all of which are affected by water projects. These river flows are largely controlled by dams and water diversion projects. The resulting significant reduction of flow (especially during the dry years) may result in changes of water quality (temperature and dissolved gases) and primary and secondary productions, which may affect the availability of food resources for larval and juvenile green sturgeon (Moyle et al., 1994). Predation is considered to be the main cause of mortality during the embryo and yolk-sac larval stages, whereas starvation plays an important role during transition to exogenous feeding, thus, increasing the vulnerability of larvae to predation (Kamler, 1992; Iguchi and Mizuno, 1999). Temperature may influence the time-interval for larvae to establish successful feeding, by changing the rate of metabolism and the pace at which the yolk reserves are consumed and first feeding occurs (Bisbal and Bengtson, 1995). Various, temperature-dependent periods of food deprivation may result in abnormal behaviour and development, including the degeneration of the alimentary tract and trunk musculature, reduced food utilization efficiency and feeding activity patterns (Heming et al., 1982).

The effects of experimental food deprivation on fish larval conditions have been described using morphometric and gravimetric (e.g. Elrich et al., 1976; Mookerji and Rao, 1999), biochemical (e.g. Robinson and Ware, 1988; Clemesen, 1993; Suneeetha et al., 1999), and histological (e.g. Oozeki et al., 1989; Theilacker and Watanabe, 1989; Green and McCormick, 1999) criteria, or a combination of those (Bisbal and Bengtson, 1995). The detection of starvation conditions is important for studies of both natural and cultured populations. However, such studies must be preceded by an experiment, where the starvation indicators are validated for fish of known nutritional history (Bisbal and Bengtson, 1995).

Acipenseriformes (sturgeon and paddlefish) differ from the modern teleosts by holoblastic cleavage, intracellular platelet yolk in the embryo, and by differentiation of the digestive system from the yolk-sac anlagen (Dettlaff et al., 1993). Development of the digestive system has been studied in Russian sturgeon *Acipenser gueldenstaedti* (Dettlaff et al., 1993), white sturgeon *A. transmontanus* (Gawlicka et al., 1995) and Siberian sturgeon *A. baerii* (Gisbert et al., 1998, 1999). Deng et al. (2002) recently described early development in green sturgeon, however, there has been no comprehensive study on the digestive system in larval stages of this species.

Using the artificial spawning of green sturgeon (Van Eenennaam et al., 2001), we initiated the studies on cultured

fish to better understand the early life history and environmental physiology of this species. The objectives of this study are to characterize, using light microscopy, the development of the digestive system in green sturgeon larvae and to evaluate the effects of food deprivation on the histopathology of the digestive tract and accessory digestive organs from the onset of exogenous larval feeding.

## 2. Materials and methods

### 2.1. Supply and maintenance of fish

Green sturgeon gametes were obtained from one female (35 kg) and male (25 kg), caught in May of 2001 by gillnets and held in cages in the Klamath River (Weitchpec, California). Ovulation was induced by two intramuscular injections (0.6 and 20  $\mu\text{g kg}^{-1}$ , with 8-h interval) of GnRH $\alpha$  ([D-Ala<sup>6</sup>, Des-Gly<sup>10</sup>]-LH-RH Ethylamide). Domperidone (2 mg  $\text{kg}^{-1}$ ) was administered with the second injection. Spermiation was induced by a single injection of GnRH $\alpha$  (10  $\mu\text{g kg}^{-1}$ ). The ovulation occurred within 15 h after second injection at river temperature 18.4–19.8 °C. Gamete collection, artificial fertilization, and the silt treatment of fertilized eggs were conducted as described by Van Eenennaam et al. (2001). The eggs (fertilization rate 80% at second-third cleavage) were transported to UC Davis in oxygenated and cooled (13–14 °C) water and incubated at 14–15 °C for 7 d before hatching. One thousand newly emerged larvae were held in two circular tanks (120 l, ca. 500 larvae per tank) of the indoor semi-recirculation system and supplied with well water (dissolved oxygen at saturation, pH 7.8–8.2, constant 16 °C temperature, and 12L:12D artificial photoperiod). A small amount of food (semi-moist Silver Cup, Nelson & Sons, Utah, and live chopped Tubifex) was presented to one group (tank) at 12 days post-hatching (dph), while the other group was deprived of food during the entire experiment (31 dph). Initiation of feeding was determined by direct examination of larvae. Characteristics such as distended stomachs and characteristic internal coloration associated with the dry commercial diet and Tubifex acted as good indicators (Gisbert and Williot, 1997). Once the first feeding was detected at 14–16 dph, the larvae were fed ad libitum and uneaten feed, faeces, and mortalities were removed daily.

### 2.2. Fish growth measurements and histology procedures

Larvae were sampled ( $n = 10$ ) daily from hatching to 20 dph and then with 3-d intervals to 31 dph. At sampling, fish were euthanized (overdose of tricaine methanesulphonate), and fixed in phosphate-buffered formalin. Large sampling mortality did not allow for a survival analysis. After 1 month of storage in formalin, larvae were measured (total length TL,  $\pm 0.01$  mm, stereoscopic microscope with a camera lucida and a digital pad Micro-Plan II, Laboratory Computer systems, Inc.), weighed (BW,  $\pm 0.01$  mg, digital microbalance), and five specimens from each sample were dehy-

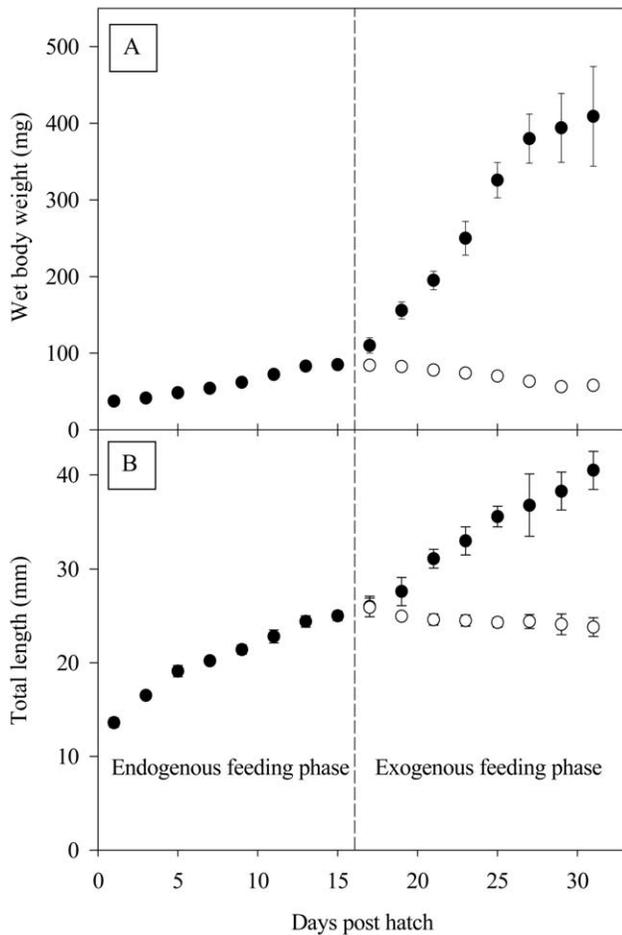


Fig. 1. Wet weight (A) and total length (B) (means  $\pm$  S.D.,  $n = 10$ ) of fed ( $\bullet$ ) and starved ( $\circ$ ) green sturgeon larvae.

drated in graded ethanol, embedded in paraffin, and sectioned at 4–6  $\mu\text{m}$  (LKB Historange Microtome). Sections were stained by haematoxylin and eosin (HE) for histological observations, periodic acid-Schiff (PAS) for neutral mucosubstances, and Alcian Blue (AB) at pH 2.5, 1.0 and 0.5 for carboxyl-rich and sulphated (weakly and strongly ionized) glycoconjugates and sialic acid (HCl hydrolysis + AB, pH 2.5) (Gisbert et al., 1999). Melanin pigment granules were identified as previously reported (Gisbert and Sarasquete, 2000). Height of the epithelial cells from different regions of the digestive tract was measured at 600 $\times$  under a microscope with an ocular micrometer (Bisbal and Bengtson, 1995), and compared among feeding and starving groups, using a Student's *t*-test ( $P < 0.05$ ).

### 3. Results

Food deprivation during 15–31 dph resulted in a loss of body wet weight and no increase of length in the food-deprived group (Fig. 1). Unfed larvae continued swimming along the bottom and walls of the holding tank, exhibited food searching behaviour, and were reactive to external stimuli. However, most food-deprived larvae died between

28 and 31 dph, whereas no mortality was observed in green sturgeon larvae that were fed.

#### 3.1. Histodifferentiation of the digestive system in fed larvae

At hatching, the larval digestive system was represented by two rudiments, a large endodermal yolk sac and a primordial hind-gut, neither were open to the exterior (mouth and anus were not differentiated). The endodermal yolk sac (volume  $16.9 \pm 2.9 \text{ mm}^3$ ,  $n = 20$ ) was filled with yolk platelets and lined with a simple, squamous epithelium, which would differentiate into the walls of stomach and intestine (Fig. 2A). The hind-gut, containing a small amount of yolk, appeared as an undifferentiated straight and narrow rudiment, which would differentiate into the spiral valve and rectum. The accessory digestive organs (liver and pancreas) were absent at hatching. Histological differentiation of the digestive system after hatching is described below.

##### 3.1.1. Buccopharynx

At hatching, the buccopharynx was closed and its lumen was filled with small yolk platelets (Fig. 2A). Clusters of basophilic cells (future gill arches) were seen in a circular position in the posteroventral region of the buccopharynx (Fig. 2B). Between 1 and 2 dph, the mouth opened with two differentiated oral valves that were composed of a stratified squamous epithelium in differentiation. The buccopharyngeal mucosa consisted of a stratified squamous epithelium with connective tissue fibres (Fig. 2C). Epithelial cells contained supranuclear eosinophilic (HE) and PAS-positive yolk platelets and some melanin granules (bleachable with hydrogen peroxide), which disappeared at age 4–5 dph. Ciliated cells (scattered through buccopharyngeal epithelium) were present from hatching until 12–14 dph (Fig. 3A). First goblet cells (unreactive to stain) appeared at 6 dph. At age 7–8 dph, they were stained by the AB (pH 2.5, 1.0, 0.5) and PAS stains, indicating the presence of neutral and acidic (carboxylated and sulphated) glycoconjugates (Table 1). The number and size of goblet cells in the buccopharyngeal epithelium increased as larvae developed. Taste bud cells (basophilic) were differentiated between 8 and 9 dph, and taste buds were fully developed at 10–11 dph. Differentiation of canine-like teeth proceeded from the base of the buccopharyngeal epithelium at 6–7 dph (Fig. 3B), with the teeth protruding into the oral valves and pharyngeal lumen at age 11–12 dph (larval dentition was present at 31 dph). Between 11 and 12 dph, the ventral and dorsal fungiform and filiform papillae developed in the anterior and central part of the buccopharynx, respectively (Fig. 3C). Numerous mucous cells and taste buds developed in the surface of the papillae.

##### 3.1.2. Oesophagus

The oesophagus was not differentiated to 6 dph, and the posterior region of buccopharyngeal cavity remained filled with the residual yolk. At 7 dph, the primordial oesophagus wall consisted of a pseudostratified columnar epithelium

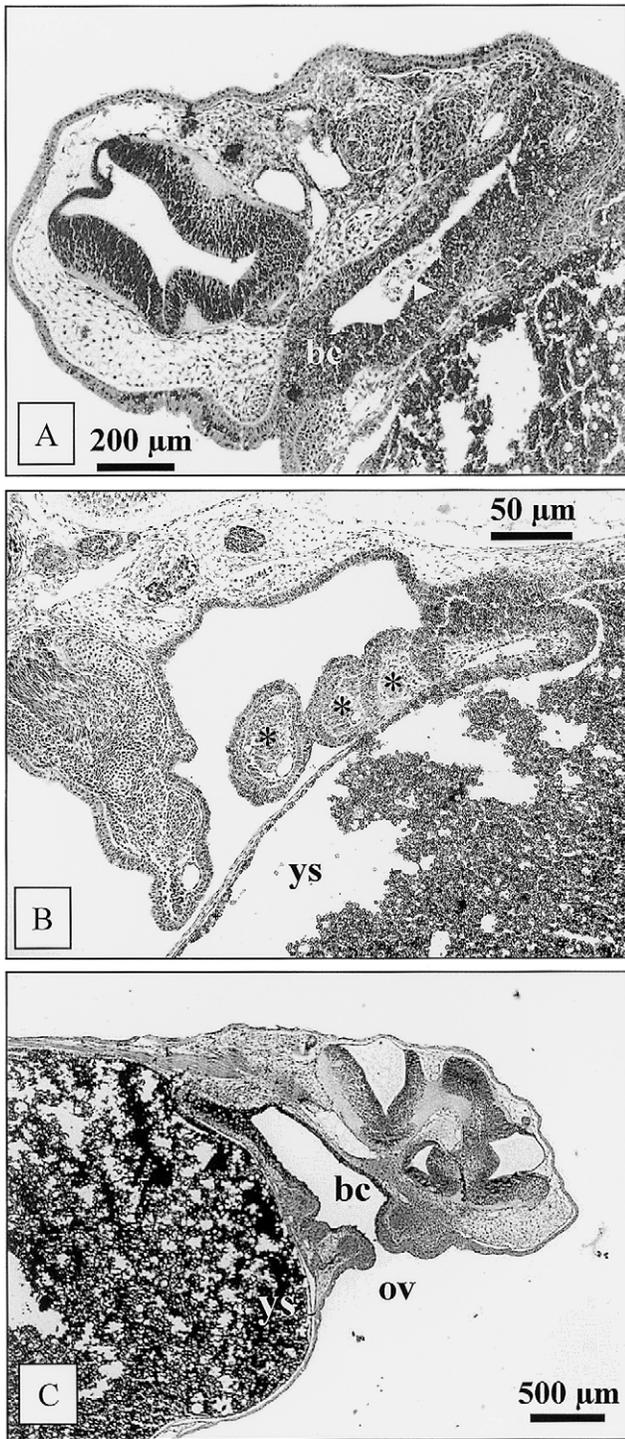


Fig. 2. (A) Head of a green sturgeon larva at hatching; note the closed buccopharynx (bc) and the buccopharyngeal epithelium containing yolk inclusions (white arrow head). (B) Differentiation of gill arches (asterisks) in the posteroventral region of the buccopharyngeal mucosa of a newly hatched larva. (C) Differentiation of oral valves (ov) and mouth opening at 2 dph; note the posterior pharynx filled with yolk (arrow) and the large yolk sac (ys).

with numerous cells containing yolk inclusions and melanin granules (Fig. 4A), which disappeared between 8 and 9 dph.

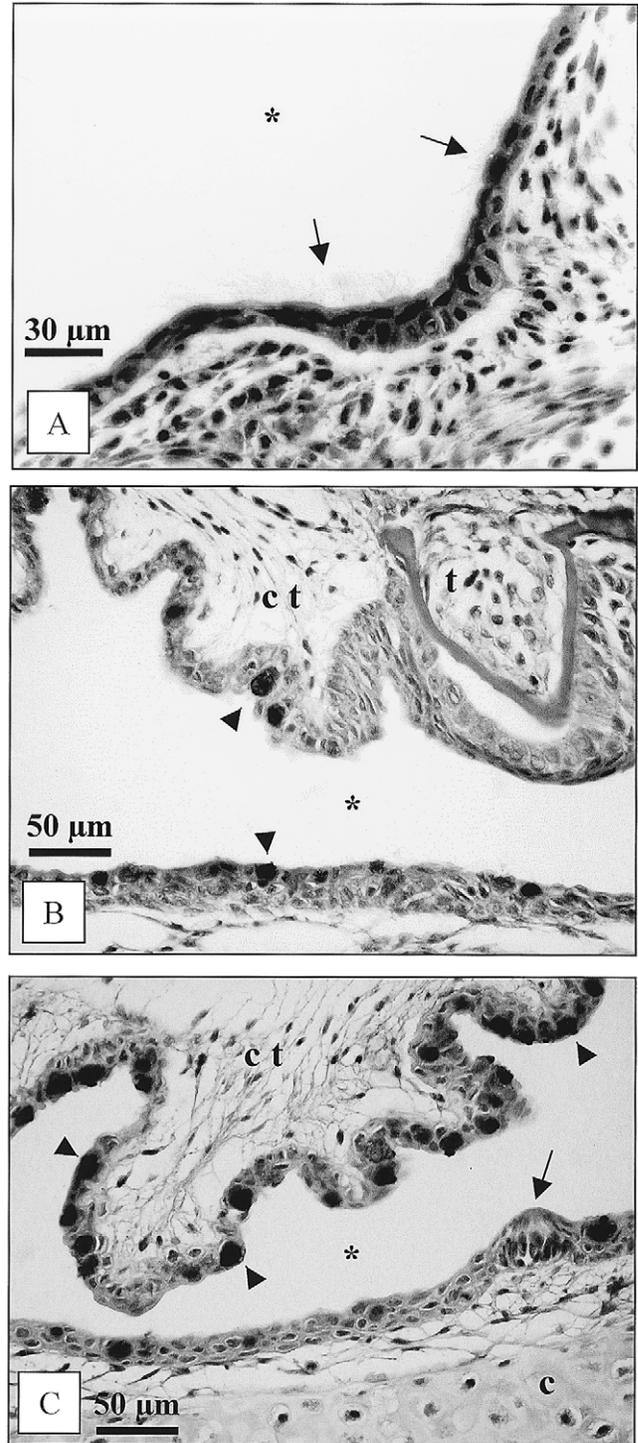


Fig. 3. (A) Ciliated cells (arrows) in the buccopharyngeal epithelium at 6 dph. (B) Details of the stratified squamous epithelium with functional goblet cells (arrow heads) lining the buccopharynx at 7 dph. Note the presence of a tooth (t) in differentiation before protruding into the buccopharyngeal lumen (\*) and the connective tissue (ct) surrounding the buccopharyngeal epithelium. (C) Details of pharyngeal papillae with goblet cells (arrow heads) at 12 dph. Note the presence of a taste bud (arrow) in the ventral buccopharyngeal epithelium and the presence of the Meckel's cartilage (c).

Differentiation of the oesophagus proceeded from the posterior region to buccopharynx. At 10 dph, the oesophagus wall

Table 1

Distribution of acid and neutral glycoproteins produced by goblet cells in different regions of the digestive tract during early development of fed green sturgeon larvae

	Buccopharynx	Oesophagus	Stomach		Intestine		
			Cardiac	Pyloric	Anterior	Intermediate	Spiral valve
Neutral glycoproteins (PAS)	3	3	3	2	2	2	3
Carboxyl-rich glycoproteins (sulphated or not) (AB pH 2.5)	2	2	0	0	2–3	2–3	2–3
Sulphated proteins (weakly ionized) (AB pH 1.0)	2	2	0	0	2	2	2
Sulphated glycoproteins (strongly ionized) (AB pH 0.5)	1	2	0	0	1–2	1–2	2
Sialic acid (HCl hydrolysis-AB pH 2.5)	0	1	0	0	2	2	2

Results are reported considering the intensity of the histochemical reactions: 0, negative; 1, weak; 2, moderate; 3, intense.

was composed of a mucosa with a *lamina propria* (loose connective tissue and a layer of musculature), a submucosa (connective tissue fibres with some blood vessels), and a serosa lined by a thin layer of squamous epithelium. Two regions of the oesophagus were distinguished by histological characteristics of the epithelia. The anterior region was lined with two cell layers: the inner layer of a simple, cuboidal epithelium and the outer layer of a simple, columnar epithelium with abundant goblet cells staining for neutral mucosubstances (PAS), carboxylated and sulphated acidic mucins (AB pH 2.5 and AB pH 1.0, 0.5), and for sialic acid (HCl hydrolysis + AB pH 2.5) (Fig. 4B, Table 1). The posterior region was lined with a ciliated columnar epithelium with very few goblet cells (Fig. 4B). Some mucosal folds were detected in the region connecting the oesophagus and glandular stomach.

### 3.1.3. Stomach

At hatching, the large endodermal yolk sac of green sturgeon was surrounded by a thin squamous basophilic epithelium. Two furrows (invaginating epithelium) appeared at 2 dph in the dorsal and ventral posterior regions of the yolk sac (Fig. 5A), merging and dividing the yolk sac into two compartments between 3 and 4 dph (Fig. 5B). The anterior wall of the furrow lined with a squamous epithelium became the ventral lining of the stomach, while its posterior wall lined with a columnar epithelium became the dorsal lining of the intestine.

The pyloric (non-glandular) stomach started differentiation at 6 dph in the anterior ventral region of the yolk sac from a fold of stratified squamous epithelium (Fig. 5C) and was well differentiated at 11–12 dph, with mucosal folds surrounded by a prominent *tunica muscularis* (Fig. 6A). The epithelial lining of the pyloric lumen consisted of ciliated columnar cells with supranuclear vacuoles containing eosinophilic (HE) and neutral (PAS-positive, AB pH 2.5, 1.0 and 0.5-negative) mucosubstances (Table 1). A wall of the pyloric stomach was composed of submucosa with connective fibres (AB pH 2.5 and 1.0-positive), some blood vessels, circular muscle fibres, and a thin serosa with basophilic squamous cells (Fig. 6B). The organ was separated from the anterior intestine by the pyloric sphincter (Fig. 6A). Platelet yolk was present in the lumen of pyloric stomach until the age 14 dph.

The cardiac (glandular) stomach started to differentiate at 8 dph, with cytoarchitectural changes (squamous to columnar cells) in the epithelium of the yolk-sac. Gastric glands in the cardiac stomach wall were not detectable by the PAS staining at 10 dph, but they were prominent at 12 dph, as the multicellular tubular glands composed of a single-type secretory cells with eosinophilic and PAS-positive apical borders and the secretory products containing neutral (PAS-positive) mucosubstances (Fig. 6C, Table 1). These glands were surrounded by compact layers of connective tissue stained for acidic mucins (AB pH 2.5, 1.0 and 0.5), smooth circular musculature, and a thin serosa. The number of gastric glands and thickness of mucosa layers increased during larval feeding phase (15–31 dph), while their histochemical properties remained the same. The platelet yolk was present in the glandular stomach until 14 dph.

### 3.1.4. Anterior and intermediate intestine

Differentiation of the intestinal wall started at 2–3 dph, progressing in a posteroanterior direction. However, the anterior region of the intestine was filled with yolk and did not differentiate until 7 dph (Fig. 5A). The differentiation of the intestinal mucosa was concomitant with the disappearance of yolk in the supranuclear vacuoles of epithelial cells. The mucosa had generally similar histological structure along the length of intestine, with the exception of number and size of intestinal folds, which were less abundant and smaller in the posterior region (Fig. 7A). During yolk resorption, supranuclear lipidic vacuoles in the cells of the intestinal epithelium increased in size and number, and were present until 16–17 dph (Fig. 7B).

The first goblet cells appeared at age 6–10 dph, in the posterior and anterior regions of the intestine, respectively. The number of goblet cells increased with differentiation of the mucosa, and they were more abundant in the posterior region. Goblet cells contained carboxylated and sulphated glycoconjugates (AB-positive at pH 2.5, 1.0, 0.5) and sialic acid (HCl hydrolysis + AB pH 2.5). Most goblet cells exhibited dark-blue staining (AB and PAS positive staining), but some exhibited magenta or purple staining, suggesting the presence of acid mucosubstances in the majority of cells and the neutral (magenta) or neutral and acid (purple) glycoconjugates in some of the cells (Table 1).

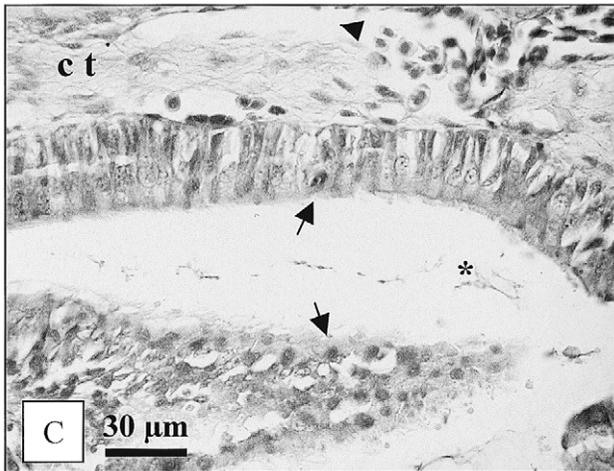
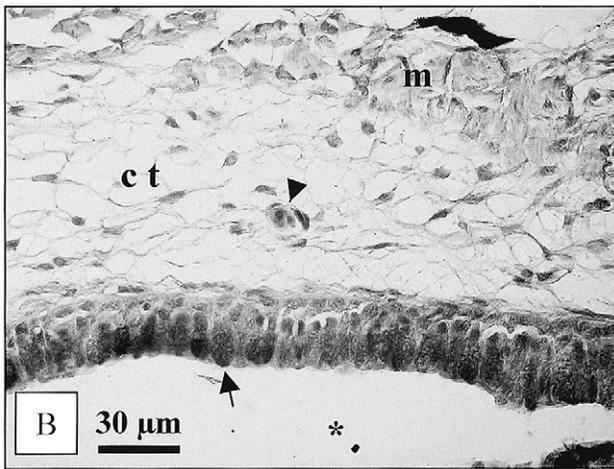
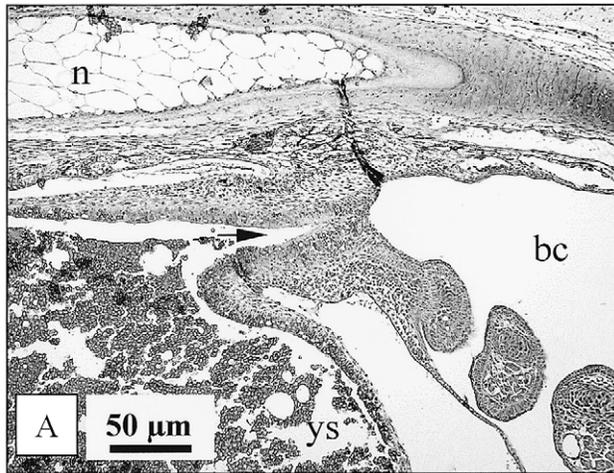


Fig. 4. (A) Primordial oesophagus (arrow) with epithelial cells containing yolk inclusions at 7 dph. (B) Anterior region of the oesophagus lined by a columnar epithelium with abundant goblet cells (arrow) at 7 dph. (C) Posterior region of the oesophagus with few goblet cells (arrows). Blood vessel (arrow head), buccopharynx (bc), connective tissue (ct), muscular layer (m), notochord (n), oesophageal lumen (asterisk), yolk sac (ys).

### 3.1.5. Spiral valve and rectum

Differentiation of spiral valve (posterior intestine) occurred soon after hatching (1–2 dph) (Fig. 5A). The lumen of spiral valve was lined with a simple ciliated columnar epithelium containing yolk inclusions in the supranuclear vacuoles

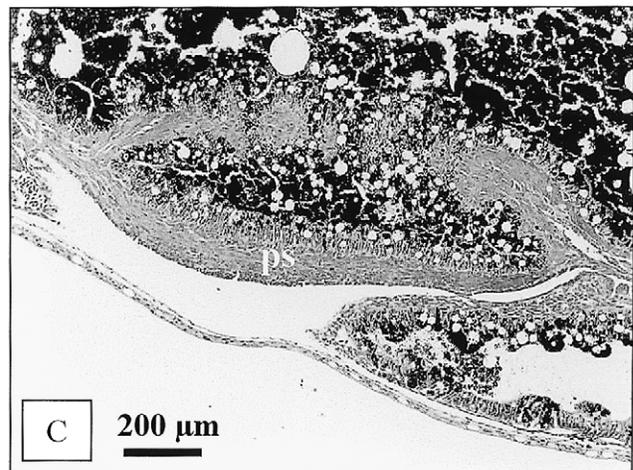
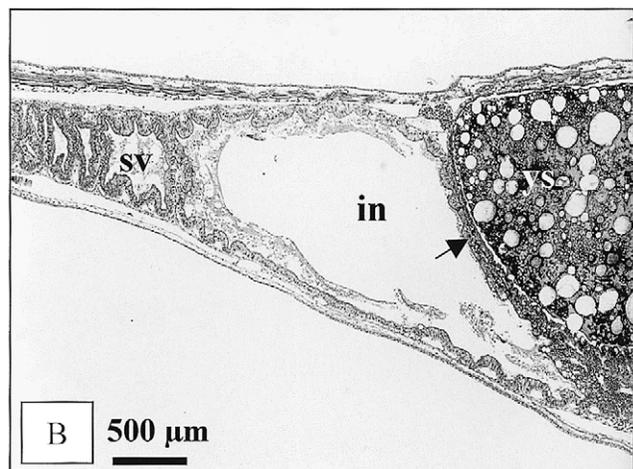
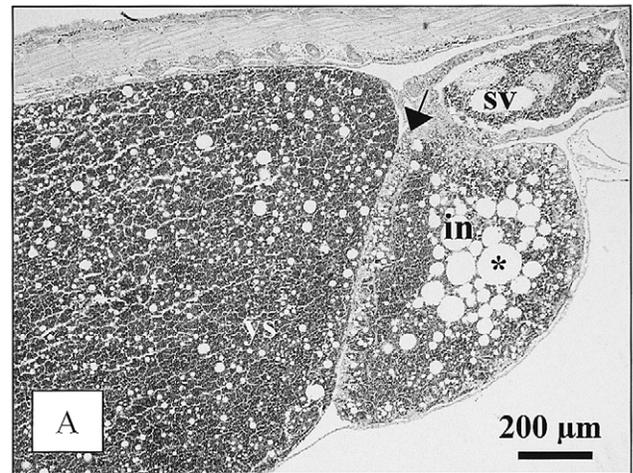


Fig. 5. (A) Division of the yolk sac (ys) by a furrow (arrow) separating it from the intestine (in) and spiral valve (sv) at 2 dph. Note lipid inclusions (asterisk) in the yolk and the rudimentary intestine and spiral valve filled with yolk. (B) Yolk sac (anlagen of the stomach), intestine (in), and spiral valve (sv) of a green sturgeon larva at 4 dph. The arrow indicates the furrow dividing the future stomach filled with yolk and the intestine. (C) Differentiation of pyloric stomach (ps) at 6 dph, from a fold of stratified squamous epithelium surrounded the yolk sac.

lium containing yolk inclusions in the supranuclear vacuoles (Fig. 7C). The first goblet cells appeared at 2 dph, and they

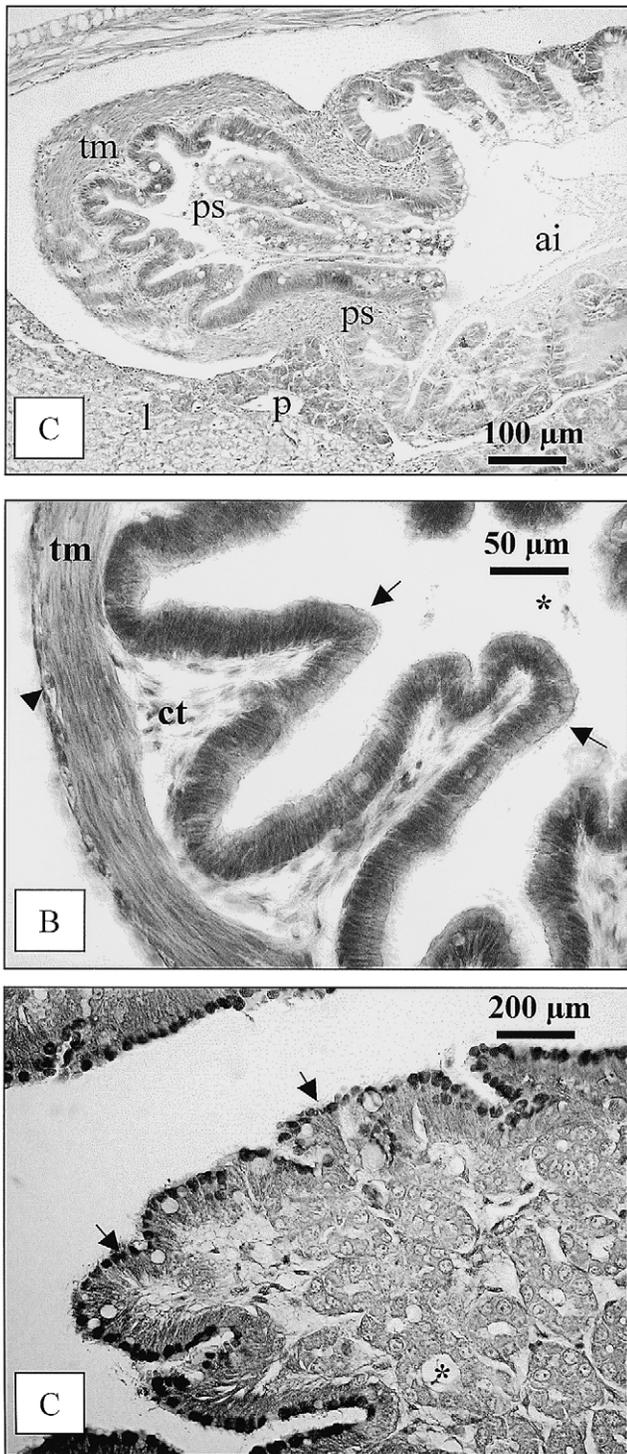


Fig. 6. (A) General view of the pyloric stomach (ps) at 15 dph. Anterior intestine (ai), liver (l), pancreas (p), pyloric sphincter (ps), *tunica muscularis* (m). (B) Details of the pyloric stomach with prominent mucosal folds (arrows) surrounded by a *tunica muscularis* (tm). Blood vessel (arrow head), connective tissue (ct), pyloric lumen (asterisk). (C) Cardiac stomach with multicellular tubular glands (asterisk) with eosinophilic apical borders (arrows) secreting neutral mucosubstances.

were similar in their histochemical properties to those in the anterior and intermediate intestine (Table 1). The lumen of the spiral valve was initially filled with yolk but, as larvae

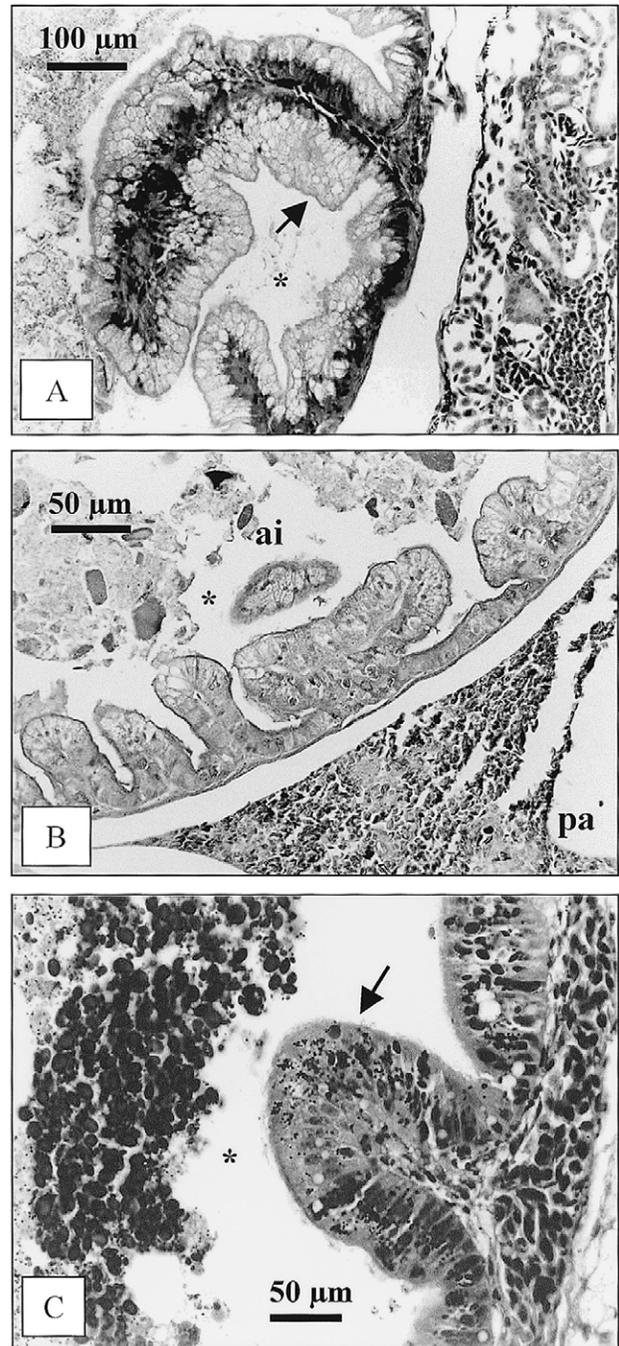


Fig. 7. (A) Epithelium (arrow) of the intermediate intestine at 14 dph, with the large supranuclear lipid vacuoles. (B) Anterior intestine (ai) and pancreas (pa) at 20 dph. (C) Enterocytes (arrow) of the spiral valve containing yolk platelets in supranuclear vacuoles, at 2 dph; note the presence of free yolk in the intestinal lumen (asterisk).

developed, it became devoid of yolk and accumulated a dark pigment (Fig. 5A). The posterior region of a hind-gut primordium differentiated into the rectum at 5 dph. The rectal mucosa was lined with a ciliated columnar epithelium containing few goblet cells (Fig. 7B). The anus opened at 9–10 dph, after the complete resorption of the residual yolk in the spiral valve.

### 3.1.6. Liver and pancreas

The liver rudiment appeared at 2 dph in a ventral portion of endodermal yolk sac, and the rudiment of exocrine pancreas developed dorsally to the furrow dividing yolk sac into the stomach and intestine. At 4 dph, polygonal hepatocytes containing large supranuclear lipidic vacuoles (not stained by HE, PAS and AB) and glycogen inclusions (PAS and diastase-PAS positivity), were arranged along hepatic sinusoids (Fig. 8A). The lipidic vacuolization and density of glycogen granules in hepatocytes increased with larval development, especially in feeding larvae. Exocrine pancreatic cells were arranged in acini at age 4–5 dph, around the small intercellular lumina. Acinar cells had eccentric (basal) nuclei and strongly basophilic cytoplasm. Zymogen granules were detected (HE and PAS) in acinar cells before the onset of first feeding (at 14 dph) (Fig. 8C).

### 3.2. Effect of food deprivation on digestive system

The digestive system of food-deprived larvae exhibited a progressive deterioration, starting after 5 d of starvation. Starvation-induced changes in tissues and cytoarchitecture of the digestive system were summarized in Table 2 and Fig. 11.

In the buccopharynx, filiform and fungiform papilla decreased in size and number between 2 (17 dph) and 16 (31 dph) days of starvation (Fig. 9A), while the ventral papillae were gradually resorbed and disappeared entirely at 16 d of starvation (31 dph). The epithelium became thinner and taste buds and larval teeth strongly protruded into the lumen. The cytoplasm of epithelial cells became hyaline after 5 d of fasting (20 dph) and pycnotic nuclei were seen at 14 d (29 dph). The histochemical properties of goblet cells in buccopharynx did not change throughout the starvation period.

Similar to the buccopharyngeal epithelium, epithelial cells of the oesophagus shrunk at 5–10 d of starvation (Fig. 11). From day 5 of fasting (20 dph) to the end of the study, the oesophageal mucosa and submucosa decreased in thickness, due to the shrinkage of the smooth muscle and connective tissue fibres. Scattered pycnotic nuclei in the oesophageal epithelium were observed at 10 d of starvation, and they were abundant after day 16 of starvation (31 dph). The histochemical properties of the goblet cells (secretion of neutral and acidic mucins with sialic acid) did not change throughout the starvation period.

Epithelial cells of the cardiac and pyloric stomach shrunk after 5–10 d of starvation (Fig. 11), and their brush borders lost their smooth appearance as starvation progressed. Scattered pycnotic nuclei were seen in the pyloric stomach after 10–12 d of starvation (25–27 dph). Mucosal folds of the pyloric stomach flattened and disappeared after 15–16 d of starvation (30–31 dph) (Fig. 9B). At the end of food deprivation period, the smooth muscle fibres separated to form intercellular spaces, and fibroblasts became atrophic or necrotic, resulting in the shrinkage of the stomach submucosa (Fig. 10A). In the cardiac stomach, gastric glands collapsed and their lumen almost disappeared, pycnotic nuclei were

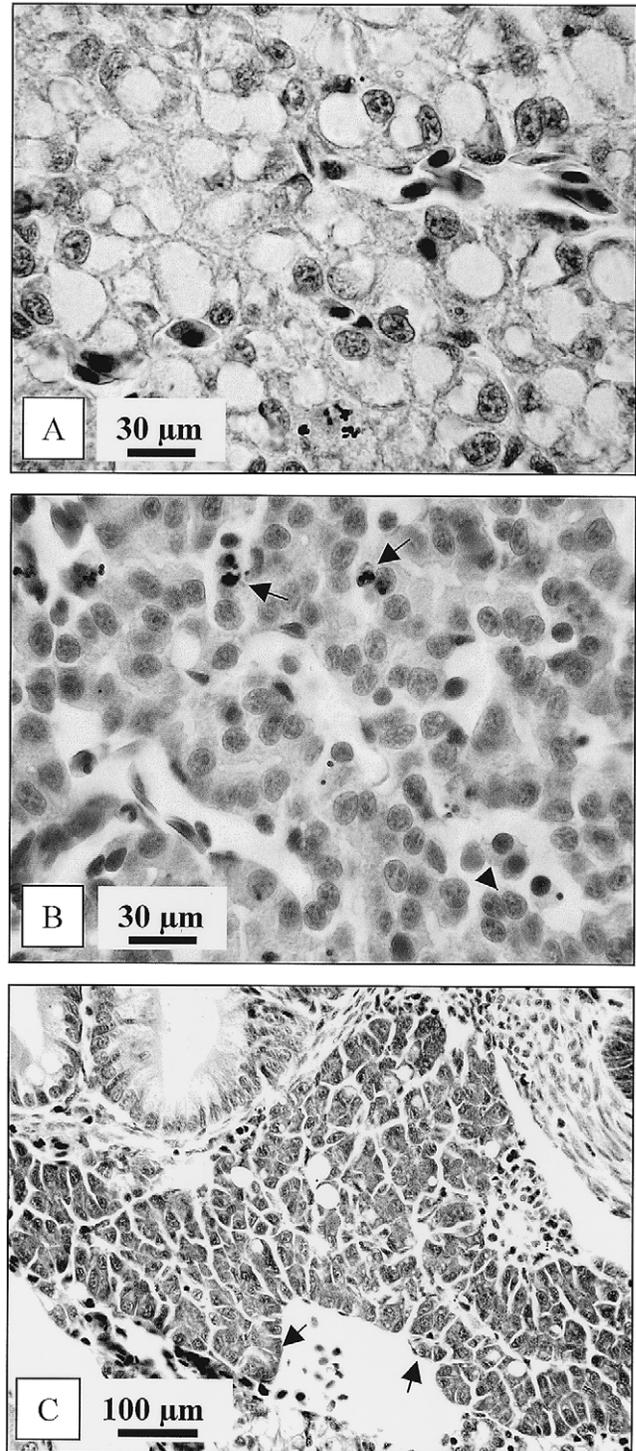


Fig. 8. (A) Liver of a feeding green sturgeon larva at 25 dph, with polygonal hepatocytes arranged along the sinusoids and containing large lipid vacuoles. (B) Liver of a larva starved for 12 d (27 dph); note large intercellular spaces, collapsed cytoplasm (arrow head) and pycnotic nuclei of hepatocytes (arrows). (C) Exocrine pancreas.

seen in the gastric glands after the 13-d starvation period (28 dph) (Fig. 9C). No changes in histochemical properties of the glycoconjugates of the different regions of the stomach were observed throughout the 16-d fasting period.

Table 2  
Summary of histological changes in the digestive system of food-deprived green sturgeon larvae (starvation day 0 = 15 dph)

Starvation days	Buccopharynx	Oesophagus	Stomach	Intestine	Liver	Pancreas
0	Squamous stratified epithelium, taste buds, goblet cells, canine teeth, filiform and fungiform papillae	Differentiated mucosa, submucosa, serosa, secretory, and food transport regions	Compact muscle fibres and connective tissue; gastric glands and epithelial cells with microvilli	Numerous mucosal folds, enterocytes with smooth microvilli; lipidic vacuoles in enterocytes	Polygonal hepatocytes arranged along sinusoids with large lipidic vacuoles and glycogen inclusions	Exocrine pancreatic cells with peripheral nuclei, basophilic cytoplasm, arranged in acini; zymogen granules present
5	Reduction in size and number of dorsal and ventral papillae	Slight decrease in epithelial cells height	Microvilli are separated; no changes in mucosa and submucosa	Disappearance of lipidic vacuoles, decreased height of enterocytes	Lipidic vacuoles disappear, glycogen decreases; hepatocytes with large cytoplasm and peripheral nuclei	Shrinkage of acinar cells and enlarged intercellular spaces
10	Thinning of mucosa, shrinkage of epithelial cells, protrusion of taste buds and teeth	Thinning of mucosa, submucosa, pycnotic nuclei; disarray of microvilli	Pycnotic nuclei in pyloric region; degeneration of connective tissue in submucosa	Flattened mucosal folds and detached microvilli; strong secretion of acid mucosubstances (AB pH 2.5, 1, 0.5 positive)	Glycogen granules disappear, hepatocytes shrink, some with pycnotic nuclei	Acinar cells with darkly pigmented nuclei, condensed cytoplasm; zymogen granules present
15	Resorption of ventral papillae, numerous pycnotic nuclei	Collapsed cytoplasm and numerous pycnotic nuclei in epithelial cells	Collapsed gastric glands, no pyloric folds, disarrayed microvilli, separated muscle fibres	Disappearance of mucosal folds; autolytic processes and epithelial desquamation; melanin plug is present	Large intercellular spaces, collapsed cytoplasm, atrophic tissue	Disarray of acinar structure, tissue degeneration; zymogen granules present

Changes in the intestinal mucosa were seen after 8–10 d of starvation (23–25 dph), including a flattening of folds in the intestine and spiral valve and shrinking of epithelial cells (Fig. 10B). Enterocytes exhibited collapsed hyaline cytoplasm and darkly pigmented elongated nuclei at 10–12 d of starvation (25–27 dph). After 14 d of starvation (29 dph), the brush borders of enterocytes were not smooth and detached from their apical borders. Pycnotic nuclei were also apparent in the intestinal mucosa after 13 d of starvation (28 dph), and desquamation processes of the intestinal mucosa were observed after 16 d (31 dph). In contrast to fed larvae, the intestinal lumen of food-deprived larvae contained a large amount of mucosubstances, mainly acidic mucins (AB pH 2.5, 1.0 and 2.5). Food-deprived larvae had the melanin plug in the spiral valve in the advanced stages of starvation (Fig. 10C).

Lipidic vacuoles and glycogen granules in hepatocytes decreased after only 2 d of starvation (17 dph) and disappeared after 5 d of starvation (20 dph), but cells retained their normal appearance (prominent eosinophilic cytoplasm, polygonal shape, and organization along hepatic sinusoids). Hepatocytes with pycnotic nuclei appeared after 10 d of starvation (25 dph) and increased in number thereafter. The cytoplasm of hepatocytes collapsed and the intercellular spaces increased in size, giving a disordered appearance to the hepatic tissue (Fig. 8B). Food deprivation resulted in a disarray of the acinar structure of the pancreas, acinar cells shrunk, developed darkly pigmented nuclei, condensed cytoplasm, and apical zymogen granules (PAS-positive). At the end of starvation period, the exocrine pancreas lost its acinar organization.

#### 4. Discussion

Anatomically and histologically, the development of the digestive system in larval green sturgeon was similar to other acipenserids (Dettlaff et al., 1993; Gawlicka et al., 1995; Gisbert et al., 1998; Boglione et al., 1999), except for the greater amount of yolk and slightly slower rate of development (Deng et al., 2002). The histological differentiation of the alimentary canal in green sturgeon proceeded from the posterior to anterior, with the spiral valve differentiating at 2 dph and the gastric stomach at 14 dph, just before first feeding. At the onset of exogenous feeding, the general anatomy and histology of larval digestive system was similar to that in juvenile or adult sturgeon species (Dettlaff et al., 1993; Gisbert et al., 1998, 1999). The buccopharynx was lined with a stratified squamous epithelium with numerous fungiform and filiform papillae, epidermal teeth and taste buds. The lumen of the oesophagus was lined with ciliated and mucous cells, with abundant goblet cells secreting neutral and acidic mucosubstances in the anterior region, and the ciliated epithelium performing food transport function in the posterior region. The epithelium of cardiac stomach was composed of cuboidal cells, with numerous simple and tubular gastric glands. The neutral secretory products present in this region of the digestive tract may serve to protect the epithelium of the stomach from auto-digestion processes caused by hydrochloric acid and enzymes produced in gastric glands (Gisbert et al., 1999). The pyloric stomach was lined with a simple columnar ciliated epithelium containing supranuclear vacuoles filled with neutral mucosubstances and organized in folds surrounded by a prominent *tunica muscu-*

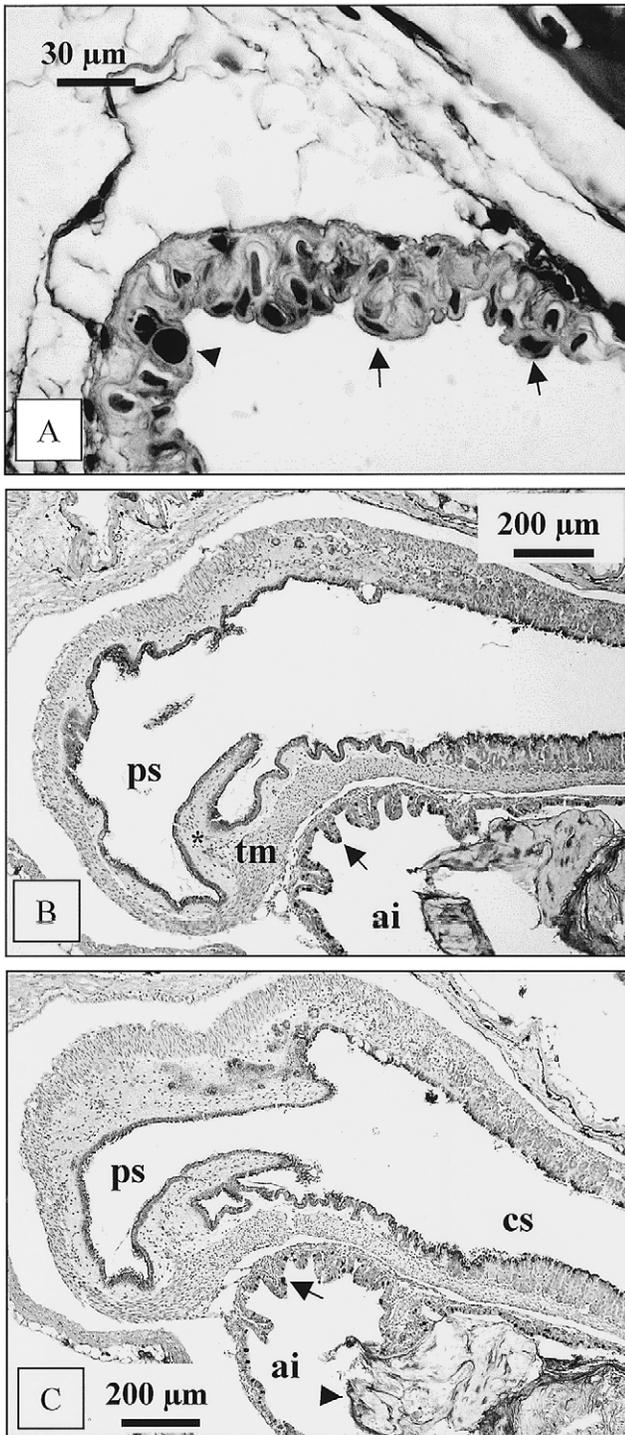


Fig. 9. (A) Resorption of dorsal pharyngeal papillae in a larva starved for 12 d (27 dph); note the presence of functional goblet cells (arrow head) and pycnotic nuclei (arrows) in the buccopharyngeal epithelium. (B) Pyloric stomach of a larva starved for 15 d (30 dph); note the thinning of the mucosa and *tunica muscularis* (tm) and disappearance of pyloric folds. (C) Cardiac stomach (cs) and anterior intestine (ai) of a larva fasted for 13 d (28 dph); note the reduction of folding and thinning of the mucosa (arrow).

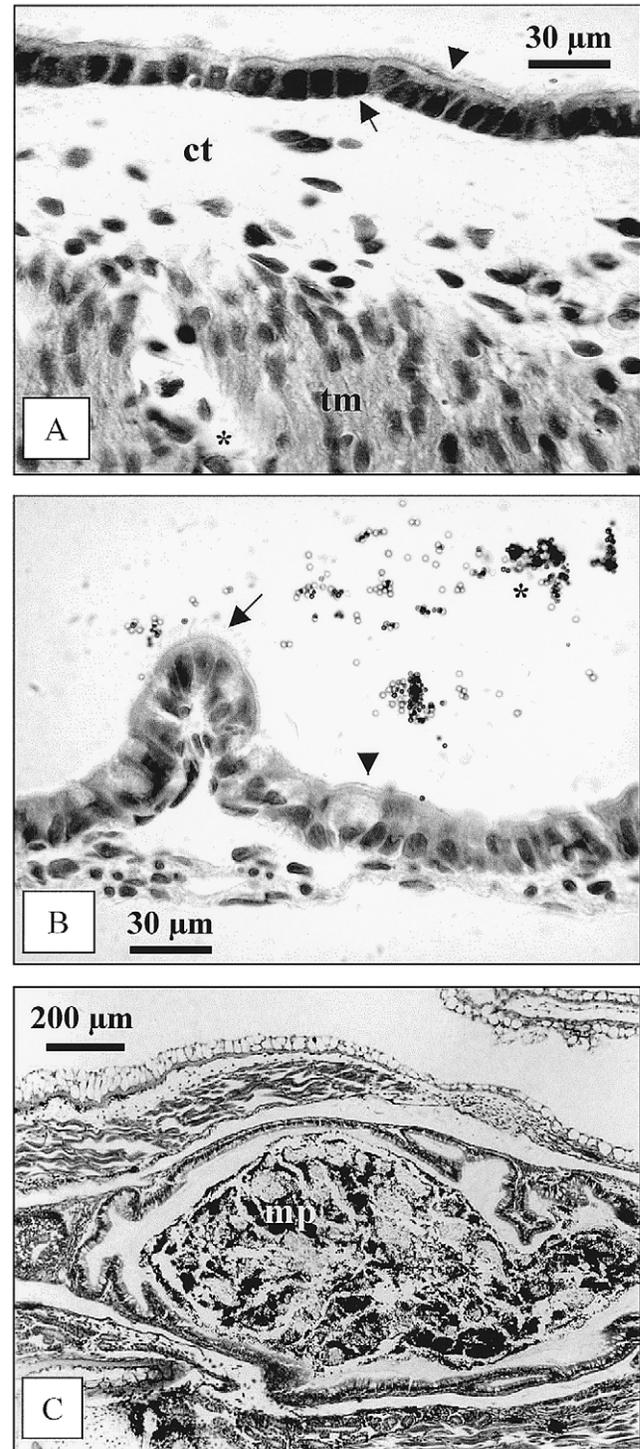


Fig. 10. (A) Details of the pyloric stomach of a larva starved for 12 d (27 dph) showing the enterocytes with a collapsed cytoplasm (arrow) and disarrangement of their brush border (arrow head). Note the onset of separation of muscle fibres (asterisk) in the *tunica muscularis* (tm) and the lax connective tissue (ct). (B) Details of the anterior intestine mucosa of a larva fasted for 13 d (28 dph); note the presence of goblet cells (arrow head), the disarrangement of the enterocytes' brush border (arrow) and the presence of melanin granules (asterisk). (C) Spiral valve of a larva starved for 12 d (27 dph); note the presence of the melanin plug (mp), the thinning of the mucosa and reduction in folding.

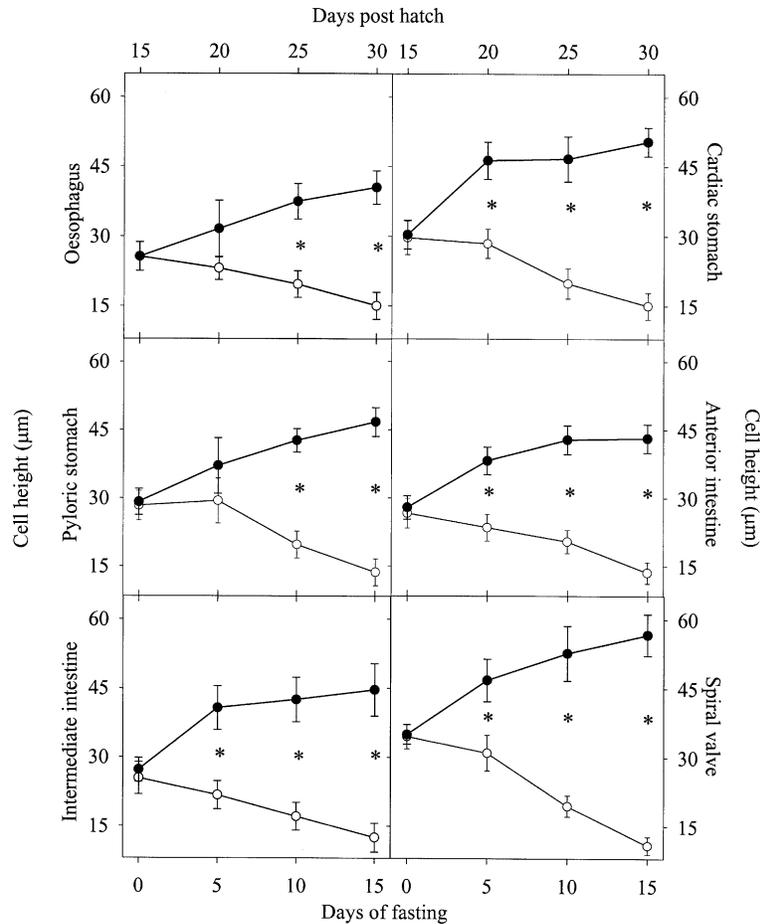


Fig. 11. Height (means  $\pm$  S.D.,  $n = 10$ ) of the epithelial cells in different regions of the digestive tracts of fed (●) and starved (○) green sturgeon larvae. Asterisks indicate significantly different means ( $P < 0.05$ ).

*laris*. The secretion of neutral mucosubstances, in conjunction with thick mucosa of pyloric region, may serve to protect the underlying layers from chemical and physical damages during trituration processes (Gisbert et al., 1998). The histological organization of the intestine was generally similar in different regions, with a simple columnar ciliated epithelium and numerous goblet cells that secreted neutral and acidic mucosubstances. At the onset of feeding, the cells of intestinal mucosa were filled with large lipid vacuoles that gradually disappeared after onset of feeding. The accumulation of lipids in the intestinal mucosa occurring during endogenous feeding phase may explain the ability of sturgeon larvae to survive long periods of food deprivation in laboratory experiments (Gisbert and Williot 1997; Gisbert et al., 1998).

While the green sturgeon larvae stayed alive during a prolonged period of food deprivation, starvation had a marked effect on the histological organization of the digestive system. In the endogenous feeding phase of sturgeon larval development, the proteins and carbohydrates of yolk were utilized for growth and metabolic energy, while lipids stored in the liver and intestinal epithelium served as an energy source for 16–17 dph (Wang et al., 1987; Gisbert et al., 1999). When lipid reserves were exhausted, the body tissues of food-deprived larvae were catabolized, resulting in

a progressive degeneration of the digestive tract and accessory organs. Histopathological changes in the digestive system of food-deprived green sturgeon larvae were similar to those observed in larval teleosts, including: (a) changes in the liver organization, decrease in glycogen and lipids stored in hepatocytes (Margulies, 1993; Green and McCormick, 1999; Crespo et al., 2001); (b) reduction in the height of enterocytes (Margulies, 1993; Bisbal and Bengtson, 1995; Theilacker and Porter, 1995; Green and McCormick, 1999; Gwak et al., 1999), and (c) degeneration of the exocrine pancreatic tissue (Yúfera et al., 1993; Gwak et al., 1999; Crespo et al., 2001).

In teleost species, liver glycogen and lipids are the first energy sources mobilized by fasted larvae (O'Connell and Paloma, 1981). The mobilization of these nutrients under the conditions of continued fasting results in the reduction of energy available to larvae (Green and McCormick, 1999). Similar results that were associated with a moderate to severe deterioration of hepatocytes of green sturgeon larvae starved for 10–15 d were observed under current experimental conditions. Proteolysis of the intestinal mucosa is also an important response to starvation. For this reason, the enterocyte height has been used as a reliable indicator of starvation or sub-optimal feeding in teleosts (Theilacker and Watanabe, 1989; Theilacker and Porter, 1995; Bisbal and Bengtson,

1995; Theilacker et al., 1996; Green and McCormick, 1999). Enterocyte degeneration implies a reduction of the absorption surface area, particularly in the spiral valve, which is the main site of nutrient absorption in sturgeons (Gawlicka et al., 1995; Gisbert et al., 1999). This fact may compromise the digestive capabilities of re-feeding larvae and directly affect their growth and survival. The intestine is not the only region, where proteolytic processes take place during starvation. Degeneration and separation of muscle fibres in the oesophagus and stomach indicate catabolic processes to provide the energy for starving larvae when food supplies are limited (Green and McCormick, 1999). Histopathological changes in digestive mucosa, pancreas and liver, caused by starvation, may also affect food digestion in green sturgeon larvae, which resumed feeding. Pancreatic enzymes appear to be particularly sensitive to food deprivation in teleost larval fish (Zambonino Infante and Cahu, 2001). Gwak et al. (1999) reported the decline of trypsin and amylase activities in starving *Paralichthys olivaceus* to very low levels, which was associated with a reduction of pancreatic volume and partial necrosis of the exocrine pancreas. In our study, we observed a progressive degeneration of exocrine pancreas due to starvation, but zymogen granules were still present in larvae starved for 10–15 d, as it has been described in teleost species (Yúfera et al., 1993). While, the stomach tissues deteriorated progressively with starvation in green sturgeon larvae, the presence of mucosubstances in pyloric region and intestinal lumen seemed to suggest that gastric glands were still secreting pepsinogen and hydrochloric acid (Buddington and Doroshov, 1986), hence the secretion of this mucous may protect the digestive mucosa from auto-digestion.

In conclusion, the histological differentiation of digestive system in green sturgeon followed patterns as reported for other acipenserids. Some chronological differences in development could be related to the rearing temperature and greater reserve of yolk in green sturgeon larvae. Food deprivation resulted in progressive deterioration of the digestive system, with the first pathological signs after 5 d of starvation, followed with severe atrophic changes in the digestive organs after 10–15 d of starvation. The histological analysis of larval digestive system may provide sensitive indicators of the nutritional condition of green sturgeon larvae, and can be used in stock management and studies on nursery habitat affected by water projects.

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