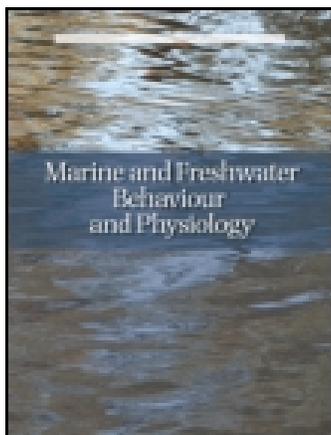


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## Osmoregulatory capabilities of the gray snapper, *Lutjanus griseus*: salinity challenges and field observations

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We investigated the osmoregulatory responses (plasma osmolality and blood hematocrit) displayed by the gray snapper 6–192 h after abrupt changes in ambient salinity. Fish were challenged with six different salinity treatments including a control (0, 5, 30, 50, 60, and 70 ppt) and blood samples were collected at various time points post-transfer. Gray snapper across all size classes tested (13.5–24.5 cm total length) acclimated successfully to hypo- and hyper-saline environments (0–60 ppt) after an adjustment period of ~96 h. However, abrupt transfers to 70 ppt resulted in 100% mortality within 48 h. Laboratory results were then compared with field measurements obtained after fish were captured in low salinity (0–4 ppt) or marine (~30 ppt) habitats, suggesting that osmoregulatory processes occurred similarly in both settings. Overall, findings suggest that gray snapper possess similar or higher osmoregulatory capabilities compared to many euryhaline species examined to date, and thus should be considered a euryhaline species.

**Keywords:** tolerance; osmoregulation; gray snapper; *Lutjanus griseus*; acclimation; euryhalinity; Everglades restoration

### Introduction

Salinity acclimation is a complex process that involves a set of physiological responses in multiple osmoregulatory organs (i.e. gills, intestine, and kidneys; Lin et al. 2004; Marshall and Grosell 2005), and is known to induce changes in parameters such as plasma osmolality (e.g. Crocker et al. 1983; Nonnotte and Truchot 1990; Varsamos et al. 2002), Na<sup>+</sup>/K<sup>+</sup>-ATPase activity (e.g. Jensen et al. 1998; Arjona et al. 2007), and blood hematocrit (e.g. Leray et al. 1981; Brown et al. 2001; Denson et al. 2003), among others. To date, much of the work on acute osmoregulatory responses of fish to salinity change has been conducted on euryhaline species that either encounter different salinity levels in their habitat or move among distinct habitats throughout their life history (e.g. salmonids). Typically, these responses begin with a “crisis” period characterized by an increase or decrease in plasma osmolality, followed by a “regulatory” phase as ions reach

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steady-state levels, usually within 2 weeks post-transfer (e.g. Ferraris et al. 1988; Mancera et al. 1993; Arjona et al. 2007); unless acclimation to the new salinity level is unsuccessful. These phases occur because to counteract the large passive forces that dominate ion and water movement in the “crisis” period, the permeability of plasma membranes and tight junctions must be altered, and ion uptake (or extrusion) systems must be activated during the “regulatory” phase (Ferraris et al. 1988).

Estuaries are generally characterized by wide salinity fluctuations over short time scales that may vary seasonally with rainfall, river discharge, tidal fluctuations, evaporation (Tabb and Manning 1961) and/or anthropogenically-driven alterations of freshwater flow (Wakeman and Wohlschlag 1983; Serafy et al. 1997). As a result, the success of many species that are either facultative or obligate users of estuaries may depend on the species-specific capacity to tolerate changes in body fluid osmolality, osmoregulate and/or engage in more immediate behavioural responses (Serafy et al. 1997; Serrano et al. 2010). The expectation is that species with juvenile stages that inhabit estuaries (e.g. Sciaenidae) will be efficient osmoregulators (Varsamos et al. 2005). In contrast, species with juvenile stages that prefer more stable salinity regimes are expected to show more limited osmoregulatory abilities (Dall 1981). Further, while working in Louisiana estuaries, Yokel (1966) contended that young individuals from different species tended to be more tolerant of low salinities, whereas adults were less dependent on estuarine areas (spent more time at sea), and therefore, were expected to be more tolerant of high salinities.

Gray snapper *Lutjanus griseus*, a species of high economic and ecological importance in South Florida, USA (Tilmant 1989; Burton 2001; Denit and Sponaugle 2004; Serafy et al. 2007), is characterized as an estuarine transient (Ley et al. 1999), but has been included in a list of species that are marine as adults, but euryhaline as larvae and juveniles (Tabb et al. 1962; Beck et al. 2001). While juveniles occupy a variety of nearshore habitats with relatively low salinities (down to freshwater), adults are predominantly marine, but also frequent estuaries and nearshore habitats, particularly to feed (Starck and Schroeder 1970; Chester and Thayer 1990; Serafy et al. 2003; Wuenschel and Martin 2006). It is then expected that as the gray snapper performs inshore–offshore migrations throughout its life span, the change in external salinity results in physiological (osmotic) stress. Ley et al. (1999) reported the most extensive salinity range for gray snapper across all sizes (0–60 ppt) and Rutherford et al. (1989) the highest salinity (66.6 ppt); however, these values were based strictly on field observations. Serafy et al. (1997), however, reported that juvenile gray snapper (7.3–8.2 cm total length, TL) survived a brief (24 h) exposure to freshwater with 0% mortality in the laboratory, but this study only examined survival and did not target larger size classes, especially those that are vulnerable to hook-and-line fishing. This information is relevant for gauging downstream effects of Florida Everglades Restoration (focused on changing the quantity, quality and timing of freshwater flow in the region; Serafy et al. 2007), since adults have been suggested to be less tolerant of salinity fluctuations than younger fish (Starck 1964; Starck and Schroeder 1970).

The main objective of this study was to advance the current understanding of the basis and limits of the gray snapper euryhalinity. This constitutes the first assessment of the acute osmoregulatory responses following salinity change of a reef fish, using size classes that are directly vulnerable to hook-and-line fishing. Overall, we used a combination of laboratory and field observations on gray snapper to: (1) characterize the osmoregulatory responses following changes in environmental salinity;

(2) determine its limits of salinity tolerance; and (3) assess potential differences associated with the size class of fish tested. For gray snapper to be considered a truly euryhaline species, we expected transient or no osmoregulatory disturbances in plasma osmolality and/or blood hematocrit after transferring fish from seawater to various hypo- and hyper-saline media. In addition, we expected responses to be unrelated to fish size. Finally, fish collected in the field were expected to display plasma osmolalities not significantly different from fish in the laboratory at similar salinities. As such, this is the first ever attempt to compare osmoregulatory laboratory measurements with field results obtained directly after fish capture.

## Materials and methods

### *Experimental animals*

Subadult and adult gray snapper ranging from 13.5 to 24.5 cm TL were collected from nearshore marine (30–34 ppt) habitats within Biscayne and Florida Bays using hook-and-line fishing gear. Upon collection, fish were transported to the laboratory in coolers and held in outdoor tanks with flowing, aerated seawater for a period of 2–3 weeks prior to experiments. Water temperature and salinity in the tanks averaged 27.8°C and 31.5 ppt. Live juvenile pink shrimp *Farfantepenaeus duorarum* were provided as food, three times per week (~3% body weight per feeding).

### *Experimental protocol*

Five different salinity treatments were chosen to encompass the widest known range reported for this species. The treatments were 0, 5, 30 (full-strength seawater), 50, and 60 ppt. A sixth treatment was selected (70 ppt) outside the range reported for this species to test for an upper lethal salinity limit. Individuals maintained in full-strength seawater (30 ppt) throughout the duration of the experiment were considered the control group. Elevated salinities were achieved by addition of natural sea salts (Instant Ocean mix) to seawater, while lower salinities were established by adjusting a mix of seawater and dechlorinated Miami city tap water to the desired salinity. In all cases, transfer of fish to various salinities was completed within 10 min. To avoid crowding stress, disease or mortality associated with high ammonia levels, fish were randomly sorted and transferred individually into 30 L aquaria equipped with biofilters and aeration. Fish were starved for 24 h before and after transfers, after which feeding was resumed according to the schedule described above. Fecal matter and other debris were siphoned from tanks 1 day after feeding and a 25% volume water change was performed at 48, 96, and 144 h. Prior attempts to draw multiple blood samples from the same individuals over time resulted in excessive mortalities, especially at extreme salinities. Therefore, individual fish were sampled only once per salinity treatment as described below. Table 1 presents the number and size range of fish sampled at each time point within each salinity treatment.

### *Sample collection from abrupt transfers*

Fish were lightly anesthetized with a 0.1 gL<sup>-1</sup> MS-222 (3-aminobenzoic acid ethyl ester, Argent Labs) prior to blood sampling. One fish from each salinity treatment

Table 1. Number and size ranges (TL) of fish sampled at each time point within each salinity treatment.

Salinity treatment (ppt)	Water osmolality (mOsm kg <sup>-1</sup> )	Sample time point (h)	N	Fish size range (cm)
0	25	6	7	13.5–20.5
		24	7	14.5–24.5
		48	6	14.0–21.0
		96	5	14.5–22.5
		192	6	13.5–21.0
5	143	6	6	14.0–20.5
		24	6	14.5–21.0
		48	6	14.0–20.0
		96	6	14.3–21.0
		192	6	14.0–22.0
30 (control)	934	6	6	14.3–22.5
		24	8	16.0–21.5
		48	8	16.0–24.0
		96	7	15.5–19.0
		192	6	16.0–24.0
50	1522	6	6	14.7–21.5
		24	6	14.5–22.5
		48	6	15.5–21.5
		96	6	17.5–21.0
		192	6	17.5–20.5
60	1857	6	6	13.5–23.0
		24	6	14.3–16.7
		48	6	19.0–21.5
		96	6	13.5–15.5
		192	5	15.5–22.5
70	2150	6	6	16.5–22.0
		24	6	15.0–23.0

Notes: Individual fish were sampled only once at any given time point.

was sampled at 6, 24, 48, 96, and 192 h post-treatment by caudal puncture using a 1 mL heparinized syringe fitted with a 21 gauge needle. Approximately 200–400  $\mu$ L of blood was obtained from each fish, a portion of which was extracted into 75  $\mu$ L capillary tubes for hematocrit determination. The capillary tubes were centrifuged for 3 min and the volume of red blood cells was then measured as a percentage. The rest of the sample was then centrifuged at 16,000 $\times$ g to separate plasma and stored at  $-20^{\circ}$ C until analysis. Plasma osmolality was measured using a Wescor Vapro 5520 vapor pressure osmometer (Wescor Inc., Logan, UT). Treatment water osmolality was also determined as reference values (Table 1).

### *Sample collection in the field*

Using the same methods described above, additional fish were sampled in the field within 15 min of capture by hook-and-line. Salinity at each capture site was recorded

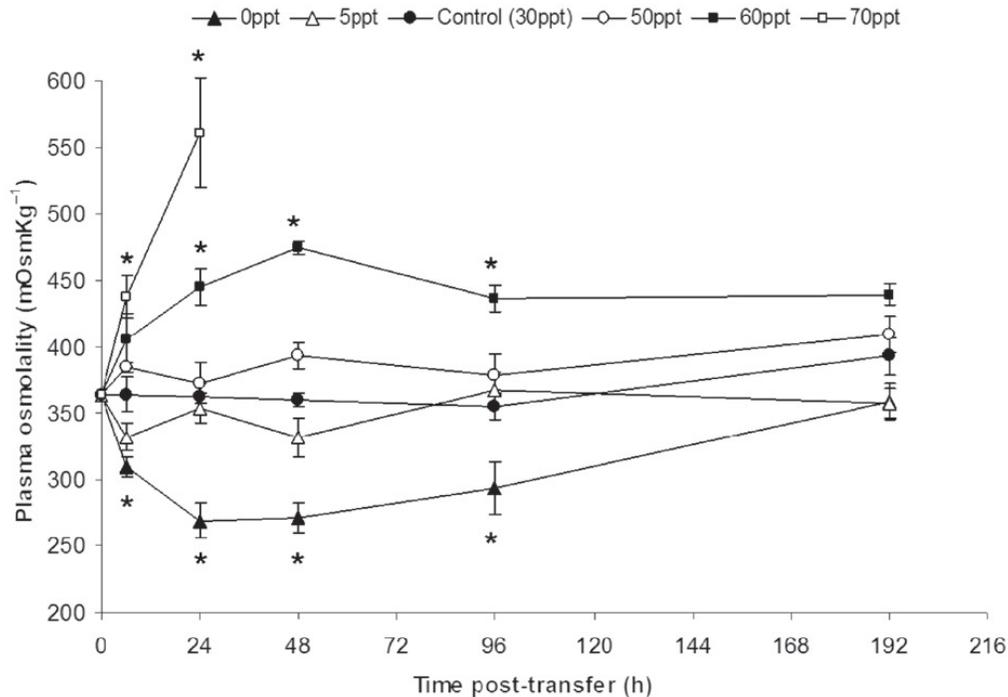


Figure 1. Changes in plasma osmolality for gray snapper *Lutjanus griseus* following abrupt transfer to different experimental salinities. All fish exposed to 70 ppt died within 24–48 h post-transfer. Asterisks correspond to significant statistical differences with respect to controls ( $P < 0.05$ ; analysis of variance and Dunnet's *post hoc* comparison test).

using a calibrated refractometer. This approach was used to compare plasma osmolalities obtained in the laboratory after abrupt transfer with values observed in fish in low salinity (0–4 ppt) versus marine (~30 ppt) habitats.

### Data analysis

Examination of data indicated normality and homogeneity of variances; thus laboratory and field values were reported as means  $\pm$  1 standard error. The significance of differences among salinities was determined using one-way ANOVA, with salinity as the main factor. When statistical significance was revealed (i.e.  $P < 0.05$ ), a Dunnet's *post hoc* test was used for multiple comparisons of the means. Finally, backward stepwise regression was used to evaluate the possible effect of fish size in relation to plasma osmolality and salinity treatment.

## Results

### Abrupt transfers

Overall, no mortalities occurred in fish from salinity treatments ranging from 0 to 60 ppt; however, all fish exposed to 70 ppt died within 48 h of transfer. Mean plasma osmolalities (Figure 1) ranged from 269 to 475 mOsm kg<sup>-1</sup> for fish transferred to salinities from 0 to 60 ppt, consistent with ranges observed in many other fresh, estuarine and marine teleosts (~260–400 mOsm kg<sup>-1</sup>; Varsamos et al. 2005).

In control fish (30 ppt), osmolality was maintained at  $367 \pm 1.32 \text{ mOsm kg}^{-1}$  ( $n=35$ ). In transfers to treatments between 5 and 50 ppt, plasma osmolality was maintained at levels very similar to controls throughout the duration of the experiment. Transfers to 0 ppt, however, significantly decreased plasma osmolality to  $310 \pm 7.53 \text{ mOsm kg}^{-1}$  ( $n=7$ ) by 6 h post-transfer, followed by a further decrease to  $269 \pm 12.75 \text{ mOsm kg}^{-1}$  ( $n=7$ ) at 24 h. By 48 h, values increased to  $271 \pm 11.46 \text{ mOsm kg}^{-1}$  ( $n=6$ ), and were no longer different from the controls at 192 h, with a value of  $359 \pm 13.73 \text{ mOsm kg}^{-1}$  ( $n=6$ ). In contrast, transfer to 60 ppt significantly increased plasma osmolality to  $445 \pm 13.39 \text{ mOsm kg}^{-1}$  ( $n=6$ ) at 24 h post-transfer, with a further increase to  $475 \pm 5.45 \text{ mOsm kg}^{-1}$  ( $n=6$ ) at 48 h. Mean osmolality had decreased by 96 h to  $436 \pm 10.09 \text{ mOsm kg}^{-1}$  ( $n=6$ ), and was no longer different from the control at 192 h ( $439 \pm 7.71 \text{ mOsm kg}^{-1}$ ;  $n=5$ ), despite water osmolality being around  $1850 \text{ mOsm kg}^{-1}$  (Table 1). Finally, fish transferred to 70 ppt significantly increased plasma osmolality to  $437 \pm 15.64 \text{ mOsm kg}^{-1}$  ( $n=6$ ) at 6 h post-transfer, a value that increased to  $561 \pm 41.29 \text{ mOsm kg}^{-1}$  ( $n=6$ ) by 24 h, and resulted in death for all fish before 48 h. Overall, for all six salinity treatments, backwards stepwise regression analysis suggested that plasma osmolality was only related to salinity treatment, was unrelated to fish size and that there was no salinity treatment by size interaction effect.

Mean hematocrit measurements (Figure 2) ranged from 31% to 43% for fish transferred to salinities from 0 to 60 ppt, consistent with normal ranges observed in many other species (32–43%). In control fish (30 ppt), hematocrit was maintained at  $35 \pm 1.32\%$  ( $n=35$ ). In transfers to 5, 50 or 60 ppt, blood hematocrit was maintained

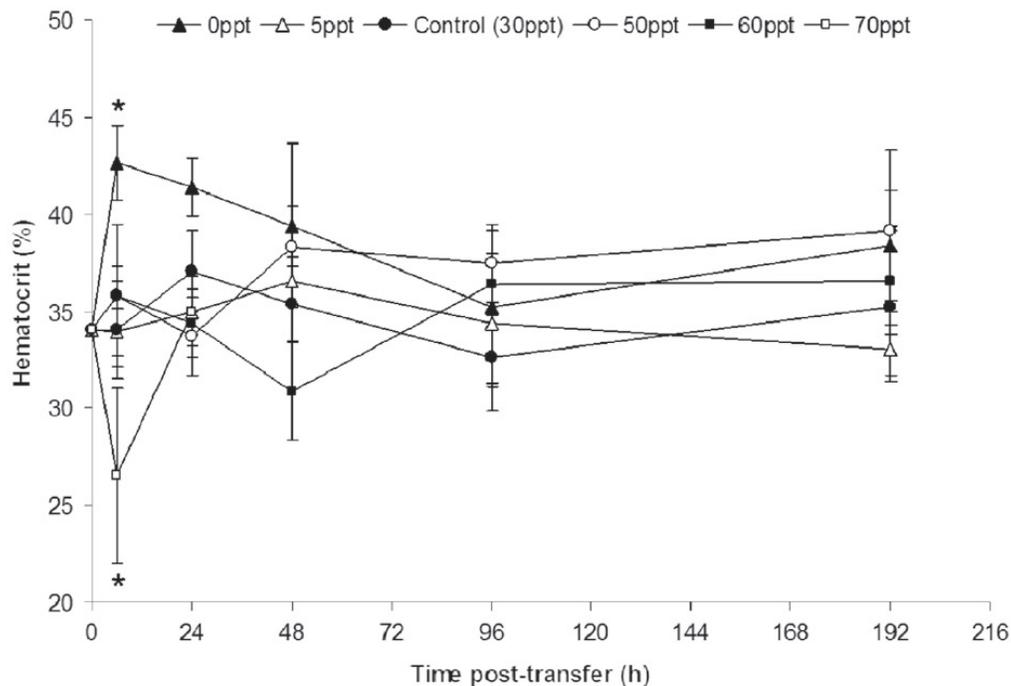


Figure 2. Changes in blood hematocrit for the gray snapper *L. griseus* following abrupt transfer to different experimental salinities. All fish exposed to 70 ppt died within 24–48 h post-transfer. Asterisks correspond to significant statistical differences with respect to controls ( $P < 0.05$ ; analysis of variance and Dunnett's *post hoc* comparison test).

at levels very similar to controls throughout the duration of the experiment, and no significant differences among treatments were observed. Although transfers to 0 ppt significantly increased blood hematocrit to  $43 \pm 4.1\%$  ( $n=7$ ) at 6 h post-transfer, values had returned to control levels by 24 h ( $41 \pm 1.53\%$ ;  $n=7$ ). The opposite occurred upon transfer to 70 ppt – a significantly reduced blood hematocrit ( $27 \pm 4.5\%$ ;  $n=6$ ) was observed at 6 h post-transfer. However, by 24 h post-transfer, hematocrit levels had returned to control levels ( $35 \pm 1.16\%$ ;  $n=6$ ) even though all fish died. Because post-mortem blood sampling was not possible hematocrit values after 24 h are unknown.

### Field collections

Overall, fish collected in low salinity habitats (0–4 ppt) displayed a high variability in plasma osmolality values, but these values were not significantly different from those observed in the laboratory after transfer to freshwater (Figure 3). On the other hand, fish collected in marine habitats displayed low variability in plasma osmolality values that were significantly higher than those observed in the laboratory controls.

## Discussion

### Abrupt transfers

This study investigated the osmoregulatory responses in plasma osmolality and blood hematocrit displayed by the gray snapper after abrupt changes in ambient salinity. Fish were challenged with six different salinity treatments including a

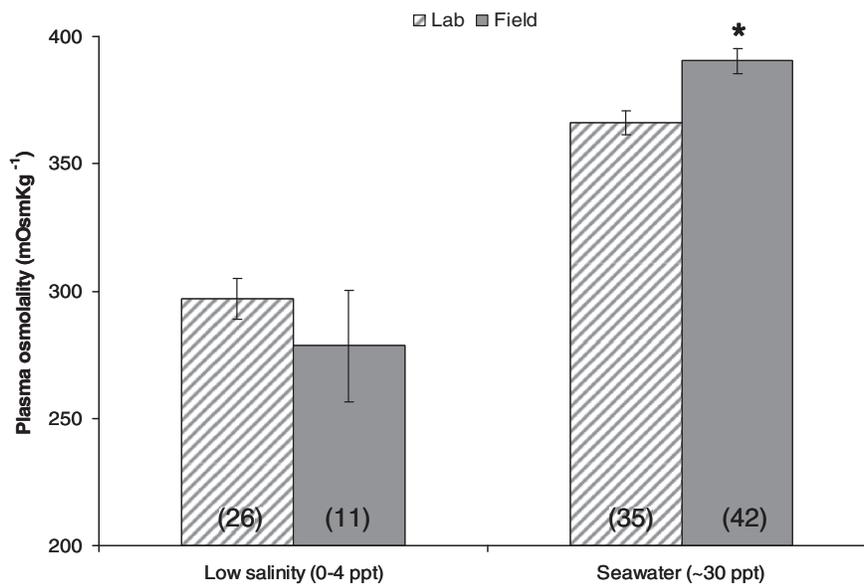


Figure 3. Comparison of plasma osmolalities from gray snapper *L. griseus* in the laboratory after transfer to either low salinity (0 ppt) or seawater (30 ppt, control) with gray snapper after capture in the field at low salinities (0–4 ppt) and full-strength seawater (~30 ppt). Asterisks correspond to significant statistical differences between field and laboratory results within each salinity ( $P < 0.05$ ; analysis of variance and Dunnett's *post hoc* comparison test).

control (0, 5, 30, 50, 60, and 70 ppt) and blood samples were collected at various time points post-transfer. Overall, results indicated no significant osmoregulatory disturbances in the salinity range of 5–50 ppt. In contrast, at extreme salinities of 0 and 60 ppt, significant but transient changes in osmolality and/or hematocrit were observed. However, by the end of the 192 h experimental period, both parameters showed no significant differences with respect to control values, suggesting a successful adaptation to these new salinity levels despite the large changes in environmental salinity. Finally, the lethal salinity, defined by the concentrations where a constant osmolality cannot be maintained (Foss et al. 2001), was observed at 70 ppt. However, the ability of the gray snapper to recover hematocrit to control values within 24 h post-transfer to 70 ppt, even preceding 100% mortality, suggests that fish were able to regain water balance even when they were unable to recover salt balance.

From the few studies that have examined the effect of body size in euryhalinity, the effect appears to be species-dependent. For example, after transfers from seawater to both hypo- and hyper-saline media, Ferraris et al. (1988) found that smaller milkfish *Chanos chanos*, not only had longer recovery times, but larger deviations from control osmolality than larger fish (260 g) compared to milkfish (120 and 40 g). In contrast, Jensen et al. (1998) found no difference in parameters observed during salinity acclimation when comparing large versus small European sea bass *Dicentrarchus labrax* (89 g compared to 6.2 g sea bass), suggesting that this species is euryhaline at all developmental stages. In this study, the range of sizes of gray snapper tested varied greatly in every treatment (mean length in each ranged from 14.1 to 23 cm TL, thus comprising both sub-adults and adults), but results indicated no significant relationships between size and osmolality of fish tested in any of the salinity treatments. Overall, these findings contradict previous field-based observations (i.e. Starck 1964; Starck and Schroeder 1970), and suggest that larger size classes of gray snapper may be equipped with the same efficient osmoregulatory capabilities that juveniles possess. Thus, based on the results from this study, we contend that gray snapper is a truly euryhaline species.

There is a paucity of literature on the immediate osmoregulatory responses for a single species when abruptly transferred from seawater to *both* hypo- and hyper-saline experimental media. Such information is available for more commonly studied species including the European sea bass (Jensen et al. 1998; Varsamos et al. 2002), red drum *Sciaenops ocellatus* (Crocker et al. 1983; Wakeman and Wohlschlag 1983), milkfish (Ferraris et al. 1988) and Gulf toadfish *Opsanus beta* (Serafy et al. 1997; Genz et al. 2008), among others. From these studies, it appears that the salinity range to which gray snapper was able to acclimate during this study exceeded corresponding ranges for the species listed above, particularly at the lower end of salinity. For example, the Gulf toadfish, which is an important component of gray snapper diet (Starck and Schroeder 1970), can successfully acclimate to hyperosmotic salinities of up to 60 ppt with a slightly faster recovery time than the gray snapper (<96 h; Genz et al. 2008), but cannot survive in salinities of or lower than 0.5 ppt for more than a week even after gradual acclimation (McDonald and Grosell 2006). Similarly, the European sea bass can osmoregulate well over the same wide range of salinities used in this study (0 to 60 ppt; Jensen et al. 1998). However, the few deaths recorded in the initial adjustive phase of sea bass after transfer to freshwater also suggest that the lower salinity threshold may fall between 5 and 0 ppt; contrasting with the ability of gray snapper to osmoregulate well in freshwater.

### ***Field collections***

To the best of our knowledge, this is the first study to compare measured plasma osmolalities directly from fish captured in the field with laboratory-measured osmoregulatory data. Overall, results showed that all size classes of gray snapper captured within the same salinity in the field (15.5–31 cm TL in freshwater collections, 9–30 cm TL in marine collections, respectively) displayed similar osmoregulatory profiles. In addition, fish collected in low salinity habitats (salinities ranging from 0–4 ppt) displayed osmolalities not significantly different from fish in the laboratory transferred to freshwater. The high variability in the osmolalities displayed in field-captured fish at these low salinities likely reflect differences in the time that each fish had spent at the salinity of capture (i.e. indicative of migration among habitats). In contrast, field-collected fish in full-strength seawater not only displayed a smaller variability in osmolality values (consistent with the fact that these areas tend to have stable salinities), but also unexpectedly displayed significantly higher osmolality values compared to those in the laboratory at similar salinities. These unexpected results may be explained by feeding differences in field versus laboratory fish. While in the laboratory fish were fed solely with shrimp, in the field, fish is the most prominent dietary component, particularly in larger size classes (Starck and Schroeder 1970). Supporting this idea, Taylor and Grosell (2006) found that a large meal of fish fed to the Gulf toadfish provided a substantial  $K^+$  and  $Ca^{2+}$  load that significantly increased the osmolality measured when compared to a squid-based diet. Alternatively, these higher than expected osmolality values in field-captured fish at high salinities could have resulted from capture and/or handling stress, albeit unmeasured. Further, osmolality has been shown to be affected by handling and/or transporting stress (Redding and Schreck 1983; Denson et al. 2003), which may explain why most osmoregulatory studies have an acclimation period after fish capture and/or rearing. Whichever the case, it is important to emphasize that field-measured osmoregulatory data are only suggestive of the conditions under which this species is found in nature.

### ***Ecological implications***

In South Florida, alteration of freshwater flow has changed the salinity regimes and degraded estuarine and nearshore habitats occupied by the gray snapper (Serafy et al. 1997). Further, salinity is expected to undergo more significant changes with the implementation of the Florida Everglades restoration, which aims at restoring more natural, mesohaline salinity regimes within many of the South Florida's coastal bays (Walters et al. 1992; Harwell et al. 1996; Serafy et al. 1997). Gray snapper and other species that are subjected to pulses of freshwater flow can either remain in these areas, if physiologically capable, or leave and risk predation and/or food scarcity while seeking a more benign habitat (Serafy et al. 1997). This study suggests that even though freshwater pulses may represent a significant osmoregulatory challenge to the gray snapper, this in itself will not lead to death. Gray snapper faced with a freshwater pulse in their natural habitat are capable of remaining in such an area, although their preferred food items may not. Early observations that multiple blood drawings from the same individuals resulted in high mortalities (especially at salinities of 0 and 60 ppt) suggest that while this species is highly tolerant of salinity changes, when combined with other stressors (e.g. capture on hook-and-line), lesser

salinity challenges than those tested may be lethal. Thus, research on the effects of multiple stressors on the osmoregulatory capabilities of this species is warranted.

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### References

- Arjona F, Vargas-Chacoff L, Ruiz-Jarabo I, Martín del Río M, Mancera M. 2007. Osmoregulatory response of Senegalese sole (*Solea senegalensis*) to changes in environmental salinity. *Comp Biochem Physiol A*. 148:413–421.
- Beck M, Heck K, Able K, Childers D, Eggleston D, Gillanders B, Halpern B, Hays C, Hoshino K, Minello T, et al. 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. *Bioscience*. 51(8):633–641.
- Brown J, Moore W, Quabius S. 2001. Physiological effects of saline waters on zander. *J Fish Biol*. 59:1544–1555.
- Burton M. 2001. Age, growth, and mortality of gray snapper, *Lutjanus griseus*, from the east coast of Florida. *Fish Bull*. 99:254–265.
- Chester A, Thayer G. 1990. Distribution of spotted seatrout (*Cynoscion nebulosus*) and gray snapper (*Lutjanus griseus*) juveniles in seagrass habitats of western Florida bay. *Bull Mar Sci*. 46:345–357.
- Crocker P, Arnold C, DeBoer J, Holt G. 1983. Blood osmolality shift in juvenile red drum, *Sciaenops ocellatus* L. exposed to freshwater. *J Fish Biol*. 23:315–319.
- Dall W. 1981. Osmoregulatory ability and juvenile habitat preference in some penaeid prawns. *J Exp Mar Biol Ecol*. 54(1):55–64.
- Denit K, Sponaugle S. 2004. Growth variation, settlement, and spawning of gray snapper across a latitudinal gradient. *Trans Am Fish Soc*. 133:1339–1355.
- Denson M, Evin R, Stuart K, Smith T. 2003. Effects of salinity on growth, survival, and selected hematological parameters of juvenile cobia *Rachycentron canadum*. *J World Aquacult Soc*. 34(4):496–504.
- Ferraris R, Almendras J, Jazul A. 1988. Changes in plasma osmolality and chloride concentration during abrupt transfer of milkfish (*Chanos chanos*) from seawater to different test salinities. *Aquaculture*. 70:145–157.
- Foss A, Evensen T, Imsland A, Oiestad V. 2001. Effects of reduced salinities on growth, food conversion efficiency and osmoregulatory status in the spotted wolfish. *J Fish Biol*. 59:416–426.
- Genz J, Taylor J, Grosell M. 2008. Effects of salinity on intestinal bicarbonate secretion and compensatory regulation of acid–base balance in *Opsanus beta*. *J Exp Biol*. 211:2327–2335.

- Harwell M, Long J, Bartuska A, Gentile J, Harwell C, Myers V, Ogden J. 1996. Ecosystem management to achieve ecological sustainability, the case of South Florida. *Environ Manage.* 20:497–521.
- Jensen M, Madsen S, Kristiansen K. 1998. Osmoregulation and salinity effects on the expression and activity of Na<sup>+</sup>, K<sup>+</sup>-ATPase in the gills of European sea bass, *Dicentrarchus labrax*. *J Exp Zool.* 282:290–300.
- Leray C, Colin D, Florentz A. 1981. Time course of osmotic adaptation and gill energetics of rainbow trout, *Salmo gairdneri* following abrupt changes in external salinity. *J Comp Physiol.* 144:175–181.
- Ley J, McIvor C, Montague C. 1999. Fishes in mangrove prop-root habitats of northeastern Florida Bay, distinct assemblages across an estuarine gradient. *Estuar Coast Shelf Sci.* 48:701–723.
- Lin C, Tsai R, Lee T. 2004. Expression and distribution of Na, K-ATPase in gill and kidney of the spotted green pufferfish, *Tetraodon nigroviridis*, in response to salinity challenge. *Comp Biochem Physiol A.* 138:287–295.
- Mancera J, Pérez-Figares J, Fernández-Llebrez P. 1993. Osmoregulatory responses to abrupt salinity changes in the euryhaline gilthead seabream (*Sparus auratus*). *Comp Biochem Physiol A.* 106:245–250.
- Marshall W, Grosell M. 2005. *Physiology of fishes.* 3rd ed. Boca Raton (FL): CRC Press.
- McDonald M, Grosell M. 2006. Maintaining osmotic balance with an aglomerular kidney. *Comp Biochem Physiol A.* 143:447–458.
- Nonnotte G, Truchot D. 1990. Time course of extracellular acid–base adjustments under hypo- or hyperosmotic conditions in the euryhaline fish *Platichthys flesus*. *J Fish Biol.* 36:181–190.
- Redding J, Schreck C. 1983. Influence of ambient salinity on osmoregulation and cortisol concentration in yearling coho salmon during stress. *Trans Am Fish Soc.* 112:800–807.
- Rutherford E, Tilmant J, Thue E, Schmidt T. 1989. Fishery harvest and population dynamics of gray snapper, *Lutjanus griseus* in Florida Bay and adjacent waters. *Bull Mar Sci.* 44(1):139–154.
- Serafy J, Faunce C, Lorenz J. 2003. Mangrove shoreline fishes of Biscayne Bay, Florida. *Bull Mar Sci.* 72:161–180.
- Serafy J, Lindeman K, Hopkins T, Ault J. 1997. Effects of freshwater canal discharge on fish assemblages in a subtropical bay, field and laboratory observations. *Mar Ecol Prog Ser.* 160:161–172.
- Serafy J, Valle M, Faunce C, Luo J. 2007. Species-specific patterns of fish abundance and size along a subtropical mangrove shoreline: an application of the delta approach. *Bull Mar Sci.* 80:609–624.
- Serrano X, Grosell M, Serafy J. 2010. Salinity selection and preference of the grey snapper *Lutjanus griseus*: field and laboratory observations. *J Fish Biol.* 76:1592–1608.
- Starck W. 1964. A contribution to the biology of the gray snapper, *Lutjanus griseus* (Linnaeus), in the vicinity of lower Matecumbe Key, Florida [PhD Dissertation]. [Coral Gables (FL)]: University of Miami.
- Starck W, Schroeder R. 1970. Investigations on the gray snapper, *Lutjanus griseus*. Coral Gables (FL): University of Miami Press.
- Tabb D, Dubrow D, Manning R. 1962. The ecology of northern Florida Bay and adjacent estuaries. *FL Bd Conserv Tech Ser.* 39:1–81.
- Tabb D, Manning R. 1961. A checklist of the flora and fauna of northern Florida Bay and adjacent brackish waters of the Florida mainland collected during the period July, 1957 through September, 1960. *Bull Mar Sci Gulf Carib.* 11(4):552–649.
- Taylor T, Grosell M. 2006. Feeding and osmoregulation: dual function of the marine teleost intestine. *J Exp Biol.* 209:2939–2951.
- Tilmant J. 1989. A history and an overview of recent trends in the fisheries of Florida Bay. *Bull Mar Sci.* 44:3–22.

- Varsamos S, Diaz J, Charmantier G, Flik G, Blasco C, Connes R. 2002. Branchial chloride cells in seabass (*Dicentrarchus labrax*) adapted to freshwater, seawater and doubly concentrated seawater. *J Exp Zool.* 293:12–26.
- Varsamos S, Nebel C, Charmantier G. 2005. Ontogeny of osmoregulation in postembryonic fish, a review. *Comp Biochem Physiol A.* 141:401–429.
- Wakeman J, Wohlschlag D. 1983. Time course of osmotic adaptation with respect to blood serum osmolality and oxygen uptake in the euryhaline teleost, *Sciaenops ocellatus* (red drum). *Contrib Mar Sci.* 26:165–177.
- Walters C, Gunderson L, Holling C. 1992. Experimental policies for water management in the everglades. *Ecol App.* 2:189–202.
- Wuenschel M, Martin G. 2006. Effect of temperature and salinity on otolith element incorporation in juvenile gray snapper *Lutjanus griseus*. *Mar Ecol Prog Ser.* 324:229–239.
- Yokel B. 1966. A contribution to the biology and distribution of the red drum, *Sciaenops ocellata*. [MS Thesis]. [Coral Gables (FL)]: University of Miami.