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Salinity selection and preference of the grey snapper *Lutjanus griseus*: field and laboratory observations

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Field observations were supplemented with laboratory experiments to reveal patterns of salinity selection and preference for grey snapper *Lutjanus griseus* (*c*. 21 cm total length, L_T), an ecologically and economically important species in the south-eastern U.S.A. Fish abundance data were examined from a long-term field survey conducted in the mangrove habitats of Biscayne Bay, Florida, where salinities ranged from <1 to 40. First, regression analyses indicated significant, positive linear relationships with salinity for both *L. griseus* frequency of occurrence and concentration (density when present). These patterns are inconsistent with physiological expectations of minimizing energetic osmoregulatory costs. Next, the salinity preference and swimming activity of 11 *L. griseus* (ranging from 18 to 23 cm L_T) were investigated using a newly developed electronic shuttlebox system. In the laboratory, fish preferred intermediate salinities in the range of 9–23. Swimming activity (measured in terms of spontaneous swimming speed) followed a parabolic relationship with salinity, with reduced activity at salinity extremes perhaps reflecting compensation for higher osmoregulatory costs. It is suspected that the basis of the discrepancy between laboratory and field observations for size classes at or near maturity ultimately relates to the reproductive imperative to move towards offshore (high-salinity) coral-reef habitats, a necessity that probably overrides the strategy of minimizing osmoregulatory energetic costs.

Key words: Biscayne Bay; electronic shuttlebox; Everglades restoration; gray snapper; osmoregulation; salinity choice.

INTRODUCTION

In estuarine environments, salinity can be a primary factor inducing changes in fish distribution patterns (Davenport *et al*., 1975; Ley *et al*., 1999; Jung & Houde, 2003), community structure (Edeline *et al*., 2005), feeding and growth rates (Fry, 1971; Lankford & Targett, 1994; Boeuf & Payan, 2001), metabolism (Fry, 1971; Haney & Walsh, 2003; Wuenschel *et al*., 2005), activity level (Swanson, 1998), swimming performance (Wakeman & Wohlschlag, 1977; Kolok & Sharkey, 1997) and survival (Johnson & Katavic, 1986; Hurst & Conover, 2002). Estuary-dwelling species rarely

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face constant salinity levels and must often cope with large fluctuations, which can represent significant stress, depending on the species-specific osmoregulatory capacity and behavioural response (Serafy *et al*., 1997).

Off southern Florida, U.S.A., one of the most important fish species utilizing coastal bay habitats is the grey snapper *Lutjanus griseus* (L.). It is among the most abundant fish species in the region (Tabb & Manning, 1961; Serafy *et al*., 2008) and is of high economic and ecological importance in local fisheries and the ecosystem at large (Tilmant, 1989; Burton, 2001; Denit & Sponaugle, 2004). Characterized as an estuarine transient (Ley *et al*., 1999), *L. griseus* is known to migrate and forage among waters of distinctly different salinities over its life span (Starck & Schroeder, 1970; Chester & Thayer, 1990; Serafy *et al*., 2003). Most published literature addressing *L. griseus* and salinity, however, has been based strictly on field observations, providing only ranges of salinities at time of sampling or collection. Ley *et al*. (1999) provide the most extensive published range for the species: 0–66·6; however, patterns of habitat selection and preference within that range have not been described. No laboratory studies have targeted the study of size classes >5 cm total length, L_T, of *L. griseus*, especially those that are directly vulnerable to hook-and-line fishing.

Salinity preference has been considered a behavioural mechanism that can keep animals within a viable salinity range, allowing them to avoid or delay exposure to adverse conditions in their natural environment (Lockwood, 1976; McGaw, 2001). Whereas salinity preference can be inferred from field data, separating salinity effects from other abiotic and biotic influences is difficult, at best. In laboratory studies of salinity preference, the species of interest have been primarily invertebrates (*i.e.* crabs), with only a few fish species assessed to date. The general assumption is that salinities with osmolalities near the osmolality of the blood (isosmotic) minimize the physiological cost of osmoregulation, allowing more energy for other processes, such as growth and reproduction (Lankford & Targett, 1994; Cardona, 2000; Hurst & Conover, 2002). Two different types of salinity preference apparatus have been used: salinity gradient tanks that present a continuum of different salinities (Davenport, 1972; Keiser & Aldrich, 1976; Moser, 1988; Chung, 2001) and salinity choice chambers with pre-established salinities which appear to work well for crustaceans (Thomas *et al*., 1981; McGaw & Naylor, 1992*a*, *b*; McGaw, 2001). The main limitation of both of these experimental set-ups is that they often require a very shallow depth to prevent vertical stratification, thus precluding the testing of relatively large, mobile organisms, such as *L. griseus*. More advanced electronic shuttleboxes (choice chambers which produce a horizontal gradient controlled by the spatial movements of the organism) have been used in several temperature preference studies (Neill *et al*., 1972; Reynolds & Casterlin, 1978; Schurmann *et al*., 1991; Mortensen *et al*., 2007), but not for determining salinity preferences.

The present study compared field-based patterns of salinity selection with laboratory-based observations of salinity preference for *L. griseus*. It was expected that given a choice of salinities in the laboratory, *L. griseus* would prefer isosmotic salinities, presumably to minimize physiological osmoregulatory costs over salinity extremes. In addition to allowing the determination of salinity preference, the automated behavioural test apparatus facilitated comparison of spontaneous swimming activity along a salinity gradient under light *v*. dark conditions. Understanding relationships between salinity and *L. griseus* abundance and preference is particularly important for gauging downstream effects of Florida Everglades restoration, which is focused on changing the quantity, quality and timing of freshwater flow in the region.

MATERIALS AND METHODS

FIELD DATA COLLECTION

To investigate relationships between *L. griseus* abundance and salinity, data were examined from an ongoing visual survey that, twice annually since 1998, has monitored fish assemblages along the mainland shoreline of Biscayne Bay, Florida, U.S.A. (Fig. 1). Details of field methods are reported in Serafy *et al.* (2003) and Faunce & Serafy (2007). Briefly, the survey follows a stratified random sampling design whereby fishes are identified and enumerated along 30 m × 2 m belt-transects that follow the prop-root edge. A single observer wearing snorkelling gear slowly swims each transect recording fish data on waterproof paper. Upon completion of each visual transect measurements of water salinity, temperature and depth are gathered using a multi-probe water quality instrument.

FIELD DATA ANALYSES

Abundances of individual fish species are often dominated by zero values and this was the case in the present study. Therefore, following the delta approach (Serafy *et al.*, 2007), a

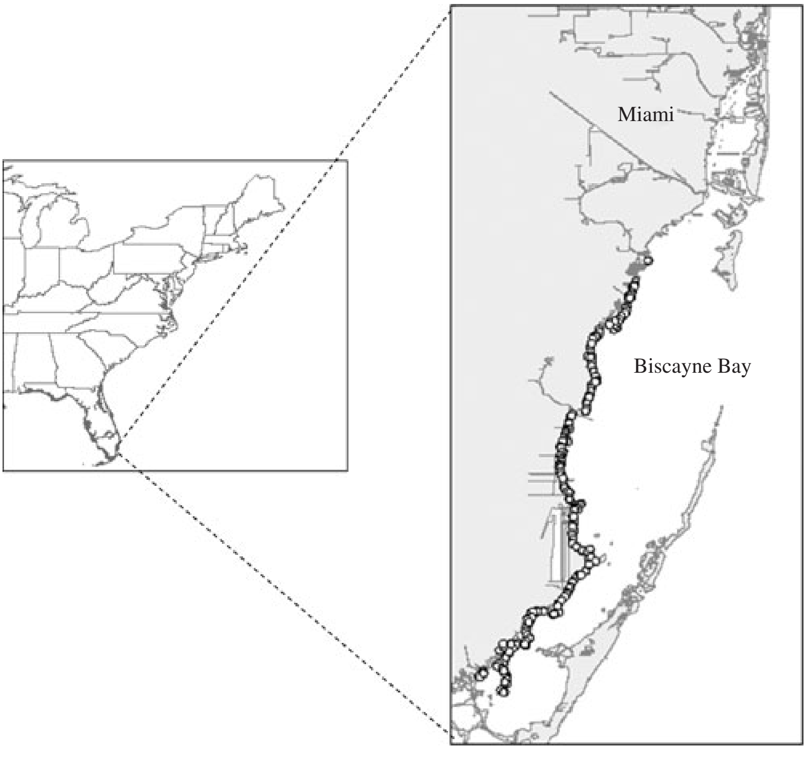

FIG. 1. Maps depicting south-eastern U.S. coast, Biscayne Bay, Florida, and visual fish survey locations (○) along its mainland shoreline.

stepwise regression was used to evaluate *L. griseus* frequency of occurrence (*i.e.* proportion positive) and concentration (*i.e.* density when present) in relation to water salinity, temperature and depth, with emphasis on revealing salinity effects. To reveal patterns in *L. griseus* occurrence along these environmental gradients, backward stepwise logistic regression was performed on binary data with the factors salinity, temperature, depth and their corresponding squared terms in the initial model. Beginning with the highest order terms, factors were removed sequentially if their P-values were $>0{\cdot}05$. Logistic model fit was judged from its final model concordance index (C), which ranges from 0·5 (poor) to 0·99 (best). The same initial model structure and backward elimination approach were used to reveal relationships between *L. griseus* concentration and the three environmental variables. In the latter analysis, however, ordinary least-squares regression on ln-transformed concentration values was performed to arrive at the final model and fit was judged from final model R^2 value. When significant, temperature and depth terms were treated as co-variables. Because their combined influence might obscure salinity effects, residual plots were examined following Neter *et al.* (1996). This involved: (1) regressing temperature and depth variables (and their square terms, if significant) against *L. griseus* presence or absence or the observed concentrations, and (2) removing their combined influence using the resulting residuals. Residual plots facilitated the visualization of the salinity effect, the main factor of interest (Neter *et al.*, 1996).

ANIMALS FOR LABORATORY EXPERIMENTS

Lutjanus griseus ranging from 18 to 23 cm L_{T} were collected from near-shore marine (salinities of 30–34) habitats within Biscayne Bay and Florida Bay using hook-and-line gear. Upon collection, *L. griseus* were transported to the laboratory in aerated coolers and held in outdoor tanks with flowing sea water for a period of 2–3 weeks before experiments. Water temperature and salinity in the tanks averaged 27·8° C and 31·5, respectively. Live juvenile pink shrimp *Farfantepenaeus duorarum* were provided as food three times per week (*c*. 3% body mass per feeding). Food was withheld for 24 h after fish were transferred to the automated salinity choice system (see below), which was housed in a environmental chamber with temperature control and 16L:8D cycle.

AUTOMATED SALINITY CHOICE SYSTEM

The automated salinity choice system was developed in collaboration with Loligo Systems (www.loligosystems.com) to allow automated testing of fish salinity preference (Fig. 2). The shuttlebox system is a modification of the device described by Schurmann *et al.* (1991) who used it to determine rainbow trout *Oncorhynchus mykiss* (Walbaum) temperature preference. The system consisted of two tanks, each 72 cm diameter, connected by a 20 cm long, 14 cm diameter tube. Water depth in the chambers was 52 cm. The system included two recirculation pumps, two shunt pumps, two dosage pumps (for freshwater and seawater inflow), four infrared floodlights, an infrared sensitive video camera, two conductivity meters to record the salinity continuously, an instrument to control the activity of the three sets of pumps (RELAY 3), and a computer with video software and a programme for data acquisition and pump control [Fig. 2(a)]. In addition, two diffusive light panels (not shown in figure) were attached to the top of the infrared lights to create a uniformly lit tank background. A video camera mounted over the shuttlebox continuously recorded the position of the fish (based on a principle of contrast) and exported x and y co-ordinates to the computer (LoliTRACK Lite version 1.1, Loligo Systems). The computer also controlled the activity of the three sets of pumps (*via* LABTECH NOTEBOOK software; www.LabTechSoft.com/), depending on the position of the fish and the difference in salinity between the two tanks. Passage of the fish into the higher salinity tank prompted the dosage pumps to increase the salinity in both tanks; the reverse happened if the fish swam into the low salinity tank. When the fish was stationary in the connecting tube, all pumps were turned off and salinity was maintained at a constant value until the fish moved again into one of the experimental tanks [Fig. 2(b)]. The salinity difference between tanks was kept at 5 in the present study regardless of overall salinity in the system by the shunt pumps, and circulatory current in each tank was ensured by recirculation pumps.

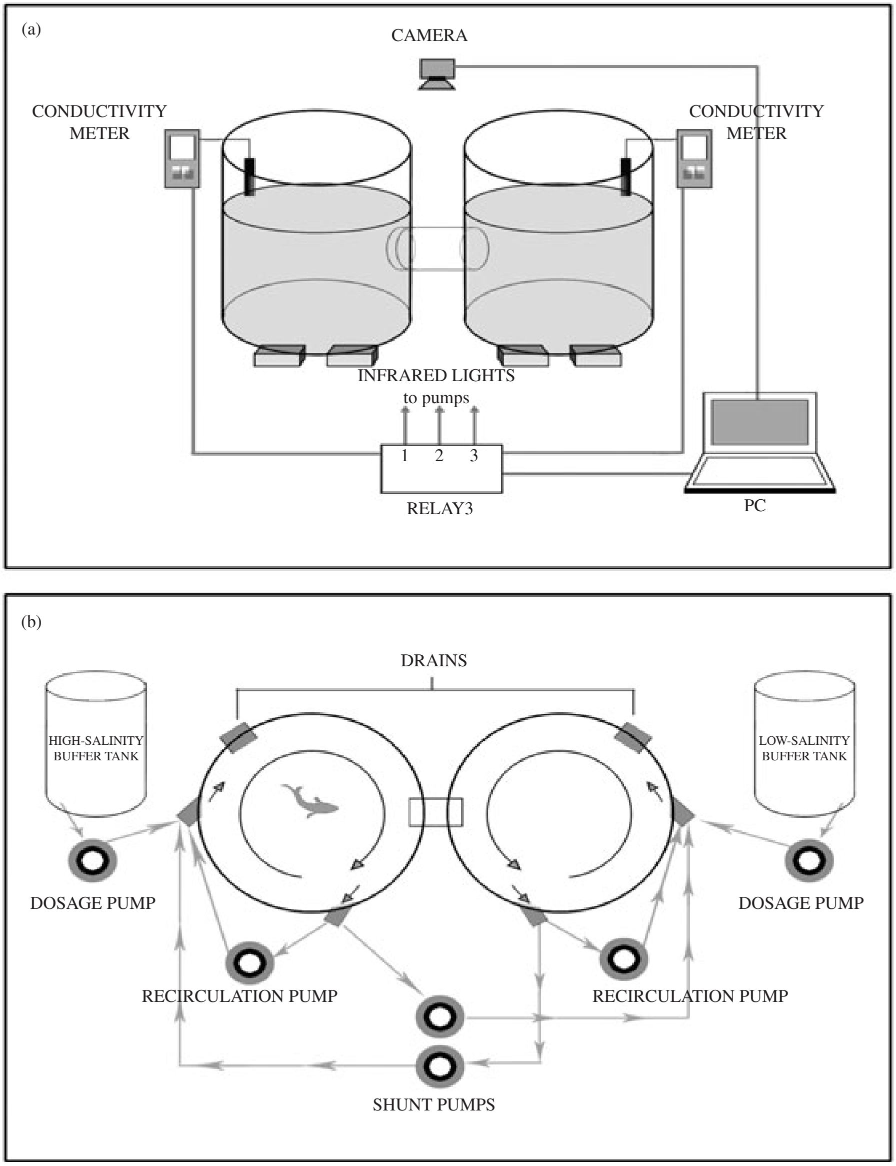

FIG. 2. Shuttlebox system for salinity preference studies depicting major components. The system consisted of two tanks, each 72 cm diameter, connected by a 20 cm long, 14 cm diameter tube. Water depth in the chambers was 52 cm. (a) Side view diagram showing electronic equipment. Figure does not show diffusive light panels attached to the top of the infrared lights. (b) Top view diagram showing the three sets of pumps and direction of water flow (→).

EXPERIMENTAL PROTOCOL

Individual fish were introduced into the shuttlebox containing air-saturated water with similar conditions to the acclimation tanks (mean temperature and salinity of 27·8° C and 31·5, respectively). Dechlorinated Miami City tap water and filtered sea water from Bear Cut (between Virginia Key and Key Biscayne, Florida) were used as freshwater and seawater sources in the low and high-salinity buffer tanks [Fig. 2(b)]. Water chemistry for these two media is given in Grosell *et al.* (2007). The 16L:8D cycle of the outdoor holding tanks was maintained throughout the duration of each experiment. To minimize transfer-associated stress on salinity preference measurements, individual fish were allowed to acclimate for 24 h to the system (while turned off) at a salinity of 31·5. After this period, salinity was controlled in response to the fish's movements in the automated salinity choice system for 48 h *via* video tracking of spontaneous behaviour. At the end of the experiment L_T was measured.

PRELIMINARY EXPERIMENTS

Fish monitoring during the 24 h acclimation period (salinity gradient absent) was conducted to test for potential behavioural effects associated with experimental surroundings. During this period, fish tended to hover along the sides of the tanks and occasional occupation of the connecting tube was also observed. These behaviours were apparent both with and without the recirculation pumps, suggesting little or no water flow influences behaviour.

LABORATORY DATA ANALYSES

Data from the automated salinity choice system were collected continuously over the duration of each 48 h trial. Most fish appeared to respond to salinity in the system at the start of the first dark period. Therefore, for consistency across fish, statistical analyses focused on data collected at the beginning of the first dark period (2000 hours) and beyond.

Following the rationale of Schurmann *et al.* (1991), the mean of the median preferred salinity was used as a measure of final salinity preference. The salinity range selected by each fish was also extracted from the dataset. An advantage of using median preferred salinities is that they tend to be less sensitive to extreme scores than the mean, and they are a better measure than the mode for highly skewed distributions (Schurmann *et al.*, 1991).

Salinity preferences for each fish were obtained for both light and dark periods. Diel differences in the mean, range, minimum and maximum preferred salinities were then tested across all fish with paired *t*-tests. Routine swimming speed (activity; S_{RS}) was calculated by measuring the distance travelled by the fish every second (in cm) during their stay in the shuttlebox system. The following equation was used: $S_{RS} = (\sqrt{\{[(X_{pos1} - X_{pos2})^2] + [(Y_{pos1} - Y_{pos2})^2]\}})t^{-1}$, where X_{pos1} and X_{pos2} are the positions of the tracked fish (cm) in the x co-ordinate at times 1 and 2, Y_{pos1} and Y_{pos2} are the positions of the tracked fish (cm) in the y co-ordinate at times 1 and 2 and t is the time (s). Data were separated by time of day (dark and light time periods) and swimming speeds were averaged for each 1 salinity bin ranging from 0 to 33. Plots of mean swimming speed *v.* salinity indicated parabolic relationships for both time periods with a tendency for elevated speeds during the dark period. Therefore, a quadratic equation was fitted to the data with the factor diel (light *v.* dark) as a dummy variable to examine whether the elevation was statistically significant. In all the analysis, statistical significance was declared at $\alpha < 0{\cdot}05$.

RESULTS

FIELD DISTRIBUTION

From July 1998 to October 2008, a total of 1157 visual fish transects were conducted along Biscayne Bay's south-western boundary (Fig. 1). Of these, 337 (29%) were positive for *L. griseus*. Salinity, temperature and depth levels associated with these transects ranged from 0·4 to 40·3, 12·3 to 35·6° C and 17 to 145 cm, respectively. Logistic regression results indicated that salinity, temperature and depth were all significant explanatory variables for *L. griseus* occurrence ($C = 0{\cdot}79$, $P < 0{\cdot}001$) [Table I(a)]. Positive linear relationships emerged between

TABLE I. Stepwise regression results indicating relationships between environmental variables and *Lutjanus griseus* (a) occurrence and (b) concentration. Final models are shown emerging after backward elimination procedure which required that *P*-values associated with explanatory variables were <0·05. Initial models included water salinity, temperature, depth and their squared terms as independent variables

(a)

Variable	Estimate	S.E.	Wald χ^2	$P > \chi^2$	Model fit *C*
Intercept	−20·264	3·543	32·717	<0·001	0·79
Salinity	0·053	0·010	26·144	<0·001	
Depth	0·056	0·005	156·273	<0·001	
Temperature	0·953	0·257	13·699	<0·01	
Temperature squared	−0·015	0·005	10·295	<0·01	

(b)

Variable	Estimate	S.E.	*t* value	$P > t$	Model fit R^2
Intercept	−1·295	0·341	−3·800	<0·001	0·23
Salinity	0·046	0·010	4·840	<0·001	
Depth	0·027	0·003	8·070	<0·001	

L. griseus occurrence and salinity [Fig. 3(a)] and depth; the relationship with temperature was quadratic with highest occurrences at intermediate temperature values. Ordinary least-squares regression results indicated that salinity and depth (but not temperature) were significant explanatory variables for *L. griseus* concentration ($R^2 = 0{\cdot}23$, $P < 0{\cdot}001$) [Table I(b)]. The relationships between *L. griseus* concentration and salinity [Fig. 3(b)] as well as depth were positive and linear.

SALINITY PREFERENCE

A representative 48 h time–course of regulatory behaviour for an individual fish tested in the shuttlebox system is shown in Fig. 4. A common observation for most fish was a *c*. 1 h period after the system was activated during which they remained almost stationary along the sides of one of the two connected tanks. After a brief period (*c*. 10 min) of exploration of the two-tank system, fish appeared to move between the tanks without hesitation. Evidence of salinity choice became apparent within *c*. 3 h after system activation and was distinct after *c*. 8 h, soon after the beginning of the first dark period. Fish appeared to strongly avoid salinities >30 and <5 during both light and dark periods, but especially during the dark phase.

Final salinity preferences were determined for 11 individual *L. griseus* for the light and dark periods. Fig. 5 shows individual salinity preferences, along with the range of salinities occupied by fish over the time–course of experiments. The lower and upper 95% CI for all fish are also shown, along with the overall mean salinity preference across all fish (15·4). In general, individual *L. griseus* displayed high variability in their salinity preference; however, intermediate salinities in the range of 9–23 were selected by all fish as their final preference. Within this range, it was generally observed that most fish that exposed themselves to low salinities tended to also

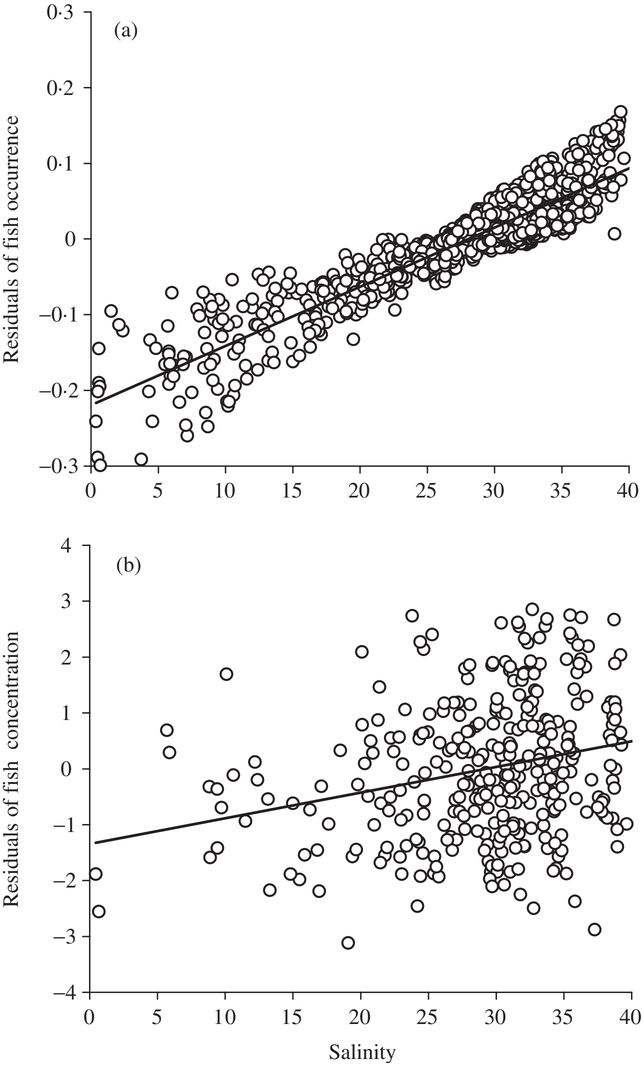

FIG. 3. Residual plots with trend lines indicating positive, linear relationships between salinity and *Lutjanus griseus* (a) occurrence and (b) concentration upon removal of depth and temperature effects (Table I).

have lower overall salinity preferences. Overall, fish displayed salinity preferences during the light phase that were slightly lower than in the dark phase (salinity means ± S.E. of 13·8 ± 1·8 and 16·9 ± 1·4, respectively); however, this difference was not statistically significant (paired *t*-test, d.f. = 10, $P > 0{\cdot}05$). Diel differences in the range, minimum and maximum salinity preference were also tested. The lower minimum salinity preference emerged as statistically lower by day *v.* night (paired *t*-test, d.f. = 10, $P < 0{\cdot}05$). A detailed inspection of the salinities preferred during the dark period (Fig. 6) revealed two distinctly different salinity preferences of fish, with about half of the specimens ($n = 6$) displaying a mean salinity preference in the range of 9–15. The others ($n = 5$) showed a salinity preference in the range of 19–23.

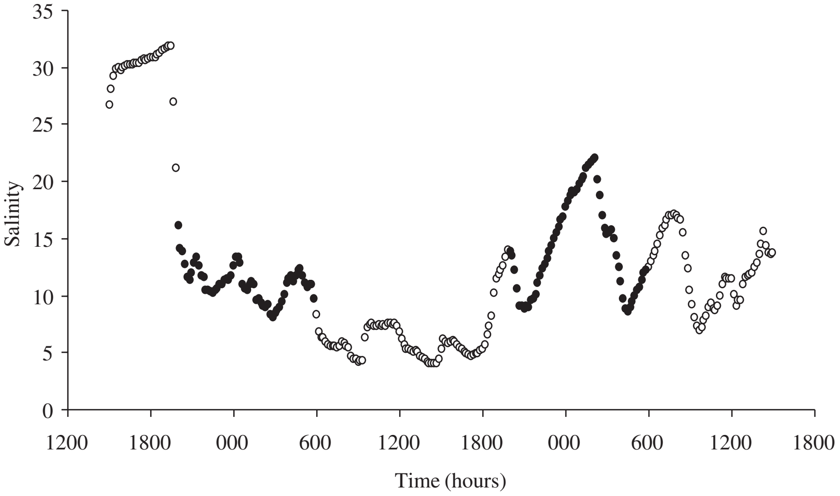

FIG. 4. Time–course example plot (salinity level *v*. time) showing diurnal regulatory behaviour (○, light period; ●, dark period) for an individual *Lutjanus griseus* tested in the shuttlebox system for a duration of 48 h.

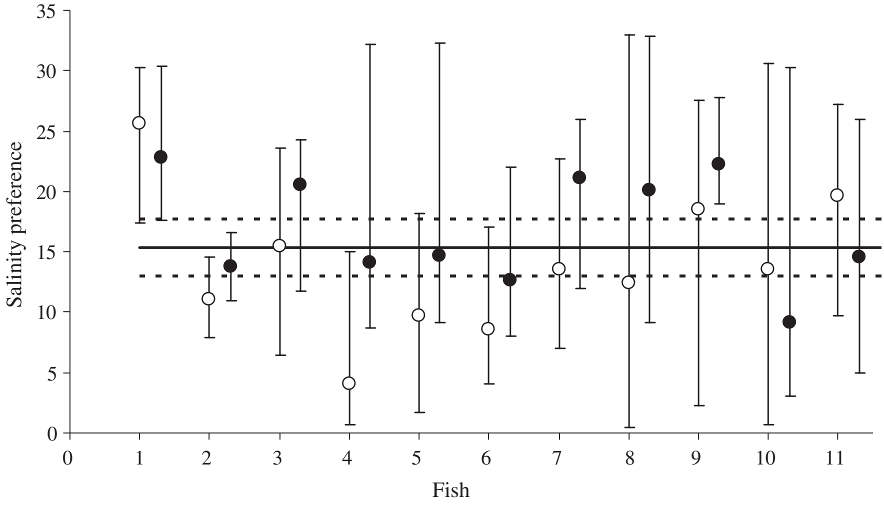

FIG. 5. Salinity preferences (mean of median preferred salinities) for individual *Lutjanus griseus* obtained during light (○) and dark (●) periods. Range of salinities occupied by individual fish during the time–course of experiments ($n = 11$) is shown. Lower and upper 95% CI for all fish are indicated (- - -), along with the overall mean across all fish (15·4, ——). Diel differences in the mean, range, minimum and maximum salinity preferences were tested for, but only the lower minimum salinity preference emerged as statistically significant (paired t-test, d.f. $= 10$, $P < 0{\cdot}05$).

SWIMMING ACTIVITY

Lutjanus griseus tested in the system displayed a marked diel rhythm in swimming activity. During the light period, fish were less active and often stationary

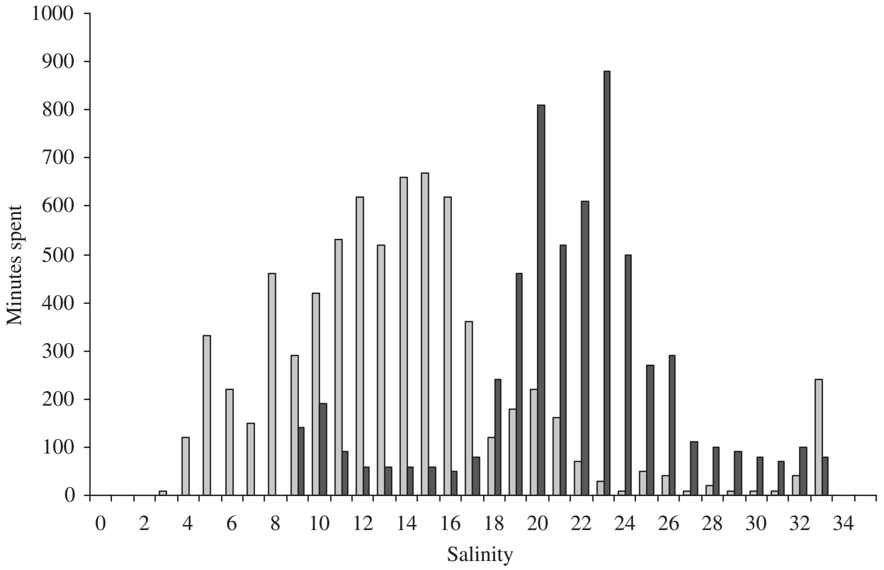

FIG. 6. Overall salinity distributions for all *Lutjanus griseus* tested ($n = 11$) by time spent at each salinity level (in min) during the more active dark period. Two distinctly different salinity preferences can be observed in the range from 9 to 15 (□, $n = 6$) and 19 to 23 (■, $n = 5$).

as compared with the dark period. During the light period, fish appeared to settle close to their final preferred salinity range, rather than making regular movements across tanks through the connecting tube, as was observed during the dark period. Fig. 7 presents mean spontaneous swimming speeds (all individuals) by salinity interval under light and dark conditions. The overall activity–salinity relationship was parabolic with reduced activity levels at extreme salinities. Further analysis of the data revealed a statistically significant diel effect, whereby across all salinity levels, *L. griseus* spontaneous swimming speeds in dark periods was significantly higher (by *c*. 0·043 L_T s^{-1}) than during light periods.

DISCUSSION

Ultimately, the performance of any aquatic species depends on the physiological suitability of the habitats it occupies (Huey, 1991; Serafy *et al*., 1997). Therefore, the success of many euryhaline species that enter estuaries and freshwater habitats probably depends on their species-specific capacity to tolerate changes in body fluid osmolality, osmoregulate and engage in more immediate behavioural responses (Serafy *et al*., 1997). *Lutjanus griseus* have long been considered estuarine transients; juveniles and sub-adults have been observed in a variety of near-shore habitats with salinities ranging from fresh water to hypersaline. Throughout its life span, *L. griseus* migrates among brackish and marine waters (and *vice versa*), and these changes in external salinity have energetic costs. Successful acclimation to a new salinity involves a set of physiological responses by multiple osmoregulatory organs (*i.e*. gills, intestine and kidneys; Lin *et al*., 2004; Marshall & Grosell, 2005) and as such,

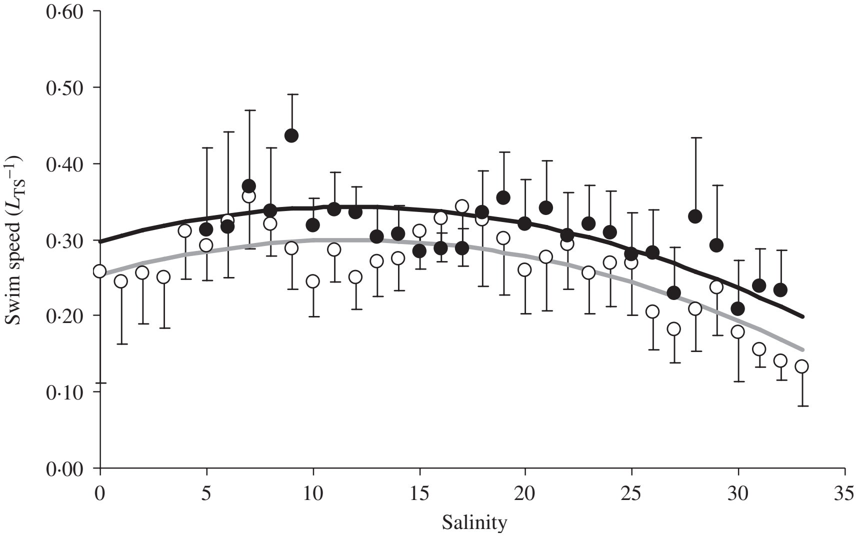

FIG. 7. Mean ± S.E. swimming speeds recorded for *Lutjanus griseus* under dark (●) and light (○) conditions by salinity level (interval ranging between 0 and 33). A quadratic salinity–speed relationship ($R^2 = 0.68$) was fitted to both sets of data (dark and light periods), revealing statistically significant differences in the swimming activity of fish between the light and dark periods ($P < 0.05$) and suggesting an elevation of swimming speeds by 0·043 total lengths (L_T) s^{-1} during the dark periods over corresponding light values (at the same salinities) The curves were fitted by: light $y = -0.0003x^2 + 0.0076x + 0.2545$ and dark $y = -0.0003x^2 + 0.0076x + 0.2977$.

requires energy expenditure, often at the cost of many other vital processes, such as growth and reproduction (Lankford & Targett, 1994; Cardona, 2000; Hurst & Conover, 2002).

The present study examined empirical data collected from Biscayne Bay to assess whether *L. griseus* (*c*. 21 cm L_T) abundances were evenly distributed along the full salinity range (0·4–40·3) at which samples were collected. Results revealed significant relationships for both *L. griseus* occurrence and concentration, both of which increased linearly across the range of salinities sampled, with highest values at salinities ≥35. Together, these field results yielded patterns for *L. griseus* that were inconsistent with the expectation that hypo and hyperosmotic salinities would be avoided to reduce energetic costs associated with osmoregulation (Lankford & Targett, 1994; Cardona, 2000; Hurst & Conover, 2002) although the findings differed from Saoud *et al*. (2007). These field-based patterns also helped in establishing hypotheses of habitat selection that could be subsequently tested with laboratory experiments.

In the laboratory, an electronic shuttlebox system was developed and tested to assess the salinity preference of *L. griseus*. Not only was this system the first of its kind for the study of salinity preference, but it also allowed measurement of spontaneous swimming activity during the testing period. By virtue of a relatively deep testing area, the system allowed the testing of large, highly mobile individuals, which is a major limitation of other salinity preference systems (Myrick *et al*., 2004). With minor modification, the system can be used for other types of preference experiments (*e.g*. temperature, dissolved oxygen, dissolved contaminants and individual ions) and to study the effects of additional factors (such as feeding or predation) on salinity preference. Notably, the observations of *L. griseus* salinity preference and swimming behaviour in this system confirm reasonable expectations that salinity preference is centred on relatively isosmotic salinities. These observations thus suggest that the automated system is capable of capturing behaviour of environmental relevance to field-caught specimens.

Overall, it was hypothesized that given a choice of salinities, *L. griseus* would tend to select isosmotic salinities that would minimize osmoregulatory costs compared with salinity extremes. Consistent with the expectations, *L. griseus* preferred intermediate salinities in the range of 9–23. Although it is uncertain why fish displayed two different salinity preferences (in the range of either 9–15 or 19–23), in both cases fish preferred salinities where osmoregulatory energy costs presumably were less than the >30 waters from which they were captured. Salinity preference differences were unassociated with the order that fish were captured or tested, fish size, or season (at time of trials), but, unfortunately, fish maturity and sex were not assessed or determined in experimental fish. Thus, the two distinct preferred salinity ranges that emerged could potentially be explained by sex and maturity stage differences. Specifically, fish tested had a very narrow size range (18–23 cm L_T), near the sizes expected for sexual differentiation and maturity for this species (Claro *et al.*, 2001). As far as is known, however, no studies have addressed the effect of maturity or sex differences on salinity preference. From the limited number of studies that have examined whether temperature preferences differed by sex and maturity stages of the organism tested (Hesthagen, 1979; Stauffer *et al.*, 1985), only one study reported significant temperature preference differences for different maturity stages (Stauffer *et al.*, 1985). On balance, it is suspected that the salinity history (prior capture) of the fish tested is the most likely cause for the two different salinity preferences observed in the present study. In the wild, fish may select salinities other than isosmotic based on other abiotic or biotic factors, and these preferences may endure for some time after they are brought to the laboratory. It is possible, therefore, that fish that had previously experienced and acclimated to low salinities in their natural habitat (prior capture) may have shown less 'reluctance' to choose low salinities in the present system.

A useful feature of the preference system is that it allowed the simultaneous recording of both salinity preferences and routine activity in a single experiment. Most studies have correlated maximum swimming speeds with salinity levels during continuous forced exercise in respirometers, and therefore these data are not directly comparable with the present ones. The interpretation of salinity effects on swimming activity of fish (regardless of time of day) was based on the rationale that metabolic scope for activity is usually reduced at less than optimal conditions (Fry, 1947). Thus, it might be expected that the highest routine swimming speeds would be obtained near isosmotic salinities with lower spontaneous activity at extreme salinities. This response will tend to minimize overall energy consumption at extreme salinities and thus favours energetic costs needed for osmoregulation. The present findings are consistent with those of Wuenschel *et al.* (2004), which demonstrated that the gross growth efficiency of small (2·5–5 cm L_T) juvenile *L. griseus* was significantly decreased at salinities ≥35. Wuenschel *et al.* (2005) later attributed these findings to increased energetic costs as evident from higher oxygen consumption rates at these salinities. The present findings differ from those of Saoud *et al.* (2007), who found that rabbitfish *Siganus rivulatus* Forsskål performed optimally at a salinity of 35, displaying the lowest Na^+ K^+-ATPase (NKA) gill activity at a salinity of 35 and no changes in growth or plasma osmolality at salinities in the range of 25–40. It is possible that these findings reflect a compensatory reduced activity at higher salinities, as seen in the present study, allowing for high growth rates despite elevated metabolic costs of osmoregulation. Due to significant differences in experimental design between the present study and that of Saoud *et al.* (2007), however, it is unclear the degree to which these apparently disparate results reflect species *v*. methodological differences.

Significant diel differences in the mean, maximum or range of salinity preferences were not detected within the experimental conditions tested. The tendency for fish to have a lower salinity preference during the light period, however, is consistent with daytime observations of *L. griseus* distribution in the field. Specifically, *L. griseus* tend to congregate along mainland mangrove shorelines during the daytime (Luo *et al.*, 2009), which due to their proximity to freshwater sources are characterized by wide salinity variation (Serafy *et al.*, 2003). Furthermore, swimming speed was found to differ significantly between the light and dark periods. The observation that active regulatory behaviour was increased during darkness may reflect the natural nocturnal foraging behaviour reported for larger size classes of this species over seagrass beds and along shorelines (Starck & Davis, 1966; Starck & Schroeder, 1970). A recent study (Luo *et al.*, 2009) employing electronic tags and video photography found *L. griseus* to exhibit distinct diel migrations among shallow water (<3 m) habitats. They found that individuals were relatively inactive by day, when they occupied mangroves or rock-ledge habitats, but made predictable, nightly migrations to nearby seagrass beds, presumably to forage for benthic prey.

INTEGRATION OF FIELD AND LABORATORY RESULTS

In nature, fishes are not always found at their preferred conditions with respect to individual environmental variables, even though they might be available. Numerous abiotic and biotic factors (*e.g.* temperature, depth, turbidity, predation and competition) may combine to define and influence the species-specific distribution patterns observed in the natural habitat. Results suggest this was the case in the present study, where field abundance patterns of *L. griseus* clearly differed from the salinity preferences determined in laboratory experiments. It is suspected that the basis of this discrepancy in size classes at or near maturity ultimately relates to minimizing distance to offshore spawning habitats. The patterns observed in the field, therefore, probably reflect the reproductive imperative to move towards offshore (high-salinity) coral reef habitats, an imperative that overrides the strategy of minimizing osmoregulatory energy costs. Furthermore, incongruence between the results of physiological studies and field surveys probably increases with ontogeny, as adults shift from maximizing their own growth and survival to optimizing conditions for egg fertilization and subsequent survival of their larval progeny.

Finally, it is unclear how coastal organisms will respond to ongoing Everglades restoration efforts, which aim to restore more natural freshwater flows within many of the South Florida's coastal bays (Walters *et al.*, 1992; Harwell *et al.*, 1996; Serafy *et al.*, 1997). These efforts will probably change salinity regimes, which in turn could have consequences for the bays' habitats and aquatic fauna (Serafy *et al.*, 2007). Therefore, an important element of this restoration is to gauge its impact on the fishes of the area before, during and after implementation (Serafy *et al*., 2007). In general, the present results point to benefits of coupling field and laboratory-based observations for gaining insight into how changes to freshwater flow will affect the species that occupy these shallow, coastal habitats. Relying on abundance–salinity relationships derived solely from field observations could have lead to the conclusion that a reduction in near-shore salinities from 35 to 15 would have detrimental effects on *L. griseus*, amounting to a decrease in suitable habitat. By virtue of the laboratory observations, however, it seems unlikely that such a salinity reduction (35 to 15) would have direct negative effects on this ecologically and economically important species. Rather, if salinity reductions in this range prove to affect near-shore *L. griseus* populations, it is suspected that it will be *via* indirect means, perhaps through salinity-induced changes to prey, predator or competitor abundances or some other element of the near-shore habitat, such as changes to water turbidity, depth profiles or the type and quantity of submerged aquatic vegetation. This stresses the importance of continuing to conduct multi-faceted monitoring of near-shore biological communities and their physical environment as restoration ensues.

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