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Acclimation trials of juvenile Italian sturgeon to different salinities: morpho-physiological descriptors

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In this study the first experimental trials of acclimation to salinities of the Italian sturgeon *Acipenser naccarii* are described. Preliminary observations regarding some morphological features of the gut, kidney and gills following exposure to 20 and 30‰ salinity are reported, and compared with those displayed by many teleosts in hyper- and hypoosmoregulation. Results are discussed with regard to the osmoregulatory mechanisms and to the physiological limits of adaptability of this species in the second year of its life cycle. Within the Acipenseridae, *Acipenser naccarii* is generally considered a euryhaline species, but nothing is known about its mechanisms of osmotic homeostasis. This species could be a good candidate for aquaculture in fresh and brackish waters owing to the recent success obtained in artificial reproduction.

Key words: osmoregulation; saltwater adaptability; *Acipenser naccarii*; oesophagus; kidney; gills.

INTRODUCTION

The Italian sturgeon *Acipenser naccarii* Bonaparte, a species about whose biology comparatively little is known (Tortonese, 1989), was once abundant in all the rivers tributary to the North Adriatic Sea. Today its distribution range has been greatly reduced, owing to the impact of factors such as pollution and overfishing. Interest in this species was aroused recently by the success obtained in artificial reproduction (Arlati *et al.*, 1988), which suggests that *A. naccarii* may be suitable both for fish farming and for restocking.

A. naccarii must be a euryhaline fish as it is found in the sea although it breeds in fresh water (Tortonese, 1989; Bernini & Nardi, 1990; Rossi *et al.*, 1992). However, when in the sea, the Italian sturgeon seems to remain near the estuaries (Paccagnella, 1948). Rochard *et al.* (1990) identified three types of life history for the sturgeons and assigned the Italian species to type 2. After spawning, the adults migrate to brackish waters, later followed by the juveniles. In a later work, Rochard *et al.* (1991) defined this sturgeon as a mainly freshwater species.

Knowledge of the ability of the Italian sturgeon to adapt to salinity variations is of particular interest in relation to its estuarine ecology and to aquaculture development. If its euryhalinity is ascertained, this could open up the way to extensive culture of sturgeons in low salinity environments, which are abundant in the north of Italy, where Adriatic ‘ Vallicoltura ’, an extensive culture in brackish lagoons, plays an important role.

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In general, little is known about the mechanisms of osmotic homeostasis in sturgeons. Some Russian species seem to have a narrow range of euryhalinity (Shelukin *et al.*, 1990; Natochin *et al.*, 1985). Few morphological studies have described cell and tissue features in relation to the osmoregulatory function. Organs involved in osmoregulation in teleosts are kidney, gills and digestive tract. Their morphofunctional features have been described in fishes under different natural and experimental conditions of salinity, and used to evaluate the adaptability of the different species. For the first time an experimental acclimation of the Italian sturgeon to varying salinity was achieved and is described in the present paper. Preliminary observations on structural adaptive changes of gut, gills, and kidney are compared with those displayed by many teleosts in order to gain initial insight into the adaptability of this species and its osmoregulatory mechanisms.

MATERIALS AND METHODS

ACCLIMATION

Juveniles of *A. naccarii* from artificial reproduction were used. Considering the difficulties in finding this species and maintaining such large animals in acceptable experimental conditions, only a small number of fishes was used.

Trial 1

During autumn 1992, two fishes (14 months old, 932 ± 101 g) were acclimated gradually from fresh water (FW: 0‰, 7·5 mOsm kg^{-1}) to artificial brackish water (BW: 20‰, 689 mOsm kg^{-1}) in closed 400 l aquaria equipped with filters (10 l) and having a water turnover of 1500 l h^{-1}. Temperature was maintained at 20° C. Fish were maintained at 20‰ salinity for 15 days.

Trial 2

In spring 1993, six fishes (20 months old, 1·9 ± 0·2 kg) were acclimated from FW (0‰, 7·5 mOsm kg^{-1}) to natural BW (20‰, 664 mOsm kg^{-1}) in a closed 3 m^3 raceway equipped with a filter room (0·4 m^3) and having a water turnover of 3500 l h^{-1}. Temperature was maintained at 20° C. After a month at 20‰, salinity was gradually increased to 30‰ (SW: 945·3 mOsm kg^{-1}). In both trials, final salinities were reached in steps of 3‰ increases per day.

During the acclimation phases, fish were starved and then fed once a day with commercial pellet food. Dissolved oxygen, ammonium, nitrites and nitrates were kept under control. For both trials, controls in FW were maintained at the same experimental conditions.

Acclimated specimens were examined at the end of Trial 1. In Trial 2 they were examined after the period in BW and after 15 and 60 in SW. FW controls were examined as well. Fish were anaesthetized (3 ppm 2-phenoxy-ethanol) and blood samples were obtained directly from the heart. Gills, oesophagus and kidney were fixed for light microscopy.

SAMPLE PROCESSING

Blood was centrifuged at 3000 rpm for 10 min. Serum osmolality was determined by the cryoscopic method (One-ten Osmometer, Fiske). Electrolyte composition was measured. Na^+ and K^+ were determined by flame photometry (GDV Digiflame 2000 with air butane flame). Cl^- concentration was measured by the mercuric thiocyanate method (Iwasaki *et al.*, 1952).

TABLE I. Ionic concentrations and osmolality values (means) of plasma in freshwater-, brackish water- and seawater-acclimated *A. naccari* in the two trials

	Na^+ (mequiv. l^{-1})	K^+ (mequiv. l^{-1})	Cl^- (mequiv. l^{-1})	Osmolality (mOsm kg^{-1})	Water osmolality (mOsm kg^{-1})	No. of fishes
Trial 1						
FW	140·7	3·6	126·1	285·2	13·0	7
BW	166·0	2·2	172·5	352·1	674·2	2
Trial 2						
FW	142·2	3·7	137·1	290·9	12·0	5
BW	146·5	3·8	154·5	292·3	666·5	2
SW (15 days)	155·0	3·8	209·0	303·7	951·0	1
SW (60 days)	141·0	3·0	133·5	320·0	951·0	2

Kidney and gut were fixed for light microscopy in Dubosq-Brazil liquid, dehydrated, embedded in paraffin and cut to 7 µm. Sections were then stained with haematoxylin–eosin and Alcian blue. Filaments of the second gill arch were fixed for 20 h in osmium tetroxide and zinc iodide 2 : 8 according to Garcia-Romeu & Masoni (1970). After rinsing and embedding in paraffin, the filaments were sectioned to a thickness of 5 µ. No staining followed, as this fixative stains chloride cells selectively.

MEASUREMENTS

Glomerular volumes and surfaces were measured by means of image analysis (QUANTIMET 970, Cambridge Instruments). Non-parametric (Kruskal–Wallis) one-way analysis of variance was performed on glomerular measurements to detect significant differences between the two acclimation conditions.

Chloride cell counts were made on histological sections of filaments at corresponding levels, i.e. when the section of a cartilaginous projection of the gill ray appeared in the middle of a filament. Two separate kinds of cells were considered: (1) cells located on both lamellae and interlamellar spaces, contacting the lamellar vessel (L); and (2) cells located in the interlamellar spaces, and extending towards the filament vessels (S). For each fish, 168 lamellae and 112 interlamellar spaces were examined.

RESULTS

During both the acclimation trials, *A. naccarii* showed normal swimming and feeding activities. No mortalities were recorded in Trial 1, while one specimen died after a few days in BW in Trial 2.

SERUM

The sturgeons, hyperosmotic to FW, were hypoosmotic to BW and SW (Table I). Comparisons of electrolyte composition in individual fishes showed that the levels of Na^+ and Cl^- concentrations were independent of osmolality values. In Trial 1 osmolality values of BW-adapted sturgeons were much higher than in FW-adapted specimens; in Trial 2 higher values were observed only in SW-adapted fishes. A progressive increase can be observed in connection with the acclimation duration.

GUT

The mucosa of the short oesophagus was organized into distally oriented papillae. The epithelium was stratified throughout. The first portion was rich in mucous cells, and many taste buds were present. After acclimation to BW and SW, the oesophageal epithelium maintained the same structure. No structural changes were observed on examining the intestine except for some degenerative aspects in sturgeons kept for 60 days in SW. The latter presented an intestine that was highly dilated by a large volume of luminal fluid, that was slightly hypoosmotic to serum.

KIDNEY

The nephron of FW *A. naccarii* [Fig. 1(a)] consisted of a corpuscle with a highly vascularized, often multilobed glomerulus, a short neck segment, two proximal tubule segments, distinguishable by the different heights of the cells and of the brush border, a long distal segment and the collecting tubule connected to the system of collecting ducts. Following exposure to BW, a significant decrease ($P<0{\cdot}001$) for both glomerular surface and volume was observed (Table II). Light microscopy observation showed only a reduction in the vascular supply to the glomeruli. After 60 days in SW [Fig. 1(b)], most glomeruli appeared further reduced, even if the decrease in size was not quantified, while vascularization was often absent. Furthermore, the examination of serial sections showed that empty capsules were present, without glomeruli. Cells of the tubules were lower than in FW, and were cuboidal or flat. The brush border also was lower and sparser. It was often difficult to distinguish among the different regions of the tubules. Moreover, many cells showed nuclear degeneration.

GILLS

Chloride cells [Fig. 2(a)] were present on the secondary lamellae and in the interlamellar region of the filament (primary lamella). They were scarce or absent on the afferent and efferent edges of the filament. On the lamellae, cells were generally round in shape, protruding from the epithelium. In the interlamellar region of the filament, chloride cells were pear-shaped or cylindrical. The cells located on the sides of the space were in contact with the lamellar vessels, while the central ones seemed to extend towards the filament vessels. After acclimation to both salinities [Fig. 2(b)], the lamellar chloride cells more often appeared flattened. A decrease in the number of the lamellar chloride cells is evident in BW-adapted sturgeons in both trials (Table III), while for the interlamellar chloride cells the differences between individuals overlapped the differences between the two acclimation conditions.

DISCUSSION

In SW, teleosts compensate for osmotic water losses by drinking and desalting water in the oesophagus and absorbing it in the intestine (Kirsch *et al.*, 1984). Passive and/or active transport of ions from the mucosal to serosal side of the oesophagus is made possible by the fact that in SW a simple columnar epithelium, consisting of transporting type cells, replaces the stratified freshwater

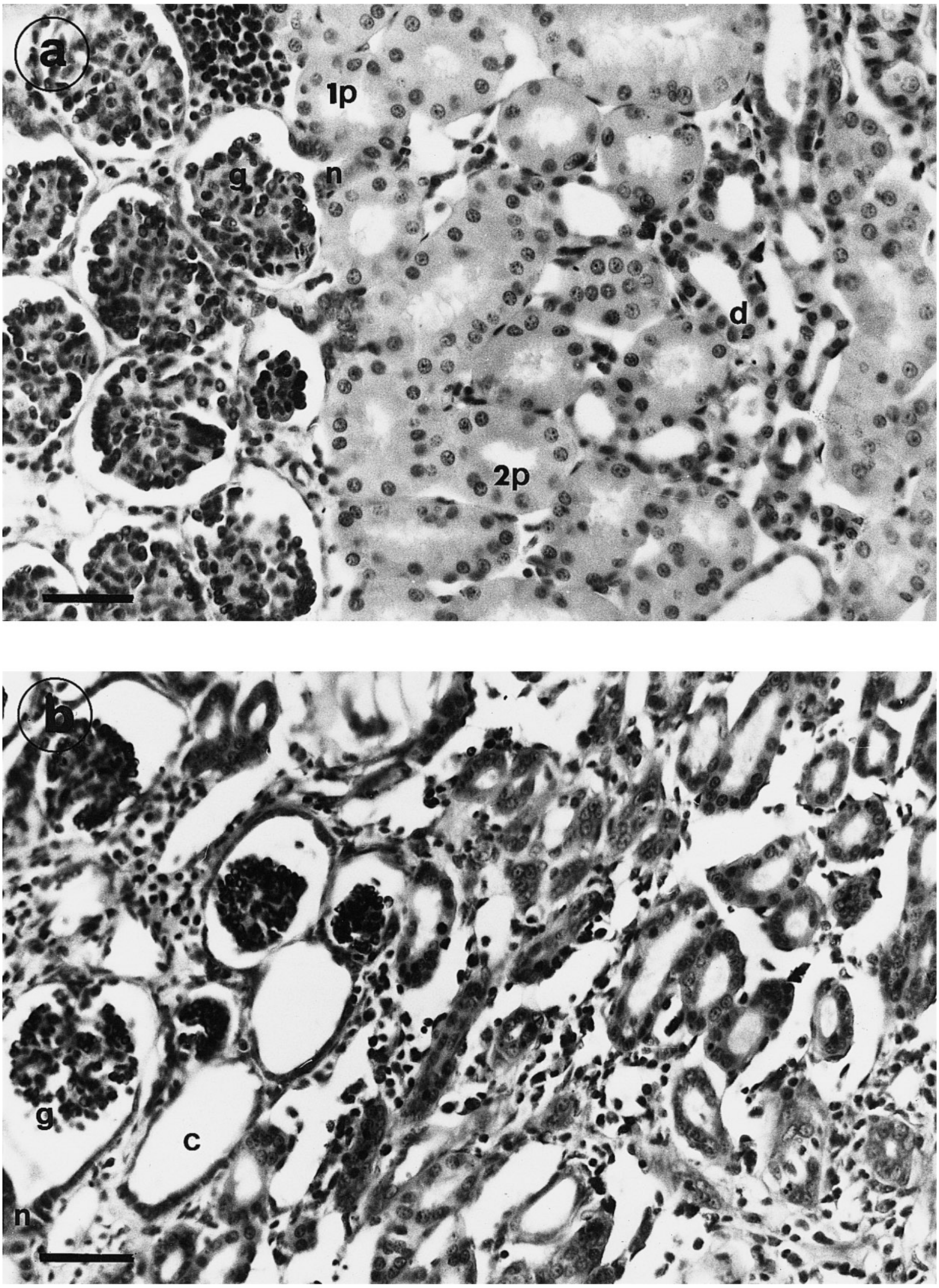

FIG. 1. Cross-section of kidney of *Acipenser naccarii* (a) living in fresh water (b) acclimated to sea water from 60 days. g, Glomerulus; n, neck segment; 1p, 2p, first and second proximal tubules; d, distal segment; c, capsule without glomerulus. Scale bar, 50 μm.

oesophageal mucosa. In *Anguilla anguilla* L. (Yamamoto & Hirano, 1978), and in *Oreochromis mossambicus* Peters (Cataldi *et al.*, 1988*a,b*) considerable changes are already evident after 7 days in SW. The oesophageal epithelium of *Acipenser*

TABLE II. Glomerular surface and volume of nephrons (mean ± S.D.) of freshwater- and brackish water-acclimated *A. naccari* in the two trials (no measures were performed on the seawater-acclimated specimens)

	Surface (μm^2)	Volume (μm^3)	No. of glomeruli (No. of fishes)
Trial 1			
FW	30 576 ± 9680	489 999 ± 218 644	17 (2)
BW	14 906 ± 2531	155 526 ± 43 346	15 (2)
Trail 2			
FW	18 924 ± 3918	222 088 ± 61 858	10 (1)
BW	12 372 ± 1581	122 067 ± 24 048	14 (2)

naccarii did not show any modifications even after 60 days in SW. The abnormal fluid content observed in the posterior intestine could be sea water ingested, not desalted in the oesophagus and diluted by osmotic losses from tissues. Water ingestion is induced in fish, even in stenohaline freshwater fishes (Lahlou *et al.*, 1969), by the transfer to a hypertonic medium (Hirano, 1974). The two-step scheme of the mechanism responsible for water recovery in teleosts cannot apply to sturgeons: from these preliminary observations it is not possible to say how water balance, that took place somehow as the fishes did survive, is carried out in the sturgeons.

In SW, teleosts reduce the amount of urine excreted. A decrease in glomerular size was observed in many euryhaline teleosts acclimated experimentally to higher salinities (Wendelaar Bonga, 1973; Oliverau & Oliverau, 1977; Colville *et al.*, 1983; Cataldi *et al.*, 1991; Cataudella *et al.*, 1991). A decrease in nephron size was observed in BW-adapted *A. naccarii*, in agreement with observations on wild *A. gueldenstaedti* Brandt from sea and from inland waters (Gambaryan, 1988). However, in 60 day-SW-acclimated sturgeons the degenerative features observed in the whole nephron were similar to those observed in *Oreochromis niloticus* L. and *O. mossambicus*, which displayed a low degree of salt tolerance (Cataldi *et al.*, 1991).

The SW teleosts excrete ions in excess through chloride cells. An increased number and/or size of the interlamellar chloride cells is described for euryhaline teleosts during adaptation to SW (Laurent & Hebebi, 1989; Cioni *et al.*, 1991; Avella *et al.*, 1993). On the other hand, a degeneration and disappearance of lamellar chloride cells, that are linked to the ion pumping capacity of the gill in FW, was reported for the Atlantic salmon during its migration to the sea (Bornacin *et al.*, 1987). The possible coexistence of two chloride cell types with different functions linked to different compartments of the circulatory system was debated recently (Laurent & Perry, 1991). A basic pattern of the gill vascular organization in teleosts and chondrosteans was demonstrated (Dunel & Laurent, 1978). As far as sturgeons are concerned, Krayushkina *et al.* (1976) describe for *Huso huso* L., a generic increase of chloride cells after 5 days of acclimation at 12·5‰ salinity, and Laurent & Dunel (1980) report an increase

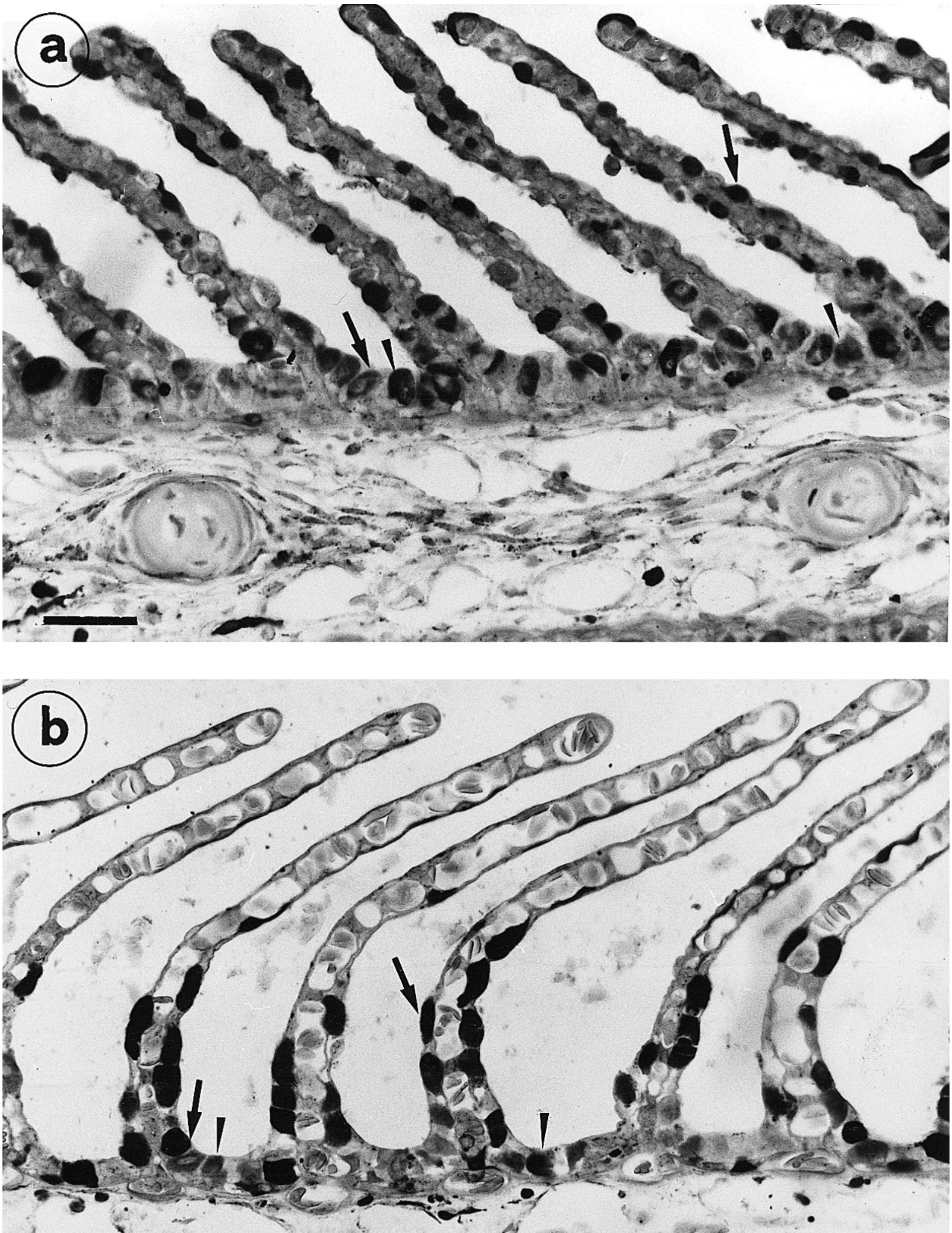

FIG. 2. Cross-section of gill filaments of *Acipenser naccarii* (a) living in fresh water, (b) acclimated to sea water from 60 days. Arrow indicates chloride cells contacting lamellar vessels, arrowhead indicates chloride cells extending towards the filament vessels. Scale bar, 50 μm.

for the euryhaline chondrosteans. In *A. naccarii*, variations in the number of chloride cells with salinity was evident only for the lamellar cells, both in BW and SW.

TABLE III. Mean numbers (± S.D.) of chloride cells (Cc) on the lamellae (*L*, *n* for each specimen = 168) and on the interlamellar spaces (*S*, *n* for each specimen = 112) in freshwater-, brackish water- and seawater-acclimated *A. naccari* in the two trials

	No. of fishes	Cc/*L*	Cc/*S*
Trial 1			
FW	2	8·83 ± 0·09	0·76 ± 0·24
BW	2	6·68 ± 0·21	1·57 ± 0·15
Trial 2			
FW	3	10·35 ± 1·90	1·30 ± 0·12
BW	2	7·22 ± 0·48	0·84 ± 0·28
SW	1	6·15 ± 0·90	1·50 ± 0·55

According to Natochin *et al.* (1985), it is possible that sturgeons follow osmoregulatory mechanisms somewhat dissimilar from those of the teleosts. However, another conclusion can be drawn from the above preliminary observations: notwithstanding the fact that during the acclimation trials young *Acipenser naccarii* survived for a long period in SW, some features suggest an increasing difficulty to adapt. In fact serum osmolality increased with exposure to SW, and an osmotic imbalance is considered a symptom of ' non-adaptation ' (Franklin *et al.*, 1992). The 20‰ salinity could represent the adaptability threshold for the Italian sturgeon as, when other stress factors, such as the non-optimal acclimation conditions of Trial 1, were added to osmotic stress osmotic imbalance emerged. Unfortunately, information concerning the biology of this species in the wild are scarce. This sturgeon may remain in estuarine brackish waters (Paccagnella, 1948), possibly enduring only brief excursions to the open sea.

On the other hand acclimation of young specimens of a single age class (1·5–2 yr old) does not allow detection of the presence of a possible smolting period, i.e. an exact period of the life cycle in which animals are physiologically prepared to adapt to salinity variations, as in salmonids (Boeuf, 1987). For young *A. transmontanus* Richardson an increased salinity tolerance with fish size has been observed, which is possibly related to ' maturational events ' (McEnroe & Cech, 1985).

Details of the patterns of osmoregulation in *A. naccarii* remain to be investigated.

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