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Glass eel *(Anguilla anguilla)* acclimation to freshwater and seawater: morphological changes of the digestive tract

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Summary

Glass eels (*Anguilla anguilla*, L. 1758) caught during ascent at the mouth of the River Tiber were kept in aquaria with freshwater and full strength salinity (35 ‰) for four months.

Morphological features of glass eels at capture and after four months of experimental rearing are described. The structure of the gut, an important osmoregulatory organ, observed in glass eels in seawater experimental rearing suggests that they undergo an irreversible process of adaptation to freshwater, despite the fact of survival in seawater.

Introduction

Numerous studies have revealed that the digestive tract plays a primary role in the osmoregulatory processes of marine and euryhaline teleosteans. In hypo-osmotic regulation, fish compensate water losses by drinking, desalting the water in the oesophagus, and absorbing it through the gut. In freshwater fish there is no evidence of the digestive tract participating in osmoregulation (see review by Kirsch et al. 1984).

The structural bases of these different mechanisms have been studied on a limited number of species. Among these, the most exhaustive studies are those carried out on eels *(Anguilla anguilla* and *Anguilla japonica)* as examples of the euryhaline species (Kirsch et al. 1984; Simonneaux et al. 1987a, 1987b, 1988). These studies, however, do not always specify the developmental stage of the individuals examined – i.e., whether they are yellow or silver eels – despite the known differences in adaptive capacity shown by this species in relation to the various migratory phases of its life cycle (Bertin 1956; Sharrat et al. 1964a, 1964b).

Notwithstanding the interest shown by a number of authors in the behaviour of glass eels during upstream migration, the literature provides no information as to the osmoregulatory mechanisms of the glass eel, i.e., the stage during which migration from the sea to freshwater takes place and ontogenetic processes are still underway. Field observations suggest that migration to inland waters requires a short-term period of adaptation to lower salinities, both for *Anguilla anguilla* and *Anguilla rostrata* (Deelder 1958; McCleave and Wippelhauser 1987). Laboratory research has also established a longterm preference of glass and yellow eels for freshwater (Schultz 1975).

Aim of the present study is to provide some morphological data with regard to capacity for and modes of acclimation to various salinities in the early period of eel life. Since the eel may live and be reared in freshwater, saltwater or in intermediate salinities during the various phases of its life cycle, the understanding of the osmoregulatory mechanisms may prove to be of use in optimizing rearing techniques.

Materials and methods

Glass eels were caught during ascent at the mouth of the River Tiber, and individuals were selected depending on their pigmentation stage (A, B and occasionally C, according to the classification by Boëtius 1976). Water salinity at the site of capture was around 5 ‰.

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Two acclimation trials were carried out: one at 0 ‰ and the other at 35 ‰. Some glass eels were stocked directly into well water (FW). Others were adapted gradually over 72 hours to 35 ‰ salinity (SW), experimental conditions being kept similar as regards stocking density (100 g/m^3), feeding (fresh fish followed by artificial fish food) and lighting (natural circadian rhythm). The acclimation trials were replicated at 15 °C and 25 °C. Mortality was recorded daily; length, weight and pigmentation of 70 individuals from each aquarium were examined every fortnight.

Histological observations were carried out on newly-caught glass eels and then on six individuals from each experimental batch, respectively after two and four months of acclimation. Samples were chosen at the pigmentation stage prevailing at the moment of fixing, i.e., B and C after two months, C and D after four months. For every pigmentation stage, several individuals of different sizes were chosen.

After starving for four days, glass eels were fixed in Bouin, embedded in paraffin for sectioning at a thickness of 7 µ, and stained with hematoxylin-eosin and Alcian blue.

Results

a) Morphology of wild specimens

Glass eels used in this study at stages A and B of pigmentation showed similar digestive tracts and exhibited a viscous fluid in the peritoneal cavity, as has been described by WILLIAMSON (1989), remaining from the stage prior to metamorphosis. Teeth have not appeared yet.

The mucosa of the anterior oesophagus is multilayered and rich in mucous cells and taste buds. The posterior region presents at the apices of the folds closely-packed columnar cells, beneath which blood circulation is particularly rich (Figs. 1a, b).

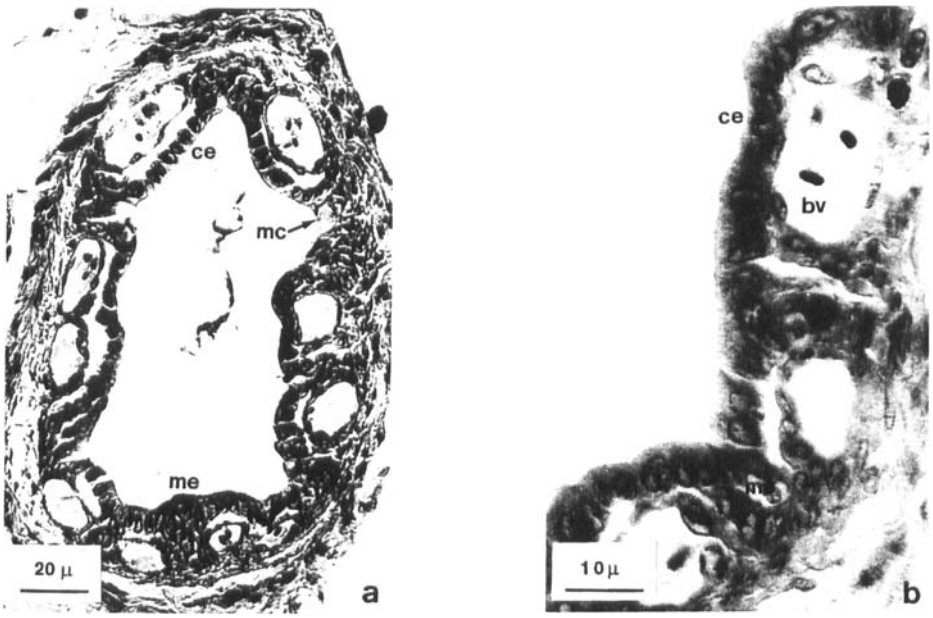

Fig. 1, a and b. Posterior oesophagus of a newly-caught glass eel (stage B). bv: blood vessel; mc: mucous cell; me: multilayered epithelium; ce: columnar epithelium

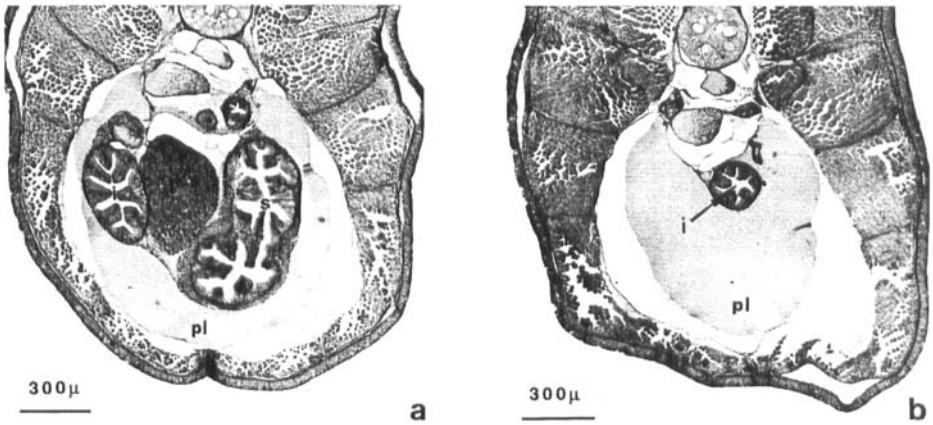

Fig. 2. Intestine of a newly-caught glass eel (stage B). a: anterior intestine, b: posterior intestine. i: intestine; l: liver; pl.: peritoneal liquid; s: stomach

The mucosa of the stomach *fundus* has few glands (Fig. 2a). The intestinal mucosa (Figs. 2a, b) rises to form a small number of folds closing the narrow lumen almost completely. It is formed of tall columnar cells; mucous cells are very rare.

b) Survival, pigmentation and development

In the first three weeks of acclimation, in all the aquaria mortality never exceeded 1.5 % per week. The acclimation of glass eels to freshwater and to full strength salinity therefore presented no problems. At the end of the second month total mortality ranged between 19.2 % (FW) and 12.6 % (SW), at both temperatures.

During the first two months of acclimation no growth was observed in the glass eels, but rather an initial reduction in weight and length (Table 1), a phenomenon that normally occurs in experimental rearing (ELIE and DAGUZAN 1976; YAHYAOUI 1988).

At the end of the experiment, glass eels showed a considerable size variation, particularly at 25 °C.

Pigmentation (Fig. 3) showed a progressive increase in the percentage of individuals at stage C both in FW and SW. This stage became predominant in the third week for the eels kept at 25 °C (89.7 % and 88 %, in FW and SW respectively) and in the sixth week for those kept at 15 °C (45.1 % and 53.2 % in FW and SW respectively) and remained such until the end of the experiment.

Table 1. **Mean total length (mm, ± SE) and weight (g, ± SE) at start, after two months and after four months of adaptation (n = 70)**

		Start	Two months	Four months
FW – 15 °C	TL	66.51 (0.379)	62.61 (0.470)	63.07 (0.342)
	W	0.27 (0.006)	0.16 (0.006)	0.16 (0.005)
SW – 15 °C	TL	»	61.73 (0.499)	62.15 (0.904)
	W	»	0.14 (0.006)	0.11 (0.007)
FW – 25 °C	TL	»	63.07 (0.494)	67.85 (0.772)
	W	»	0.12 (0.006)	0.27 (0.017)
SW – 25 °C	TL	»	61.58 (0.397)	67.62 (0.993)
	W	»	0.09 (0.003)	0.24 (0.026)

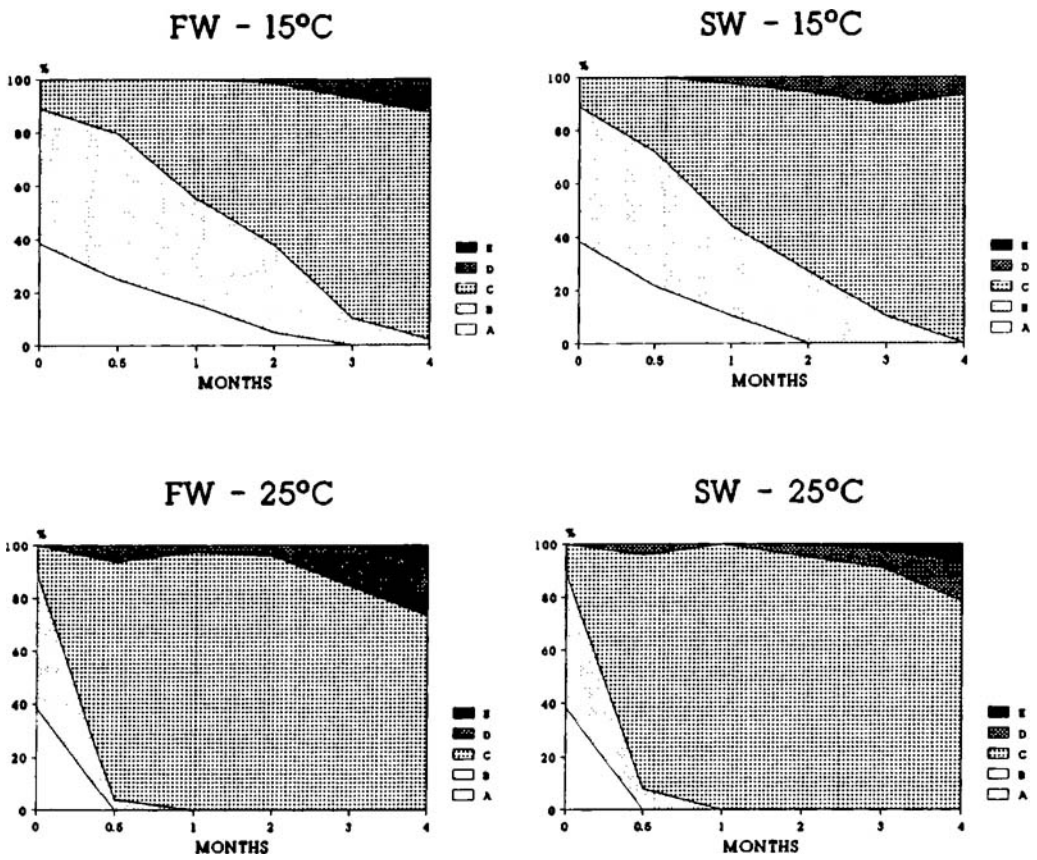

Fig. 3. Pigmentation rates (%) of the glass eels in the four batches. Pigmentation stages: from A to E, according to BOËTIUS (1976)

Both in FW and SW, a progressive development of dentition was observed together with the reduction and disappearance of the peritoneal liquid and the enlargement and elongation of the intestine, which presented the loop described in adult eels (WILLEMSE 1979) after four months. A thickening of the *tunica muscularis* was noted in the gut, together with an increased complexity of the mucosa layers. The oesophageal mucosa presented more complex folds and club cells (Figs. 4a, b; 5a, b); the gastric mucosa showed a considerable increase in the number of glands. The intestine presented numerous and elongated folds (Figs. 4c, d; 5c, d). An increase was also noted in the number of mucous cells.

In all four batches, morphological comparison of stage C after two months of acclimation, and stage C after four months, generally shows a higher development in the latter. Moreover, by the end of the experiment the same development was reached by both the small-sized glass eels and by the larger ones.

c) Morphology of glass eel gut in fresh- and seawater

Some structural differences appeared in the various regions of the digestive tract between glass eels kept for four months in FW and SW, at both temperatures.

In FW, the posterior oesophageal mucosa (Fig. 4b) became multilayered, with dense accumulation of mucous cells on the luminal side. In SW, single-layered stretches composed

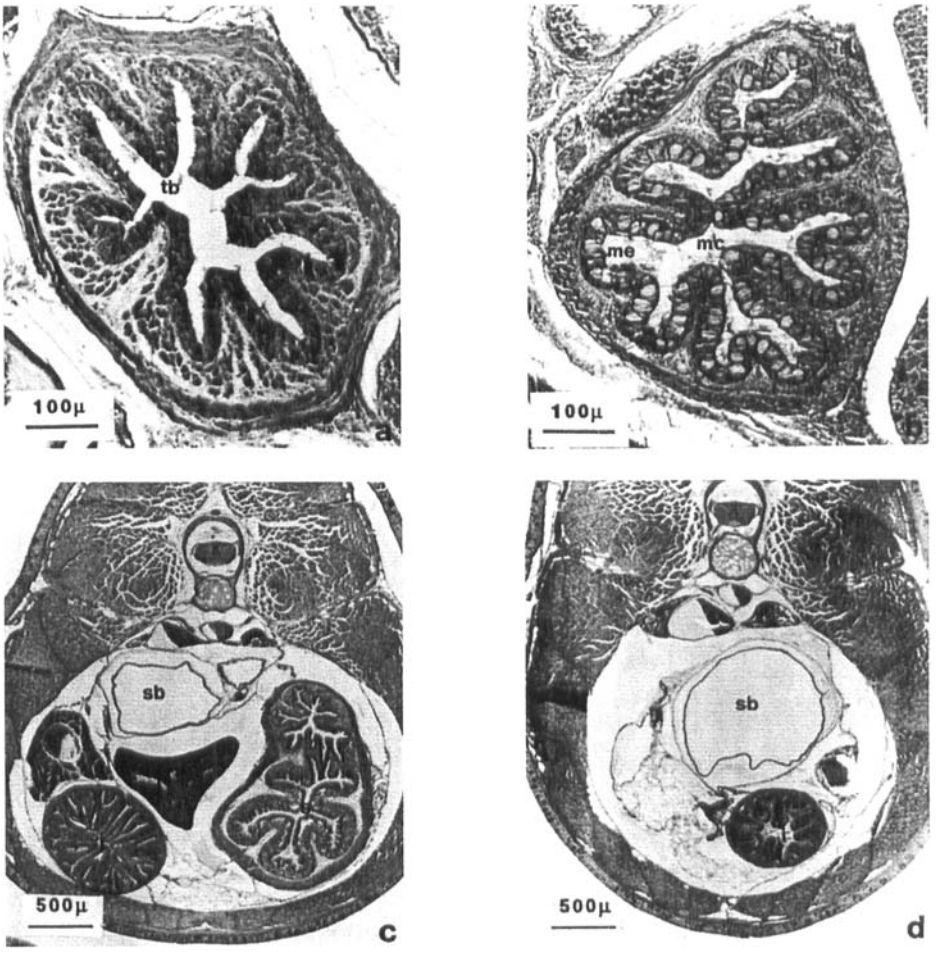

Fig. 4. Glass eel (stage D) acclimated to FW for four months.
a: anterior oesophagus, b: posterior oesophagus. mc: mucous cells; me: multilayered epithelium; tb: taste buds, c: anterior intestine, d: posterior intestine. i: intestine; l: liver; s: stomach; sb: swimbladder

of columnar cells with large intercellular spaces remained at the apices of the folds and on the large blood vessels (Figs. 5a, b).

In FW, the folds of the anterior intestine (Fig. 4c) were expanded so as to fill the lumen completely, whereas in SW they were smaller in size (Fig. 5c).

In SW, the posterior intestine (Fig. 5d) was dilated so as to occupy the whole of the peritoneal cavity. In some cases this would appear to bring about a compression and displacement of the swimbladder. The dilatation of the intestinal lumen was also brought about by the distended aspect of the muscular layers and mucosa, the folds of which were either very small or non-existent. The epithelial cells were irregular in size and generally not as tall as in FW. In some cases they even took on a distorted and flattened appearance.

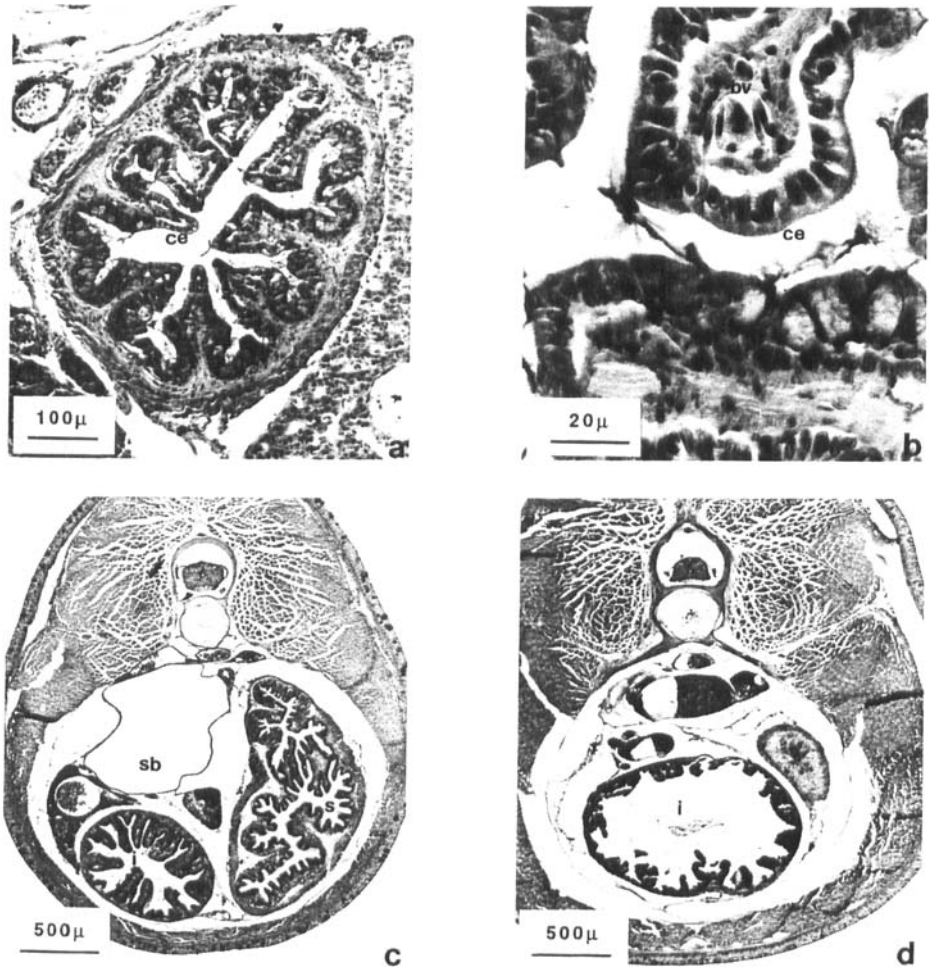

Fig. 5. Glass eel (stage D) acclimated to SW for four months.
a and b: posterior oesophagus. bv: blood vessel; ce: columnar epithelium
c: anterior intestine, d: posterior intestine. i: intestine; s: stomach; sb: swimbladder

Discussion

Acclimation to full strength salinity and to freshwater showed similar survival rates, demonstrating an "osmoregulatory ability" of glass eels.

At both temperatures and salinities the transition from typical postmetamorphic structures (Berndt 1938) to those similar to the adult morphology (Willemse 1979; Clarke and Witcombe 1980) was obvious. Development continued irrespective of the environment, but probably time elapsed since metamorphosis varied between individuals.

In contrast to suggestions by several authors (Vilter 1944, 1945a; Lecomte Finiger 1983; Cantrelle 1984) in our experiment morphological development of the intestine proceeded independent from the pigmentation process. Pigmentation developed very slowly. This process is expected to take from four to eight weeks (Lecomte Finiger 1983); however the time required to complete pigmentation has never been specified.

The correlation between development and pigmentation, never established on the basis of morphological studies but only inferred from field observations, must be the object of further investigations.

In FW, the oesophageal mucosa, multi-layered throughout, confirms an hyper-osmotic regulation (MEISTER et al. 1983). In wild specimens and in the SW samples, the oesophageal mucosa was instead characterized by areas with simple columnar epithelium. Prominent intercellular spaces – indicative of ion transport activity (YAMAMOTO and HIRANO 1978; MEISTER et al. 1983; SIMONNEAUX et al. 1987a) – were always observed in the oesophagus of glass eels in SW.

Thus glass eels, transferred into SW at a moment when they have activated an hyperosmotic regulation only from a short lapse of time, are probably able to reactivate the mechanism of drinking, and desalting the water ingested at the oesophagus level.

In contrast to the oesophagus, the intestine showed a modification that cannot be interpreted as a functional adaptation to salinity. In eels and other marine teleosts, the anterior region of the intestine is most efficient in water absorption (KIRSCH and MEISTER 1982).

During the transition of the adult eel to SW, the folds, whose structure seems to be important for the intestine osmoregulatory function (SIMONNEAUX et al. 1988), increase their surface by infoldings and the serosal spaces become very narrow (KIRSCH et al. 1984). In glass eels acclimated to SW this change was not observed, while the posterior intestine was enormously dilated, a modification never described for the adult eel (KIRSCH et al. 1984). Since the glass eels kept at both salinities received the same feeding and had been starved for four days before fixing, this dilation may be attributed to abnormal water retention, probably owing to lower transepitelial ion and water transporting efficiencies.

Our data indicate that the process of acclimation to FW has started, and is irreversible despite the fact of glass eels remaining in SW. The mechanism of osmotic pre-adaptation to higher salinity is well known in silver eels in the pre-migratory phase (UTIDA et al. 1967; KIRSCH and MAYER-GOSTAN 1973; KIRSCH et al. 1975; THOMPSON and SARGENT 1977).

The prolonged capacity to live in high salinity, in spite of a preacclimation to freshwater of the gut, must be ensured by the complex feedback inter-relations of the many organs of the hydrosaline regulatory system. This capacity guarantees to glass eels migrating in inland waters a period of tolerance in case of unavoidable delays, or enables them to move in salinity stratified water in estuaries during the first phase of migration (MCCLEAVE and WIPPELHAUSER 1987).

It could thus be interesting to ascertain whether, like the anadromous Salmonids, the eel also has an optimum interval ("fenêtre": BOEUF 1987) for transition from one level of salinity to another.

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