Please note that this is an author-produced PDF of an article accepted for publication following peer review. The definitive version is available on the publisher Web site

Development, Growth & Differentiation
April 2006, Volume 48 Issue 3, Pages 139 - 151
http://dx.doi.org/10.1111/j.1440-169X.2006.00852.x
© 2006 Blackwell Publishing, Inc.

Archimer, archive institutionnelle de l'Ifremer
http://www.ifremer.fr/docelec/

The definitive version is available at www.blackwell-synergy.com

Digestive tract ontogeny of *Dicentrarchus labrax*: Implication in osmoregulation

Ivone Giffard-Mena[1], Guy Charmantier[1], Evelyse Grousset[1], Fabien Aujoulat[1] and René Castille[1, *]

[1] Adaptation Ecophysiologique et Ontogenèse, UMR 5171, GPIA, Université Montpellier II, Place E. Bataillon, CC 092, 34095 Montpellier, Cedex 05, France

*: Corresponding author : R. Castille, email address : castille@univ-montp2.fr

Abstract:

The ontogeny of the digestive tract (DT) and of Na^+/K^+-ATPase localization was investigated during the early postembryonic development (from yolk sac larva to juvenile) of the euryhaline teleost Dicentrarchus labrax reared at two salinities: seawater and diluted seawater. Histology, electron microscopy and immunocytochemistry were used to determine the presence and differentiation of ion transporting cells. At hatching, the DT is an undifferentiated straight tube over the yolk sac. At the mouth opening (day 5), it comprises six segments: buccopharynx, esophagus, stomach, anterior intestine, posterior intestine and rectum, well differentiated at the juvenile stage (day 72). The enterocytes displayed ultrastructural features similar to those of mitochondria-rich cells known to be involved in active ion transport. At hatching, ion transporting cells lining the intestine and the rectum exhibited a Na^+/K^+-ATPase activity which increased mainly after the larva/juvenile (20 mm) metamorphic transition. The immunofluorescence intensity was dependent upon the stage of development of the gut as well as on the histological configuration of the analyzed segment. The appearance and distribution of enteric ionocytes and the implication of the DT in osmoregulation are discussed.

Keywords: enteric ionocyte • fish larva • immunolocalization • Na^+/K^+-ATPase • osmoregulation

1 **Introduction**

2

3 Water absorption and ionic regulation activities enable aquatic animals to adapt
4 to external salinity medium fluctuations. In diluted media, the organism is
5 subjected to water invasion and ion loss. Hyper-osmoregulatory mechanisms
6 compensate these movements, with a low water intake, active absorption of Na^+
7 and Cl^- by the gills, and production of abundant and hypotonic urine by the
8 kidneys which reject water excess and retain most of the filtered electrolytes. In
9 sea-water, hypo-osmoregulatory mechanisms compensate water loss and ionic
10 invasion. Dehydration is avoided by an important drinking rate during which ions
11 and secondarily water are absorbed by the intestine; gills reject the excess of
12 ions (Kirsch *et al.* 1981; Jensen *et al.* 1998; Hawkins *et al.* 2004).

13

14 The sea bass *Dicentrarchus labrax* is a euryhaline marine teleost, able to live
15 within a rather wide salinity range, between fresh water and media where
16 salinity reaches 60‰ (Jensen *et al.* 1998; Varsamos, 2002), although spawning
17 and hatching occur in sea water. Eggs and pre-larvae drift passively towards
18 coastal zones, larvae actively search for low salinity environment and juveniles
19 tend to enter lagoons and estuarine zones (Moustakas *et al.* 2004). At the end
20 of the juvenile period, individuals engage in sporadic and occasional migrations,
21 before swimming offshore and migrating long distances (Jennings & Pawson,
22 1992; Picket & Pawson, 1994). For aquaculture purposes, the species is also

reared in environments of fluctuating salinity conditions (Barnabé *et al.* 1976; Shields, 2001).

Although most osmoregulatory organs are not yet developed, eggs and embryos are able to maintain an osmotic equilibrium towards the environment. In teleost larvae, these organs are less developed than in adults. The regulatory capabilities tend to increase during the post-embryonic development, along with the differentiation of osmoregulatory sites as the tegument, gills, kidney and digestive tract, all of which contain ion-transporting cells or ionocytes (Alderdice, 1988). In the sea bass, the ability to osmoregulate is present at hatching and increases during the larval phase (Varsamos *et al.* 2001), mainly based first on tegumentary ionocytes, then on gill mitochondria-rich cells (MRC) (Varsamos *et al.* 2004), on the urinary apparatus (Nebel *et al.* 2005) and probably on the digestive tract. Yet, the role of the latter in osmoregulation, particularly during larval and juvenile steps, remains a research subject.

The structure of the digestive tract of teleosts varies with different factors. Its functions include digestion, nutriment absorption, hormone secretion, immune protection and water and salt transfers for hydro mineral homeostasis. It regulates energy and material exchanges between the environment and the internal medium. Its structure is also variable according to the nature of the diet. Rather short in carnivorous species (20% of body length), the digestive tract is long in herbivorous fish (20 times the body length) (Buddington & Kuz'mina,

1 2000). It comprises distinct portions, the mouth cavity, the esophagus, the
2 stomach, the anterior and posterior intestine and the rectum, each one playing a
3 role in ion and water regulation (Loretz, 1995; Ando *et al.* 2003).
4
5 With regard to its particular cytological characteristics, the intestinal epithelium,
6 in addition to nutriment absorption, serves also osmoregulatory functions
7 (Ostos-Garrido *et al.* 1993; Abaurrea-Equisoain & Ostos-Garrido, 1996; Verri *et*
8 *al.* 2000; Aoki *et al.* 2003). It accomplishes water and NaCl coupled transports
9 between the lumen and the serosal side, as it has been reported in the
10 saltwater-adapted eel *Anguilla anguilla* (Alves *et al.* 1999; Ando *et al.* 2003).
11 Na^+/K^+-ATPase, basolaterally located in MRCs, is the main enzyme involved in
12 osmoregulation; it provides ionic and electrical gradients for Na^+/K^+ exchange,
13 enabling ion and water transport across the intestine cells, as demonstrated in
14 *Anguilla anguilla*, *A. japonica*, *Pseudopleuronectes americanus*, *Gadus morhua*
15 and *Solea solea* (Loretz, 1995; Cutler *et al.* 1996).
16
17 The characterization and localization of chloride cells have been studied in the
18 tegument, branchial epithelium and urinary system of a variety of adult teleosts,
19 sea bass adults and larvae in particular (review in Varsamos *et al.* 2005).
20 However, despite several in-depth studies on the early development of the
21 digestive tract in the sea bass (Zambonino-Infante & Cahu, 2001), there is a
22 paucity of information concerning the ontogeny of the ion-transporting cells in
23 this organ. The aim of this study is thereof to investigate the development of the

ion-transporting cells of the digestive tract during the ontogeny of *D. labrax*, reared at different salinities. It points out the immunolocalization of Na^+/K^+-ATPase, as well as the appearance and distribution of enteric ionocytes in different sections of the digestive tract during the ontogenesis.

Materials and methods

Animals and rearing conditions

Dicentrarchus labrax young stages were provided by a local fish farm (Poissons du Soleil, Balaruc/Hérault, France). Hatching occurs in full sea water (34 ‰) at a temperature of 15°C. Individuals were collected at different developmental stages, and immediately separated into two series which were progressively conditioned to two different salinity strengths: sea water (SW) (37‰ = 1088 $mOsm.kg^{-1}$) and diluted sea water (DSW) (5‰ = 147 $mOsm.kg^{-1}$) obtained by addition of dechlorinated tap water. The osmotic pressure of the media was measured with a micro-osmometer Model 3300 (Advanced Instruments, Needham Heights, MA, USA). The animals were maintained during 48h before sampling at a temperature of 17°C, with a 12L/12D circadian rhythm. Samples were processed at hatching (day 0 = D0: 3.5 mm), then at 2 (D2: 4 mm), 5 (D5: 5 mm), 29 (D29: 10 mm), 51 (D51: 20 mm) and 72 (D72: 25 mm) days of development, for light microscopy and immunological studies. These stages

were selected according to previous results which showed significant steps in the acquisition of the capacity to osmoregulate (Varsamos *et al.* 2001).

Following the mouth opening (D5), the individuals received *Artemia* nauplii and fine particle fish artificial meal (Gemma/Nutreco Aquaculture, Vervins, Picardie, France), whose diameter was related to the size of the animal (50 to 250 µm from 10 mm, 180 to 400 µm from 20 mm and 315 to 500 µm from 25 mm).

The experiments were conducted according to the French law concerning animal scientific experimentation. All fish were anesthetized using of phenoxy 2 ethanol (150µg/L).

Light microscopy and Na^+/K^+-ATPase immunolocalization

The fish were fixed by immersion into Bouin solution, during 48h. Rinsed with 70% ethanol, the fixed material was dehydrated using increasing grades of ethanol baths (95 and 100 %) and butanol-1, before the treatment with Histochoice clearing agent preparing the tissues to absorb the embedding medium (Paraplast). Histological sections of 4µm obtained with a Leitz Wetzlar microtome, were divided into two alternate series, A and B. A series were stained using the Masson's trichrome method for tissular topography. On B sections, Na^+/K^+-ATPase activity was investigated through immunofluorescence. Sections were mounted on Poly-L-Lysine coated slides

1 for the immunolocalization of Na^+/K^+-ATPase (Varsamos *et al.* 2002a, b). A
2 treatment with 150 mM $NaCl^-$ and 0.01% Tween in 10 mM phosphate buffer
3 solution (PBS), pH 7.3, allowed the permeabilization of the tissues. Aldehyde
4 groups were hidden using 50 mM NH_4Cl in 20 mM PBS, pH 7.3. Non-specific
5 sites were blocked with 1% bovine sero-albumin solution (BSA) and 0.1%
6 gelatin in 20 mM PBS, pH 7.3. The slides, placed in a wet chamber during 2h,
7 were submitted to a mouse monoclonal antibody at 10 µg/mL diluted in BS (1L
8 of 20 mM PBS, pH 7.3, 10g BSA and 0.95g of gelatin) raised against the α-
9 subunit of avian Na^+/K^+-ATPase (mouse anti-chicken IgG α5, Takeyasu *et al.*
10 1988) that cross-reacts with fish tissue (Van Der Heijden *et al.* 1999), and is
11 highly conserved among fish species, including the site for binding fluorescein
12 5' isothiocyanate (FITC) (Schonrock *et al.* 1991). The anti-Na^+/K^+-ATPase
13 antibody, commonly used, was obtained from DSHB (Developmental Studies
14 Hybridoma Bank, University of Iowa, USA). The slides, rinsed with BS in order
15 to remove the excess of primary antibody, were submitted during 1h in dark
16 conditions to a goat-anti-mouse antibody and FITC diluted to 1/200 in PBS-BSA
17 (1L 20 mM PBS, pH 7.3 with 10g BSA). After a final rinsing with BS, the slides
18 were mounted with an aqueous support (Biomedia Co.), and examined with a
19 Leitz Diaplan fluorescence-fitted microscope with the appropriated filter set
20 (450-490 nm). A Leica digital camera adapted to the microscope and a Leica
21 FW4000 I software (Leica Microsystems, Rueil-Malmaison, France) were used
22 to obtain images from the tissues. The fluorescent images were subjected to
23 analysis for the quantification of fluorescence intensity using Optimas version

6.51, image analysis software (MediaCybernetics, Silver Spring, MD, USA). The signal intensity was measured as the fluorescent epithelium surface, compared to that of whole epithelium. Analysis of variance (ANOVA) and student's *t*-test were used for statistical comparisons of the mean values ($p<0.05$) for three animals and three images of each gut segment.

Transmission Electron Microscopy

Two groups of animals at stages D2, D6, D34 (11 mm) and D51 were maintained during 48h in SW and DSW before sampling for transmission electronic microscopy (TEM). In addition, a second experiment was run with 44 days-young juveniles (D44: 15 mm) conditioned during 10 days to SW and DSW.

Lethargized D2 and D6 individuals were processed entirely, while D34, following a short fixation and death, were transversely divided into four pieces; the dissection of the digestive tract was possible in D44 and D58 animals. Samples were doubly fixed at 4°C (320 mOsm.kg^{-1}, pH 7.2). The pre-fixation was carried out in a mixture (1:1, v/v) of 4% glutaraldehyde in 0.1 M sodium cacodylate buffer for 18 h. The post-fixation was performed in a mixture (1:1, v/v) of 1% osmium tetraoxide and 0.1 M sodium cacodylate buffer for 2 h at 4°C. Ethanol-dehydrated samples where embedded in EPON 812. Ultra-thin sections (50-150 nm) of the anterior intestine were contrasted with uranyl

acetate and lead citrate prior to examination on a JEOL 1200 EX transmission electron microscope at 70 kV. Semi-thin sections (0.5-1 µm) stained with toluidine blue were used for light microscopy studies.

Results

Digestive tract ontogenesis

At day 0 (D0), the digestive tract (DT) of newly-hatched larvae is observed above the yolk-sac; pancreas and liver sketches appear surrounding a bulky vitellin vesicle (Fig. 1A). The DT is a tube made of an undifferentiated single cellular layer, opened at the posterior end only (Fig. 1B), running parallel to urinary tubules.

At D2, two DT parts are observed. The anterior part includes the mouth cavity, the pharynx, the esophagus and the developing stomach which begins to extend (Fig. 1C). Urinary tubules are located between the chord and the digestive tract. The posterior part comprises the intestine (anterior and posterior) and the rectum where cells contain an ovoid irregular predominantly basal nucleus (Fig. 1D). The anterior intestine is lined by a single cellular coat with large nuclei and a well developed apical brush-border including ciliated structures (Fig. 1E). The cytoplasm of enteric ionocytes contains a number of oval-round shaped mitochondria and free ribosomes; the endoplasmic reticulum

is well developed, junctional complexes join the cells. The 48h acclimation period to the different experimental salinities had no marked effect on this configuration.

At D5, the DT, opened at the mouth end (Fig. 1F), increases in length and diameter, showing bends and markedly differentiated regions; the yolk vesicle is not completely resorbed and the esophagus exhibits a two-layer folded epithelium, including interspaced goblet cells (mucocytes) (Fig. 2A). These are lacking in the stomach mucosa, which is characterized by a number of “U” profiled folds (Fig. 2B); the diameter of the anterior intestine is smaller than that of the posterior region. A valvule appears at the transition between the stomach and the anterior intestine whose epithelium is also lacking goblet cells. The epithelium of the posterior intestine is a folded single layer, deprived of mucocytes and showing an apical brush-border. A second valvule is located at its junction with the rectum, whose inner stratified layer shows long folded walls. Epithelial cells show a well developed apical brush-border, a central long polymorphic nucleus, free ribosomes, and several mitochondria with noticeable crests; a well developed tubular system occurs at the apical region (Fig. 2C). Lamellar structures surround the mitochondria located at the basal side (Fig. 2D). This configuration is observed in both SW and DSW-acclimated animals.

Among the modifications affecting the young fish within the interval D7-D29, the exhaustion of the yolk and the transition to exotrophy are key events. At D29,

1 the DT shows well defined distinct zones. The mouth cavity contains a
2 number of small mucocytes, branchial arches and developing teeth. The
3 esophagus exhibits an apparent muscular coat, a *lamina propria* and several
4 large epithelial mucocytes (Fig. 2E). The stomach mucosa is thick, mucocytes
5 are not observed; pancreas and liver are more evident as well as the forming
6 swimming-bladder. Small mucocytes occur in the anterior intestine as well as in
7 the rectum (Fig. 2F). Enterocytes of D34 larvae, higher than those reported in
8 precedent stages, have an ovoid nucleus and a ribosome-rich cytoplasm.
9 Junction complexes, tubular system, and microvilli are better developed than in
10 previous stages; large mitochondria are located at the basal side (Fig. 3A),
11 associated with lamellar structures (Fig. 3B). No difference was observed after
12 2 days of acclimation (SW and DSW).
13
14 Tissular layers differentiated as muscular layer, *lamina propria* and mucosa
15 characterize the DT at D51. The mouth contains developed teeth; large
16 mucocytes are observed in the esophagus but not in the stomach. The anterior
17 intestinal epithelium is a single layer of apical brush-border columnar cells and
18 few goblet cells; the posterior section is still undeveloped (Fig. 3C, D). The
19 rectum shows also a luminal brush-border, and few mucocytes. Enterocytes of
20 D51 young juveniles acclimated to SW and DSW show numerous mitochondria;
21 different other cell types (including mucocytes and digestive endocrine cells) are
22 observable. The Golgi apparatus as well as the rough endoplasmic reticulum is
23 well developed. The apical mitochondria, more numerous, show low developed

1 crests; at the basal side, the lamellar structures surrounding mitochondria are
2 well developed in both experimental media (Fig. 3E).
3
4 Cytological differences between individuals exposed to the two experimental
5 media were found only after 10 days of acclimation. The density of the tubular
6 system, the number of microbodies and of mitochondria were higher in SW than
7 in DSW-acclimated individuals (Fig. 4A, B). Mitochondria were lacking in the
8 area just below the brush border where numerous small densely packed
9 vesicles formed a tubule-vesicular system underneath the apical membrane. In
10 SW, this area appeared narrower than in DSW (Fig. 5A, B). SW enterocytes
11 exhibited an extensive smooth-surfaced tubular system forming a network from
12 the basal to the apical part of the cell; lamellar structures surrounding basal
13 mitochondria were also reported (Fig. 5C). This system was less developed in
14 DSW (Fig. 5D).
15
16 At D72, the increase in size and the appearance of gastric glands represent the
17 main morphological modifications of the digestive tract (Fig. 6A).
18
19 *Na^+ /K^+ -ATPase immunolocalization*
20
21 No fluorescence was observed in control sections deprived of primary antibody
22 (not illustrated). At D0, the enzyme detected in the posterior section of the gut
23 showed an immunocytochemical response higher than that observed in the

urinary tubules. Fluorescence was evident at the basolateral side of the intestinal epithelial cells; some fluorescent tegumentary ionocytes were also observed (Fig. 6B).

At D2, an increased fluorescence response was systematically observed in the posterior section of the DT (Fig. 6C); tegumentary ionocytes and urinary tubules were also fluorescent in both experimental media, while mouth and esophagus were not. The developing pyloric region, close to the stomach, appeared weakly fluorescent in both SW and DSW media. Similar observations were made in D5 samples.

Some few epithelial cells occurring in the mouth cavity and in the esophagus were fluorescent at D29 (Fig. 6D). In SW the ATPase response observed in both anterior and posterior intestine (Fig. 7A, B) was higher than that recorded in the rectum (Fig.7C), as suggested by the intensity of fluorescence at the epithelium basal side. On the other hand, anterior and posterior acclimated intestine showed a higher immunoreaction in SW than in DSW. In DSW, the fluorescence observed in the anterior intestine was higher than that of the rectum (Fig. 7D, E, F).

An increased number of fluorescent cells were observed in the mouth cavity (Fig. 8A) and in the esophagus of D51 fish; however their number declined near the stomach. Moderate fluorescence intensity was observed at the basolateral

1 compartment of the pyloric region and in the forming pyloric caeca (Fig. 8B).
2 Na^{+}/K^{+}-ATPase labeling showed by the anterior intestine in SW was higher than
3 that recorded for the posterior sections (posterior intestine and rectum) (Fig. 8C,
4 D, E). In DSW the anterior intestine exhibited a response higher than that of the
5 rectum (Fig. 8F, G, H).

6

7 At D72, the number of fluorescent cells in the mouth cavity and in the
8 esophagus increased, particularly in SW-acclimated individuals; the amount of
9 isolated ionocytes decreased in the vicinity of the stomach. The gastric mucosa
10 appeared fluorescent in both SW and DSW (Fig. 8I). As observed for previous
11 stages, the enzyme labelling in the intestine was primarily located in the
12 basolateral region of the enterocytes.

13

14 The results of quantification for Na^{+}/K^{+}-ATPase in D29 larva show a significantly
15 higher intensity in the anterior and posterior intestine in SW compared to DSW.
16 While the intensity is lower in the rectum and it does not change with salinity
17 (Fig. 9). In SW D51 young juvenile, Na^{+}/K^{+}-ATPase intensity is significantly
18 different in each gut segment, with the highest value in the anterior intestine,
19 and decreasing values in the posterior intestine and the rectum (Fig. 10). In
20 DSW, no difference was found between the anterior and posterior intestine, with
21 the rectum, displaying the lowest fluorescence.

22

Discussion

The sea-bass osmoregulatory capability increases along the development (Varsamos *et al.* 2001). Under natural conditions in coastal zones, the sea bass early development occurs in a range of salinity well-fitted for the limited osmoregulatory abilities of the larvae (Dendrinos & Thorpe, 1985; Johnson & Katavic, 1986; Saillant *et al.* 2003; Varsamos *et al.* 2005). At the beginning of the juvenile phase at about 20 mm in length, migratory movements to lagoons and estuaries (low salinity sites) are reported to start (Kelley, 1988; Saillant *et al.* 2003). This change in behavior is very likely related to the enhancement in osmoregulatory capabilities occurring at this stage (Varsamos *et al.* 2001), based itself on the ontogeny of different osmoregulatory sites (Varsamos *et al.* 2004, 2005; Nebel *et al.* 2005) including the digestive tract. In teleosts, the development of the digestive tract starts early, followed by progressive differentiation steps during which nutriment absorption is linked to osmoregulation (Zambonino-Infante & Cahu, 2001). In addition to the description of the digestive tract, the chronology of events related to the ontogeny of osmoregulation most be considered when establishing sequential appearance and distribution of ionocytes.

In early larvae (D0-D2), when the mouth is still closed and the gills are not developed, the digestive tract is represented by a straight tubular structure open only at the posterior end. At this stage, feeding depends upon the yolk vesicle

1 (Mani-Ponset *et al.* 1995) which persists beyond the mouth opening (D5) until
2 the end of endotrophy (D7). The observed epithelial ciliated structures suggest
3 a motion activity in a forming digestive tract devoid of peristaltic contractions
4 (Tytler *et al.* 1990). No cilia have been observed in later stages. The
5 appearance of mucocytes in the anterior intestine may be related to the
6 digestion of ingested food, since mucus may also act as an enzyme support,
7 and not only as a lubricant (Vu, 1980). However, it does not represent a barrier
8 preventing water diffusion, being moreover an ion exchanger interface
9 (Varsamos *et al.* 2005). Structures typical of the adult gut do not appear before
10 D71. Our observations are in agreement with other in the sea-bass (Vu, 1976,
11 1980; Connes & Benhalima, 1984; García-Hernández *et al.* 2001).
12
13 These results indicate that Na^+/K^+-ATPase occurs at hatching in the intestine
14 and rectum, starting later (D29) in the mouth cavity and in the esophagus. The
15 quantification of fluorescence intensity corroborates the histochemical
16 observations. These reveal high levels of fluorescence in the anterior and
17 posterior intestine of SW-acclimated young juveniles and the highest intensity of
18 the protein in the anterior intestine in juveniles, thus indicating the progressive
19 involvement of this organ in ion transport and potentially in hydro-mineral
20 regulation. From D0, fluorescence was located in the baso-lateral compartment
21 of both intestinal and rectal epithelial cells, as reported in the trout (Seidelin *et*
22 *al.* 1999). Such a distribution is consistent with the models already proposed in
23 SW-adapted fish for the intestine concerning the flux of Na^+ from the lumen to

1 the intercellular compartment and the serosa, creating thereby a favorable
2 gradient for Na^+ influx at the apical membrane, followed by water absorption
3 (Gibson *et al.* 1987; Loretz, 1995; Marshall & Bryson, 1998; Schettino &
4 Lionetto, 2003; Trischitta *et al.* 2004). The abundance of fluorescence along the
5 intestinal brush-border suggests a function restricted to water and nutriment
6 absorption (Buddington & Kuz'mina, 2000; Aoki *et al.* 2003).
7
8 The lack of fluorescence in the mouth and esophagus during the early
9 development (D5) may be linked to the fact that during this period,
10 osmoregulation is accomplished by tegumentary ionocytes whose numbers
11 decrease at D29 (Varsamos *et al.* 2002b, 2005), whereas this type of cells
12 begins to appear in other osmoregulatory sites, most notably along the gills
13 (Varsamos *et al.* 2002a and b). This situation corresponds to the starting point
14 of a low osmoregulatory activity in the esophagus. Moreover, it suggests that
15 ingested water is probably desalted in the esophagus by both passive diffusion
16 and limited ionic active transport, and then absorbed by the intestine following
17 an active ionic transfer (Kirsch & Laurent, 1975; Venturini *et al.* 1992; García-
18 Hernández *et al.* 2001; Ando *et al.* 2003; Aoki *et al.* 2003). Although the
19 immunofluorescence intensity observed in the stomach at D2 is feeble
20 compared to that of other sections of the gut, it suggests an ionic regulation
21 persisting until the juvenile status, at least in certain sections of the organ. The
22 osmoregulatory role of the stomach probably precedes the installation of the
23 digestive function, since mature gastric glands do not appear before D72. In

1 addition, the fluorescence in the digestive tract at D0, and also 24 hours
2 before hatching, indicates that osmoregulation is closely related to nutriment
3 absorption (Noaillac-Depeyre & Gas, 1973) because the ionocytes are well
4 developed in the digestive tract, appearing intensely immunoreactive to the
5 Na^+/K^+-ATPase antibody. The progressive development of such a capability
6 could explain the transposition of chloride cell functionality from the skin to the
7 gills and kidney (review in Varsamos *et al.* 2005) and to the gut.
8
9 The ATPase labelling observed in the pyloric caeca at the larva-juvenile
10 transition, also reported for the trout *Oncorhynchus mikiss* (Abaurrea-Equisoain
11 & Ostos-Garrido, 1996), and the brown trout *Salmo trutta* (Seidelin *et al.* 1999),
12 indicates ion transport occurring at this level. The intense labelled signal
13 observed for the intestine inner wall at the juvenile stage in SW indicates the
14 prominent role of the enzyme in ion absorption, accompanied probably by water
15 uptake from the lumen, very likely facilitated by the occurrence of aquaporins as
16 indicated in the american sea-bass *M. saxatilis* (Madsen *et al.* 1994), the trout
17 *S. trutta* (Seidelin *et al.* 1999; Fuentes *et al.* 1997) and the eel *A. anguilla*
18 (Cutler & Cramb, 2001; Lignot *et al.* 2002; Aoki *et al.* 2003). The response
19 intensity depends upon the stage of development of the gut as well as on the
20 histological nature of the analyzed section. Considering the intensity of
21 fluorescence, the anterior intestinal portion appears more particularly involved in
22 ion absorption because it develops early within a period of 25 to 30 days
23 following hatch.

1

2 The general aspect of enterocytes is characterized by regular microvilli, a well
3 developed tubular network, an endoplasmic reticulum and numerous
4 mitochondria whose shape and number change in relation to the developmental
5 status of the fish and the salinity. Similar ontogenetical changes in gill chloride
6 cells have been reported (review in Varsamos *et al.* 2005). The abundance of
7 basal mitochondria associated with a developed tubular system is clearly
8 related to ion transport. Morphological modifications appear following changes
9 in salinity. In SW the tubule-vesicular system is more developed than following
10 an acclimatation to DSW. Microbodies and mitochondria are abundant, whereas
11 the sub apical part of the cell is less developed. All these changes may be
12 interpreted as an increase of the exchange area enabling ion and water
13 transport across the epithelium. The abundance of mitochondria suggests
14 enhanced cellular activity to produce ATP, providing thereof the substrate for
15 Na^+/K^+-ATPase activity, followed by the accumulation of sodium ions in the
16 tubular system as well as in the basolateral spaces between intestinal columnar
17 cells. Chloride ions would diffuse down their electrochemical gradient from the
18 cell to these local regions of high sodium concentration, creating an osmotic
19 gradient enabling water to diffuse from the lumen into fish tissues (Cutler *et al.*
20 1996).

21

22 The zone deprived of mitochondria, located just beneath the brush border, is
23 reminiscent of a similar area in gill chloride cells and it has already been

hypothesized as a transient communication channel between the internal and external environment (Sardet *et al.* 1979). The difference in thickness of this zone, between SW and DSW adapted material, could to some extent be involved into the osmoregulatory capabilities of the cell.

Regulation of the drinking rate occurs after the mouth opening (D5), but it seems likely that water absorption in the gut occurs earlier, and osmoregulatory performances increase within the interval D18 to D51 (Varsamos *et al.* 2001, 2005). A significant increase of the osmoregulatory capacity occurs at the larva-juvenile transitional step (D63), since blood osmolality is strictly regulated 63 to 86 days after hatching (17 to 26 mm in length) (Varsamos *et al.* 2001, 2004, 2005; Nebel *et al.* 2005). In SW conditions, drinking rates increase (Varsamos *et al.* 2004, 2005) in order to compensate chronic water loss. The increment of osmotic water permeability of the intestine, two to six folds that reported in other species, following SW transfer (Lignot *et al.* 2002; Aoki *et al.* 2003), could be in relation with the high development of the tubule-vesicular system.

During the post-embryonic period the digestive tract regulates the metabolic responses to environmental fluctuations like salinity variations. Its implication in osmoregulation increases from D29 (10 mm), mainly from the larva/juvenile (20 mm) metamorphic transition; this increase in euryhalinity enables the individual to tolerate drastic salinity changes. The capacity of the European sea bass to osmoregulate originates from a synergy of the gills, the urinary system and the

1 digestive tract, well developed in juveniles. From an ecological point of view,
2 this capacity is essential for the migratory movements into estuaries and
3 lagoons, accomplished at some precise moments of the development. The
4 involvement of the digestive tract in osmoregulation results from its anatomical
5 as well as functional changes. From these results, one may assert that starting
6 from D0, Na^+/K^+-ATPase occurs at the baso-lateral side of the enteric
7 ionocytes, in the intestine and the rectum of *D. labrax*, probably in relation to
8 both nutrient absorption and osmoregulation. The location of the enzyme is
9 consistent with the flux of ions and water, already proposed for the gut of
10 marine adult teleosts.
11
12 **Acknowledgements**

13 IGM is very indebted to CONACyT for the allocation of a grant, to Dr. Mireille
14 Charmantier for electronic microscopy advisory, to Dr. Catherine Nebel for
15 valuable suggestions, to Dr. François Bonhomme, Director of the SMEL
16 biological station of Sète for laboratory facilities and to “Poissons du Soleil” fish
17 hatchery for providing the living material.

1 **References**

2 Abaurrea-Equisoain, M. A. & Ostos-Garrido, M. V. 1996. Enterocytes in the
3 anterior intestine of *Oncorhynchus mykiss*: cytological characteristics
4 related to osmoregulation. *Aquaculture* **139**, 109-116.

5 Alderdice, D. F. 1998. Osmotic and ionic regulation in teleost eggs and larvae.
6 In *Fish physiology*, Vol. XIA (Eds. W. S. Hoar & D. J. Randall), pp. 163-
7 251. Academic Press, New York.

8 Alves, P., Soveral, G., Macey, R. I. & Moura, T. F. 1999. Kinetics of water
9 transport in eel intestinal vesicles. *J. Membr. Biol*. **171**,177-182.

10 Ando, M., Mukuda, T. & Kozaka, T. 2003. Water metabolism in the eel
11 acclimated to sea water: from mouth to intestine. *Comp. Biochem. Physiol.*
12 **136B,** 621-633.

13 Aoki, M., Kaneko, T., Katoh, F., Hasegawa, S., Tsutsui, N. & Aida, K. 2003.
14 Intestinal water absorption through aquaporin 1 expressed on the apical
15 membrane of mucosal epithelial cells in sea water-adapted Japanesee eel.
16 *J. Expl. Biol*. **206**, 3495-3505.

17 Barnabé, G., Boulineau-Coatanea, F. & René, F. 1976. Chronologie de la
18 morphogenèse chez le loup ou bar *Dicentrarchus labrax* (L.) (Pisces,
19 Serranidae) obtenu par reproduction artificielle. *Aquaculture* **8**, 351-363.

1 Buddington, R. K. & Kuz'mina, V. 2000. Digestive system. In *The Laboratory*
2 *Fish* (Ed. G. K. Ostrande), pp. 379-384. Academic Press, New York.

3 Connes, R. & Benhalima, K. 1984. Ultrastructure de l'intestin du loup
4 *Dicentrarchus labrax* L. au cours du développement larvaire. *Bull. Soc.*
5 *Zool. Fr.* **109**, 19-33.

6 Cutler, C. P., Sanders, I. A., Luke, G., Hazon, N. & Cramb, G. 1996. Ion
7 transport in teleosts: identification and expression of ion transporting
8 proteins in the branchial and intestinal epithelia of the European eel. In
9 *Gene Expression and Manipulation in Aquatic Organisms*, Vol. 58 (Eds.
10 S.J. Ennion & G. Goldspink), pp. 43-74. *Soc. Exp. Biol.* Seminar Series.

11 Cutler, C. P. & Cramb, G. 2001. Molecular physiology of osmoregulation in eels
12 and other teleosts: the role of transporter isoforms and gene duplication.
13 *Comp. Biochem. Physiol.* **130A**, 551-564.

14 Dendrinos, P. & Thorpe, P. J. 1985. Effects of reduced salinity on growth and
15 body composition in the European bass *Dicentrarchus labrax* (L.).
16 *Aquaculture* **49**, 333-358.

17 Fuentes, J., Soengas, J. L., Rey, P. & Rebolledo, E. 1997. Progressive transfer
18 to seawater enhances intestinal and branchial Na^+/K^+-ATPase activity in
19 non-anadromous rainbow trout. *Aquaculture International* **5**, 217-227.

1 García-Hernández, M. P., Lozano, M. T., Elbal, M. T. & Agulleiro, B. 2001.
2 Development of the digestive tract of sea bass *Dicentrarchus labrax* L.
3 Light and electron microscopic studies. *Anat. Embryol.* **204**, 39-57.

4 Gibson, J. S., Ellory, J. C. & Lahlou, B. 1987. Salinity acclimation and intestinal
5 salt transport in the flounder: the role of the basolateral cell membrane. *J.*
6 *Exp. Biol.* **128**, 371-382.

7 Hawkins, G. S., Galvez, F. & Goss, G. 2004. Seawater acclimation causes
8 independent alterations in Na^+/K^+- and H^+-ATPase activity in isolated
9 mitochondria-rich cell subtypes of the rainbow trout gill. *J. Exp. Biol.* **207**,
10 905-912.

11 Jensen, M. K., Madsen, S. S. & Kristiansen, K. 1998. Osmoregulation and
12 salinity effects on the expression and activity of Na^+K^+-ATPase in the gills
13 of European sea bass *Dicentrarchus labrax* (L). *J. Exp. Zool.* **282**, 290-
14 300.

15 Jennings, S. & Pawson, M. G. 1992. The origin and recruitment of bass,
16 *Dicentrarchus labrax*, larvae to nursery areas. *J. Mar. Biol. Assoc. UK.* **72**,
17 199-212.

18 Johnson, D. W. & Katavi, I. 1986. Survival and growth of sea-bass
19 *Dicentrarchus labrax* larvae as influenced by temperature, salinity and
20 delayed initial feeding. *Aquaculture* **52**, 11-19.

1 Kelley, D. F. 1988. The importance of estuaries for sea-bass, *Dicentrarchus*
2 *labrax* (L.). *J. Fish. Biol.* **33**, 25-33.

3 Kirsch, R. & Laurent, P. 1975. L'oesophage, organe effecteur de
4 l'osmorégulation chez un téléostéen euryhalin, l'anguille *Anguilla anguilla*.
5 *C.R. Acad. Sc. Paris* **280**, 2013-2015.

6 Kirsch, R., Meens, R. & Meister, M. F. 1981. Osmorégulation chez les
7 téléostéens marins: rôle des branchies et du tube digestif. *Bull. Soc. Zool.*
8 *Fr.* **106**, 31-37.

9 Lignot, J. H., Cutler, C. P., Hazon, N. & Cramb, G. 2002. Immunolocalisation of
10 aquaporin 3 in the gill and the gastrointestinal tract of the European eel
11 *Anguilla anguilla*. *J. Exp. Biol.* **205**, 2653-2663.

12 Loretz, A. C. 1995. Electrophysiology of ion transport in teleost intestinal cells.
13 In *Cellular and molecular approaches to fish ionic regulation*, (Eds. C. M.
14 Wood & T. J. W. Shuttle), pp. 25-56. Academic Press. London.

15 Loretz, A. L. & Pollina, C. 2000. Natriuretic peptides in fish physiology. *Comp.*
16 *Biochem. Physiol.* **125A**, 169-187.

17 Madsen, S. S., McCormick, S. D., Young, G., Endersen, J. S., Nishioka, R. S. &
18 Bern, H. A. 1994. Physiology of seawater acclimation in the striped bass,
19 *Morone saxatilis* (Walbaum). *Fish Physiol. Biochem.* **13**, 1-11.

1 Mani-Ponset, L., Guyot, E., Diaz, J. P. & Connes, R. 1996. Utilization of yolk
2 reserves during post-embryonic development in three teleostean species:
3 the sea-bream *Sparus aurata*, the sea-bass *Dicentrarchus labrax* and the
4 pike perch *Stizostedion lucioperca*. *Mar. Biol.* **126**, 539-447.

5 Marsall. W. S. & Bryson, S. E. 1998. Transport mechanisms of teleost chloride
6 cells: an exclusive model of a multi-functional cell. *Comp. Biochem.*
7 *Physiol.* **119**, 97-106.

8 Moustakas, C. T., Watanabe, O. W. & Copeland, K. A. 2004. Combined effects
9 of photoperiod and salinity on growth, survival and osmoregulatory ability
10 of larval southern flounder *Paralichthys lethostigma*. *Aquaculture* **229**, 159-
11 179.

12 Nebel, C., Nègre-Sadargues, G., Blasco, C. & Charmantier, G. 2005. Morpho-
13 functional ontogeny of the excretory system of the European sea-bass
14 *Dicentrarchus labrax*. *Anat. Embryol.* **209**, 193-206.

15 Noaillac-Depeyre, J. & Gas, N. 1973. Mise en évidence d'une zone adaptée au
16 transport des ions dans l'intestin de la carpe commune *Cyprinus carpio*.
17 *C.R. Acad Sci. Paris* **280D**, 2013-2015.

18 Ostos-Garrido, M. V., Nunez-Torres, M. I. & Abaurrea-Equisoain, M. A. 1993.
19 Lipid absorption by enterocytes of the rainbow trout, *Oncorhynchus*
20 *mykiss*: diet induced changes in the endomembranous system.
21 *Aquaculture* **110**, 161-171.

1 Picket, G. D. & Pawson, M. G. 1994. Sea bass: biology, exploitation and
2 conservation. In *Fish and Fisheries Series*, Vol. 12 (Ed. T. J. Pitcher), pp.
3 1-235. Chapman and Hall, London.

4 Saillant, E., Fostier, A., Haffra, P., *et al.* 2003. Effects of rearing density, size
5 grading and parental factors on sex ratios of the sea-bass (*Dicentrarchus*
6 *labrax* L.) in intensive aquaculture. *Aquaculture* **221**, 183-206.

7 Sardet, C., Pisam, M. & Maetz, J. 1979. The surface epithelium of teleostean
8 fish gills. Cellular and functional adaptations of the chloride cell in relation
9 to salt adaptation. *J. Cell. Biol.* **80**, 96-117.

10 Schettino, T. & Lionetto, M. G. 2003. Cl^- absorption in European Eel intestine
11 and its regulation. *J. Exp. Zool.* **300A**, 63-68.

12 Schonrock C., Morley S.D., Okawara Y., Lederis K. & Richter D. 1991. Sodium
13 and potassium ATPase of the teleost fish *Catostomus commensoni.*
14 Sequence, protein structure and evolutionary conservation of the alpha-
15 subunit. *Biol. Chem.* **372,** 279-286.

16 Seidelin, M., Madsen, S. S., Byrialsen, A. & Kristiansen, K. 1999. Effects of
17 Insulin-like Growth Factor-I and Cortisol on Na^+, K^+-ATPase expression in
18 osmoregulatory tissues of brown trout (*Salmo trutta*). *Gen. Comp.*
19 *Endocrinol.* **113**, 331-342.

1 Shields, R. J. 2001. Larviculture of marine finfish in Europe. *Aquaculture* **200**,
2 55-88.

3 Takei, Y. 1993. Role of peptide hormones in fish osmoregulation. In *Fish*
4 *Ecophysiology* (Eds. Rankin and Jensen), pp. 136-159. Chapman and
5 Hall, London.

6 Takeyasu K., Tamkun M.M., Renaud K.J. & Fambourg D.M. 1988. Ouabain
7 sensitive (Na^{+}+K^{+})-ATPase activity expressed in mouse L cells by
8 transfection with DNA encoding the alpha-subunit of avian sodium pump.
9 *J. Biol. Chem.* **25**, 4347-4354.

10 Trischitta, F., Denaro, M. G. & Faggio, C. 2004. Ion transport in the intestine of
11 *Gobius niger* in both isotonic and hypotonic conditions. *J. Exp. Zool.* **301A**,
12 49-62.

13 Tytler, P., Tatner, M. & Findlay, C. 1990. The ontogeny of drinking in the
14 rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Fish Biol.* **36**, 867-875.

15 Van Der Heijden, A. J. H., Van Der Meij, J. C. A., Flik, G. & Wendelaar-Bonga,
16 S. E. 1999. Ultrastructure and distribution dynamics of chloride cells in
17 tilapia larvae in fresh-water and sea-water. *Cell. Tissue Res.* **297**, 119-130.

18 Varsamos, S., Conne,s R., Diaz, J-P., Barnabé, G. & Charmantier, G. 2001.
19 Ontogeny of osmoregulation in the European sea bass *Dicentrarchus*
20 *labrax. Mar. Biol.* **138**, 909-915.

1 Varsamos, S. 2002 Tolerance range and osmoregulation in hypersaline
2 conditions in the european sea bass *Dicentrarchus labra*x. L. *Mar. Biol.*
3 **138**, 909-915.

4 Varsamos, S., Diaz, J-P., Charmantier, G., Flik, G., Blasco, C. & Connes, R.
5 2002a. Branchial chloride cells in sea bass *Dicentrarchus labrax* adapted
6 to freshwater, seawater and doubly concentrated seawater. *J. Exp. Zool.*
7 **293**, 12-26.

8 Varsamos, S., Diaz, J-P., Charmantier, G., Blasco, C., Connes, R. & Flik, G.
9 2002b. Location and morphology of chloride cells during the post-
10 embryonic development of the European sea bass *Dicentrarchus labra*x.
11 *Anat. Embryol.* **205**, 203-213.

12 Varsamos, S., Wendelaar-Bonga, S. E., Charmantier, G. & Flik, G. 2004.
13 Drinking and Na^+/K^+-ATPase activity during early development of
14 European Sea Bass, *Dicentrarchus labrax* ontogeny and short-term
15 regulation following acute salinity changes. *J. Exp. Mar. Biol. Ecol.*
16 **311**,189-200.

17 Varsamos, S., Nebel, C. & Charmantier, G. 2005. Ontogeny of osmoregulation
18 in postembryonic fish: A review. *Comp. Biochem. Physiol.* **141**, 401-429.

19 Venturini, G., Cataldi, E., Marino, G., Pucci, P., Garibaldi, L., Bronzi, P. &
20 Cataudella, S. 1992. Serum ions concentration and ATPase activity in gills,
21 kidney and esophagus of european sea bass *Dicentrarchus labrax*

1 (Pisces, Perciformes), during acclimation trials to freshwater. *Comp.*
2 *Biochem. Physiol.* **103A**, 451-454.

3 Verri, T., Maffia, M., Danieli, A., *et al.* 2000. Characterization of the H^+ peptide
4 cotransporter of eel intestinal brush border membranes. *J. Exp. Biol.* **203**,
5 2991-3001.

6 Vu, Tan-Tue. 1976. Etude du développement du tube digestif des larves du bar
7 *Dicentrarchus labrax*. *Arch. Zool. Exp.* Gén. **117**, 493-509.

8 Vu, Tan-Tue. 1980. Etude histologique de l'épithélium du tube digestif du bar
9 *Dicentrarchus labrax*. L., au cours du développement post-embryonnaire.
10 *Arch. Zool. Exp. Gén.* **121**, 91-206.

11 Zambonino-Infante, J. L. & Cahu, C. L. 2001. Ontogeny of the gastrointestinal
12 tract of marine fish larvae. *Comp. Biochem. Physiol.* **130C**, 477-487.

Figure legends

Fig. 1. *Dicentrarchus labrax,* D0 SW newly hatched larvae (A) digestive tube above the oil drop, pancreas and liver sketches (B) open at the posterior end. D2 SW yolk sac larvae digestive tube (C) anterior part (D) posterior part (E) ciliated structures in the lumen of the digestive tube anterior section. D5 SW larvae (F) head. A-D transverse sections, F lateral section, Masson's trichrome. E TEM. Ap, anterior part; Bb, brush border; C, chord; Ci, cilia; DT, digestive tube; E, esophagus; Ey, eye; L, liver; Lu, lumen; M, Muscle; Mo, mouth; NT, neural tube; P, pancreas; Pe, Posterior end; Pp, posterior part; T, tegument; UT, Urinary tubules; Y, yolk.

Fig. 2. D5 SW larvae esophagus (A) goblet cells (B) stomach "U" folds. D6 SW larvae anterior section (C) epithelial cell apical region showing developed tubular system and apical junction (D) basal mitochondria surrounded by lamellar structures. D29 SW larvae. (E) esophagus (F) rectum in DSW showing goblet cells. A, B, E, F transverse sections, Masson's trichrome. C, D TEM. Aj, Apical junction; Bb, brush border; E, esophagus; Gc, goblet cell; Lp, *Lamina propria*; Ls, lamellar structure; m, mitochondria; M, Muscle; R, rectum; S, stomach; Ts, tubular system; UT, Urinary tubules; Y, yolk.

Fig. 3. D34 SW larvae anterior intestine epithelial cell. (A) large mitochondria are predominantly located at the basal side (B) lamellar structures surrounding

1 basal mitochondria. D51 SW young juvenile (C) anterior intestine with goblet
2 cells; (D) posterior intestine. (E) anterior intestine epithelial cell with
3 mitochondria surrounded by lamellar structures. C, D transverse sections,
4 Masson's trichrome. A, B, E TEM. Ai, anterior intestine; Bb, brush border; Bm,
5 basal membrane; Gc, goblet cell; Lm, lateral membrane; Lp, *Lamina propria*; Ls,
6 lamellar structure; m, mitochondria; MUQ, mucosa; N, nucleus ; Pi, posterior
7 intestine.
8
9 Fig. 4. D44 young juvenile anterior intestine. Mature and immature epithelial
10 cells; (A) SW-acclimation (B) DSW-acclimation. TEM. Bb, brush border; Ic,
11 immature cell; Lm, lateral membrane; m, mitochondria; Mc, mature cell; N,
12 nucleus; Ts, tubular system.
13
14 Fig. 5. D44 young juvenile anterior intestine. Apical region, developed tubular
15 system; (A) SW-acclimation (B) DSW-acclimation. Lamellar structures
16 surrounding basal mitochondria; (C) SW-acclimated (D) DSW-acclimated. TEM.
17 Az, apical zone; Bb, brush border; Bm, basal membrane; Bs, basal side; Jc,
18 junction complex; Ls, lamellar structure; m, mitochondria; Sv, small vesicles; Ts,
19 tubular system.
20
21 Fig. 6. D72 SW-acclimated juvenile. (A) Stomach, appearance of gastric glands.
22 (B) D0 SW newly hatched larvae. D2 SW-acclimated larvae (C) Fluorescent gut,
23 excretory system and tegumental ionocytes, fluorescent digestive tube, urinary

1 tubules and tegumentary ionocytes. D29 larvae. SW-acclimated (D)
2 esophagus. A, B transverse section, Masson's trichrome. C whole animal
3 sagittal section. D transverse section, fluorescent microscopy. As, anterior
4 section; Bch, branchial chamber; C, chord; DT, digestive tube; E, esophagus;
5 Ey, eye; Gc, goblet cell; Gg, gastric glands; I, ionocyte; M, Muscle; MUQ,
6 mucosa; NT, neural tube; P, pancreas; Ps, posterior section; T, tegument; TI,
7 tegument ionocytes; UT, Urinary tubules; Y, yolk.
8
9 Fig. 7. D29 larvae. SW-acclimated: (A) anterior intestine (B) posterior intestine
10 (C) rectum. DSW-acclimated (D) anterior intestin (E) posterior intestine (F)
11 rectum. Transverse sections, fluorescent microscopy. Ai, anterior intestine; Pi,
12 posterior intestine; R, rectum.
13
14 Fig. 8. D51 SW-acclimated young juvenile. (A) mouth (B) pyloric stomach with
15 fluorescent pyloric sac. SW-acclimated (C) anterior intestine (D) posterior
16 intestine (E) rectum. DSW-acclimated (F) anterior intestine (G) posterior
17 intestine (H) rectum. D72 SW-acclimated juvenile (I) esophagus-stomach
18 transition, note the fluorescence at the stomach level. Transverse sections,
19 fluorescent microscopy. Ai, anterior intestine; E, esophagus; L, liver; Mo, mouth;
20 Pc, pyloric caeca; Pi, posterior intestine; Pr, pyloric region; R, rectum; S,
21 stomach.
22

Fig. 9. Quantitative analysis of Na^{+}/K^{+}-ATPase intensity. Data were determined with Optimas software version 6.51 and expressed as the fluorescence epithelium surface percentage ± SD for three samples at each time point. (AI) Anterior intestine, (PI) posterior intestine, (R) rectum, (D29) day 29, length in millimeters, (SW) 35 ppt sea water, (DSW) 5 ppt diluted sea water. Different letters besides the bars indicate significant differences ($p < 0.05$).

Fig. 10. Quantitative analysis of Na^{+}/K^{+}-ATPase intensity. Data were determinate with Optimas software version 6.51 and expressed as the fluorescence epithelium surface percentage ± SD for three samples at each time point. (AI) Anterior intestine, (PI) posterior intestine, (R) rectum, (D51) day 51, length in millimeters, (SW) 35 ppt sea water, (DSW) 5 ppt diluted sea water. Different letters besides the bars indicate significant differences ($p < 0.05$).

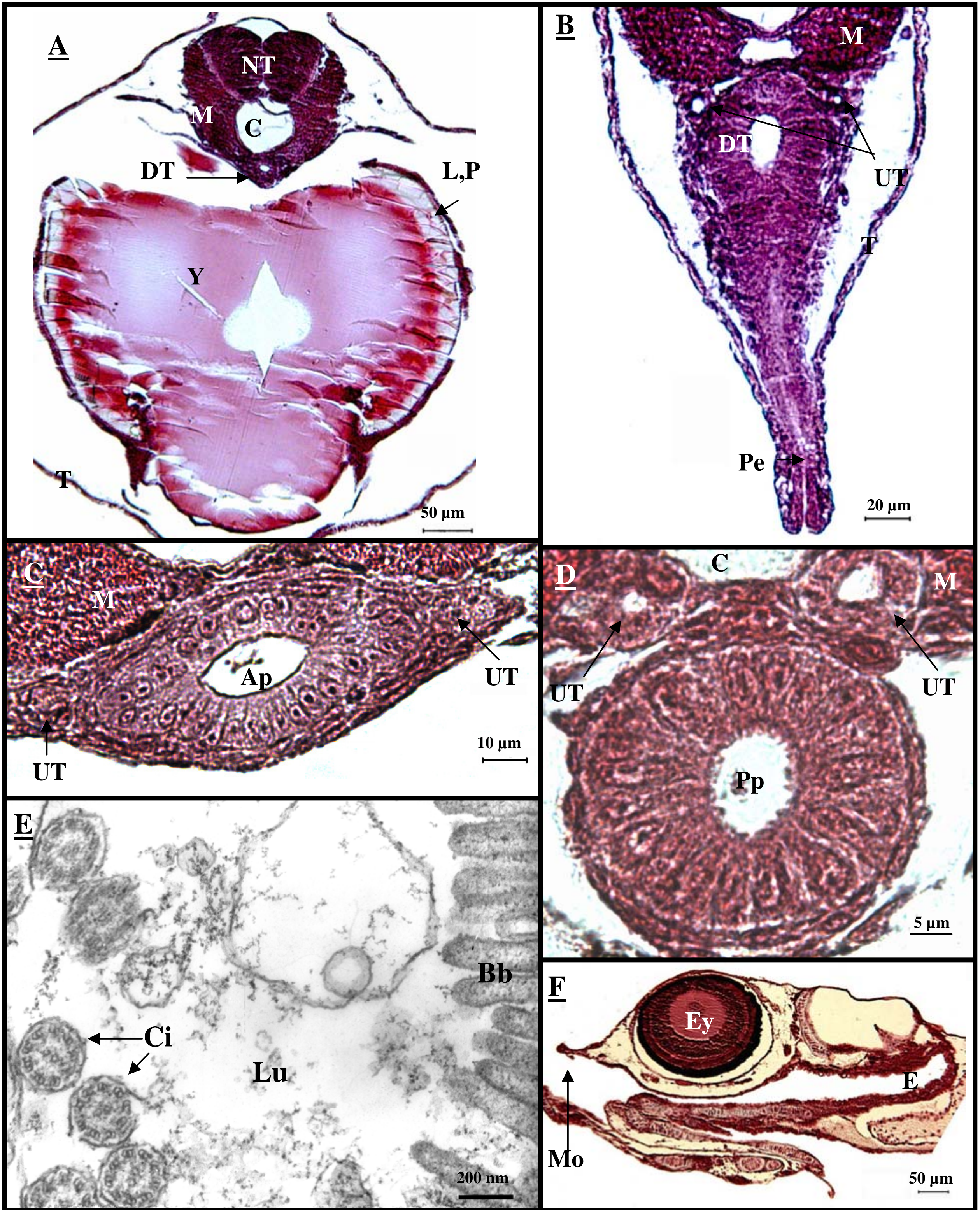

Fig. 1.

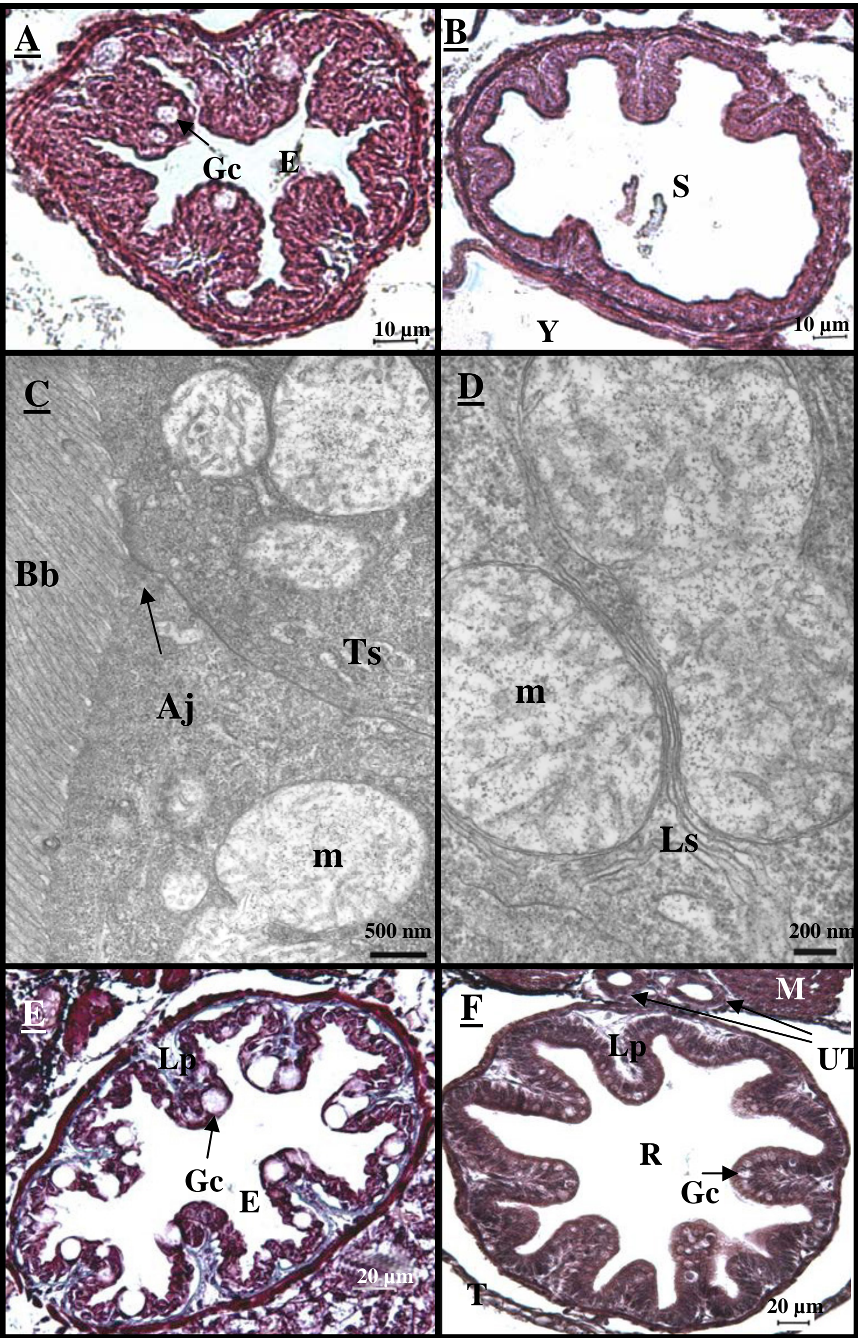

Fig. 2.

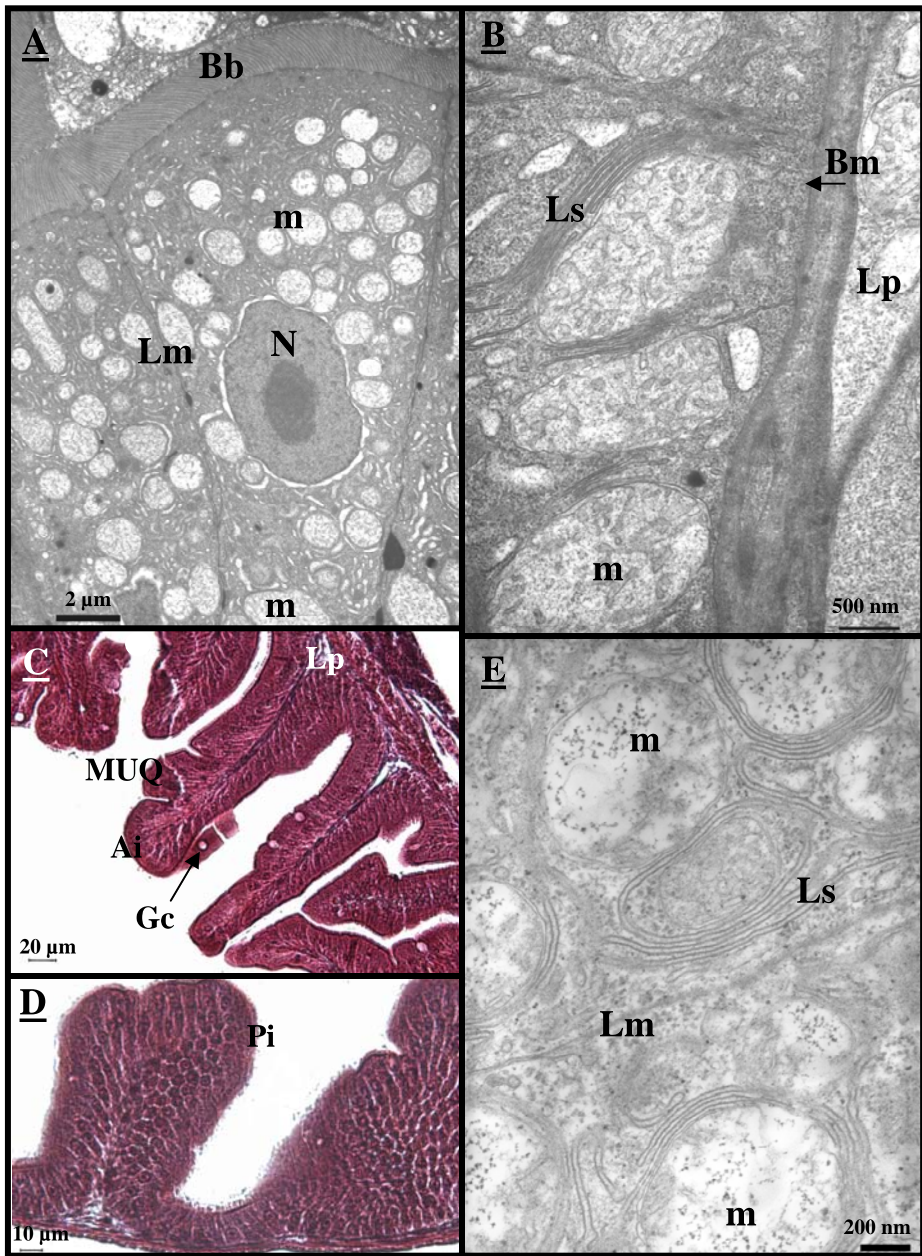

Fig. 3.

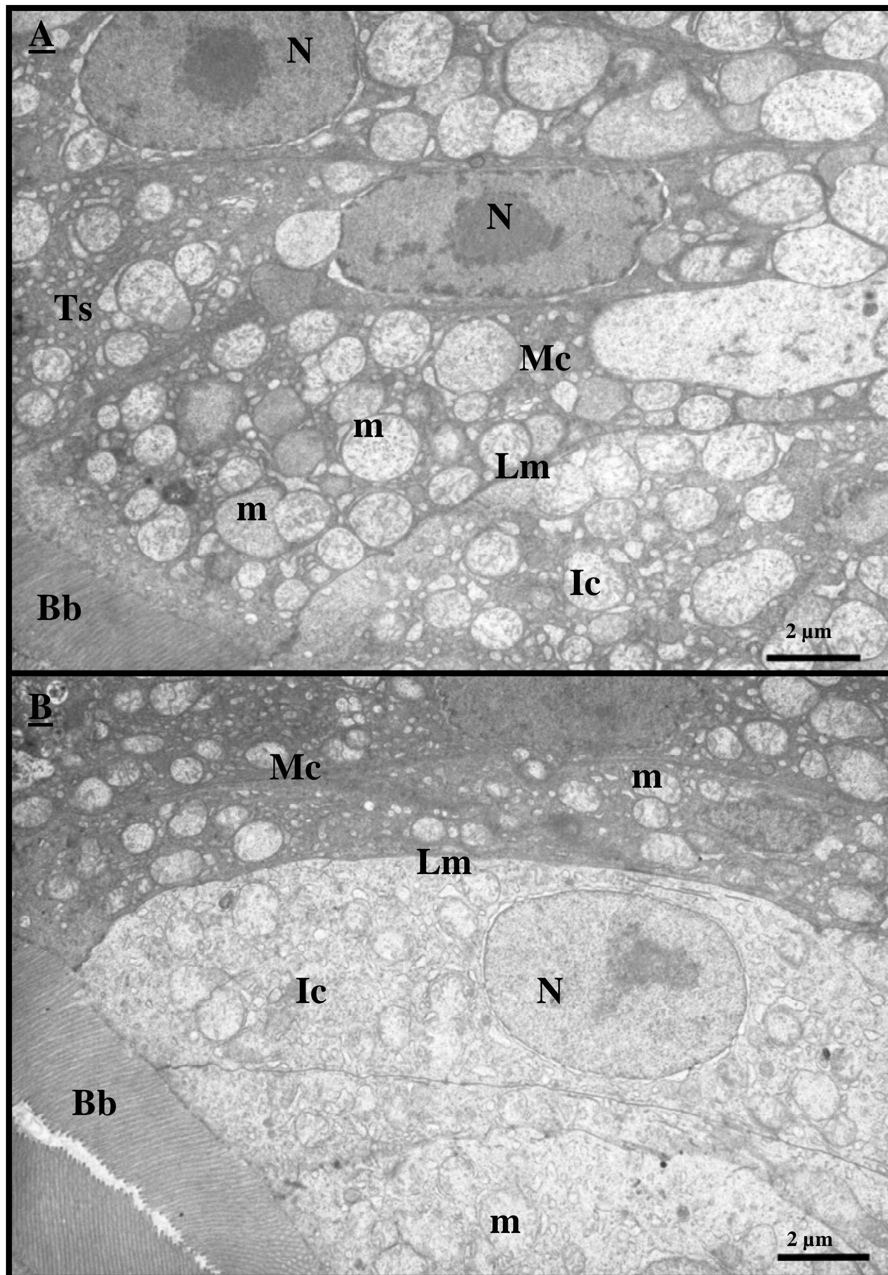

Fig. 4.

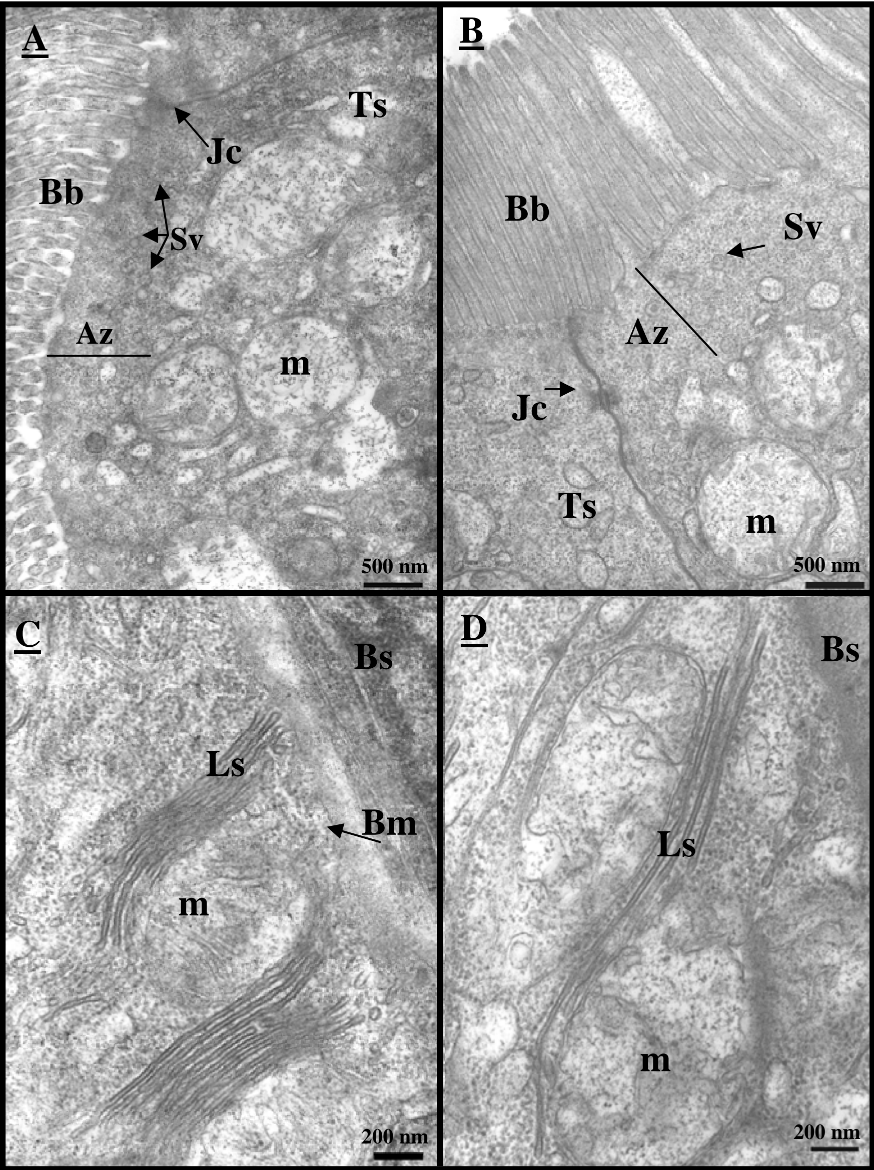

Fig. 5.

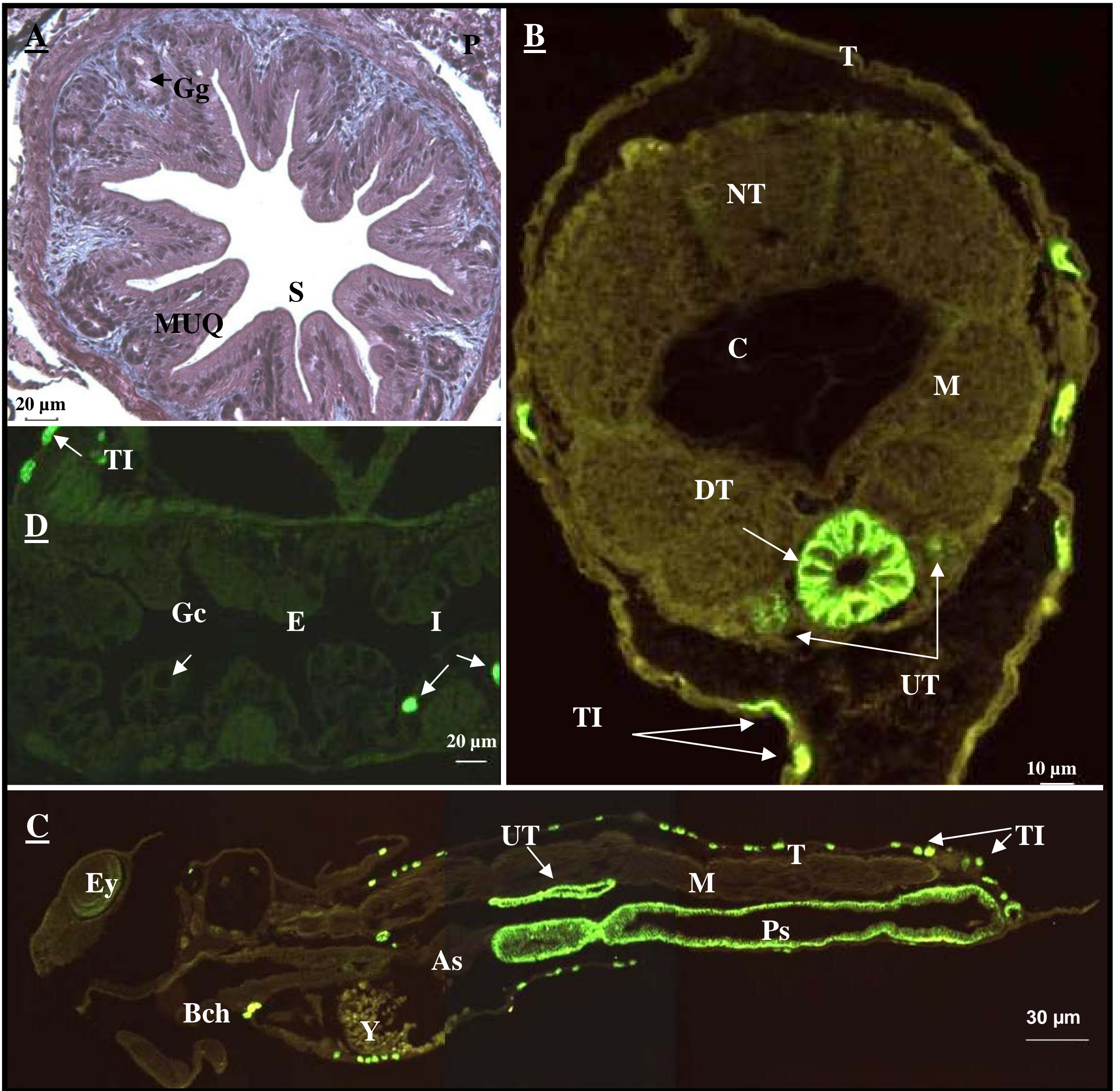

Fig. 6.

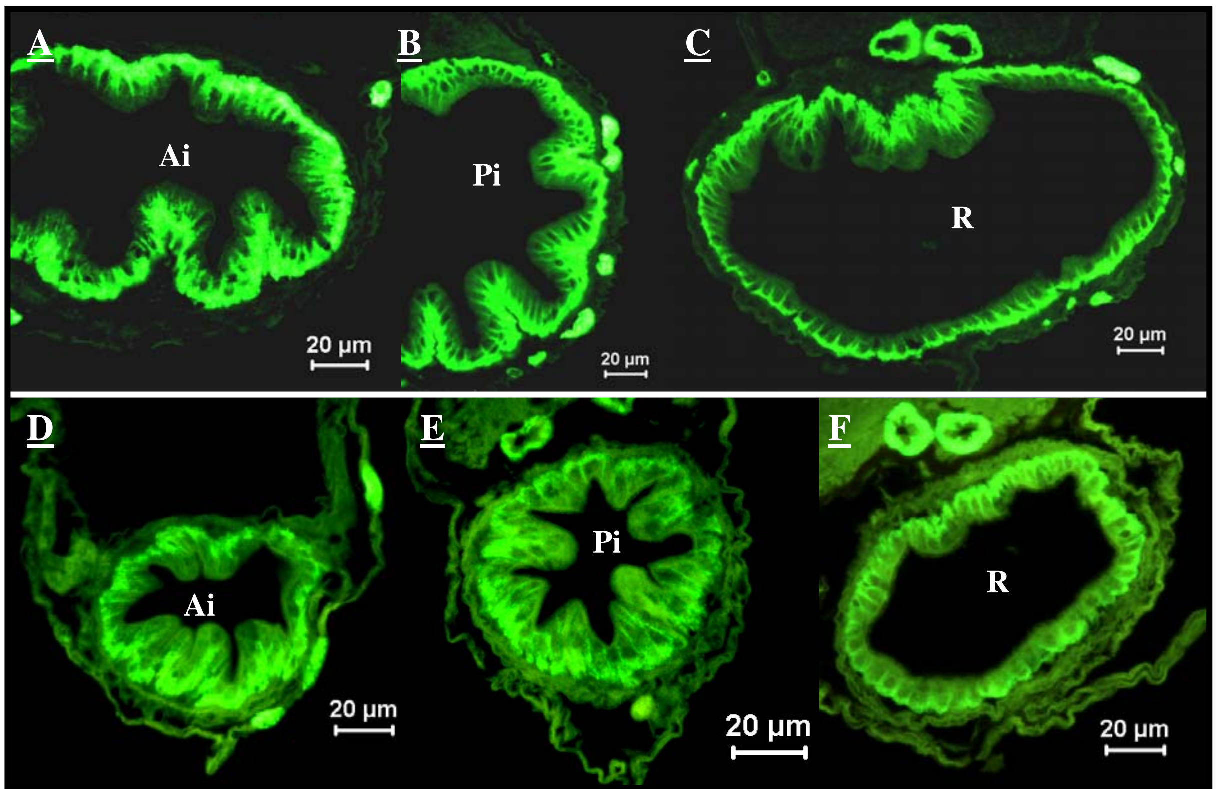

Fig. 7.

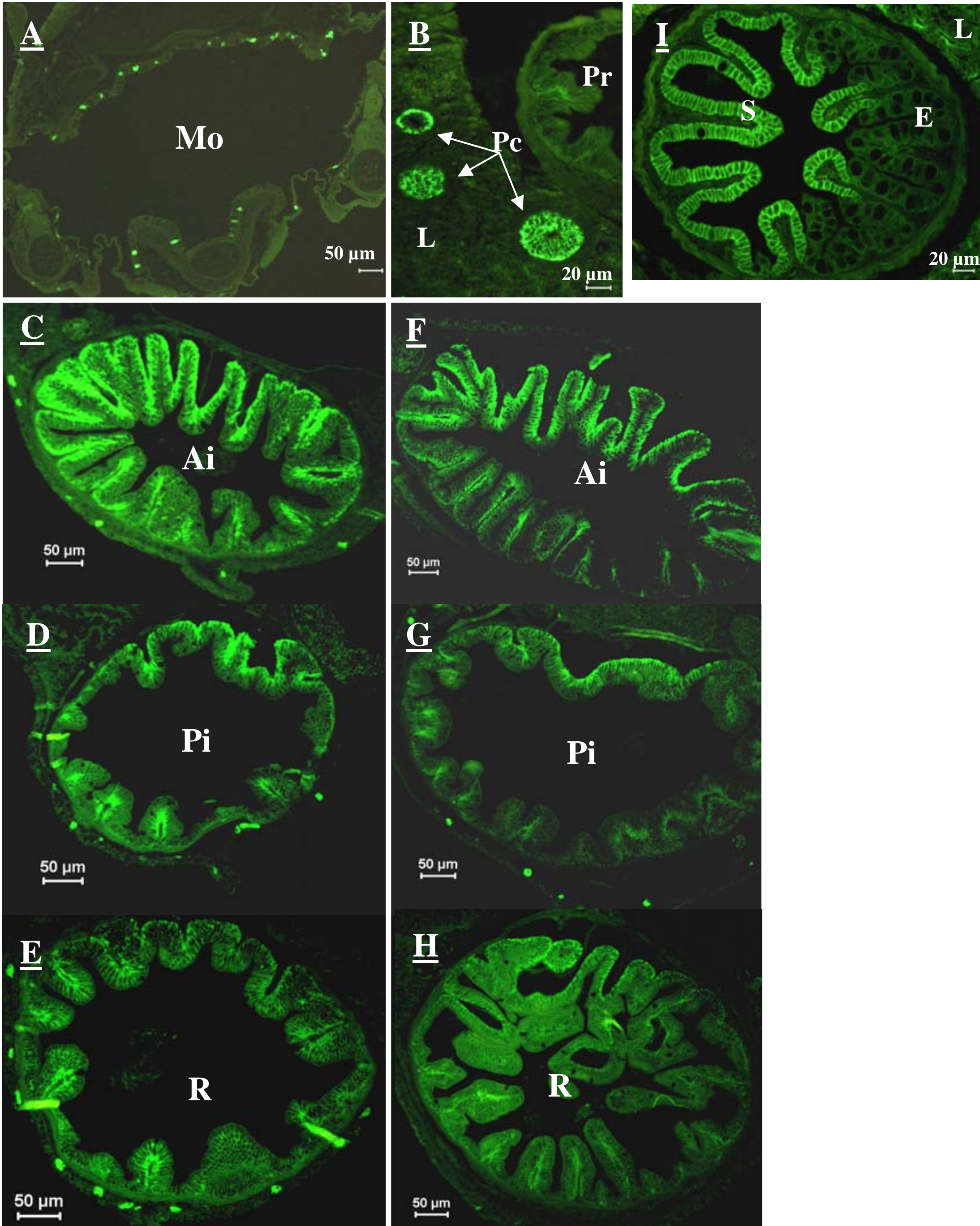

Fig. 8.