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Acclimation trial of *Mugil cephalus* juveniles to freshwater: morphological and biochemical aspects

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Synopsis

Acclimation trials of *Mugil cephalus* juveniles (SL = mean 28.05, sd = 3.54 mm) were performed by means of a gradual transfer to freshwater in 48 h. The adaptation capabilities of *M. cephalus* were evaluated using descriptors such as tissue osmolality, chloride cell number, Na^+-K^+ ATPase activity and morphological aspects of oesophagus and gills. The high survival rate and tissue osmolality regulation demonstrate the adaptation ability of striped mullet of this size. Chloride cell number and osmolality proved to be good markers of functional adaptation. The morphological and biochemical aspects of oesophagus and gills in juveniles are similar to those of the adult, suggesting that osmotic regulatory mechanisms are precociously developed to allow the colonization of eutrophic inland waters.

Introduction

Mullets are of great interest to aquaculture, in particular in the Mediterranean. Their euryhalinity and feeding behaviour make them good candidates also for polyculture in eutrophic freshwater.

Mugil cephalus is known to be highly tolerant to salinity variations: the adult survives both in freshwater and at salinities as high as 90‰ (Wallace 1975) and can withstand abrupt salinity changes from 0 to 45‰ and viceversa (Hossler et al. 1979).

There is evidence of mullets spawning in seawater (Arnold & Thompson 1958, Liao 1969, Shehadeh & Ellis 1970, Fitch 1972, De Silva 1980) and freshwater (Yashouv 1969, Johnson & McClendon 1970), naturally or induced.

Eggs can tolerate salinity changes over a wide range, as reported by Lee & Menu (1981) and Walsh et al. (1989). Conversely, a limited degree of salinity tolerance was experimentally found in embryos and newly hatched free embryos (Sylvester et al. 1975, Hu & Liao 1981, Murashige et al. 1991).

Juvenile striped mullets spend most of their first year of life in waters that vary in salinity from near freshwater to full seawater (Kilby 1949, 1955). Limited information is available on *M. cephalus* adaptability to freshwater, notwithstanding the fact that a knowledge of environmental effects on the juveniles is of interest to both the ecologist and the aquaculturist.

Acclimation to freshwater in experimental conditions was never successfully achieved for *M. cephalus* juveniles less than 30 mm SL (Mires et al. 1974, Nordlie et al. 1982), although juveniles longer than 40 mm were successfully acclimated by Nordlie et al. (1982). On the basis of adaptation trials on juveniles of different size groups, the former authors concluded that hypo-osmotic regulatory ca-

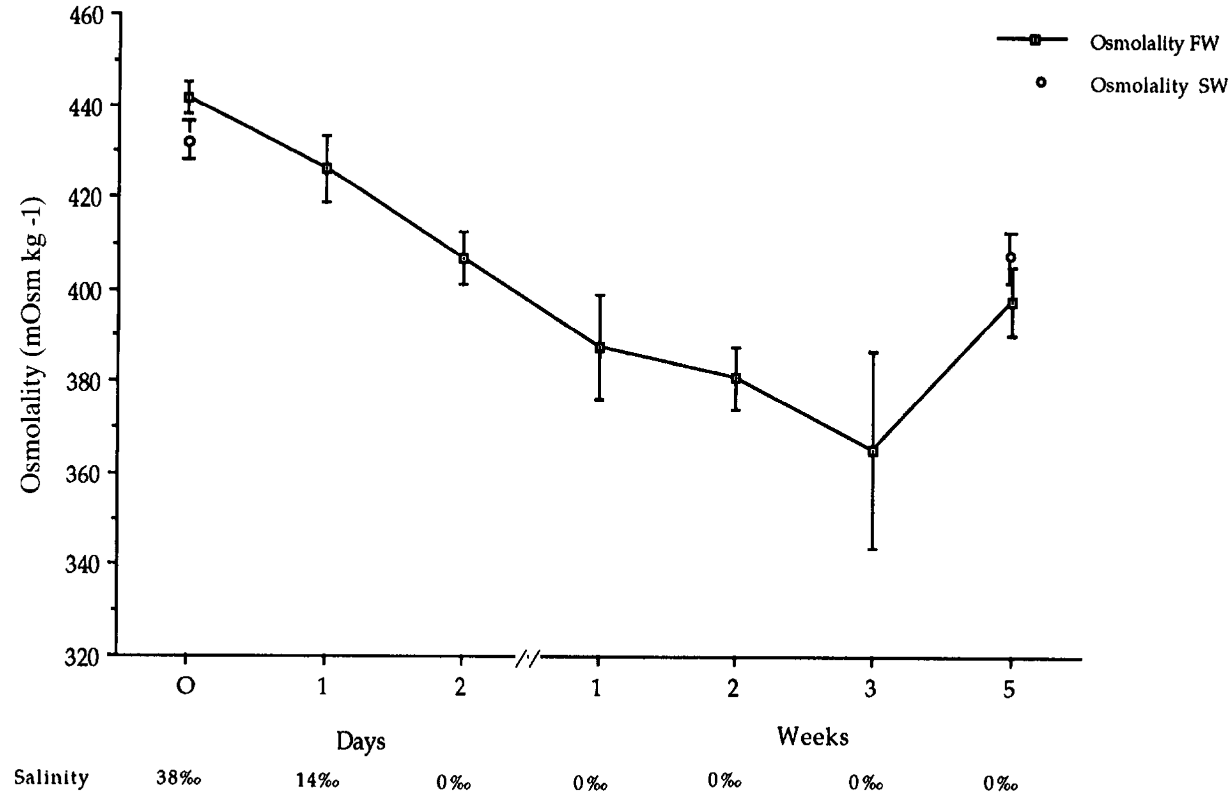

Fig. 1. Tissue osmolality (mean ± s.d.) as a function of salinity and time of exposure to freshwater.

pabilities are not in a definite state at a size smaller than 40 mm SL.

Little information is available on *Mugil cephalus* osmoregulatory mechanisms. Gill chloride cells have been described with regard to their ultrastructural, biochemical and quantitative aspects (Hossler et al. 1979, Abraham et al. 1991, Cioni et al. 1993). Recently Cataldi et al. (1993) described a peculiar feature of the oesophagus, different from the commonly accepted model (Kirsch et al. 1985), for freshwater-adapted mullet and sea bass.

The present paper reports the results of juvenile *Mugil cephalus* adaptation trials to freshwater. The adaptation ability was evaluated taking into consideration some morphological and biochemical aspects of two osmoregulatory organs, oesophagus and gills, as well as tissue osmolality.

Materials and methods

Acclimation

Mugil cephalus juveniles (n = 5000) were caught in the Tyrrhenian Sea (Mediterranean Sea) and kept in rearing tanks (1000 l) at 38‰ salinity for two months. At the beginning of the trial, juveniles (SL = mean 28.05, sd = 3.54 mm) were stocked in three square PVC tanks (500 l) equipped with an open freshwater system (flow rate 0.2 l sec^{-1}), at a density of 2.5 ind l^{-1}. Three identical acclimation trials were simultaneously carried out, gradually replacing seawater (SW: 38‰, 1113 mOsm kg^{-1}; 14° C) with freshwater (FW: 0‰, 18 mOsm kg^{-1}) in 48 h. Acclimation in freshwater was then protracted for 36 days. Temperature ranged between 11–17° C and dissolved oxygen was above 7 mg l^{-1}. During the transition to FW the fish were starved, and then fed once a day ad libitum with commercial food. Mortality was recorded daily. The same experimental

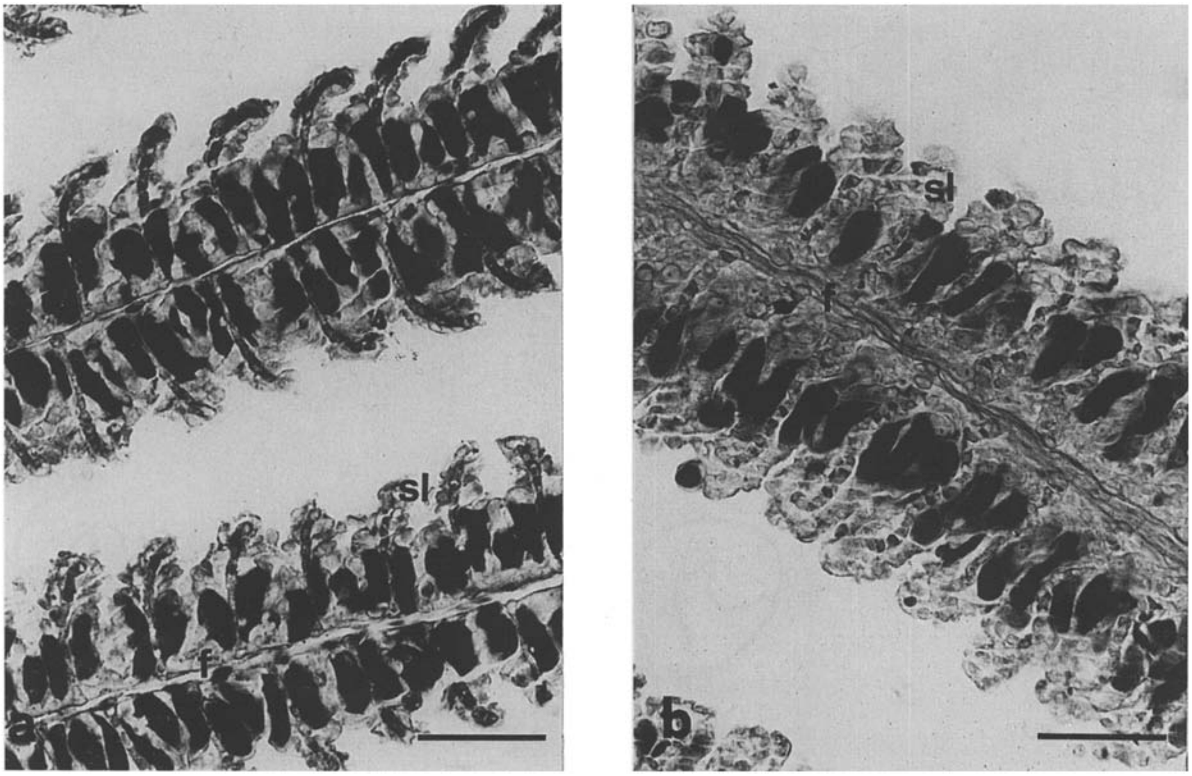

Fig. 2. Gill arches of SW (a) and FW (b) acclimated *Mugil cephalus*. Chloride cells appear strongly stained in black in SW, while they are more heterogeneously stained in FW. f = filament, sl = secondary lamellae. Bar = 40 µm.

conditions were followed for a control group maintained in seawater (SW).

Sampling

All samplings were carried out on fish starved for 24 h, and measured for standard length (mm) and weight (g).

At one day from the start of the trial (salinity 14‰), after 2 d (0‰), and then at weekly intervals, ten individuals were randomly sampled, and whole body osmolality and Na^+-K^+ ATPase activity in oesophagis and gills were measured. Three individuals were sampled and fixed for histological observation of oesophagus and gills at weekly intervals.

At the beginning and at the end of the trial the same samplings were carried out on the SW group.

Biochemical and histological methods

Tissue **osmolality** was measured on homogenates of the whole body, centrifuged at 5000 rpm for 15 min. Osmolality was then determined using a cryoscopic method (One-ten Osmometer, Fiske).

Table 1. Mean number of chloride cells per inter-lamellar space in SW and FW adapted *Mugil cephalus* juveniles.

	SW	FW 9 d	FW 22 d	FW 36 d
mean	2.120	2.285	2.387	2.497
s.d.	0.060	0.085	0.085	0.080
p	* vs. 9, 22, 36 d	* vs. SW	* vs. SW	* vs. SW

* = $p < 0.05$.

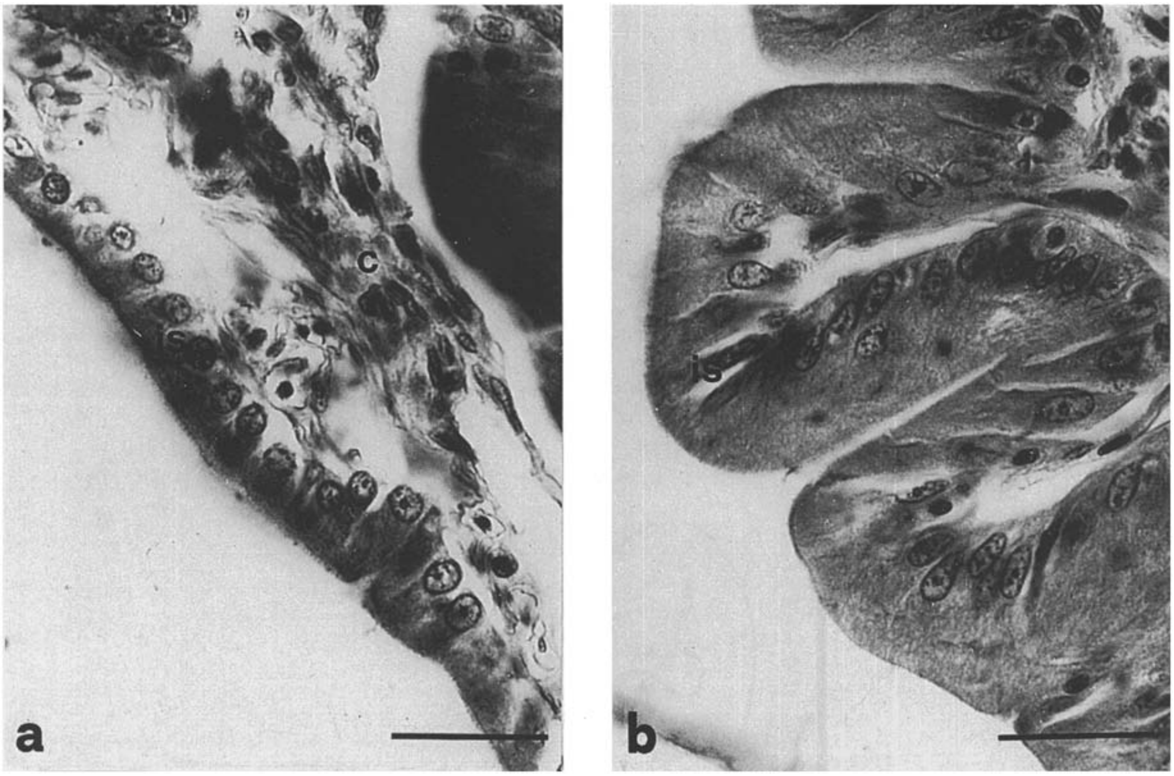

Fig. 3. Posterior oesophageal mucosa of SW (a) and FW (b) acclimated *Mugil cephalus*. c = capillaries, is = intercellar spaces. Bar = 20 µm.

ATPase activity was measured on fresh gill arches and oesophagus. The method is described in detail in Venturini et al. (1992).

For **histological observation** oesophagus was fixed in Bouin's solution, dehydrated, embedded in paraffin, sectioned at 7 µm thickness and stained with hematoxylin, eosin and alcian blue.

Gill arches 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, gill arches were sectioned at 5 µm thickness. No staining followed, as this fixative selectively stains chloride cells.

Chloride cells were counted on 90 intra-lamellar spaces, randomly selected on different gill arches, for each fish.

Statistical analysis

Osmolality, oesophageal and gill ATPase values and chloride cell numbers were processed by a one-way ANOVA test. The same variables were subjected to a multiple regression test, in order to identify the variable best correlated to freshwater. Within the stepwise regression model, the time of exposure to freshwater was considered as the dependent variable.

Chloride cell numbers in the different acclimation conditions were compared by means of t-tests.

Results

During the adaptation trial *Mugil cephalus* juveniles showed normal behaviour, swimming and feeding activities. Final size reached after 36 d was similar in FW and SW (SL = mean 36.6, sd 2.4 and mean 36.8, sd 2.7 mm, respectively). Low mortality rates were recorded both in FW and SW groups during the 48 h acclimation (0, 0.54 and 0.72% in FW, 0% in SW), and in the following period as well (5.27, 6.55 and 7.09%, in the three FW groups, 1.64% in the SW group).

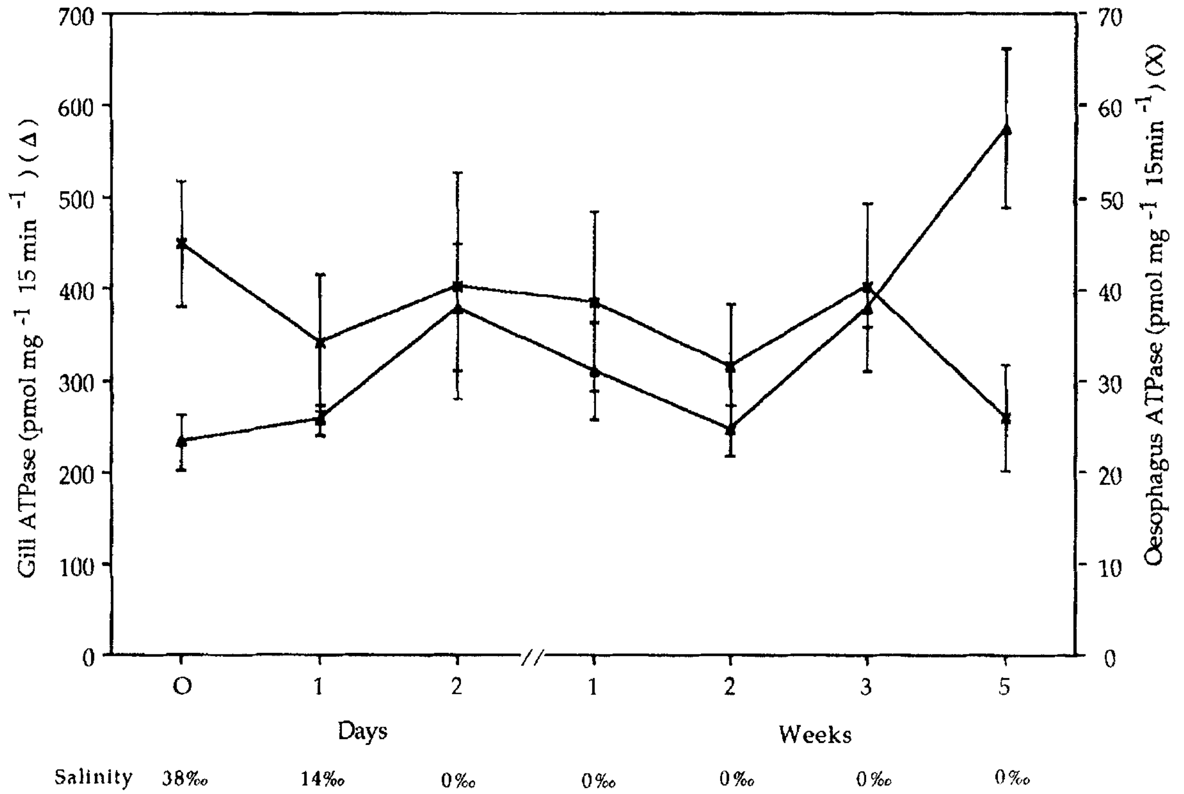

Fig. 4. Gill (Δ) and oesophagus (X) Na^+-K^+ ATPase activity (mean ± s.e.) as a function of salinity and time of exposure to freshwater.

Tissue osmolality decreased in fish held in FW for two weeks, then significantly increased towards SW values (Fig. 1). Tissue osmotic concentration values after 1 d of acclimation (14‰) were not significantly different from SW values, but were always higher ($p < 0.05$) than those observed in FW (2, 9, 16, 22 and 36 d).

Gills (Fig. 2) of juveniles held in SW exhibited chloride cells located on the filament, on the afferent side and in the inter-lamellar spaces, leaning against the lamellae. These strongly stained cells had a basal nucleus and an apical pit. Their shape was cylindrical and elongated, and they generally extended as far as the lamellar capillaries.

In FW-adapted juveniles, chloride cells appeared rounder in shape, and were heterogeneously stained. Mean number of chloride cells per inter-lamellar space increased significantly ($p < 0.004$) following transfer to FW (Table 1). No significant differences were observed at the different times of exposure to FW.

The **oesophageal** mucosa (Fig. 3) of juveniles in SW was completely multilayered in the anterior portion, with many mucous cells and taste buds. Behind this portion the epithelium became thinner, but mucous cells and taste buds were still present. Areas of richly vascularized, monolayered epithelia were present, composed of cuboidal cells with large inter-cellular spaces. This epithelium became predominant in the posterior region. Following adaptation to FW, the monolayered epithelium was still present at the top of the folds.

Gill **Na^+-K^+ ATPase activity** (Fig. 4) strongly increased in specimens immediately after exposure to FW ($p < 0.02$). After 16 d in FW, during which no significant decrease was observed, enzymatic activity increased again: after 36 d in FW, gill Na^+-K^+ ATPase activity reached values about 2.6 times higher than those observed in SW ($p < 0.003$).

Oesophagus ATPase values (Fig. 4) in SW did not differ from those observed in FW. However, it must be noted that ATPase activity displayed strong variations within both SW and FW groups, irrespective of the time spent in FW, as shown by the large standard error values.

Stepwise regression analysis showed that, within the regression model, chloride cell numbers ($F = 20.43$) and tissue osmolality ($F = 10.84$) best described adaptation to FW.

Discussion

The results obtained in this study suggest that a gradual acclimation to FW (in 48 h) ensures complete survival in *Mugil cephalus* juveniles ranging in size from 20 to 30 mm SL.

Apart from some empirical knowledge, there is no evidence in the literature concerning the adaptability of *M. cephalus* juveniles to FW.

Osmolality values measured on tissue homogenates were similar to plasma osmolality values reported by Nordlie et al. (1982) for *M. cephalus* juveniles in SW, thus supporting the validity of the method. Tissue osmolality was found to be a good marker of functional adaptation to FW in *M. cephalus*: its decreasing trend followed the salinity decrease, although a significant recovery was observed over the 3 week period in FW. Plasma osmolality recovery is considered indicative of acclimation capability (Parry 1960a, Boeuf 1987, Franklin et al. 1992).

The oesophagus in striped mullet juveniles adapted to FW did not undergo any drastic modifications, retaining areas of monolayered epithelium as in the adult (Cataldi et al. 1993) and in sea bass (Cataudella et al. 1991). Also, the number of chloride cells was found to be significantly higher in FW adapted *Mugil cephalus* juveniles than in SW, as recently reported for adult sea bass and striped mullet that were fully adapted to FW (Cioni et al. 1993). Conversely, previous works reported a higher number of chloride cells in SW for other euryhaline species (Pisam 1981, Laurent & Hebebi 1989).

Oesophageal ATPase activity did not seem to be related to environmental salinity, unlike what was observed in *Dicentrarchus labrax*, in which the oesophageal ATPase enzymatic activity was the variable best correlated with decreasing salinity (Venturini et al. 1992).

With regards to the branchial Na^+-K^+ ATPase in *M. cephalus* juveniles, our results cannot be compared to others because of differences in the acclimation protocols and biochemical methods (striped grey mullet, Hossler et al. 1979, Hossler 1980). Lasserre (1971), Lasserre & Gallis (1975) and Gallis & Bourdichon (1976) reported that *Chelon labrosus* showed strongly increased gill Na^+-K^+ ATPase activity in FW, with values twice those in SW, as we observed for *M. cephalus* juveniles, while results for *Liza ramada* were discordant. Furthermore, a high Na^+-K^+ ATPase activity in FW has been reported in several marine euryhaline species (Kirschner 1968, Towle et al. 1977, Doneen 1981, Stagg & Shuttleworth 1982). A reverse pattern has been observed in other species (De Renzis & Bornancin 1984).

The interpretation of the morphological and biochemical changes observed in oesophagus and gills after acclimation to FW is hampered by the scarcity of studies on the specific osmoregulatory mechanisms of *M. cephalus*. Everything considered, morphological and biochemical aspects of oesophagus and gill structure of striped mullet juveniles, in FW and SW, are in accordance with those of the adult and of other euryhaline marine species. This, together with the high survival rates observed, supports the assumption that the osmoregulatory mechanisms at work in the adults are already present in the juveniles of 28 mm SL, as reported also by De Silva (1980). This assumption does not agree with Nordlie et al. (1982), who suggested for *M. cephalus* smaller than 40 mm a low tolerance to low salinities and FW, coupled with the fact that ontogenesis of osmotic regulatory capabilities is not in a definitive state at this size. It is clear that smaller young seem to be more susceptible to salinity changes, as in salmonids (Johnsson & Clarke 1988), red drum (Weirich & Tomasso 1991), tilapias (Villegas 1990) and sea bass (Marino et al. 1994). This has been explained in terms of gill surface/body volume ratios (Parry 1960b, Villegas 1990, Bjerknes et al. 1992). Smaller fish are subjected to a higher osmotic imbalance due to passive exchanges, since their ratio of gill surface to body weight is higher than in larger fish (Muir 1969). Thus, a gradual acclimation to FW could be better endured by juveniles than an abrupt transfer. Sea bass juveniles, in fact, do not tolerate direct exposure to FW (Chervinski 1974), but show high survival rates when gradually acclimated (Marino et al. 1994).

M. cephalus juveniles actually migrate to estuaries at a size of 15 mm (Tang 1975, De Silva & Silva 1979). In estuarine environments salinity changes do not occur instantaneously, but take some time, owing to salinity stratification hampering the mixing of different layers of water. *M. cephalus* young might thus be better able to tolerate slow acclimation times, such as those tested in this trial, than direct transfer to FW. This finding may possibly prove to be of practical interest in improving adaptation protocols for *M. cephalus* rearing in freshwater ponds.

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