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Acclimation of the sturgeon, *Acipenser naccarii* Bonaparte 1836 to saltwater: Effect of age and weight

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SUMMARY – Sturgeons of four ages/weights (1+, 2+, 3+, 4+) were subjected to a progressive change from freshwater to saline waters (33 ppt) and then maintained 30 days at this salinity level. Parameters as survival rate, weight changes, body composition, osmolality and main ionic and urea concentration in blood samples were periodically monitored. As a whole, physiological parameters mainly related with blood osmolality increased as environmental salinity did, until they reached a plateau between 21st and 32nd days (salinities, 26.8 ppt and 30.5 ppt, respectively). An overall consideration of the results led us to conclude that age 2+ results the most favourable for these purposes. Although more in depth studies are required, this first approach resulted in a very encouraging balance.

Key words: Sturgeon, *Acipenser naccarii*, marine aquaculture.

RESUME – *"Acclimatation de l'esturgeon,* Acipenser naccarii *Bonaparte 1836, à l'eau de mer : Effet de l'âge et du poids". Des esturgeons de quatre âges/poids (1+, 2+, 3+, 4+) sont soumis à un changement progressif de l'eau douce à l'eau salée (33 ppt) et ils sont maintenus pendant 30 jours dans cette salinité. Les paramètres comme taux de survie, changement de poids, composition corporelle, osmolarité et concentrations principales des ions et urée dans le sang sont déterminés périodiquement. Toutes les valeurs des paramètres physiologiques en rapport avec l'osmolarité du sang ont subi un accroissement en accord avec la salinité de l'environnement, jusqu'à atteindre la stabilité entre les jours 21 et 32 (salinités 26,8 ppt et 30,5 ppt respectivement). Une considération générale des résultats permettrait de conclure que l'âge 2+ est le plus favorable pour ces propos, bien que de nouvelles études soient nécessaires.*

Mots-clés : *Esturgeon,* Acipenser naccarii*, aquaculture marine.*

Introduction

Until later 60' there was an important sturgeons fishery activity in the Guadalquivir River (Southern Spain) which was used for caviar production. Since 80', *Acipenser sturio* has been considered as the only sturgeon species autochthonous from Iberian Peninsula, although there were important references supporting the existence of three autochthonous species: *Huso huso* (Linnaeus, 1758), *A. naccarii* (Bonaparte, 1836) and *A. sturio* (Linnaeus, 1758). Nowadays this assertion has been fully demonstrated (Domezain *et al.*, 1997; Garrido-Ramos *et al.*, 1997; Hernando *et al.*, 1997).

A. naccarii is being the object of freshwater intensive culture with a notable success. In order to consider this species as an exploitable marine resource, a first problem to solve is the achievement of the control of their induced acclimation to the seawater. The studies at this respect are very scarce in this and other related species (McEnroe and Cech, 1985; Cataldi *et al.*, 1995, 1997; Sánchez de Lamadrid and Vioque, 1995). The experiment now presented is included into a wider Project aimed to the development of the most suitable methodology for the forementioned controlled seawater adaptation.

Material and methods

Nine experimental groups (eight fish each one) were made from sturgeons (*A. naccarii*) grown in a freshwater farm (Piscifactoría "Sierra Nevada", Riofrío, Granada, Spain). Four of them (groups CF) were maintained, as controls, in the facilities of the fish farm and corresponding to the age/sizes 1+, 2+, 3+ and 4+, respectively. Other four groups (S), with the same age/size composition were moved to the saltwater adaptation facilities (CICEM "El Toruño", Cádiz, Spain). Finally, a ninth group (1+, CF/S) was also maintained in saltwater facilities but in a pond exclusively provided with freshwater acting as an *in situ* control. The initial mean weights corresponding to each fish size were 387 (1+), 1242 (2+), 3627 (3+) and 5410 (4+) g.

Saltwater acclimation was induced by a progressive increase in the salinity levels of the respective ponds according to a temporal pattern displayed in Fig. 2. As can be seen, the changes were initially more pronounced and then became slower. Once reached the planned salinity top (33 ppt, at day 44th) the surviving fish were maintained at this salinity an additional period one month long.

Throughout the experimental period, all the groups were fed on a semi-moist pellet manufactured in the fish farm at a daily ration size on 1% body weight. All the fish were individually labelled.

Parameters measured: fish weight evolution (all the fish), blood [red blood cells count (RBC), haemoglobin, haematocrit], and plasma parameters (Osmolality, Na^{+}, K^{+}, Cl^{-}, Mg^{2+}, Ca^{2+}, phosphate, glucose, urea) were measured. These analysis were performed at the beginning and the end of the experimental period in CF lots. In lots S and CF/S the analyses were repeated when environmental salinities reached the values 0, 20, 27, 30 and 33 ppt and, also, at the end of the experiment (one moth after the salinity 33 ppt was reached). The blood analyses were performed on three fish of each lot. Initial values of CF lots were used as corresponding to salinity 0 in S lots.

In acclimation ponds salinity, temperature, oxygen, ammonia, nitrites and pH were daily measured by using a data-logger GRANT-YSI 3800 colourimetric assay-kits (Merck). Throughout the experiment oxygen concentration in these ponds was 6-8 ppm while temperature ranged 20-23°C. Nitrogenous wastes never reached toxic levels.

Analytical methods: fish were weighed after sedation. Then, blood was extracted from the caudal vein; a fraction was used for RBC count, haemoglobin and haematocrit determination by using standard techniques. The remaining blood was centrifuged and plasma separated. Electrolytes were measured by using selective electrodes in an automatic system (SYNCHRON CX3, Beckman Instruments); the other parameters in a discrete flow colourimetric automatic system (SYNCHRON CX4CE, Beckman Instruments), osmolality by cryoscopy with a semiautomatic osmometer (OSMOMAT OM620).

Health of fishes was continuously evaluated by means of a series of analytical and clinical controls; blood and external microbiological cultures were also performed. All the controls did not show pathological problems.

Results and comments

If the survival rate is considered as an index of the success/failure of the process, the age/size 2+ revealed as the most favourable for starting the process of saltwater adaptation (survival rate = 88%). These fish also exhibited the most favourable weight evolution (Fig. 1) followed by 4+ and 1+ fish, although these groups exhibited higher mortality rates. Fish selection and moving to the experimental ponds could imply a considerable degree of stress and so, all the fish, including CF, underwent initial weight decreases that were progressively attenuated and, even, reverted. In contrast, the mortality rates in S fish were higher as water salinity was. So, it could be concluded that a fraction of fish became relatively well adapted (survival and growth), while another fraction failed in this purpose and died as the experiment went forwards. The relative importance of the former fraction was the highest in fish 2+.

Blood cells parameters displayed higher values as the fish age/size increased (Table 1).

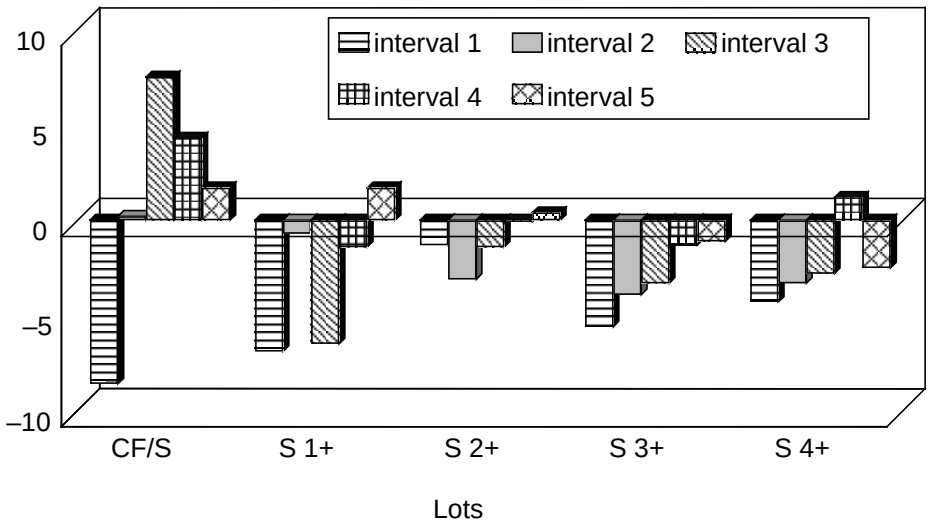

Fig. 1. Evolution of weight changes (% 10 days) in the intervals between samplings during the experimental period.

Table 1. Effect of sturgeon age on some blood parameters

Lots	Age/size	RBC count (thousands/mm^3)	Haemocrit (%)	Haemoglobin (g/100 cm^3)
CF initial	1+	368.3	18.7	5.3
	2+	655.0	20.3	7.2
	3+	583.3	27.3	8.0
	4+	685.3	28.0	7.4
CF final	1+	445.0	18.2	3.8
	2+	541.7	19.7	5.7
	3+	655.0	26.0	7.2
	4+	585.0	25.8	6.7

A certain fall of these indices was detected as the experiment advanced both in CF and S fish, (Table 2) so this fact can not be attributed, at least in full, to the salinity changes. Repeated sampling effects on the same fish were not detected in these indices.

Table 2. Effects of saltwater induced acclimation on some blood parameters

Lot	Age	RBC count (thousands/mm^3)		Haemocrit (%)		Haemoglobin (mg/100 cm^3)	
		Initial	Final	Initial	Final	Initial	Final
CF/S	1+	368.3	515.0	18.7	19.3	5.3	4.8
S	1+	368.3	506.7	18.7	16.7	5.3	4.2
S	2+	655.0	590.0	20.3	17.0	7.2	4.2
S	3+	583.3	607.5	27.3	24.5	8.0	7.0
S	4+	685.3	598.3	28.0	16.3	7.4	5.4

Concerning the parameters measured in plasma, glucose was not affected by the environmental increasing salinity nor the age/size.

In CF fish, maintained in freshwater, there was no significant age differences in osmolality and electrolytes concentration, although a slight trend to present more reduced values in younger fish was evident (Table 3) reflecting a comparatively more "diluted" blood as expected in strictly adapted freshwater fish, being reasonable to assume a later progressive preparation to more concentrated waters determined by some type of endogenous conditioning.

Table 3. Effect of fish on plasma osmolality and main electrolytes concentration

	Osmolality (mOsm/l)	Sodium (mEq/l)	Chloride (mEq/l)
Sturgeons 1+	272.7	133.3	120.7
Mean other three ages	289.2	138.9	122.0

Total osmolality as so as plasmatic levels of sodium, potassium, chloride and magnesium ions increased in the fish subjected to increasing salinity with respect to both controls (CF and CF/S) (Figs 2, 3, 4 and Table 4), reaching respective peaks in the samples corresponding to salinities 26.8-30.5 ppt. Then, these values trend to become stable or, even, to reduce (sampling #6). Plasma calcium levels were also increased during seawater adaptation, while phosphate ones moved in opposite sense. This whole pattern was common to the four ages/sizes tested, suggesting a closely similar effectiveness/failure of the osmoregulatory mechanisms, irrespective of age. So, this criterion seems not to be enough to decide the age/size more suitable for starting seawater acclimation process. The ability of fish to bear with success the unavoidable changes in their body fluids composition might be the determining factor.

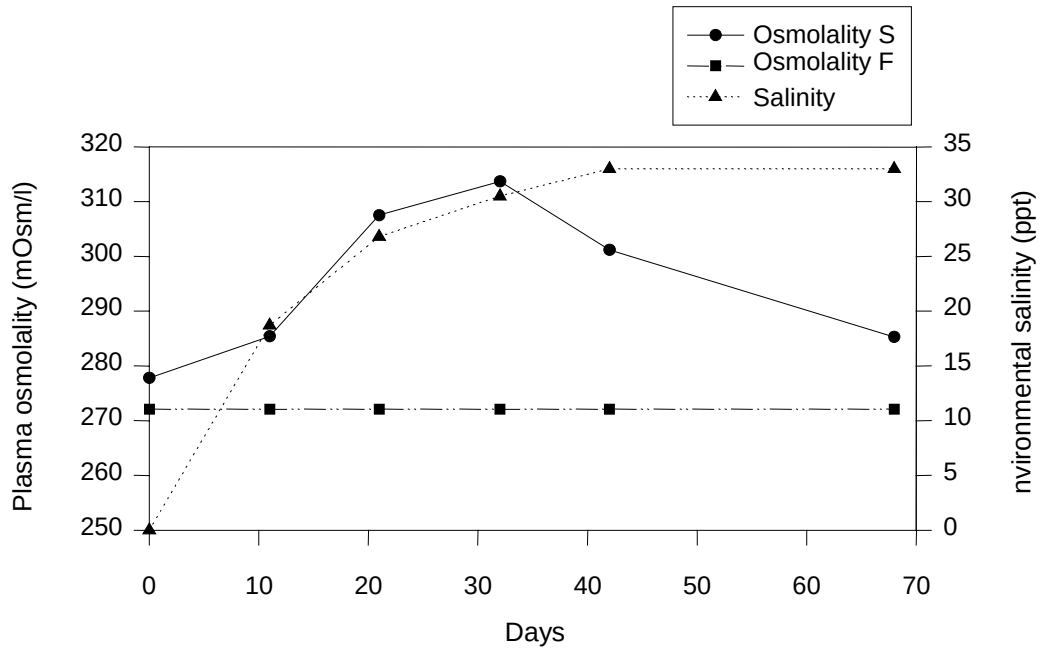

Fig. 2. Effect of environmental salinity on plasma osmolality of sturgeons maintained in freshwater (F) or in increasing salinity water (S).

It results highly significant and interesting the increase of urea plasma levels during seawater adaptation, remaining the levels of this metabolite high even when inorganic osmolytes trend to

decrease. The role of several nitrogenous metabolites (urea, free amino acids, trimethylamine oxide) has been clearly established in several aquatic animals including some fish.

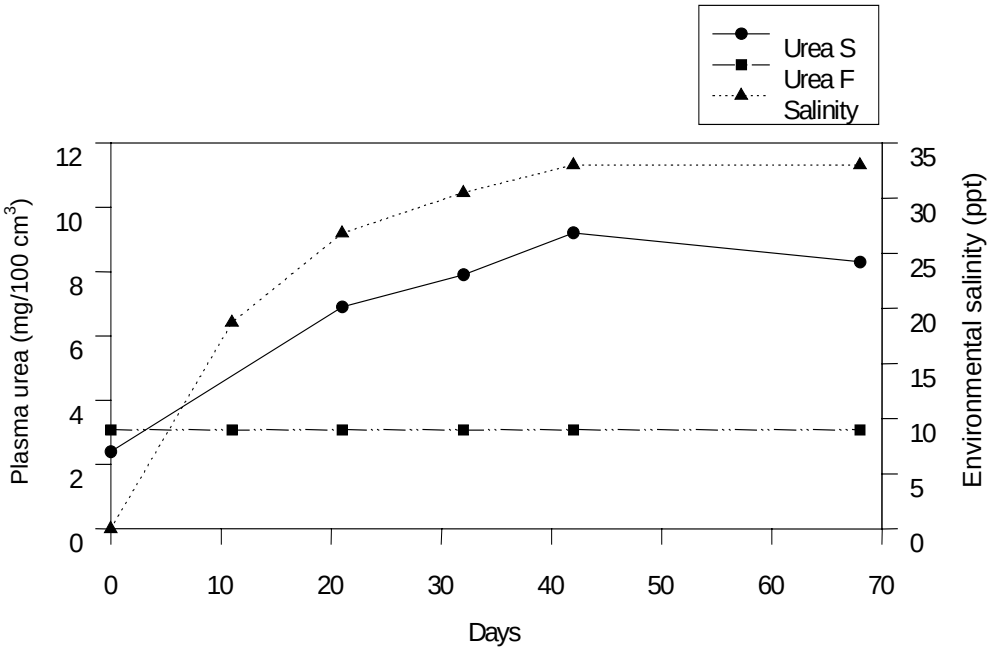

Fig. 3. Effect of environmental salinity on plasma urea concentration of sturgeons maintained in freshwater (F) or in increasing salinity water (S).

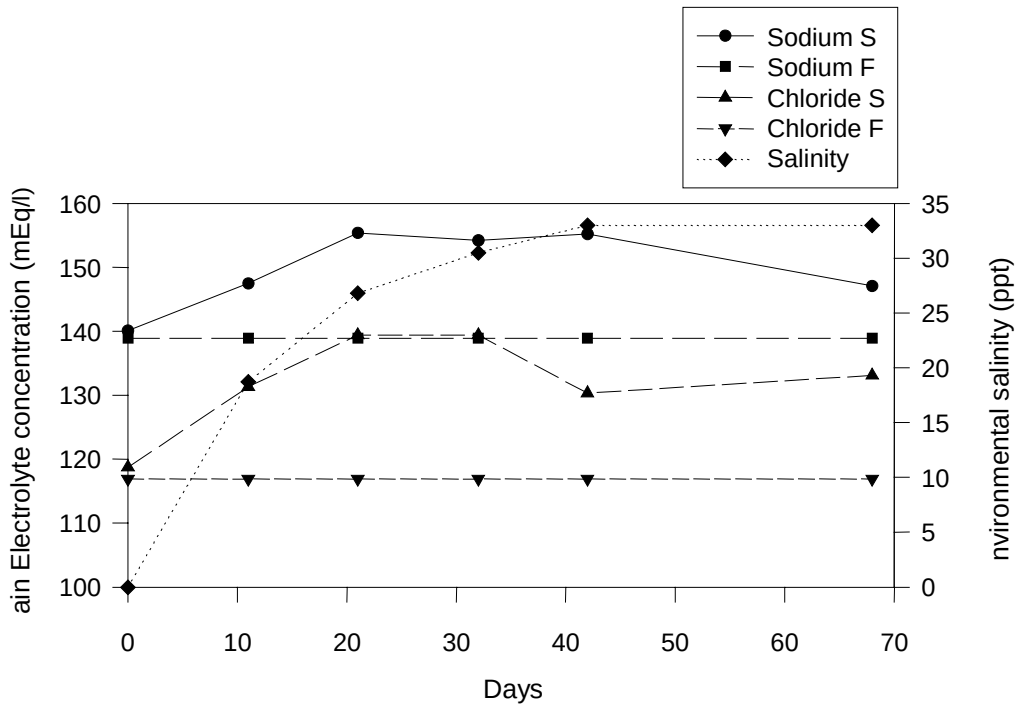

Fig. 4. Effect of environmental salinity on chloride and sodium ions concentration on plasma of sturgeons maintained in freshwater (F) or in increasing salinity water (S).

Overall, from the results it becomes clear from this experiment the possibility of getting an artificial induction of saltwater adaptation of this species taking advantage of the its diadromous character.

More studies are required to go deep into the physiological basis of the process, in order to design a successful methodology.

Table 4. Evolution of plasmatic concentration of minorities electrolytes (mEq/l)

	Day of the experiment/environmental salinity					
	0/0	11/18.7	21/26.8	32/30.5	42/33	68/33
Mg^{2+}	2.4	2.7	3.5	3.5	n.a.†	n.a.
Ca^{2+}	8.6	9.3	9.6	9.3	n.a.	8.2
K^{+}	2.9	3.0	2.7	2.6	3.0	3.5
Phosphates	11.2	11.6	11.0	9.5	n.a.	n.a.

†n.a. = no available.

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