

**EFFECTS OF ACUTE SALINITY AND WATER QUALITY CHANGES
ON JUVENILE GREENBACK FLOUNDER, *RHOMBOSOLEA
TAPIRINA* (GÜNTHER, 1862)**

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Background. Greenback flounder (*Rhombosolea tapirina*) is an Australian species with an aquaculture potential. The main aim of this study was to define the effects of acute salinity- and water quality changes on the blood characteristics and the gill structure of the greenback flounder.

Material and methods. Juvenile greenback flounder cultured in sea water (33‰) were transferred directly to salinities of 0, 3, 7, 15, and 40‰.

Results. Fish responded similarly following transfer to 3, 7, and 15‰. In these salinities significant decreases in plasma osmolality occurred during the first four hours, after which effective osmoregulation began stabilising osmolality within 24 hours. Plasma sodium and magnesium concentrations mirrored plasma osmolality, while succinate dehydrogenase activity increased up to three-fold 24 hours after transfer. Fish failed to regulate plasma osmolality following direct transfer to fresh water (pH = 6.5; total hardness 20 mg · l⁻¹). Plasma osmolality decreased rapidly for the first 10 hours post transfer at which time plasma osmolality temporarily increased before resuming its decline. Low water pH (6.2–6.5) was an important contributor to the mortality observed following transfer to fresh water.

Conclusion. Results of this study confirm the greenback flounder to be very tolerant of direct transfer to low- or high salinity and are directly applicable to aquaculture of this species, in particular freshwater treatments against parasites, including trichodinids.

Key words: fish, greenback flounder, *Rhombosolea tapirina*, salinity challenge, osmoregulation

INTRODUCTION

The greenback flounder, *Rhombosolea tapirina* is considered a potential commercial aquaculture species in southern Australia because of a high consumer acceptance, reasonable market price, and defined culture techniques (Purser and

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Thomas 1995). Adaptation to environmental factors was identified as one area of research necessary for flounder culture development (Hart et al. 1993) but to date has been studied little. In the wild, juvenile flounder are estuarine but as adults move offshore to spawn (Crawford 1984). In a culture environment, salinity tolerance has only been studied in early life stages (Hart and Purser 1995) with no data available for juveniles. To assess the growth rate of this species at different salinities, the tolerance range must first be clarified. This tolerance range is also essential to assess the suitability of estuarine or hypersaline land-based farming sites.

Euryhalinity may be used in selected aquacultural species to improve growout performance. Juvenile turbot, *Scophthalmus maximus* have shown better growth and feed conversion efficiency between 15 and 25‰ (Scherrer 1984, Gaumet et al. 1995), summer flounder, *Paralichthys dentatus* at 20‰ (Malloy and Targett 1991), and southern flounder, *Paralichthys lethostigma* larvae (Daniels et al. 1996) than in sea water. At these lower salinities, it has been suggested that oxygen consumption is significantly reduced (Waller 1992), indicating a lower metabolic demand due to decreased energy partitioning for osmoregulation (Kirschner 1995).

The changes in the branchial epithelium in response to salinity changes are rapid and intensive. To compensate, a marked mitochondrial proliferation in chloride cells is common in many species following salinity transfer (Sargent et al. 1975). Succinate dehydrogenase (SDH) is a primary enzyme in the oxidative catabolism of sugars (Lehninger et al. 1993) and as such is used effectively as a marker of mitochondrial abundance and activity. This enzyme is concentrated in the chloride cells within fish gills and has been used as an indicator of the osmoregulatory activity (Sargent et al. 1975, Langdon and Thorpe 1984).

The main aim of this study was to define the effects of acute salinity and water quality changes on the blood characteristics and the gill structure of the greenback flounder. These results may also be used to determine the suitability of this species for use as an example of an osmoregulatory system highly optimised for rapid salinity fluctuations. In terms of aquaculture, the results will improve our understanding of the reaction of flounder to salinity changes at a production site or alternatively, provide guidelines for the selection of suitable production sites.

MATERIAL AND METHODS

Cultured juvenile flounder (80–190 g) were maintained in two 1200-l tanks in a temperature- and photoperiod-controlled recirculating holding facility. Water quality parameters were maintained with temperature at $17 \pm 0.5^\circ\text{C}$, salinity at 32–35‰, pH at 7.9 ± 0.4 , and a L:D of 12:12. The salinity was measured using a conductivity/salinity meter (Yeo-Kal salinity bridge model 602), pH using an Activon pH meter, and ammonia was measured by the ammonia salicylate method and a HACH DR/2000 spectrophotometer. Fish were transferred directly from the holding facility to a series of 100-l experimental tanks filled with 60 l of water adjusted to the

desired salinity. Five flounder were randomly allocated to each of the experimental tanks from the holding tanks. All five fish in each tank were sampled at the one time and were not re-sampled during the experiment to ensure independence of data. Fish from one tank were used as a measure at each time point for each salinity treatment. Each experimental tank was aerated by a single air-stone, which maintained the dissolved oxygen level above 95% saturation. Water exchange was not conducted during the trials, as total ammonia and pH levels were not found to alter significantly in this time. Fish were not fed for 24 h prior to the beginning of the trials.

Experiment 1

This trial tested the plasma osmolality of the flounder following transfer to the salinities of 40, 33, 15, 7, 3, and 0‰. The salinity was adjusted using an appropriate mixture of sea water and aged tap water. Tap water was aged by vigorous aeration for at least 24 hours, and was found to be soft (total hardness 20 mg·l⁻¹, calcium 12 mg·l⁻¹), with pH = 6.5. Salinity greater than 33‰ was achieved by the addition of laboratory-grade NaCl to sea water. Total ammonia remained below 0.2 ppm during all trials, pH ranges can be found in Table 1. Temperature remained constant at 16.5°C.

Table 1

Range of water pH in different treatments

Treatment	Minimum pH	Maximum pH
33‰	8.2	8.3
40‰	8.2	8.4
15‰	7.3	7.6
7‰	7.2	7.4
3‰	6.9	7.3
0‰	6.2	6.5

Experiment 2

In experiment 1 there was an adverse effect on the fish after transfer to 0‰. This experiment is a supplementary trial to experiment 1 investigating more closely the effect of fresh water transfer on the flounder. In the first trial a characteristic of the fresh water (FW) was a low conductivity. In this trial, calcium carbonate (FW + CaCO₃), a combination of calcium carbonate and magnesium sulphate (FW + CaCO₃/MgSO₄) or sodium chloride (FW + NaCl), were added to the fresh water to increase conductivity (see Table 2). Fish were transferred as above from 33‰ to each of these treatments.

Table 2

Summary of water composition for the three trials conducted in experiment 2, and the transfer to fresh water (FW) (experiment 1)

Ions added to FW	pH	Total hardness [$\text{mg}\cdot\text{l}^{-1}$]	Osmolality [mOsm]
CaCO_3	8.0	40	9
$\text{CaCO}_3/\text{MgSO}_4$	7.4	200	8
NaCl	7.3	20	8
none	6.5	20	0

In both experiments 1 and 2, blood (five fish/treatment/time) was sampled from individual fish at 2–4 hour intervals for up to 48 hours (Fig. 1). Time zero (T_0) samples were taken from the fish remaining in sea water (33‰). Blood was extracted from the caudal vein using a heparinised 2-ml syringe and 25-gauge needle following anaesthesia of the fish in $50 \text{ mg}\cdot\text{l}^{-1}$ benzocaine. Plasma was separated from the blood samples by centrifugation at 6000 rpm for three minutes. Plasma was analysed for osmolality (mOsm) using an Advanced™ freezing-point depression osmometer. Plasma from fish transferred to 0, 3, and 15‰ was analysed for sodium and magnesium ion concentrations ($\text{mg}\cdot\text{l}^{-1}$) using a Varian AA-300 atomic absorption spectrophotometer. Fish sampled 24 hours post transfer were sacrificed by benzocaine overdose and whole gill arches from the pigmented side of the fish excised. The first gill arches were fixed (Davidson's fixative) for routine histology, whilst the third and fourth gill arches were frozen at -20°C for SDH analysis. Control gills were sampled at the same time from fish remaining in sea water. Gill samples taken from experiment 2 trials were also stained for the presence of mucus using alcian blue. Morphology of histological sections was assessed using an Olympus BH-2 stereomicroscope at $400\times$ magnification, whilst determination of chloride cell size (diameter) and density (number of cells per lamellar unit) was undertaken using a Cue-2 image analyser at $400\times$ magnification. Cell density was determined following the method of Speare et al. (1997).

Statistical analyses were conducted using JMP v 3.2.1 (SAS Institute INC) statistical package. ANCOVA was used to compare plasma osmolality changes over time for a range of trials simultaneously, whereas ANOVA was used for comparisons between results taken at a single time point. All data were tested for normality using the Shapiro-Wilk W test on residuals, and for homogeneity of variance using Cochran's test. A significance level of $P < 0.05$ was considered significant in all tests. ANCOVA tests fitted time versus the response variable (e.g. plasma osmolality) as a full factorial combination with treatment as the covariate.

RESULTS

Experiment 1

Plasma osmolalities of fish were significantly reduced after transfer from sea water to salinities ranging from 0 to 15‰ (Fig. 1; ANCOVA $P < 0.001$). No significant difference was detected between treatments at T_0 (ANOVA; $P = 0.872$), whereas osmolalities of fish from each treatment, except transfer to 40‰, were found to be significantly lower than the control across the entire sampling period (ANCOVA; $P < 0.001$). Although readings from fish exposed to 15, 7, and 3‰ differed significantly from the control, these treatments were not found to be significantly different from each other (ANCOVA; $P = 0.185$).

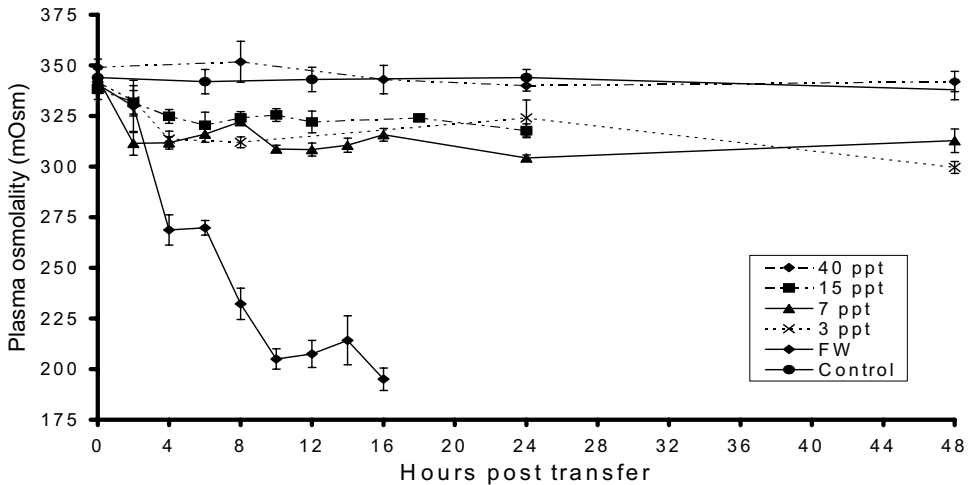


Fig. 1. Plasma osmolalities ($\bar{x} \pm SE$; $n = 5$) following direct transfer from 33‰ to various salinities

No mortalities were recorded following transfer to salinities greater than FW. In contrast, transfer to fresh water resulted in fish mortality and the trial was stopped 16 hours post transfer. This response was characterised by appearance of mucus on the water surface of the tanks 4 hours post transfer, and by 12 hours post transfer the fish were in an unresponsive state with a visibly depressed and irregular ventilation rate. At 16 hours there were two mortalities (6% of the fish remaining in fresh water) and the trial was aborted with the remaining fish transferred to 15‰ for recovery. Recovery was slow, with some improvement in ventilation rate and responsiveness after four to six hours. Further mortalities were recorded from the recovery tanks; 43% of those fish exposed for 16 hours to fresh water did not successfully re-acclimate to sea water following an eight-hour phase in 15‰.

In fresh water, the osmolality did not fall faster than other treatments, with no significant difference detected between the values at two hours post transfer for either the 15, 7, 3, or 0‰ treatments (ANOVA; $P = 0.284$).

Plasma samples from fish transferred to 0, 3, and 15‰ were analysed for sodium and magnesium concentrations. Following transfer to fresh water, sodium and magnesium concentrations dropped steadily for 10 hours, before increasing significantly to 12 hours post transfer (ANOVA; $P = 0.019$). Concentrations then decreased, becoming significantly lower 16 hours post transfer (Fig. 2). Following transfer to 3‰, plasma sodium and magnesium dropped significantly to a minimum value at four hours (Fig. 3, ANOVA; $P < 0.001$). Sodium concentration then rose again to a significantly higher value at 14 hours post transfer (ANOVA; $P = 0.044$). Magnesium concentrations did not differ from the four-hour value for the remainder of the trial. Transfer to 15‰ also elicited a significant drop initially (Fig. 4), to a minimum value at two hours (ANOVA; $P = 0.019$). Values for sodium then rose significantly (ANOVA; $P = 0.011$) to levels not significantly different from the T_0 values at six hours post transfer (ANOVA; $P = 0.84$). Magnesium concentrations although rising sharply did not differ significantly from the two-hour values for the remainder of the trial.

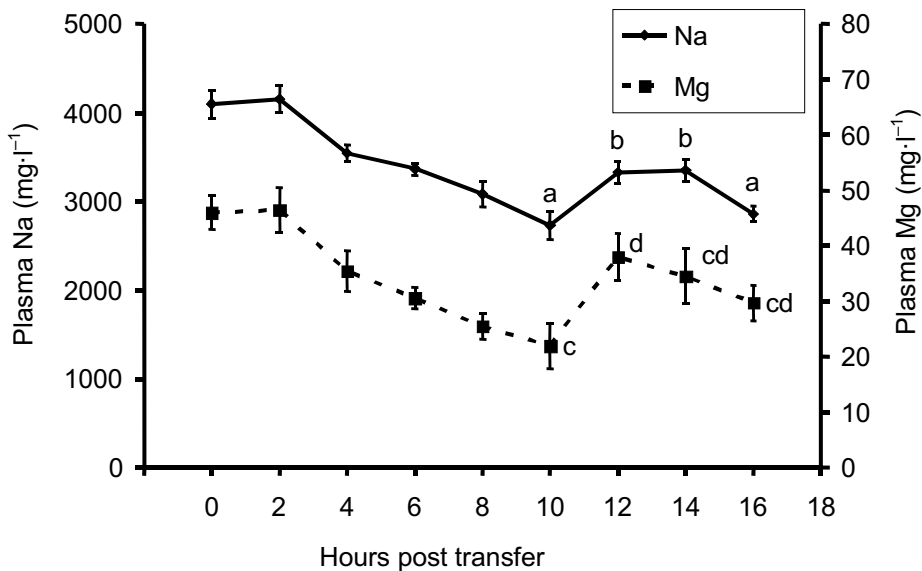


Fig. 2. Plasma concentrations of sodium and magnesium ($\bar{x} \pm SE$; $n = 5$ fish) following direct transfer to FW from 33‰. Common letters denote values not significantly different ($P > 0.05$)

The concentrations of sodium and magnesium in sea water are 10 500 mg.l⁻¹ and 1350 mg.l⁻¹, respectively (Boyd 1990). When comparing these figures with the plasma sodium- and magnesium concentrations, we find that this species regulates these two ions at completely different levels. Plasma sodium is regulated to 36% of the seawater concentration, whereas magnesium remains more than 10 fold lower at approximately 2%. However despite the large difference in concentration gradient for these two ions their response to salinity transfer was very similar (Table 3).

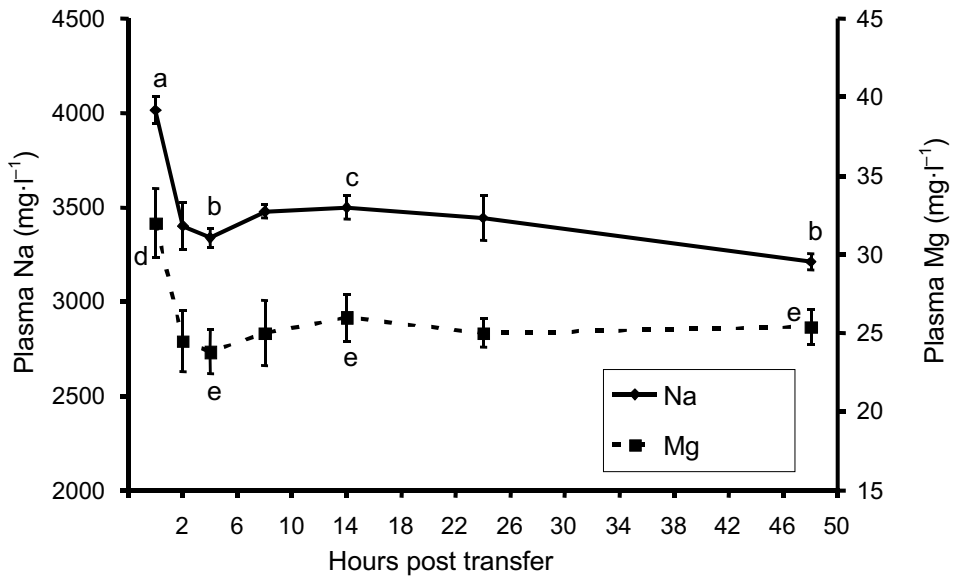


Fig. 3. Plasma concentrations of sodium and magnesium ($\bar{x} \pm SE$; $n = 5$ fish) following direct transfer to 3‰ from 33‰. Common letters denote values not significantly different ($P > 0.05$)

The statistically significant differences in ionic (Na^+ , Mg^{++}) concentration shown in Table 3, indicate a measurable treatment effect. However, when considering these differences in context with the changes in external concentrations of sodium and magnesium across the three treatments, it appears that the differences are minimal and that homeostatic regulation is overcoming the majority of external variations. For instance the difference in sodium concentration between water of 15 and 3‰ is approximately $3500 \text{ mg}\cdot\text{l}^{-1}$, whereas the difference in plasma sodium across these salinities is only $430 \text{ mg}\cdot\text{l}^{-1}$. These results confirm that homeostatic mechanisms overcame the majority of the external variation.

Table 3

Plasma sodium and magnesium concentrations ($\bar{x} \pm SE$; $n = 5$ fish) at their lowest point (as $\text{mg}\cdot\text{l}^{-1}$ and as a percentage of the initial time-zero value) during 3 different trials testing transfer from 33‰ to fresh water, 3‰, and 15‰. Common superscripts denote values not significantly different ($P > 0.05$)

Treatment	Sodium		Magnesium	
	Minimum [$\text{mg}\cdot\text{l}^{-1}$]	Minimum [% of T_0]	Minimum [$\text{mg}\cdot\text{l}^{-1}$]	Minimum [% of T_0]
Freshwater	$2733^a \pm 157.4$	65.7 ± 3.9	$22.0^a \pm 4.2$	41.8 ± 7.6
3‰	$3340^b \pm 120.3$	83.2 ± 3.1	$23.8^a \pm 2.8$	79.1 ± 8.75
15‰	$3770^c \pm 124.6$	89.0 ± 2.9	$26.8^a \pm 1.9$	69.4 ± 5.6

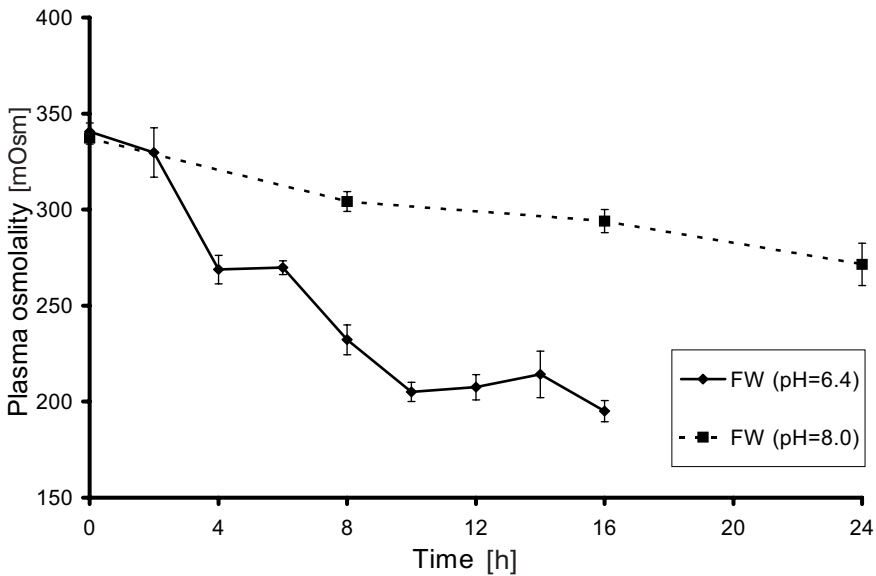


Fig. 4. Plasma concentrations of sodium and magnesium ($\bar{x} \pm \text{SE}$; $n = 5$ fish) following direct transfer to 15‰ from 33‰

Chloride cell density and size were found to decrease after 7 day exposure to 3‰ (Table 4). There were no significant differences 24 hours after transfer to various salinities.

Table 4

Average number of chloride cells per lamellar unit ($n = 150$ units), average surface area of chloride cells, μm^2 , ($n = 100$ cells) and succinate dehydrogenase activity ($n = 5$ fish) 24 hours (unless otherwise indicated) following direct transfer to a range of salinities; $\bar{x} \pm \text{SE}$ common superscripts denote no significant difference ($P > 0.05$)

Treatment	Chloride cell density [cells per lamellum]	Chloride cell size [μm^2]	SDH activity [$\mu\text{mol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$]
SW	$2.19^a \pm 0.13$	$95.1^a \pm 3.1$	$0.19^a \pm 0.13$
FW (16 hrs)	$2.11^a \pm 0.10$	$87.0^a \pm 4.5$	$0.89^b \pm 0.15$
3‰	$2.11^a \pm 0.14$	$90.0^a \pm 3.5$	$0.58^c \pm 0.11$
3‰ (7 days)	$1.45^b \pm 0.03$	$76.1^b \pm 2.1$	$0.23^{ac} \pm 0.10$
7‰	$2.08^a \pm 0.10$	$91.2^a \pm 4.5$	$0.50^c \pm 0.11$
40‰	$2.20^a \pm 0.15$	$93.6^a \pm 5.2$	$0.41^{ac} \pm 0.09$

Significant changes were observed in the SDH activity of the gills from fish transferred to a range of salinities (Table 4; ANOVA; $P = 0.014$). Activity was highest after transfer to fresh water, increasing from 0.19 to 0.89 $\mu\text{mol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$. Activities also rose significantly after transfer to 3, 7, and 15‰, whilst transfer to 40‰ did not increase the SDH activity significantly.

Experiment 2

No mortalities were recorded during these trials. All trials ran the full 24 hour duration, after which there was no visible deterioration of the external appearance or behaviour of the fish. No visible differences in ventilation rate were observed, nor loss of mucus or responsiveness.

Responses of plasma osmolalities were significantly greater after transfer to FW than to FW + CaCO₃ / MgSO₄ or FW + NaCl (Fig. 5; ANCOVA; $P < 0.001$). Transfer to FW brought a rapid and continuing decline in osmolality with fish becoming moribund from 16 hours post transfer. In contrast osmolalities of fish from both the FW + CaMg and FW + NaCl treatments did not continue to rapidly decline, but appeared to level out from 12 hours onwards. No difference was detected between these two treatments (ANCOVA; $P = 0.184$).

Transfer to FW + CaCO₃ also significantly improved tolerance to direct transfer compared to transfer to FW (Fig. 6; ANCOVA; $P < 0.001$). This treatment resulted in a gradual decline in plasma osmolality for the duration of the trial. There was no significant difference detected between any of the three trials run in this section (ANCOVA; $P = 0.793$).

Conventional histology did not reveal any significant differences between the treatments used in this section. Alcian blue staining confirmed mucus to be present on the gills after all treatments tested in this section. In contrast, very little mucus was found on gills from fish subjected to 16 hours in fresh water.

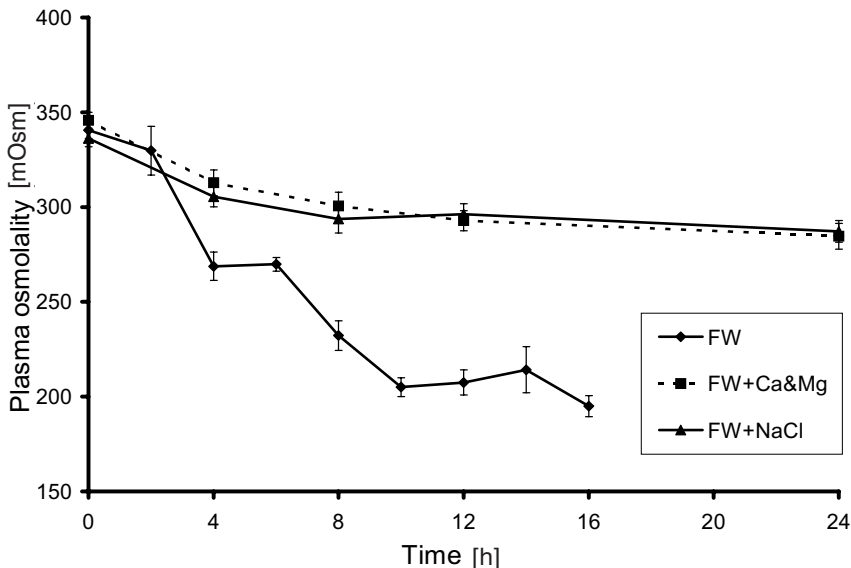


Fig. 5. Plasma osmolalities ($\bar{x} \pm SE$; $n = 5$ fish), following direct transfer to fresh water, fresh water plus calcium and magnesium (to equal 3‰ levels); and fresh water plus sodium chloride (to equal the osmolality of calcium and magnesium treatment)

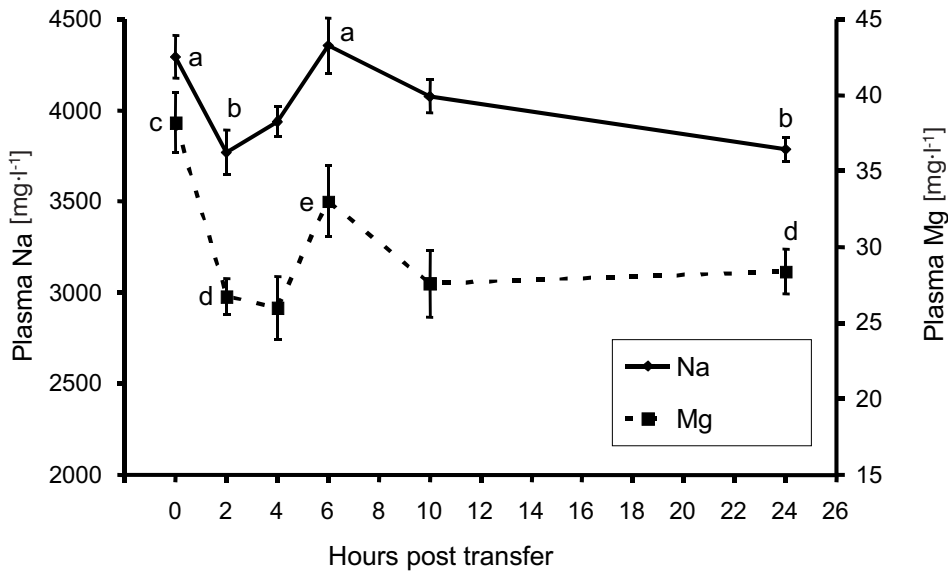


Fig. 6. Plasma osmolalities ($\bar{x} \pm \text{SE}$; $n = 5$ fish), following direct transfer from 33‰ to fresh water (pH = 6.4) and fresh water plus calcium carbonate (pH = 8.0)

DISCUSSION

Numerous phases have been described in fish following salinity challenge. Houston (1959) was the first to describe these phases, defining the crisis phase as a period of rapid and significant change leading up to the stabilisation phase in which homeostatic mechanisms regulate internal conditions to a stable value within the tolerance range.

In this study, similar osmolality responses recorded following transfer to salinities ranging from 3‰ to 15‰, all indicate a crisis phase lasting approximately four hours. This four-hour crisis phase compares favourably with studies on eels, tilapia, and barramundi which found ionic turnaround times of two hours (Kirsch and Mayer-Gostan 1973), three hours (Hwang et al. 1989), and four hours (Almendras 1996), respectively.

Direct transfer to fresh water was found to be too extreme for the osmoregulatory ability of this species. The continuous decline in plasma osmolality was briefly interrupted by short and unsuccessful attempts at regulation from four to six hours and again from 10 hours post transfer before mortalities began at 16 hours post transfer. The initial attempt to osmoregulate at four hours coincides well with the ionic turnaround time seen in other transfers, but why was it not continued and why was it attempted again 10 hours post transfer? One likely explanation lies not in ionic regulation, but in regulation of water balance.

Existing literature indicates that significant decreases in branchial and intestinal permeability would not be expected until between eight and 12 hours post transfer

(Ogasawara and Hirano 1984, Hwang 1987). Thus the four-hour turnaround of ionic mechanisms would see ionic uptake beginning before any significant decrease in permeability. The brief attempt at regulation suggests that the higher osmotic gradient associated with fresh water drives water influx at such a rate as to make any attempt at ionic regulation uneconomical until permeability has been reduced.

Responses to transfers conducted in experiment 2 indicate that only subtle changes in water chemistry are required to avert the severe response recorded following transfer to FW (experiment 1). Treatments tested the effects of extra calcium carbonate and magnesium sulphate, calcium carbonate alone, and sodium chloride. However, associated with each of these treatments was a rise in water pH and osmolality over the FW treatment. As no other water quality parameters were changed significantly, it is likely that one of these adjustments accounts for the significantly improved response.

Whilst it is unlikely that an increase in osmolality of this magnitude (8 mOsm; roughly equivalent to 0.3‰) is itself capable of altering osmotic gradients enough to have an effect, the ions comprising this change may themselves have a significant effect. Environmental hardness has been shown to significantly affect performance and survival in fresh water (Carrier and Evans 1976, Greco et al. 1995). Results of this study indicate increased FW hardness (addition of calcium and magnesium) significantly improved conditions for this species, however given that addition of calcium carbonate alone produced the same improved result indicates magnesium is unlikely to be the deciding factor. Similarly the increase in calcium can also be discounted due to the improved response following addition of sodium chloride alone. Thus these transfers rule out lack of either calcium or magnesium as the cause of severe osmoregulation stress following transfer to FW.

The last water quality variable in these trials was pH. Treatments in experiment 2 tested fresh water with pH between 7.3 and 8.0, significantly higher than FW treatment in experiment 1 (pH = 6.5). All treatments produced a similar, improved response indicating that the pH rise from 6.5 to 7.3 was sufficient to enable successful osmoregulation following transfer directly from sea water.

One of the key effects of low pH is increased ionic efflux (McDonald et al. 1991), thus acidic environments exacerbate the problems associated with transfer to hypotonic media. If sufficiently acidic, ionic efflux will be too extreme and the compensatory mechanisms will not be powerful enough to conserve internal osmolality. Environmental calcium levels play a major role in pH toxicity, because sufficient calcium will reduce branchial permeability masking the effects of low pH (Carrier and Evans 1976). From these results it may appear that at 12 mg·l⁻¹ of environmental calcium, branchial permeability is sufficiently low to enable successful transfer to FW, with pH of 7.3. However this calcium concentration is not sufficient for the lower pH of 6.5, at which level internal osmolality was not regulated following direct transfer from sea water.

Although calcium levels undoubtedly play a significant role in the success of a transfer to fresh water, mucus layers also play a critical role in low pH tolerance. Mucus

is a highly multifunctional material which has been implicated in a wide range of functions, including respiration, excretion, disease resistance, and osmoregulation (Shephard 1994). It is comprised of polyanionic glycoproteins which bind cations such as calcium and has been found to significantly impede ionic diffusion, to as low as 15% of the rates through distilled water (Marshall 1978). These properties enhance ion retention, thus it follows that mucus hypersecretion is a common response to low pH environments (Jago and Haines 1990, McDonald et al. 1991, Laurent and Perry 1991).

Immediately following transfer to FW, mucus began sloughing from the fish. This mucus began to appear on the water surface two hours after transfer, and by 16 hours at the first mortality fish were tangibly coarse to the touch, devoid of mucus as confirmed by alcian blue staining. A similar response was described by Handy et al. (1989) in rainbow trout. They found that at low pH, mucus was dialysed much more easily resulting in increased ionic efflux. Further lowering of the pH ended in dissolution and sloughing of mucus from the body of the fish. Following mucus loss, fish failed to osmoregulate properly and mortality followed.

These studies indicate that the increased ionic efflux under acid conditions is greatly increased at pH levels causing loss of mucus. Thus, defining the minimum pH of fresh water as that which permits mucus retention rather than defining the minimum hardness may more accurately represent the deciding factor in survival following transfer to fresh water.

Chloride cell size and density were both found to decrease after 7 day exposure to low salinity. This response has long been associated with the freshwater adaptive effect of prolactin (Ogasawara and Hirano 1984, Dauder et al. 1990, Richman et al. 1991) and reflects the reduced requirement for active ion extrusion. However, it is worth noting that recent work has proved conclusively that chloride cells are the primary sites for calcium and chloride uptake in hypotonic media (Flik et al. 1993, Li et al. 1997). Thus, when the media become extremely dilute e.g. fresh water, especially soft water, a proliferative response is observed in the chloride cells.

SDH activity is regularly used as an indicator of mitochondrial activity and can be used as an indirect measure of chloride cell activity (M. Powell pers. comm.). The activity of SDH increased following transfer of flounder to 0, 3, and 7‰, but it was not statistically significant for other salinity transfers or after 7 days in 3‰. This trend of activity of osmoregulatory enzymes is similar to the trend shown by some species of the families Serranidae and Mugilidae as well as Australian bass, but opposite to the trend reported in Salmonidae and Anguillidae or golden perch (Lasserre 1971, Sargent et al. 1975, Langdon and Thorpe 1984, Langdon 1987). The three to four fold increases found here support existing work on eels (Sargent et al. 1975) and pinfish (Bahn and Mansuri 1978) where two or four fold increases were found following FW to SW and SW to FW transfer, respectively. The greater SDH activity in flounder from lower salinities than sea water suggests that the uptake and retention of ions from lower salinity water requires greater osmoregulatory activity by the gills than the

excretion of excess salt in sea water or that the transformation processes are more drastic. This is typical of marine teleosts investigated up to date. The decrease in SDH activity following 7 day acclimation to 3‰ indicates that the mitochondrial abundance and/or activity decreases with acclimation time.

This study has confirmed the greenback flounder to be a truly euryhaline species. The duration of the crisis phase following salinity challenge is comparable with other major euryhaline species, which coupled with rapid re-stabilisation of internal conditions suggests this species to be highly adapted for a rapidly altering environment. The tolerance of soft water ($20 \text{ mg}\cdot\text{l}^{-1} \text{ CaCO}_3$) observed here is very rare in marine species other than flounders, emphasising the greenback flounder as an excellent candidate for further investigation of effective osmoregulation strategies.

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