Killifish habitat suitability as a measure of coastal restoration performance: Integrating field data, behavioral trials and simulation

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ABSTRACT

One of the goals of the Comprehensive Everglades Restoration Plan (CERP) is to restore more natural salinity regimes to the coastal bays of South Florida (USA) with the aim of improving their degraded estuarine function (e.g., as productive feeding and nursery areas). Here, we examined the occurrence (presence–absence) and concentration (presence-only fish density) of goldspotted killifish (*Floridichthys carpio*) observed in an ongoing fish survey as potential indicators of restoration performance in Biscayne Bay, Florida. To determine *F. carpio* salinity affinity, we applied regression analyses to field data and performed behavioral choice trials in the laboratory, whereby individuals were exposed to a salinity gradient (10–50). Analysis of field data indicated a parabolic relationship between killifish occurrence and salinity, where occurrence is maximized at salinity ~22. Killifish concentration was unrelated to salinity. Experimental salinity choice results tended to agree with field observations of occurrence and demonstrated a preference for intermediate salinity levels (10–30). Finally, we performed a simulation exercise to compare seasonal changes in habitat suitability within our study domain over the course of one year. Seasonal comparisons were performed as a proxy for assessing different freshwater management alternatives. We applied the relationship between killifish occurrence and salinity that resulted from analyses of field data to the output of a published hydrodynamic salinity transport model. This exercise provided areal estimates of suitable habitat gained or lost, as well as maps indicating the geographic distribution of habitat quality by season. We conclude that: (1) continued field monitoring of goldspotted killifish occurrence is warranted for its utility as a biological indicator of nearshore salinity conditions in an ecosystem-based management framework and (2) the regression relationships revealed by the present study may be useful in habitat suitability modeling exercises, especially those that compare impacts of different freshwater flow scenarios via simulation.

1. Introduction

Coastal ecosystems around the world have been degraded as a consequence of fisheries overexploitation and habitat alteration (Lotze et al., 2006), with the latter driven by anthropogenic increases in freshwater consumption, watershed development and channelization for flood and mosquito control (Kennish, 2002). In Southern Florida, profound changes to nearshore salinity regimes occurred over a span of about 100 years with the construction and operation of an extensive inland and coastal canal system. Built by the US Army Corps of Engineers (USACE), the system effectively transformed many of the region's coastal bays from genuine subtropical estuarine systems to freshwater-pulsed, occasionally hypersaline lagoons (Serafy et al., 1997). To address this problem, several management and conservation entities, including the USACE, are striving to re-establish more natural salinity regimes along the region's mainland shorelines through modification of the quantity, quality and timing of freshwater flows. The vehicle for salinity regime restoration in this region is the Comprehensive Everglades Restoration Plan (CERP), a multi-agency initiative that has among its goals the improvement of the estuarine function of South Florida's coastal bays, including Biscayne Bay (Fig. 1). Freshwater delivery has been identified as one of the most critical pressures affecting the region today (Cook et al., 2014) and it
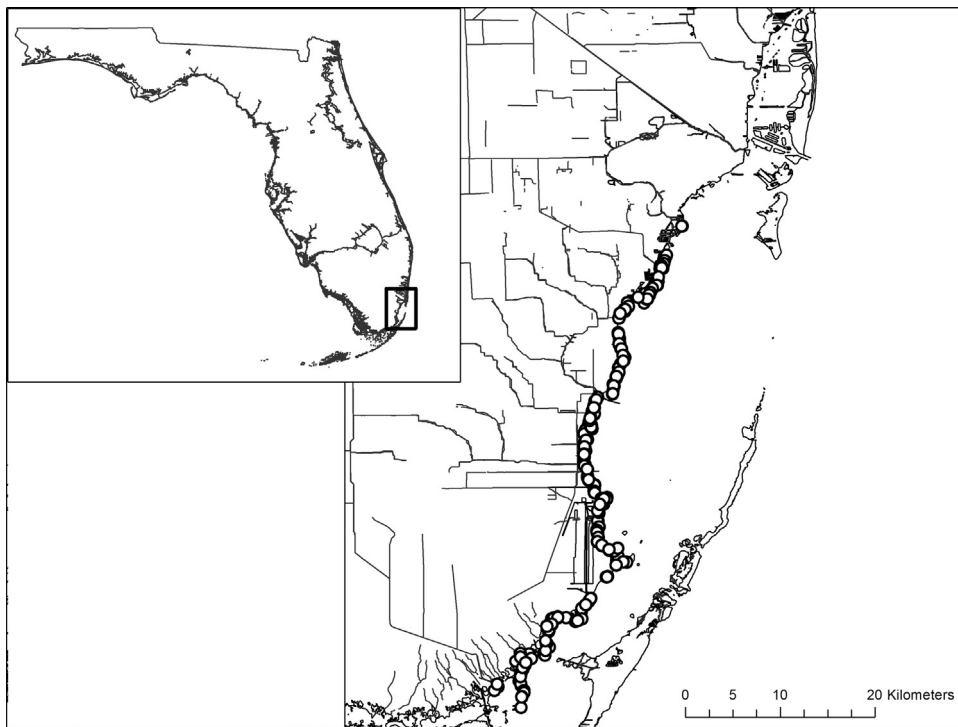
Fig. 1. Location of Biscayne Bay on the southeastern coast of Florida, USA (left panel) and map depicting sampling site locations (right panel, white circles) along the Bay's mangrove-lined mainland shoreline.

is imperative that appropriate indicators are identified and monitored in order to optimally sustain ecosystem services during and after the restoration period.

Fish-based indicators of ecosystem function are desirable because they are among the most direct, integrative measures of secondary production in a given system (Fausch et al., 1990; Soto-Galera et al., 1998; Sheaves et al., 2012). Typically, this production is communicated from lower trophic levels to top predators via small forage fish, and thus forage fish are widely considered to be essential to ecosystem function (Pikitch et al., 2012). Forage fish serve to connect coastal Florida's mangrove, seagrass and reef habitats, thus they likely mediate multiple conservation ecology pathways that link restoration efforts to ecosystem services.

Researchers currently monitoring biological communities along southern Florida's mainland shorelines are faced with the challenge of identifying candidate biological indicators of salinity change that may have utility for gauging and guiding the effectiveness of CERP efforts. Part of the challenge is that pre-canal-system biological data are almost entirely lacking and, because organisms inhabiting coastal shoreline habitats typically must cope with large fluctuations in salinity, many possess remarkable tolerance of osmotic stress. However, high tolerance, conferred by physiological robustness does not necessarily equate to lack of affinity for a narrower range of environmental conditions than tolerance studies alone might suggest (Serrano et al., 2010). For fishes, one approach to revealing these affinities, if they exist, is to couple detailed analyses of field distribution and abundance data with laboratory-based behavioral studies, whereby fish are allowed to distribute themselves along a salinity gradient (Serrano et al., 2010; Bucking et al., 2012).

The focal species in the present study is *Floridichthys carpio*, the goldspotted killifish. This diminutive, schooling cyprinodontid occurs in freshwater, brackish and marine ponds, marshes, estuaries and lagoons from southeastern Florida along the Gulf of Mexico and Yucatán Peninsula to Honduras (Robins and Ray, 1986; Smith, 1997). A seasonally abundant, resident species inhabiting

mangrove prop-root habitats of South Florida (Serafy et al., 2003; Lorenz and Serafy, 2006), *F. carpio* can maintain osmoregulatory homeostasis from 25 to 70 (Nordlie and Haney, 1998), and is an important component of the diets of wading birds and juvenile gamefishes (Schmidt, 1989; Crabtree et al., 1998; Ramo and Busto, 1993; Hammerschlag et al., 2010). Therefore, management actions that benefit *F. carpio* habitat have the potential to result in broad ecosystem gains through enhancement of the food web.

The purpose of this study was to evaluate *F. carpio* habitat suitability as a candidate indicator of salinity regime change along the mangrove-lined shorelines of Biscayne Bay and adjacent nearshore waters. We were particularly interested in finding evidence of salinity preference, a behavioral response that allows animals to remain within a suitable salinity range (Lockwood, 1976). Field data were collected and analyzed to investigate potential salinity-abundance patterns and laboratory-based, behavioral trials were conducted to test for salinity preference-avoidance responses upon exposure to a salinity gradient. Finally, we demonstrate how a fish-habitat relationship derived from the field data may be applied to the output of a numerical, two-dimensional hydrodynamic and salinity transport model (Wang et al., 2003) that simulates the changing salinity characteristics of the Bay over the course of one year. Results of this work have direct relevance to decisions surrounding resource allocation toward future field monitoring as well as to ongoing simulation exercises that seek to quantitatively compare different water management scenarios in terms of the quantity and location of suitable fish habitat.

2. Methods

2.1. Study site

Biscayne Bay (Fig. 1) is one of several subtropical coastal systems that have transitioned over the last century from estuarine to marine (Smith, 1896; Browder and Ogden, 1999; Marshall et al., 2009). The transition was a direct result of construction of the

extensive inland and coastal canal system known as the Central and South Florida Flood Control Project (Serafy et al., 1997; Browder et al., 2005). Historically, Biscayne Bay received consistent freshwater sheet flow *via* marsh and mangrove wetlands and creeks, groundwater upwelling, rainwater runoff and artesian springs within the bay: conditions that supported thriving estuarine biological communities (Smith, 1896; Kohout and Kolipinski, 1967). Currently, the majority of fresh water is diverted from the system and replaced by marine input. As a consequence, several estuarine species (e.g., oysters, black drum, red drum) are today only rarely encountered in Biscayne Bay, where euryhaline and marine species now predominate (Serafy et al., 2003).

2.2. Collection and analysis of field data

This study draws on data from an ongoing, 15-year survey designed to monitor fish assemblages associated with mangrove shorelines along the mainland and the leeward side of the northernmost Florida Keys (i.e., Ragged, Sands and Elliott Keys). Survey methodology details are provided by Serafy et al. (2003, 2007). Briefly, sampling entailed a snorkeler slowly swimming a 30 m × 2 m belt-transect and recording the species identity, number and size structure of all fishes observed within the 60 m^2 area swept. Transect site selection followed a stratified random design, where strata were shoreline type and season (Serafy et al., 2003). Upon completion of each visual transect, water temperature, salinity and depth were recorded using a multi-probe instrument (YSI model 650MDS, Xylem, Inc.), as well as a pole marked-off at 2 cm increments.

Following Serafy et al. (2007) and Serrano et al. (2010), we applied the delta approach (appropriate when counts are dominated by zero values) to reveal goldspotted killifish abundance relationships with water temperature, salinity and depth. Two abundance metrics were analyzed: "occurrence" (the likelihood of encountering one or more *F. carpio* per 60 m^2 transect) and "concentration" (number of individuals per transect when *F. carpio* was present). Logistic regression was used on the binary occurrence component and ordinary least squares (OLS) regression, after log$_e$-transformation, was applied to the concentration component. A backward stepwise regression (logistic or OLS) approach was taken in the analysis of both metrics (Y, occurrence or concentration) whereby an initial model with the factors salinity (S), temperature (T) and depth (D) and their respective squared terms was applied. Inclusion of squared terms in the model allowed for assessment of both parabolic and linear relationships between fish metrics and the habitat variables.

$$Y = S + T + D + S^2 + T^2 + D^2$$

Beginning with the squared terms, factors were successively removed if they were not significant at the $\alpha = 0.05$ level. Final model fit was judged from its concordance index (C) value for logistic regression results (values range from 0.5 to 0.99) and R^2 value for OLS results.

The significance of factors was determined from Wald Chi-Square and *t*-tests, respectively (SAS Institute Inc., 2008). After regression, $Y_{occurrence}$ values were converted to logistic probability units (0-1) (SAS Institute Inc., 2008).

The fish-habitat relationships found in these analyses were assumed to be indicative of habitat quality, where high values of Y reflected high quality (i.e., suitable) habitat. To report these habitat quality values, we adopted the stratified scoring system used in this special issue, which assigns one of five levels (optimal, good, fair, poor, critical) to the habitat, according to the relative values of fish occurrence or concentration that it is likely to produce. Categories were created by dividing the range into five, equally spaced
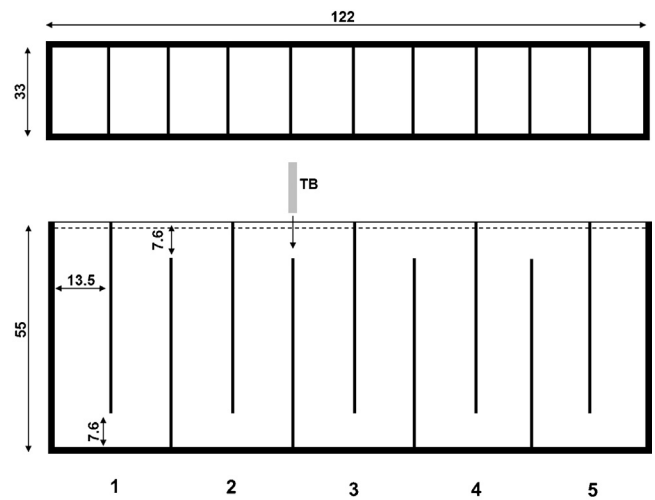

Fig. 2. Diagram of five-chamber salinity gradient aquarium (based on Staaland 1969) used to examine salinity preference-avoidance in *F. carpio*. Upper and lower panels depict top and side views; dimensions in cm. Dashed line indicates water level. Each chamber was a "U-segment" that could be temporarily disconnected from its neighbor using a slotted rubber barrier (TB = temporary barrier).

habitat suitability intervals. Color-coding of the five levels was used in graphic depictions of fish-habitat relationships and simulation exercise results (see below) to facilitate their visualization.

2.3. Collection and analysis of laboratory data

As an independent test of fish-salinity relationships, salinity choice trials were conducted using 208 L (U.S. standard 55-gallon) baffled glass aquaria (122 cm × 33 cm × 53 cm). The general aquarium design (Fig. 2) was first described by Staaland (1969) and subsequently modified and used by several others to determine salinity preferences in small fishes (Spieler et al., 1976; Fivizzani and Spieler, 1978; Campeau et al., 1984; Audet et al., 1985; Lankford and Targett, 1994; Bos and Thiel, 2006). Our experimental set-up followed Bos and Thiel (2006) in that each aquarium was partitioned into five, U-shaped chambers by nine alternating, vertically oriented, glass baffles. Adjacent chambers were connected at the top of the "U-segment" via a water column 7.6 cm deep.

Salinity gradient and control aquaria were prepared with levels in the former being 10, 20, 30, 40, and 50 in each chamber and levels in the latter being those observed at time of fish capture (mean salinity ± 1 S.E.: 35.8 ± 0.26, N = 39). Salt water from Bear Cut (between Virginia Key and Key Biscayne, Florida) averaging 34 was combined with de-chlorinated City of Miami tap water to produce the lower levels (10, 20, 30), while Instant Ocean Sea Salt (Spectrum Brands, Madison, Wisconsin) was dissolved in Bear Cut salt water for the higher levels (i.e., >34). Orientation of the salinity gradient (i.e., left–right) within each gradient aquarium was alternated between trials. Each tank was lined with opaque, blue aquarium trade paper to prevent visual interaction between fish in the four adjacent tanks. Chambers were temporarily disconnected with rubber dividers prior to the start of each trial while the salinity gradient was prepared and fish were introduced to the tank. Before each trial, tanks were aerated to near 100% DO saturation with air stones.

Goldspotted killifish were collected from the mainland shoreline of Biscayne Bay using a 7 m × 1 m haul seine with 3 mm mesh. Individuals ranged 25–67 mm total length, and collection salinities ranged 32.4–39.1. Fish were collected in daylight hours and were transported to the laboratory within an hour of collection. To allow for recovery from stressors associated with handling and transport (Rottlant and Tort, 1997; Robertson et al., 1988), and to ensure acclimation to laboratory conditions, fish were held overnight in one of

Table 1
Results of the stepwise regression on *F. carpio* field observations as they relate to salinity, depth, and/or temperature. Shown are parameter estimates for final models describing fish occurrence (a) and concentration (i.e., density when present, b).

(a) Fish occurrence

Parameter	Estimate	S.E.	Wald X^2	$P > X^2$	Model fit (C)
Intercept	5.2997	0.566	87.578	<0.0001	0.754
Salinity	0.0693	0.031	4.920	0.0265	
Depth	−0.0403	0.003	179.478	<0.0001	
Temperature	−0.1504	0.011	175.074	<0.0001	
Salinity^2	−0.00156	0.001	6.049	0.0139	

(b) Concentration (i.e., density when present)

Parameter	Estimate	S.E.	t-Value	$P > |t|$	Fit (R^2)
Intercept	−2.165993	1.390	−1.560	0.1195	0.065
Depth	−0.012682	0.003	−4.030	<0.001	
Temperature	0.4818324	0.108	4.440	<0.001	
Temperature^2	−0.0103764	0.002	−4.900	<0.001	

the previously described tanks containing water of the same collection salinity. The next morning, one fish per tank was introduced into the central chamber of gradient or control aquaria, and allowed to adjust to its new surroundings for an additional 2 h, without the barriers, before its behavior was monitored for 4 h.

A digital video recording (DVR) system consisting of an overhead, 3-camera array monitored fish movement with the room fully lit. Videos were scored post-trial by an observer who recorded all movements between chambers, noting the exact time of each move (hh:mm:ss). Trials in which the fish did not move from the central chamber were assumed null behavior and excluded from analysis. Salinity, temperature and dissolved oxygen were measured in each chamber using a YSI 650 MDS multi-probe before and after each experiment. Utilization of gradient versus control aquaria was compared in terms of the proportion of time spent in each chamber. Kruskal–Wallis tests were performed on chamber utilization data (parametric statistics were not used due to non-normality and heterogeneity of variance). Specifically, following Bos and Thiel (2006), differences between mean time spent under control and salinity-gradient conditions were tested on a chamber-by-chamber basis.

2.4. Simulation and estimation of change in habitat suitability

To visualize and quantify changes in suitable habitat for the focal species, we applied the regression relationship for *F. carpio* occurrence that resulted from the analysis of field data to the output of a numerical hydrodynamic and salinity transport model of southern Biscayne Bay and adjacent sounds (Wang et al., 2003). Objectives were to: (1) simulate continuous habitat quality dynamics on a real spatial domain, with time steps of 20 min, for a one year period spanning from April, 2004, to March, 2005 and (2) quantify and map the simulation output for each of four quarters: April to June (Q1), July to Sep (Q2), October to December (Q3), and January to March (Q4). The 20-minute time step was the finest temporal resolution available for the model. The one-year time period was selected to achieve overlap between empirical data and simulation outputs. The year was partitioned into quarters to match the collection of field season data as surveys were conducted across 3-month seasons.

Our shoreline study domain was defined as the 2 m-wide belt along Biscayne Bay's western boundary extending between latitudes 25.235° N and 25.678° N, which covers a total area of about 267,000 m^2. This belt was composed of 1470 quadrilateral elements that were approximately 100 m × 2 m each. At each 20-min time-step, values for water salinity and depth were queried for each element from the hydrodynamic salinity transport model. The temperature values used were derived from empirically

measured water temperatures recorded by a 25-station array of probes located in the general vicinity of the study domain. A single mean temperature was used for all elements at each time-step because spatial variation between each station at each time step was very small (mean 95% confidence interval = 0.33 °C). Simulated habitat-suitability output was summarized as the summed area of habitat for each quality level at each time step (5 levels × 26,280 time steps) and the mean area for each quarter by level (5 levels × 4 quarters). It is important to note that this exercise was performed in lieu of a comparison of a series of water management scenarios because management alternatives for Biscayne Bay are still under development and have not yet been incorporated into the Wang et al. (2003) or any other broadly available simulation models.

3. Results

3.1. Field observations

Fish surveys along Biscayne Bay's mainland shoreline (conducted August, 1998–September, 2012) totalled 2190 visual belt-transects; goldspotted killifish was encountered in approximately 41% (888). Salinity, temperature and depth conditions during field sampling ranged 0.5–42.4, 12.5–36.6 °C, and 20–213 cm, respectively. Stepwise regression results for *F. carpio* occurrence (Table 1a) revealed that the factors salinity, temperature, depth and salinity^2 were significant (C = 0.735). Temperature had the greatest effect, followed by salinity and depth (Table 1a). Therefore, the following logistic regression model was adopted:

$$Occurrence = \text{Intercept} + \beta_1 * \text{Salinity} + \beta_2 * \text{Temperature} + \beta_3 * \text{Depth} + \beta_4 * \text{Salinity}^2$$

A matrix of *F. carpio* probability of occurrence *P(Occurrence)* was generated using the model (above). This matrix was visualized via layered surface plots whereby the predicted occurrence values with respect to salinity and temperature were stacked vertically along a depth gradient (Fig. 3). At greater depths, temperature appears to be the more constraining variable, while temperature and salinity effects appear equally strong at shallower depth. The relationship (Table 1a) predicted salinity optima for *P(Occurrence)* at salinity ~22.

In contrast to the *P(Occurrence)* result, no relationship between *F. carpio* concentration and salinity was found (Table 1b). Depth, temperature, and temperature^2 were the only significant terms in the final model ($R^2 = 0.06$) for this abundance metric.
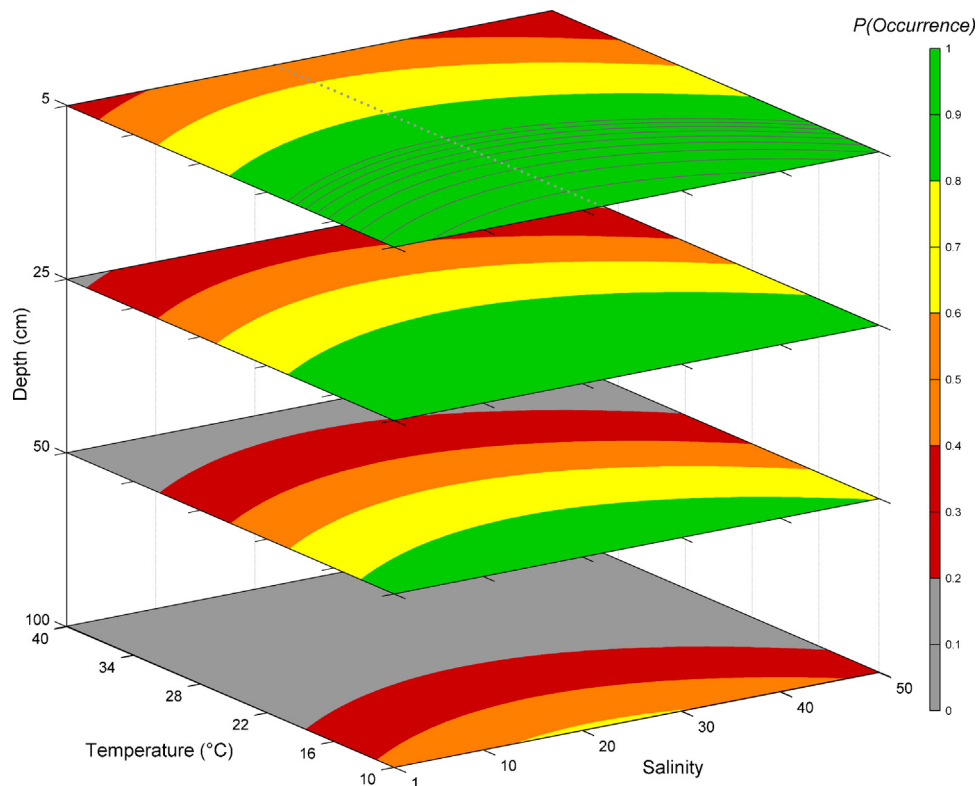
Fig. 3. Probability of *F. carpio* occurrence surfaces plotted over the range of salinities, temperatures and depths observed in Biscayne Bay field surveys. Colors correspond to the five categories of habitat suitability adopted in this special issue: 0.8–1.0 = optimal; 0.6–0.8 = good; 0.4–0.6 = fair; 0.2–0.4 = poor; and 0.0–0.2 = critical. Gray contours are plotted at probability intervals of 0.01 from 0.9 to 0.98. Gray points represent the maximum *P(Occurrence)* value at each possible temperature, from 10 to 40 °C.

3.2. Laboratory trials

A total of 39 behavioral trials were conducted, with 17 and 22 salinity gradient and control trials, respectively. Average salinity levels in control and gradient aquaria are depicted in Fig. 4. Mean (±1 SD) water temperature, dissolved oxygen (DO) levels and total length of fish monitored during trials were $25.5 \pm 1.4\,^{\circ}$C, $6.2 \pm 0.9\,\text{mg}\,\text{L}^{-1}$ and $44 \pm 6.4\,\text{mm}$, respectively. Differences between salinity gradient and control treatments in terms of mean water temperature and mean total length of fish monitored were minor, with no discernible temperature gradient from chamber 1 through 5 and no appreciable shift in temperatures over time. Mean final DO level in control aquaria trials was $6.8 \pm 0.6\,\text{mg}\,\text{L}^{-1}$
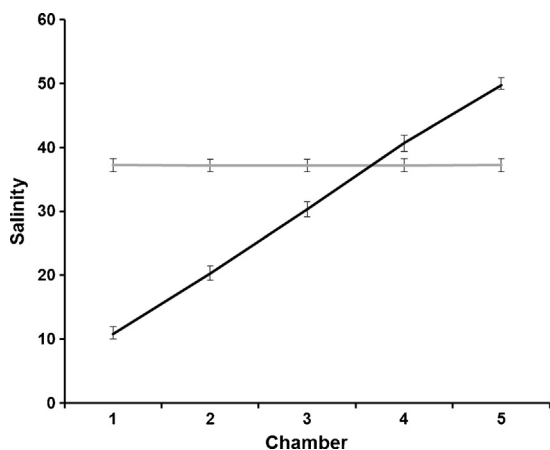
with no clear pattern from one chamber to the next. However, mean final DO levels in the salinity gradient aquaria were not identical across chambers, rather, they declined from $5.8 \pm 0.6\,\text{mg}\,\text{L}^{-1}$ in the lowest salinity chamber to $4.9 \pm 0.1\,\text{mg}\,\text{L}^{-1}$ in the highest salinity chamber. This $0.9\,\text{mg}\,\text{L}^{-1}$ DO reduction was statistically significant ($P < 0.05$) and although undesirable, was unavoidable given the inverse relationship between oxygen solubility and water salinity as well as limitations of our experimental apparatus (see next section). Similar to field studies, the highest occupancy values were observed in the intermediate salinity range and markedly different from that of the control (Fig. 5). Kruskal–Wallis tests indicated significantly lower use (i.e., avoidance; $P < 0.05$) of chambers containing the highest salinity levels (i.e., 40 and 50). Use of the chambers with the lowest salinities (i.e., 10 and 20) was relatively low, but was not statistically different from use of the corresponding chambers in the control aquaria.

3.3. Habitat suitability simulations

The dynamic habitat simulation of *P(Occurrence)* generated a large set of output data (1470 elements, 26,280 time steps). Fish concentration was not modeled because salinity, the factor most relevant to water management applications, did not emerge as having a significant influence on fish concentration in our analyses. Fig. 6 is a map of quarterly averages of *P(Occurrence)* for each element. The entire shoreline habitat was generally intermediate in quality (poor–good), with no quarterly means recording either optimal or critical, although these values were recorded for short time intervals in isolated patches (not shown). Habitat quality along the entire shoreline was a mix of mostly poor and fair habitat during Q1, predominantly poor during Q2, and improved to fair/good during Q3 and Q4. A close inspection of geographic features revealed patterns of change in *P(Occurrence)* due to changes in water depth

Fig. 4. Mean salinity levels in each chamber of control (gray line, no gradient) and salinity gradient aquaria (black line).
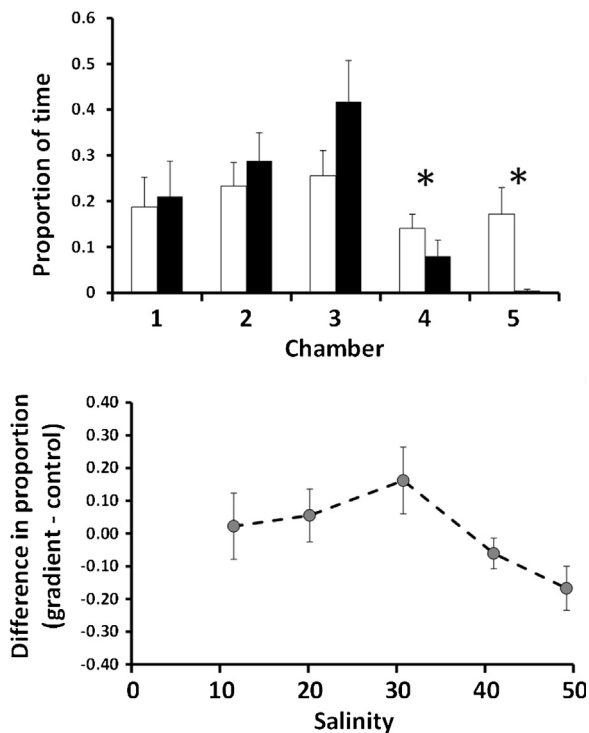
Fig. 5. Results of salinity affinity trials with single *F. carpio*. Upper panel shows mean (and 1 SE) chamber utilization (proportion of 4-hour trial period that each chamber was occupied) under control (no salinity gradient, white bars) and salinity gradient conditions (black bars). Lower panel shows difference in mean chamber utilization between control and salinity gradient treatments. Salinities in gradient chambers 1, 2, 3, 4 and 5, were 10, 20, 30, 40 and 50, respectively. Asterisks indicate significant differences between control and gradient conditions (i.e., *P* < 0.05) as determined via Kruskal–Wallis tests.

south of Mangrove Point (MP) where the slope of the shoreline is greatest, and due to salinity, along the length of shoreline where the density of freshwater point-source inputs from canals is greatest, here called the Canal Zone (CZ).

Fig. 7 shows mean habitat quality distributions (areas, m²) for each simulated quarter, pooling across all (spatial) elements. Consistent with trends shown in Fig. 6, habitat was predominantly less suitable (critical, poor, or fair) during Q1 and Q2, accounting for approximately 82% and 95% of total habitat area (i.e., 267,000 m²) respectively. As the simulated year progressed into Q3 and Q4, total habitat area shifted from less to more suitable, with the top two grades (optimal, good) totaling 48% and 65%, respectively, of the entire domain. The greatest quarterly change in suitable habitat area was from Q2 to Q3, with good and optimal habitat increasing by factors of 7.3 and 91, respectively.

4. Discussion

Altered flow patterns in South Florida due to CERP actions include a redistribution of large parcels of fresh water that were formerly diverted to the Atlantic Ocean, Gulf of Mexico and coastal bays via canals (USACE, 2010), eliciting the need for ecological indicators of salinity regime change in the region. Indicator species can include those that are correlated with specific environmental variables (Carignan and Millard, 2002). Goldspotted killifish exhibited an affinity for intermediate salinity ranges in the field and an apparent aversion for ≥40 in the laboratory trials, suggesting its potential utility as an indicator of salinity conditions, which are of major concern in the CERP (Perry, 2004).

Results of the present study suggested a parabolic relationship for *F. carpio* occurrence throughout the salinities encountered in
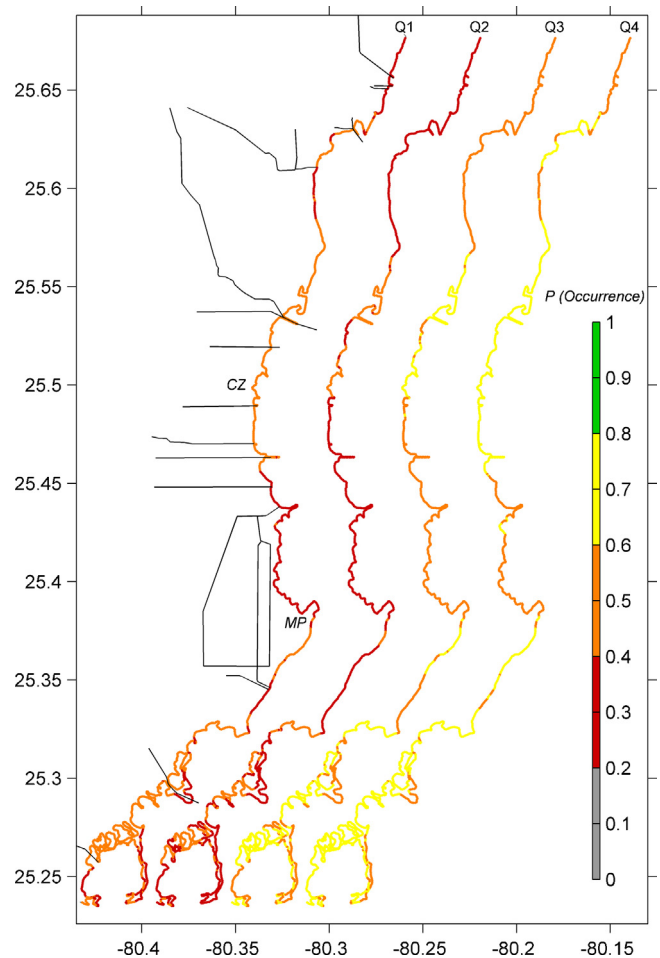

Fig. 6. Map depicting mean seasonal habitat quality values for each of the 1470, 100 m × 2 m cells along the western shore of Biscayne Bay and adjacent waters. Colors correspond to the five categories of habitat quality adopted in this special issue: 0.8–1.0 = optimal (green); 0.6–0.8 = good (orange); 0.4–0.6 = fair (yellow); 0.2–0.4 = poor (red); and 0.0–0.2 = critical (gray). Inshore canals indicated by black lines; CZ = Canal Zone, MP = Mangrove Point. Note that mean (±1 SD) canal discharge values (in cubic feet per second) by quarter were: Q1 = 4.51 ± 9.23; Q2 = 116.46 ± 165.24; Q3 = 100.05 ± 148.18; Q4 = 11.98 ± 22.92.

the field (Table 1). Although salinity is not necessarily the main driver for this distribution, it may be a proxy for other environmental gradients (e.g., sediments, nutrients, toxicants) in coastal systems due to its influence on the suspension of dissolved ions and other constituents in the water column (Day et al., 1989). *F. carpio* is most commonly found in the intermediate salinity range, with a salinity optimum for *P(Occurrence)* at ~22 for all depths and temperatures. This level falls within the 13–24 range obtained by Lorenz and Serafy (2006), also based on field observations. In addition, this finding is consistent with the expectation that hypo- and hyperosmotic salinities are avoided to reduce energetic (osmoregulatory) costs (Lankford and Targett, 1994; Hurst and Conover, 2002; Serrano et al., 2010). Conducting a similar analysis on *F. carpio* occurrence, Serafy et al. (2007) failed to detect a significant salinity effect, a result which is likely a consequence of using a model regression equation that did not include higher order terms.

Whereas a relationship between salinity and *F. carpio* occurrence was evident from our results, a relationship between salinity and *F. carpio* concentration (density when present) was not; instead killifish concentration was related only to depth and temperature. While our results tend to preclude a simple and direct salinity effect on *F. carpio* concentration, salinity may operate indirectly (i.e., in
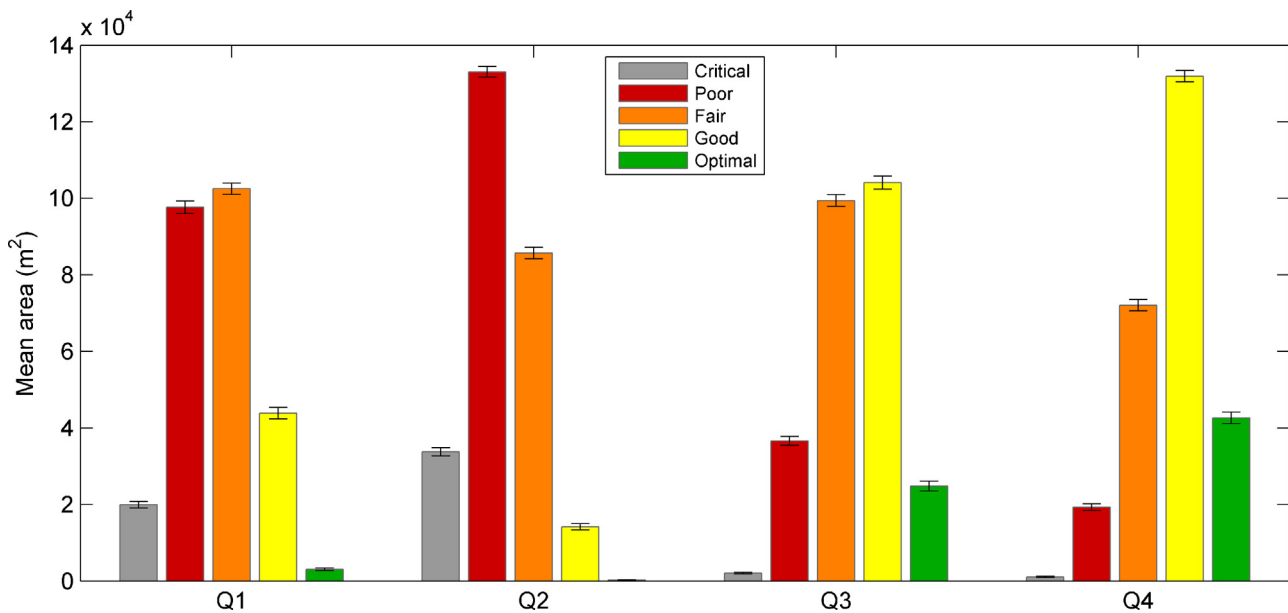
Fig. 7. Histograms depicting mean habitat quality along the entire shoreline study domain for each of the four seasons (quarters) simulated. Colors correspond to the five categories of habitat quality adopted in this special issue: 0.8–1.0 = optimal (green); 0.6–0.8 = good (orange); 0.4–0.6 = fair (yellow); 0.2–0.4 = poor (red); and 0.0–0.2 = critical (gray). Note that mean (± 1 SD) canal discharge values (in cubic feet per second) by quarter were: Q1 = 4.51 $\pm$ 9.23; Q2 = 116.46 $\pm$ 165.24; Q3 = 100.05 $\pm$ 148.18; Q4 = 11.98 $\pm$ 22.92.

combination with other unmeasured factors such as prey availability and predator densities) or over longer times scales than were considered here (e.g., see Lorenz 1999 and Faunce et al., 2004). Additionally, fish concentrations are inherently more difficult to estimate underwater than fish presence because fish are not stationary and obstacles to the underwater surveyor's line of sight are common, especially in shallow prop-root habitats. Working in Australian estuaries, Sheaves and Johnston (2010) also found it was advantageous to base fish indices on species occurrence (probability of encounter) in monitoring surveys given the high variability in density and catch-per-unit-effort in these systems.

The spatial distribution of organisms along physiochemical gradients is a key mechanism underlying fish community dynamics (Ley et al., 1999; Vega-Cendejas and De Santillana, 2004), yet few empirical studies have quantitatively determined organism distribution by taking into account the agency of motile organisms to behaviorally respond to environmental changes (Heath et al., 1993; Lankford and Targett, 1994; Serrano et al., 2010). In this study, the use of Staaland (1969) aquaria facilitated the coupling of field observations to experimental results by allowing the variable in question, salinity, to be directly manipulated. Consistent with field data, laboratory results revealed avoidance of hypersaline conditions (40 and 50; see Fig. 5). Due to their ability to maintain homeostasis from 25 to 70 (Nordlie and Haney, 1998), the tested individuals could be expected to spend the most amount of time in chambers 3, 4 and 5 (30, 40 and 50, respectively) during gradient trials, and the least amount of time in chambers 1 and 2 (10 and 20). Despite the possibility that low statistical power may have precluded the detection of significant usage differences (i.e., between chamber 1 control and chamber 1 gradient), our results suggest that when given the choice, intermediate salinities are preferred.

From a strictly experimental standpoint, our laboratory trials were not ideal due to: (1) the confounding of salinity and dissolved oxygen gradients and (2) generally lower final DO levels in the gradient aquaria. Elimination of the 0.9 mg L^{-1} DO gradient from chambers 1 to 5 presented a technical challenge that might have been addressed via graduated infusion of O$_2$ gas in each chamber. The gas infusion system would need to be gentle enough so as to not evoke vertical water flow and mixing, which would rapidly

undermine, and eventually eliminate, the salinity gradient in the aquaria. Creating ideal conditions with respect to dissolved oxygen were beyond immediate feasibility. Additionally, as water salinity increases, its oxygen solubility decreases, thus the conditions in our experiments may not be unlike those encountered in the field. Fish distribution within the tank may have also affected oxygen content due to greater consumption of more heavily visited chambers. Clearly, additional refinement of the experimental aquarium set-up that we used in this study is warranted, as is analysis of continuous temperature, salinity and dissolved oxygen levels in the field. The overall discrepancy in final DO values between control and treatment trials, may be a reflection of higher overall oxygen consumption by *F. carpio* in the gradient aquaria. Higher oxygen consumption could possibly indicate that fish were stressed by the confinement, albeit voluntary, imposed by the salinity gradient in which they were placed. A system that allows simultaneous collection of fish movement and respiratory information (e.g., video monitoring of fish position and opercular beat rate) would be ideal, but would likely take substantial time and resources to develop and perfect.

Major pressures experienced in the region include freshwater delivery and marine construction, both of which have considerable impacts on ecosystem states (e.g., coastal wetlands and oyster reefs) and services (e.g., pollution treatment and protection from storms) (Cook et al., 2014), and directly relate to future CERP efforts. The CERP aims to re-establish historical freshwater flows through the Everglades to contiguous coastal systems (Harwell et al., 1999), potentially resulting in a more estuarine fish community. However, the effects on the distribution of individual species of forage fish cannot be predicted without an understanding of species-specific salinity tolerances and affinities (Lorenz and Serafy, 2006). In this study, field observations and experimental data showed that hypersaline environments (40 and 50), although physiologically tolerable, were generally avoided by *F. carpio*. This suggests that the addition of fresh water to Biscayne Bay could have positive effects on *F. carpio* populations *via* the expansion or creation of preferred intermediate-salinity microhabitat. However, because the lowest salinity tested in experiments was 10, further trials with a hyposaline gradient (i.e., from 0 to 10) need to be performed

to establish whether *F. carpio* preference in the laboratory is consistent with field distributions for lower salinities. Salinity levels 0 and 2 have been shown to significantly decrease embryo survivorship in *Cyprinodon variegatus*, a similar species that occupies a slightly lower salinity range than *F. carpio* (Bachman and Rand, 2008; Lorenz and Serafy, 2006). Although the consequences of fresh water on *F. carpio* reproductive success is limited to speculation until appropriate field and laboratory studies are conducted, elevated mortality in adults at 0 (Nordlie and Walsh, 1989; Bachman and Rand, 2008) suggests that this species would only enter fresh water for short time periods and may avoid these areas for spawning. If more freshwater is added to the system, suitable inland marsh breeding grounds may be pushed towards the shoreline, with negative population-level effects that may influence their detection in visual fish surveys along the shoreline. Although beyond the scope of current efforts, additional fish and salinity monitoring in the semi-terrestrial marsh habitats just west of Biscayne Bay's shoreline deserves consideration.

A logical next step in this line of investigation is to model how different freshwater flow scenarios will impact *F. carpio* habitat suitability along Biscayne Bay's mainland shoreline by applying our relationships that link fish occurrence to salinity, temperature and depth variation. This step was not undertaken here because consensus has yet to be reached as to the nature and number of different scenarios to be considered, and thus relevant model configurations and outputs are unavailable for this application. Instead, we substituted seasons for scenarios to demonstrate a possible approach that begins with collecting empirical (field and laboratory) data and ends with areal estimates and maps of habitat quality for the focal species. The exercise yielded clear differences in habitat quality among seasons that would need to be considered in any future simulations of potential freshwater management scenarios. This form of habitat suitability modeling should be coupled with continued field studies that quantify the occurrence of the species and further analyses to validate model accuracy and sensitivity with new data. Results from this metric may then provide spatially explicit depictions of CERP-driven salinity effects on this important shoreline-dwelling forage species, which can subsequently be included in an overall EBM strategy for coastal South Florida. Further laboratory work would also be prudent as there are numerous salinity, temperature and dissolved oxygen combinations that are important to test for a broader understanding of *F. carpio* salinity preference and the role it plays in habitat selection.

5. Conclusions

Collectively, our field and laboratory work demonstrate that *F. carpio* has an affinity for a salinity range that is much narrower than indicated by physiological tolerance experiments and knowledge of this affinity may shed light on the ecological consequences of salinity and other changes in the habitats that they occupy. Further laboratory studies that test reproductive output as well as preference/avoidance behaviors at low salinity ranges may provide useful parameters for future habitat suitability modeling exercises. These models could then allow prediction of *F. carpio* population responses to different freshwater flow scenarios and better inform water management decisions that are relevant in an EBM framework for the sustainability of ecosystem states and services in the region. While promising, the habitat suitability work described here is simplistic in that its basis is highly correlative and does not capture myriad interactions among biotic and abiotic system components that likely determine fish presence and density. These interactions clearly deserve consideration and integration as more data become available and as complementary models (e.g., hydrodynamic, trophic, demographic) become more comprehensive and spatiotemporally resolved. For the time being, however, *F. carpio* occurrence in visual belt-transects of Biscayne Bay's western shoreline appears to represent a practical ecological indicator of present-day salinity conditions and thus has utility in ecological assessments that aim to compare how a set of proposed CERP-related salinity changes might impact this important forage species in our region. We contend that forage fish habitat suitability maps with areal estimates have particular utility as decision-making tools for those tasked with the difficult challenge of setting conservation area priorities and for performing meaningful ecosystem evaluations for coastal Florida.

References

Audet, C., Gérard, J., FitzGerald, G., Guderley, H., 1985. Salinity preferences of four sympatric species of sticklebacks (Pisces: Gasterosteidae) during their reproductive season. Copeia 1985 (1), 209–213.

Bachman, P.M., Rand, G.M., 2008. Effects of salinity on native estuarine fish species in South Florida. Ecotoxicology 17 (7), 591–597.

Bos, A.R., Thiel, R., 2006. Influence of salinity on the migration of postlarval and juvenile flounder *Pleuronectes flesus* L. in a gradient experiment. J. Fish. Biol. 68 (5), 1411–1420.

Browder, J.A., Ogden, J.C., 1999. The natural South Florida system II: predrainage ecology. Urban Ecosyst. 3 (3), 245–277.

Browder, J.A., Alleman, R., Markley, S., Ortner, P., Pitts, P.A., 2005. Biscayne bay conceptual ecological model. Wetlands 25 (4), 854–869.

Bucking, C., Wood, C.M., Grosell, M., 2012. Diet influences salinity preference of an estuarine fish, the killifish *Fundulus heteroclitus*. J. Exp. Biol. 215 (11), 1965–1974.

Campeau, S., Guderly, H., Fitzgerald, G., 1984. Salinity tolerances and preferences of fry of two species of sympatric sticklebacks: possible mechanisms of habitat segregation. Can. J. Zool. 62 (6), 1048–1051.

Carignan, V., Millard, M.A., 2002. Selecting indicator species to monitor ecological integrity: a review. Environ. Monit. Assess. 78 (1), 45–61.

Cook, G.S., Fletcher, P.J., Kelble, C.R., 2014. Towards marine ecosystem based management in South Florida: investigating connections among ecosystem pressures, states, and services in a complex coastal system. Ecol. Indic. 44, 26–39.

Crabtree, R.E., Stevens, C., Snodgrass, D., Stengard, F.J., 1998. Feeding habits of bonefish, *Albula vulpes*, from the waters of the Florida keys. Fish. Bull. 96 (4), 754–766.

Day, J.W., Hall, C.A.S., Kemp, W.M., Yáñez-Arancibia, A., 1989. Estuarine Ecology. John Wiley & Sons, Inc., New York, Chichester, Brisbane, Toronto, Singapore, 558 pp.

Faunce, C.H., Serafy, J.E., Lorenz, J.J., 2004. Density–habitat relationships of mangrove creek fishes within the southeastern saline Everglades (USA), with reference to managed freshwater releases. Wetlands Ecol. Manage. 12, 377–394.

Fausch, K.D., Lyons, J., Karr, J.R., Angermeier, P.L., 1990. Fish communities as indicators of environmental degradation. Am. Fish. Soc. Symp. 8, 123–144.

Fivizzani, A.J., Spieler, R.E., 1978. Modified Staaland device with automatic recording techniques for determining salinity preference in fishes. J. Fish. Res. Board Canada 35 (6), 910–912.

Hammerschlag, N., Ovando, D., Serafy, J.E., 2010. Seasonal diet and feeding habits of juvenile fishes foraging along a subtropical marine ecotone. Aquat. Biol. 9 (3), 279–290.

Harwell, M.A., Gentile, J.H., Bartuska, A., Harwell, C.C., Myers, V., Obeysekera, J., Ogden, J.C., Tosini, S.C., 1999. A science-based strategy for ecological restoration in South Florida. Urban Ecosyst. 3 (3), 201–222.

Heath, A.G., Turner, B.J., Davis, W.P., 1993. Temperature preferences and tolerances of three fish species inhabiting hyperthermal ponds on mangrove islands. Hydrobiologia 259 (1), 47–55.

Hurst, T., Conover, D., 2002. Effects of temperature and salinity on survival of young-of-the-year Hudson River striped bass (*Morone saxatilis*): implications for optimal overwintering habitats. Can. J. Fish Aquat. Sci. 59 (5), 787–795.

Kennish, M.J., 2002. Environmental threats and environmental future of estuaries. Environ. Conserv. 29 (1), 78–107.

Kohout, F.A., Kolipinski, M.C., 1967. Biological zonation related to groundwater discharge along the shore of Biscayne Bay, Miami, Florida. In: Lauff, G.H. (Ed.), Estuaries, 83. American Association for the Advancement of Science Publication, pp. 488–499.

Lankford, T.E., Targett, T.E., 1994. Suitability of estuarine nursery zones for juvenile weakfish (*Cynoscion regalis*): effects of temperature and salinity on feeding, growth and survival. Mar. Biol. 119 (4), 611–620.

Ley, J.A., McIvor, C.C., Montague, C.L., 1999. Fishes in mangrove prop-root habitats of northeastern Florida Bay: distinct assemblages across an estuarine gradient. Estuarine, Coastal Shelf Sci. 48 (6), 701–723.

Lockwood, A.P.M., 1976. Physiological adaptation to estuaries. In: Newell, R.C. (Ed.), Adaptation to Environment. Butterworth, London, pp. 315–393.

Lorenz, J.J., Serafy, J.E., 2006. Subtropical wetland fish assemblages and changing salinity regimes: implications for everglades restoration. Hydrobiologia 569 (1), 401–422.

Lotze, H.K., Lenihan, H.S., Borque, B.J., Bradbury, R.H., Cooke, R.G., Kay, M.C., Kidwell, S.M., Kirby, M.X., Peterson, C.H., Jackson, J.B.C., 2006. Depletion, degradation and recovery potential of estuaries and coastal seas. Science 312 (5781), 1806–1809.

Marshall, F.E., Wingard, G.I., Pitts, P., 2009. A simulation of historic hydrology and salinity in Everglades National Park: coupling paleoecologic assemblage data with regression models. Estuar. Coasts 32 (1), 37–53.

Nordlie, F.G., Haney, D.C., 1998. Adaptations in salt marsh teleosts to life in waters of varying salinity. Ital. J. Zool. 65 (Suppl. 1), 405–409.

Nordlie, F.G., Walsh, S.J., 1989. Adaptive radiation in osmotic regulatory patterns among three species of Cyprinodontids (Teleostei: Atherinomorpha). Physiol. Zool. 62 (6), 1203–1218.

Perry, W., 2004. Elements of South Florida's comprehensive everglades restoration plan. Ecotoxicology 13, 185–193.

Pikitch, E.K., Rountos, K.J., Essington, T.E., Santora, C., Pauly, D., Watson, R., Sumaila, U.R., Boersma, P.D., Boyd, I.L., Conover, D.O., Cury, P., Heppell, S.S., Houde, E.D., Mangel, M., Plagányi, É., Sainsbury, K., Steneck, R.S., Geers, T.M., Gownaris, N., Munch, S.B., 2012. The global contribution of forage fish to marine fisheries and ecosystems. Fish Fisher., http://dx.doi.org/10.1111/faf.12004.

Ramo, C., Busto, B., 1993. Resource use by herons in a Yucatan wetland during the breeding season. Wilson Bull. 105 (4), 573–586.

Robertson, L., Thomas, P., Arnold, C.R., 1988. Plasma cortisol and secondary stress responses of cultured red drum (*Sciaenops ocellatus*) to several transportation procedures. Aquaculture 68 (2), 115–130.

Robins, C.R., Ray, G.C., 1986. A Field Guide to Atlantic Coast Fishes of North America. Houghton Mifflin Company, Boston, MA, USA.

Rottlant, J., Tort, L., 1997. Cortisol and glucose responses after acute stress by net handling in the sparid red porgy previously subjected to crowding stress. J. Fish Biol. 51 (1), 21–28.

SAS Institute Inc., 2008. SAS/STAT® 9.2 User's Guide. SAS Institute Inc., Cary, NC.

Schmidt, T.W., 1989. Food habits, length-weight relationship and condition factor of young Great Barracuda, *Sphyraena barracuda* (Waldbaum), from Florida Bay, Everglades National Park, Florida. Bull. Mar. Sci. 44 (1), 163–170.

Serafy, J.E., Lindeman, K.C., Hopkins, T.E., Ault, J.E., 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.E., Faunce, C.H., Lorenz, J.J., 2003. Mangrove shoreline fishes of Biscayne Bay, Florida. Bull. Mar. Sci. 72 (1), 161–180.

Serafy, J.E., Valle, M., Faunce, C.H., 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 (3), 609–624.

Serrano, X.M., Grosell, M., Serafy, J.E., 2010. Salinity selection and preference of the gray snapper *Lutjanus griseus*: field and laboratory observations. J. Fish Biol. 76 (7), 1592–1608.

Sheaves, M., Johnston, R., 2010. Implications of spatial variability of fish assemblages for monitoring of Australia's tropical estuaries. Aquat. Conserv. Mar. Freshw. Syst. 20, 348–356.

Sheaves, M., Johnston, R., Connolly, R.M., 2012. Fish assemblages as indicators of estuary ecosystem health. Wetlands Ecol. Manage. 20, 477–490.

Smith, H.M., 1896. Notes on Biscayne Bay Florida, with a reference to its adaptability as the site of a marine hatching and experiment station. Report of the Commissioner for the year ending June 20, 1985. United States Commission of Fish and Fisheries, Government Printing Office, Washington, D.C.

Smith, C.L., 1997. National Audubon Society field guide to tropical marine fishes of the Caribbean, the Gulf of Mexico, Florida, the Bahamas, and Bermuda. Alfred A. Knopf, Inc., New York.

Soto-Galera, E., Diaz-Pardo, E., Lopez-lopez, E., Lyons, J., 1998. Fish as indicators of environmental quality in the Rio Lerma Basin, Mexico. Aquat. Ecosyst. Health Manage. 3, 267–276.

Spieler, R.E., Meier, A.H., Loesch, H.C., 1976. Photoperiodic effects on salinity selection in the Gulf killifish, *Fundulus grandis*. Copeia 1976 (3), 605–608.

Staaland, H., 1969. A device for the study of salinity preference in mobile marine animals. Comp. Biochem. Physiol. 29 (2), 853–857.

U.S. Army Corps of Engineers and South Florida Water Management District, 2010. Central and southern Florida project comprehensive everglades restoration plan Biscayne Bay coastal wetlands phase 1, vol. 1. USACE, SFWMD, Jacksonville, FL/West Palm Beach, FL.

Vega-Cendejas, M.E., De Santillana, M.H., 2004. Fish community structure and dynamics in a coastal hypersaline lagoon: Rio Lagartos, Yucatan, Mexico. Estuar. Coast. Shelf Sci. 60 (2), 285–299.

Wang, J.D., Luo, J., Ault, J.S., 2003. Flows, salinity and some implications for larva; transport in south Biscayne Bay, Florida. Bull. Mar. Sci. 72, 695–723.