2008-01-01

Ecophysiology of the Gray Snapper (Lutjanus griseus): Salinity Effects on Abundance, Physiology and Behavior

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ECOPHYSIOLOGY OF THE GRAY SNAPPER *LUTJANUS GRISEUS*: SALINITY EFFECTS ON ABUNDANCE, PHYSIOLOGY AND BEHAVIOR

By

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A THESIS

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Master of Science

Coral Gables, Florida

August 2008

UNIVERSITY OF MIAMI

A thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science

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Ecophysiology of the Gray Snapper *Lutjanus griseus*: (August 2008)
Salinity Effects on Abundance, Physiology and Behavior

Abstract of a thesis at the University of Miami.

Thesis supervised by Professor Joseph E. Serafy.
No. of pages in text. (85)

Mangroves and seagrass beds serve as "essential fish habitat" for many economically- and ecologically-valuable species. Depending on their location, these shallow-water habitats are often characterized by substantial fluctuation in salinity levels, which can represent a source of osmoregulatory stress for associated organisms. In South Florida, one of the most important fish species that utilizes these habitats is the gray snapper (*Lutjanus griseus*). Although this species constitutes a significant portion of the region's total recreational fishery harvest, the effects of salinity on its distribution, physiology and behavior remain poorly understood. The main goal of this thesis was then to investigate the ecophysiological basis of habitat selection by the gray snapper. Specific objectives include to: (1) examine patterns of distribution and abundance across gradients in environmental salinity; (2) measure physiological status and responses to controlled salinity challenges and; (3) conduct behavioral trials to examine for salinity preferenda (if any).

To begin investigating if salinity could be a primary factor structuring the gray snapper assemblages, I examined empirical data collected from Biscayne Bay to test the null hypothesis that gray snapper abundances were evenly distributed along the full

salinity range at which samples have been collected. Using the delta approach, three abundance metrics (frequency of occurrence, concentration and delta density) were used as an index for the distribution and abundance of this species. Results indicated that abundance patterns for the smaller gray snapper were consistent with a strategy of reducing osmoregulatory costs by selecting intermediate salinities. However, corresponding abundance patterns for subadult gray snapper were inconsistent with this strategy of minimizing energetic costs, suggesting that this life stage may be indifferent to the range of salinities at which they were observed. These patterns helped developed further hypotheses regarding the ecophysiology of juvenile and subadult gray snapper, the latter of which was then tested via laboratory experiments.

Subsequently, I challenged fish in the laboratory with six different salinity treatments (0, 5, 30, 50, 60 and 70ppt, including control) for 192 consecutive hours and collected blood samples at different time points. Results indicated that physiological stress to salinity changes is unlikely to occur at a salinity range of 5 to 50 ppt. At salinities of 0 and 60 ppt transient significant changes in plasma osmolality and/or blood haematocrit were observed, but were corrected after an initial adjustment period of approximately 96 hours. At the highest salinity treatment (70 ppt), a constant osmolality could not be maintained, resulting in death for all fish within 48 hours of exposure. Overall, these findings demonstrate the strong euryhalinity and extraordinary tolerance of this species to both extreme hypo- and hypersaline environments.

Finally, I investigated the salinity preference and effects on swimming behavior of the gray snapper in an automated salinity choice shuttlebox via 48-hr trials. In general, gray snapper tested displayed either one of two distinctively different salinity

preferences. Half of gray snappers displayed a salinity preference in the range of 9-15 ppt, whereas the other half displayed a salinity preference in the range of 19-23 ppt. Recorded swimming speeds in all fish tested reflected a significant, but weak negative linear relationship with salinity during both time periods of the day (light and dark); however, gray snapper were usually most active during the dark period across all salinities. Overall, these findings reveal that gray snapper prefer slightly hyperosmotic salinities that may minimize the physiological costs of osmoregulation compared to extreme salinities.

ACKNOWLEDGEMENTS

This thesis was completed thanks to the support, guidance and advice of many people at the University of Miami. First, I would like thank my two co-advisors and excellent mentors, Dr.'s Joe Serafy and David Die, for the constant support, encouragement and guidance necessary to achieve the diverse goals of this research. Joe Serafy selflessly donated his time towards directing me in the right direction and helping with the statistical analyses. My sincere thanks are also provided to the remaining members of my committee, Dr.'s Martin Grosell and Jerry Lorenz, as they contributed substantial guidance time and numerous supplies to conduct my research. More importantly, their insight and expertise in my research area greatly improved the completion of this document.

Numerous other people provided many types of support. Without these people, my research could not have been possible. In the field, I give my deepest thanks to Brian Teare, Neil Hammerschlag and the South Florida Student Shark Program, Derke Snodgrass, Craig Faunce, John Stieglitz and the rest of the researchers at the Audubon of Florida. In the laboratory, I would like to thank the members of the Grosell Lab- Sue Ebanks, Robert Gerdes, John Blanchard, Josi Taylor and Janet Genz, for their constant help and guidance. In addition, the people at Loligo Systems (Denmark) provided their expertise and valuable troubleshooting help. In the administrative side, I thank Susan Mac Mahon, Marilyn Greene and Maggie Evans. For other types of support, I owe thanks to the following people and friends. Darlene Johnson and donated a great deal of time to help me with many of the statistical analyses. My office mate Ayeisha Brinson provided consistent help and motivation. Katie Drew, Kristin Kleisner, Lauren Zamora, Nitzan

Soffer, Steve Saul and my CUFER group provided encouragement and support. Lastly, the consistent help, guidance, motivation, support and understanding from my parents and husband, Alexis Torres, made completion of this work possible. I thank my dad in particular, for constantly taking from his time by serving as a proofreader. Alexis generously provided the shuttlebox system depictions shown in Figure 4.1.

I am indebted to the people and organizations that ensured the financial support for this research. Major funding was provided through a fellowship from NOAA's Living Marine Resources Cooperative Science Center. Additional funding was provided by the Cooperative Unit for Fisheries and Education Research and grants provided to Martin Grosell and Joe Serafy.

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CHAPTER 1: AN INTRODUCTION TO RELATIONSHIPS AMONG GRAY SNAPPER (*LUTJANUS GRISEUS*) AND SALINITY

Under a variety of conditions, mangrove and seagrass beds function as valuable habitats for fish, including many ecological and recreationally important species (Thayer et al. 1987; Thayer and Chester, 1989; Parrish, 1989; Morton, 1990; Sheridan, 1992; Laegdsgaard and Johnson, 1995; Mullin, 1995; Halliday and Young, 1996; Ley et al. 1999; Nagelkerken et al., 2000ab; Nagelkerken et al., 2001; Beck et al. 2001b; Serafy et al., 2003; Faunce et al. 2004; Dorenbosch et al. 2004; Dorenbosch et al. 2005; Wuenshel et al. 2004; Cocheret de la Moriniere et al, 2004; Lugendo et al. 2005; Wuenshel et al. 2005; Faunce and Serafy, 2006). These habitats play different roles in the development and life stages by serving as daytime refuge, feeding nurseries and/or nesting areas. They also provide pre-recruits and juveniles with abundant food resources, less competition with adults and less predation (Thayer and Chester, 1989). The nursery function of these near-shore habitats is especially apparent for juvenile stages of reef fishes (Nagelkerken et al. 2001). Further, in South Florida, approximately 70-90% of the local harvested species depend on mangrove and seagrass habitats for at least part of their life cycles (Lindall and Saloman, 1977).

Mangrove and seagrass beds are near-shore shallow habitats generally structured by physical characteristics that include water temperature, salinity and depth (Sogard, 1989). Usually temperature, salinity and their interactions are the two most important environmental parameters which affect marine and estuarine teleosts (Morgan et al. 1981; Stauffer et al. 1984). However, in estuaries, fluctuations in salinity levels even in waters as deep as 25 m can be extremely sharp over brief periods (± 10 ppt; Moser and Gerry,

1989). Therefore, it has been suggested that salinity fluctuation is the most important determinant of fish distribution in estuaries (Moser and Gerry, 1989; Ley et al. 1999).

In South Florida, water flow is mainly controlled by a network of canals and levees designed to modulate the freshwater flow from Lake Okeechobee south to the Everglades and other adjacent areas (Serafy et al. 1997). This alteration of freshwater flow has changed temperature and salinity regimes and also reduced and/or degraded wetlands and other estuarine near-shore habitats (Reddering, 1988; Whitfield and Bruton, 1989; Longley, 1994; Serafy et al. 1997). As a result, Biscayne Bay has experienced drastic changes in both the amount of freshwater it receives and the way it is delivered, leading to large and abrupt changes, especially during the rainy season (Fatt, 1986; Serafy et al. 1997). In some areas, drops in salinity of about 25 ppt have been recorded within 60 minutes after canal locks have been opened (Fatt, 1986; Wang and Coffer-Shabica, 1988; Serafy et al. 1997). In Florida Bay, on the other hand, the lack of flushing and circulation and limited freshwater flow, coupled with high rates of evaporation, has likely promoted the high salinity in the area (Continental Shelf Associates, 1995). Further, in the vicinity of the Everglades, the amplitude of salinity fluctuations has led to altered natural salinity patterns (Lorenz, 1999). Overall, in both bays ecological and biomass decline has been observed as a result of both the reduction of mangrove coverage and the modification of freshwater delivery into the region (McIvor et al. 1994; Ogden, 1994, Faunce et al. 2004).

In an attempt to address these and other habitat issues, the Comprehensive Everglades Restoration Plan (CERP) is implementing the modification of water delivery into South Florida's bays and estuaries, aiming to modify and restore more natural

salinity patterns (Serafy et al. 1997; Serafy and Valle, 2006; Serafy et al. 2007). CERP comprises numerous large-scale projects designed to re-hydrate existing coastal wetlands that are now drained by the canal system and redistribute the freshwater flow to the bays (Serafy et al. 2003). These efforts will modify the timing, location and volume of freshwater flow into South Florida estuaries, and likely change salinity regimes, with possible repercussions on the area's habitats, fishes and fisheries (Serafy et al. 2003). Therefore, insight into fish response to salinity change is critical to CERP.

Gray snapper

The gray snapper (*Lujanus griseus*) is predominantly a tropical/subtropical species, much more widely distributed than its congeners in the Western Atlantic (Starck and Schroeder, 1970). Of the 14 species of *Lutjanus* in this region, 5 including the gray snapper are either facultative or obligate users of estuaries (Lindeman et al. 2001). Its habitats are known to range between inshore areas, mangroves, estuaries, lagoons, deeper channels and offshore reefs (Starck and Schroeder, 1970). Gray snapper enter estuaries as advanced larvae, so this life stage is not considered to be affected by salinity in estuaries (Rutherford et al. 1989; Serafy et al. 2008). Juveniles, however, tend to occupy a variety of near-shore shallow habitats and thus have been collected over a wide range of temperatures and salinities, and over a large latitudinal range (Starck and Schroeder, 1970; Chester and Thayer, 1990; Able and Fahay, 1998; Ley et al.1999; Denit and Sponaugle, 2004). Adults generally remain offshore (where spawning occurs), but also frequent estuaries and near-shore habitats, particularly to feed (Starck and Schroeder, 1970; Domeier et al. 1996; Lindeman et al. 2001; Allman and Grimes, 2002; Denit and Sponaugle, 2004). A three-stage ontogenetic strategy has been shown for this species in a

recent study: (1) settlement and grow-out within seagrass beds, (2) expansion to mangrove habitats at 100–120 mm total length, and (3) increasing utilization of inland mangroves during the dry seasons and with increasing body size (Faunce and Serafy, 2007).

This species is extremely important both ecologically and economically in the region. It is the most common snapper in the waters of Biscayne Bay and is collected in all areas of the Everglades National Park, from the freshwater of river tributaries to the highest salinities of Florida Bay (Tabb and Manning 1961; Serafy et al. 2008). Economically, it is exploited both recreationally and commercially in Cuba (Claro et al. 2001) and throughout the southeastern region of the US, with the majority of the landings being taken in South Florida (Burton, 2001; Faunce, 2005). In Florida Bay, gray snapper has accounted for 35% of the total harvest in the recreational fishery, where more than 86% caught have been estimated to be 3 to 4 year old fish (Tilmant, 1989). Ecologically, gray snapper is one of the top predators in seagrass beds and coral reefs (feeding primarily on shrimp, crabs and fish, particularly toadfish), thus having an important role in the marine ecosystem communities (Crocker, 1960; Starck and Schroeder, 1970; Denit and Sponaugle, 2004). Further, its removal has been associated with significant changes in the food web in both Cuba and the Florida Keys (Claro, 1991; Ault et al. 1998, Denit and Sponaugle, 2004).

Literature review

A review of the primary literature available was conducted for gray snapper, focusing on determining the range of salinities reported in the past decades. Approximately 90 papers were found in the primary literature for this species, with ~33%

of these including salinity and information on size in most cases (Table 1.1). These studies date back to the 1900s; however, the only information provided for the gray snapper before 1960s was the presence of juveniles in freshwater (0 ppt). The first salinity values were reported for this species by Crocker (1960), who observed/collected gray snappers in waters with salinities ranging from 0-47.7 ppt across all sizes. Juvenile gray snapper, however, were reported years later in salinities as high as 66.6 ppt in Florida Bay by Rutherford et al. (1989). Ley et al. (1999) reported the most extensive salinity range for gray snapper across all sizes (0-60 ppt) and categorized the gray snapper as an estuarine transient. The authors mentioned, though, that gray snapper were rarely collected at salinities >54 ppt.

From the literature, it is clear that gray snappers show a strong preference for marine habitats (particularly as adults) and salinity ranges between 20-40 ppt, as most studies have reported/collected them at these salinities. However, information regarding the distribution of this species across salinity gradients remains limited. In addition, a critical shortcoming in the available literature is the lack of any laboratory studies targeted to larger size classes of gray snapper, especially those that are vulnerable to hook-and-line fishing. Adults have been suggested to be less tolerant of salinity fluctuations than young fish (Starck, 1964), particularly to salinities higher than 50 ppt (Continental Shelf Associates, 1995), but their actual upper lethal tolerances has not been assessed yet. Only Serafy et al. (1997) reported that gray snapper were very tolerant (0% mortality) when exposed to a freshwater pulse in the laboratory for 24 hours after being acclimated to full strength seawater.

Due to the abundance and economic/ecological importance of this species in the local context, a great deal of attention has been given in many studies to the gray snapper's habitat preference, diet, mortality, age, growth and reproduction. Not until recently has this species been the focus of studies interested in understanding the physiological effects of environmental parameters such as salinity and temperature. Specifically, Wuenschel et al. (2004, 2005) assessed the effects of both temperature and salinity on the energetics of small juvenile gray snappers (25-50 mm total length) under 20 combinations of these two variables (18, 23, 28 and 33^0C) and (5, 15, 25, 35 and 45 ppt), often experienced by juveniles in nursery habitats. These studies demonstrated that juvenile gray snapper could survive and grow over this wide range and combinations of temperatures and salinities. Wuenschel et al. (2004, 2005) thus classified gray snapper as having a wide tolerance in both habitat and environment.

Research overview

The main goal of this study is to investigate the ecophysiological basis of habitat selection for the gray snapper. More specifically, this work is an attempt to gauge how salinity changes influence the distribution patterns, the physiology and the behavior of the gray snapper. A combined approach of fieldwork and laboratory studies will be used in an effort to increase the understanding of the effects of salinity on different aspects of the biology of this species. Therefore, Chapter 2 aims at determining if the gray snapper distribution and abundance is primarily influenced by salinity. Abundance and distribution of gray snapper along the mainland shoreline of Biscayne Bay will be used to determine the extent to which salinity explain the observed distribution patterns. Chapter 3 assesses the immediate physiological responses observed in the gray snapper as a result

of abrupt changes in salinity levels as reflected in plasma osmolality and blood haematocrit. This work serves as a first step towards understanding the basis and limits of the euryhalinity of the gray snapper. Chapter 4 reports the first known attempt to study the salinity preference of a reef fish. Specifically, this chapter aims at determining the salinity preference displayed by the gray snapper given a choice of salinities in an artificial salinity gradient and its possible repercussions on the swimming behavior. Finally, Chapter 5 provides a summary of this work with some implications and future research recommendations.

Year of publication	*Authors*	*Salinity reported (ppt)*	*Size reported (mm)*
1902	Eigenmann	0	<100
1928	Hildebrand and Schroeder	0	<100
1934	Breder	0	<100
1939	Hildebrand	0	<100
1949	Herald and Strickland	0	<100
1960	Croker	0-47.7	88-470
1960	Springer and Woodburn	3-35	14-164
1961	Tabb and Manning	0-37	>20
1963	Gunter and Hall	0	<100
1970	Roessler	17.9-43.3	*nd*
1987	Sogard et al.	17-44	*nd*
1987	Thayer et al.	13.2-35.5	<30-250
1989	Sogard et al.	20-50	141-187
1989	Thayer and Chester	35.2-36.5	*nd*
1989a	Rutherford et al.	25.8-66.6	72-116
1989b	Rutherford et al.	8-48	<100
1990	Chester and Thayer	35.3-36.8	>30-97.7
1992	Sheridan	31.2-36.9	*nd*
1993	Montague and Ley	11.4-33.1	*nd*
1997	Serafy et al.	0-35	28-265
1999	Thayer et al.	23.2-42	*nd*
1999	Ley et al.	0-60	48-380
2000a	Nagelkerken et al.	34.3-36.3	>25-375
2001	Nagelkerken et al.	33.1-35.7	99-169
2003	Serafy et al.	7-40	40-610
2003	Barimo and Serafy	30.2-33.5	129-310
2004	Faunce et al.	0-48	38-500
2004	Wuenschel et al.	5,15, 25, 35 & 45	25-50
2005	Wuenschel et al.	5,15, 25, 35 & 45	25-50
2006	Wuenschel and Martin	5,15, 25, 35 & 45	25-50
2007	Serafy et al.	0-42	51-210
2007	Whaley et al.	0.1-37.4	<100

Table 1.1 Chronological list of studies in the primary literature on gray snapper with salinity and size values reported.

CHAPTER 2: PATTERNS OF GRAY SNAPPER (*LUTJANUS GRISEUS*) ABUNDANCE ACROSS SALINITY GRADIENTS: AN EXAMINATION OF FIELD DATA COLLECTED FROM BISCAYNE BAY

SYNOPSIS

Data from two field-based fish surveys were examined to test the null hypothesis that gray snapper abundances were evenly distributed along the full salinity range at which samples have been collected. One dataset was associated with a trawl study, which captured young-of-the-year juveniles (~100 mm TL) in seagrass beds, and the second was associated with a visual fish survey conducted in mangroves habitats utilized by subadults (~180 mm TL). Due to the preponderance of zero catches in both datasets, the delta approach was used, whereby trends in three abundance metrics were examined along salinity gradients. This was achieved by: (1) designating each fish sample to one of a series of 5-ppt salinity intervals; (2) calculating for each interval the frequency of occurrence, mean concentration (density when present) and delta density (product of occurrence and concentration) of gray snapper; and (3) examining for trends across the range of salinity bins using standard regression models. For the smaller size class of gray snapper, a parabolic relationship emerged with respect to their occurrence, which is consistent with following a strategy of reducing osmoregulatory costs by selecting intermediate salinities. However, corresponding abundance patterns for subadult gray snapper were inconsistent with minimizing energetic costs, suggesting that this life stage may be indifferent (from both osmoregulatory and salinity preference standpoints) to the range of salinities at which it was observed.

BACKGROUND

Processes influencing patterns of fish habitat utilization are typically species- and scale-dependent (Faunce and Serafy, 2007). In the marine environment, the processes that appear to structure fish assemblages are usually the result of both abiotic and biotic factors. For example, Martino and Able (2003) reported that while large-scale (>10 km) patterns in the structure of estuarine fish assemblages appear to be primarily a result of individual species responses to abiotic factors (e.g., salinity, temperature and depth), smaller scale (<1 km) patterns appear to be the result of habitat associations that are driven by biotic factors (e.g., habitat selection, competition, and/or predation). Martino and Able (2003) suggested that physiological tolerances to abiotic factors set up the community framework, while biotic interactions refine species distribution patterns within this structure. A recent study (Wuenschel et al. 2005) also suggested that although abiotic factors may not be determinants of habitat selection *per se*, the profitability of a given habitat to its occupants can be a function of these factors. Thus, while biotic factors within specific habitats play a major role in defining fish assemblages, the physiological consequences of habitat selection within the context of the surrounding abiotic environment are also important.

Only recently have ecologists begun to quantify the density and species composition of fish assemblages living in mangrove habitats (Ley et al. 1999; Serafy et al. 2003). A recent review of mangrove-fish studies by Faunce and Serafy (2006) indicated that most analyses to date have been conducted at the assemblage- rather than the species-level. Emphasis has been placed on revealing temporal patterns and identifying assemblage-level patterns of fish use at a limited number of locations, with

few studies providing species-specific information on patterns of distribution and abundance (Serafy et al. 2003; Faunce and Serafy, 20006). Moreover, very few studies have investigated mangrove fish densities and assemblage structure along physicochemical gradients, such as salinity (Ley et al. 1999).

Compared to its benthic communities (e.g., seagrass and hard bottom), Biscayne Bay's mangrove fauna have only recently received focused attention (Serafy et al. 2003). Fish assemblages within Biscayne Bay's mainland shoreline have been monitored and quantified in recent years in an effort to determine how fish diversity, density and abundance have changed through time (Serafy et al. 2003; Serafy et al. 2007; Serafy et al. 2008). One advantage of using Biscayne Bay as a study area is that this region provides the opportunity to examine how reef fish utilization of seagrass and mangrove habitats varies across broad spatial scales and physicochemical conditions (Faunce and Serafy, 2007).

Ecologically, Biscayne Bay has been utilized by five principal species of reef fish, with one of them being the gray snapper (Serafy et al. 2003; Faunce et al. 2004; Faunce, 2005). This species is among the most abundant fishes in the region (Tabb and Manning 1961; Serafy et al. 2008), poses few identification problems in field surveys (Serafy et al. 2007), and is of high economic and ecological importance in local fisheries and the ecosystem at large (Ault et al. 1998; Tilmant, 1989; Burton, 2001; Denit and Sponaugle, 2004; Faunce, 2005). Several recent studies conducted within Biscayne Bay (Serafy et al. 2003; Serafy et al. 2007; Faunce and Serafy, 2007) indicate a progression of habitat use from seagrass beds during the first year of life to mangrove habitats during subsequent years at subadult stages.

In this chapter, I explore the extent to which patterns of gray snapper abundance are correlated with salinity with the aim of establishing hypotheses about their ecophysiology and testing them in subsequent laboratory experiments. The specific objective was to examine empirical data on gray snapper collected from Biscayne Bay to reveal patterns, if any, in abundance along salinity gradients. Two fish datasets were analyzed: one resulting from a rollerframe trawl study along the seagrass beds adjacent to the Biscayne Bay's mainland shoreline (Serafy et al. 1997); and the second resulting from ongoing visual fish surveys conducted along the Biscayne Bay's mangrove-lined shorelines (first described by Serafy et al. 2003). Patterns in three abundance metrics (frequency of occurrence, concentration and delta-density, *see Methods)* were examined. The primary question addressed was: based on empirical field observations, are gray snapper distributed evenly along the full range of salinity levels at which they have been collected/observed?

METHODS

Details of the design and methods used in the fish studies that generated the two datasets examined here can be found in Serafy et al. (1997, trawl study) and Serafy et al. (2003, visual fish survey). Brief methodological descriptions follow.

Trawl survey

Fishes were collected from eight, seagrass-dominated study sites in Biscayne Bay over 14 consecutive months (August 1993-October 1994) using a commercial live bait shrimp fishing vessel equipped with paired rollerframe trawl nets. Trawling was conducted exclusively at night, predominately in seagrass habitats and salinity,

temperature and depth were recorded at time of sampling. All fishes caught were identified to species, enumerated and measured for total length (TL). Catches from the two simultaneous tows were averaged and fish abundance was expressed as numbers per 1000 m^2. Gray snapper were the eighth most abundant fish taxon collected, and averaged 94 mm TL.

Visual fish survey

Visual fish surveys analyzed in this study were conducted twice annually from 1998 through 2007 during consecutive wet and dry seasons along the mangrove-shorelines of Biscayne Bay. Surveys were conducted exclusively during the day. A modification of the visual "belt-transect" census method of Rooker and Dennis (1991) was used. This method entailed snorkeling 30 m long transects parallel to the shore and recording species information. At each transect, salinity, depth and temperature were recorded at time of sampling. Transect width was 2 m, thus fish abundance data was expressed as numbers per 60 m^2. Gray snapper were the fourth most abundant fish taxon collected, and averaged 180 mm TL.

Data Analysis

Initial plots (Figures 2.1 and 2.2) indicated that both gray snapper abundance datasets were dominated by "zero catches" (i.e., the species was not observed > 45% of samples); therefore I adopted the delta approach (*sensu* Serafy et al. 2007) towards data analysis. Briefly, this procedure consists of analyzing three "abundance metrics" for each species of interest: (1) frequency of occurrence, (2) concentration (density when present, exclusive of zeros), and (3) delta density (product of the occurrence by the concentration). Following Serafy and Valle (2006) salinity gradients were constructed via

5-ppt binning of salinity measurements recorded at time of sampling. Specifically, each sample was designated to one of following salinity bins: (1) 0-5 ppt; (2) 5-10 ppt; (3) 10-15 ppt; (4) 15-20 ppt; (5) 20-25 ppt; (6) 25-30 ppt; (7) 30-35 ppt; and (8) 35-40 ppt. Next, gray snapper frequency of occurrence (hereafter termed occurrence), mean concentration, and delta-density were calculated for each bin. Finally, standard linear regression was performed using the salinity as the independent variable and each of the three abundance metrics as the dependent. This approach does not assume that abundance patterns are driven solely by salinity. Rather, it isolates underlying salinity trends, if present, relegating the influence of other factors to variance around each of the metric values.

RESULTS

Trawl survey

Examination of salinity data indicated that salinities during the wet season (June through October) were the most variable relative to the dry season (November through May). Therefore, analyses were limited to wet season data as they provided a more homogenous distribution of samples across salinity bins and reduced the possible confounding influence of seasonal temperature variation. A total of 132 paired tows were conducted during the wet season, of which 71 (53.8%) were positive for gray snapper (Table 2.1). One significant pattern emerged upon regression of the three metrics against the salinity interval gradient (Figures 2.3 A-C). Gray snapper occurrence showed a strong parabolic relationship (R^2= 0.98) with respect to salinity, with highest values occurring at salinities around 22.5 ppt. Gray snapper concentration increased with

salinity, but this relationship was only marginally significant ($p = 0.06$). Delta density tended to increase along the gradient, but this trend was not statistically significant.

Visual fish survey

As with the trawl data, visual fish survey analyses were limited to wet season samples as salinities during this season were more variable relative to the dry. Similarly, analyses were limited to samples obtained along the mainland shoreline of Biscayne Bay as other shorelines received only minor quantities of fresh water. A total of 544 visual transect samples were considered. Of these, 182 (33.5%) were positive for gray snapper (Table 2.1). Significant trends in both gray snapper occurrence and concentration emerged across the salinity gradient (Figure 2.4 A-C). Occurrence showed a strong linear positive relationship ($R^2= 0.96$) with respect to salinity, with highest values occurring at salinities >35 ppt. Conversely, the relationship between mean gray snapper concentration and salinity was parabolic ($R^2= 0.80$) with highest values at the salinity extremes. Like occurrence, delta density tended to increase along the salinity gradient, however this pattern was only marginally significant ($p = 0.053$).

DISCUSSION

My main goal in this chapter was to examine two empirical datasets to test the null hypothesis that gray snapper abundances were evenly distributed along the full salinity range at which samples have been collected. Given the preponderance of "zero catches", I used the delta approach to examine trends in three abundance metrics across salinity gradients that were constructed by binning samples within a series of 5 ppt intervals. Results indicate that rejection of the null hypothesis is appropriate for gray snapper

occurrence (both datasets) and also for subadult concentrations along Biscayne Bay's mainland shoreline. These patterns represent further hypotheses regarding the ecophysiology of juvenile (~100 mm TL) and subadult (~180 mm TL) gray snapper, the latter of which will be tested via laboratory experiments that are detailed in subsequent chapters.

The occurrence of gray snapper juveniles (averaging ~100 mm TL) showed a strong parabolic relationship with respect to salinity, peaking around 22.5 ppt. This pattern is somewhat consistent with physiological expectations, as extreme salinities most likely represent large energetic costs for osmoregulation. Further, at extreme salinities the energy available for other vital processes such as growth has to be diverted for osmoregulation (Lankford and Targett 1994; Cardona 2000; Hurst and Conover, 2002), which for juveniles, may protract a life stage of high predation risk. Wuenschel et al. (2004, 2005) demonstrated that the growth of juveniles (25-50 mm TL) was significantly lower under high salinity conditions (35 and 45ppt), and attributed it to increased energetic costs and higher oxygen consumption rates. Therefore, it is possible that the occurrence pattern observed for trawl-caught juveniles reflects an affinity for intermediate salinities that may ultimately enhance growth rates.

Two significant abundance-salinity relationships emerged for the subadult gray snapper – both of which differed markedly from those found for juveniles. Specifically, gray snapper occurrence increased linearly across the range of salinities in which it was observed, whereas its concentration followed the shape of an inverse parabola (Figures 2.4 A-B). As with the juveniles, the delta-density pattern for subadults was suggestive of a linear increase, but was not significant at the $p < 0.05$ level. Discrepancies in the

abundance patterns of the two size classes of gray snapper may reflect differences in sampling time (night versus day), increasing osmoregulatory capacity with age and/or the overwhelming influence of other factors, such as the reproductive imperative to move towards offshore (high-salinity) spawning sites (Starck and Schroeder, 1970). Whatever the underlying cause, this analysis yielded patterns that are inconsistent with a strategy of minimizing osmoregulatory costs. This begs the following question for subdadult gray snapper: Is this life stage essentially indifferent, from both osmoregulatory and salinity preference standpoints, to the range of salinities at which it was observed? This central question forms the basis for my thesis and is directly addressed in the remaining chapters.

Future field research might be directed at shedding light on the "salinity history" of individuals prior to their capture. Among the most significant developments in the area of electronic tagging is the pop-up satellite archival transmitting (PAT) tag (Block et al. 2001; Luo et al. 2006). This technology can help reveal aspects of fish behavior as never seen before (Sibert and Nielsen, 2001; Jonson et al. 2003; Luo et al. 2006), including habitat utilization and distribution patterns across complex physicochemical gradients. Once attached to a fish, current PAT tag technology can sample temperature, depth (pressure) and light levels at user-defined time intervals and transmit collected data after detachment. As PAT tags eliminate the need for recovery of the tag itself, they represent a truly fishery-independent means of obtaining fish movement data (Luo et al. 2006). However, their application is limited by high costs, inability to tag fish as larvae or juveniles, high rates of mortality during early life history stages and possible repercussions on fish behavior (Gillanders and Kingsford, 2000; Eldson and Gillanders, 2005).

An alternative approach is the use of otoliths as natural tags for answering ecological questions regarding the life history of fishes (Campana et al. 1995, Thorrold et al. 1997; Thorrold et al. 1998; Elsdon and Gillanders 2003; Eldson and Gillanders, 2005). Key assumptions underlying the use of otoliths as environmental "recorders" are that fish incorporate elements from their environment that are permanently deposited in their otoliths (Campana 1999; Martin and Wuenschel, 2006). Therefore, fish otoliths can be removed at any life stage to obtain, in theory, a detailed chronological record of the environment to which the fish was previously exposed (Campana, 1999). One drawback from this method, however, is that reconstructing environmental histories from otolith chemistry requires detailed knowledge of how physical and biological factors influence the elemental signatures, making interpretation of results complex (Eldson and Gillanders 2003; Eldson and Gillanders, 2004; Eldson and Gillanders, 2005; Martin and Wuenschel, 2006). Martin and Wuenschel (2006) examined the otolith microchemistry of juvenile gray snapper that had been held in the laboratory under a broad range of known temperatures and salinities. While some promising results emerged in terms of relationships between salinity and Mg and salinity and Ba, individual variation was high in both cases (Martin and Wuenschel, 2006). Clearly, answers to questions surrounding the salinity tolerances, preferences and histories of gray snapper will require researchers to explore multiple lines of investigation for several years to come.

Fish dataset	*Total number of tows/transects wet season*	*Number of positive tows/transects*	*% Positive*
Trawl survey (1993-1994)	132	71	53.8%
Fish surveys (1998-2007)	544	182	33.4%

Table 2.1. Sampling effort (in number of transects or tows), number and percent of positive samples (where gray snapper was present) observed for the wet seasons of the trawl and fish surveys in Biscayne Bay.

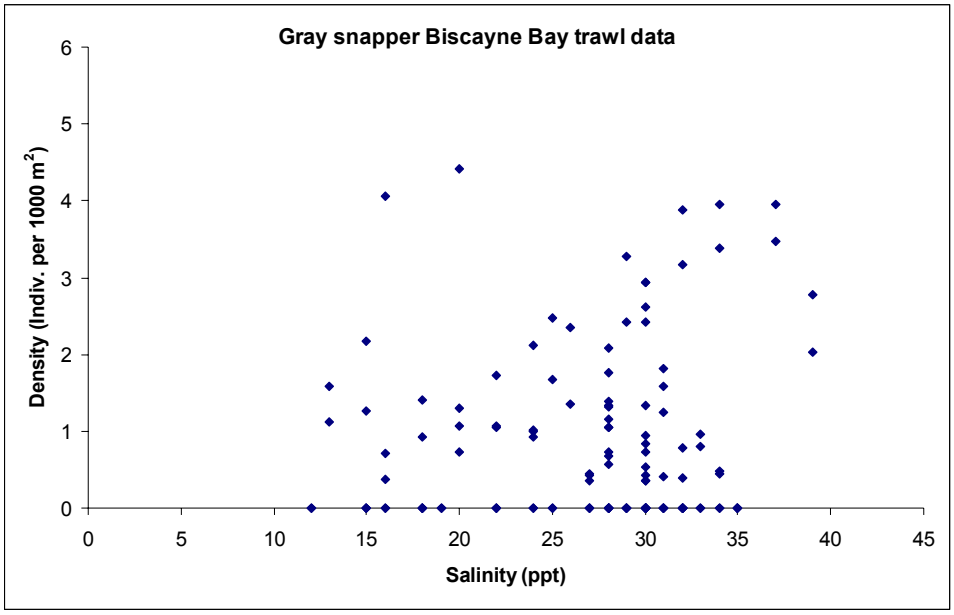

Figure 2.1. Gray snapper *Lutjanus griseus* specific densities observed by salinity at time of sampling in the trawl survey.

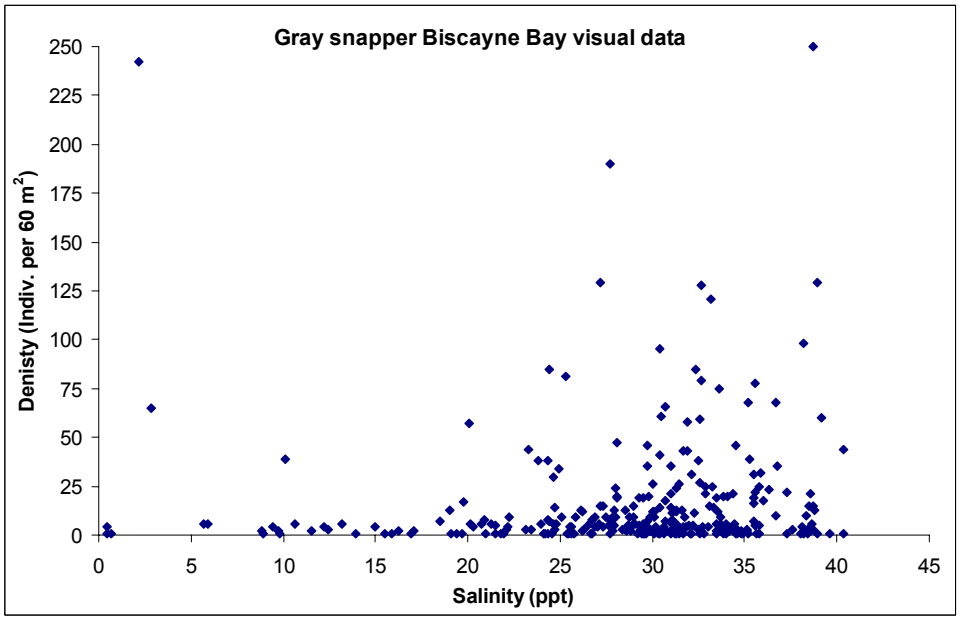

Figure 2.2. Gray snapper *Lutjanus griseus* specific densities observed by salinity at time of sampling in visual surveys.

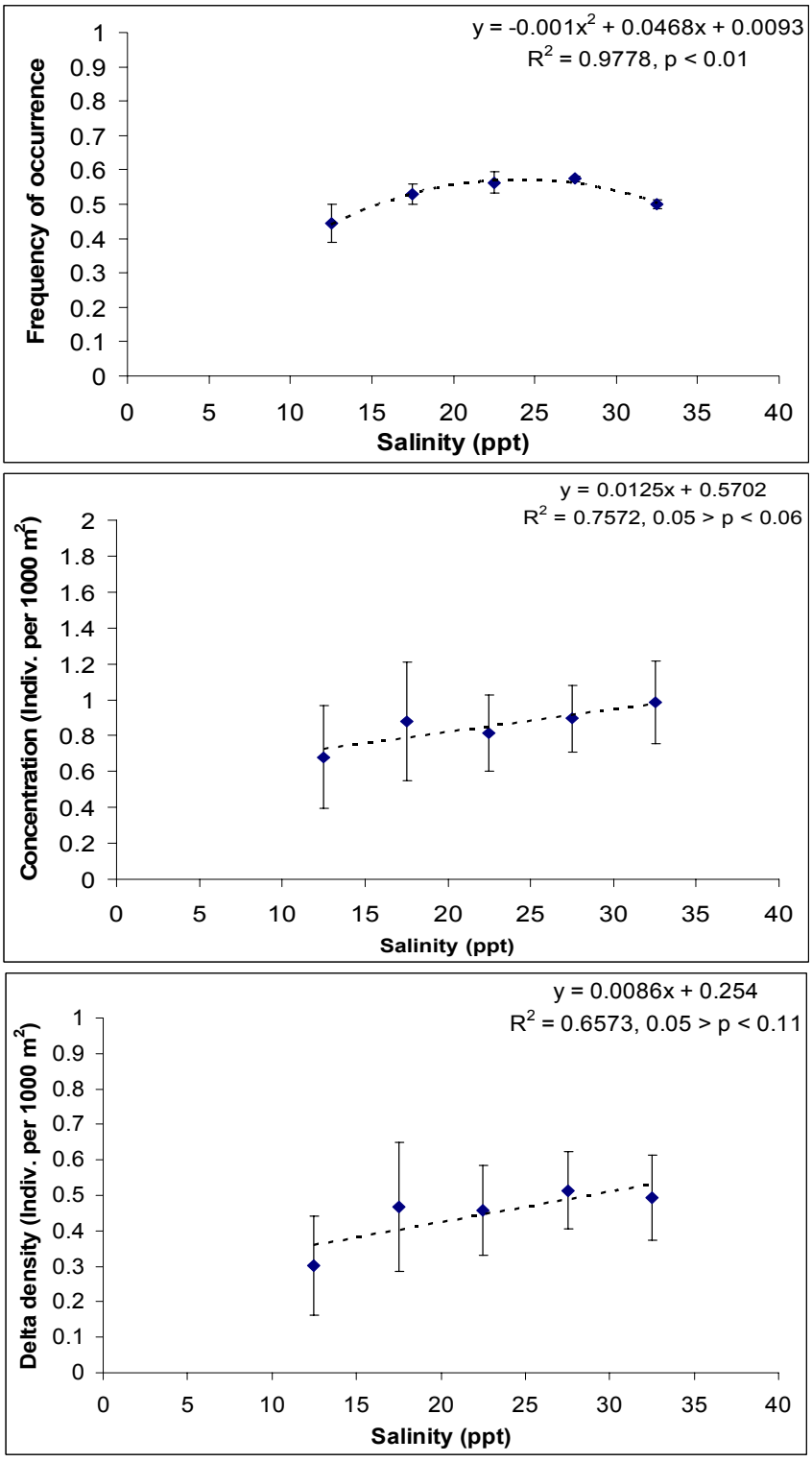

Figure 2.3 (A-C). Abundance metrics for gray snapper *Lutjanus griseus* by salinity level in Biscayne Bay seagrass-trawls during the wet seasons. Regression plots are shown, along with the equation of the relationship, strength of the trendline (R^2) and p-value. Error bars are standard errors of the mean values.

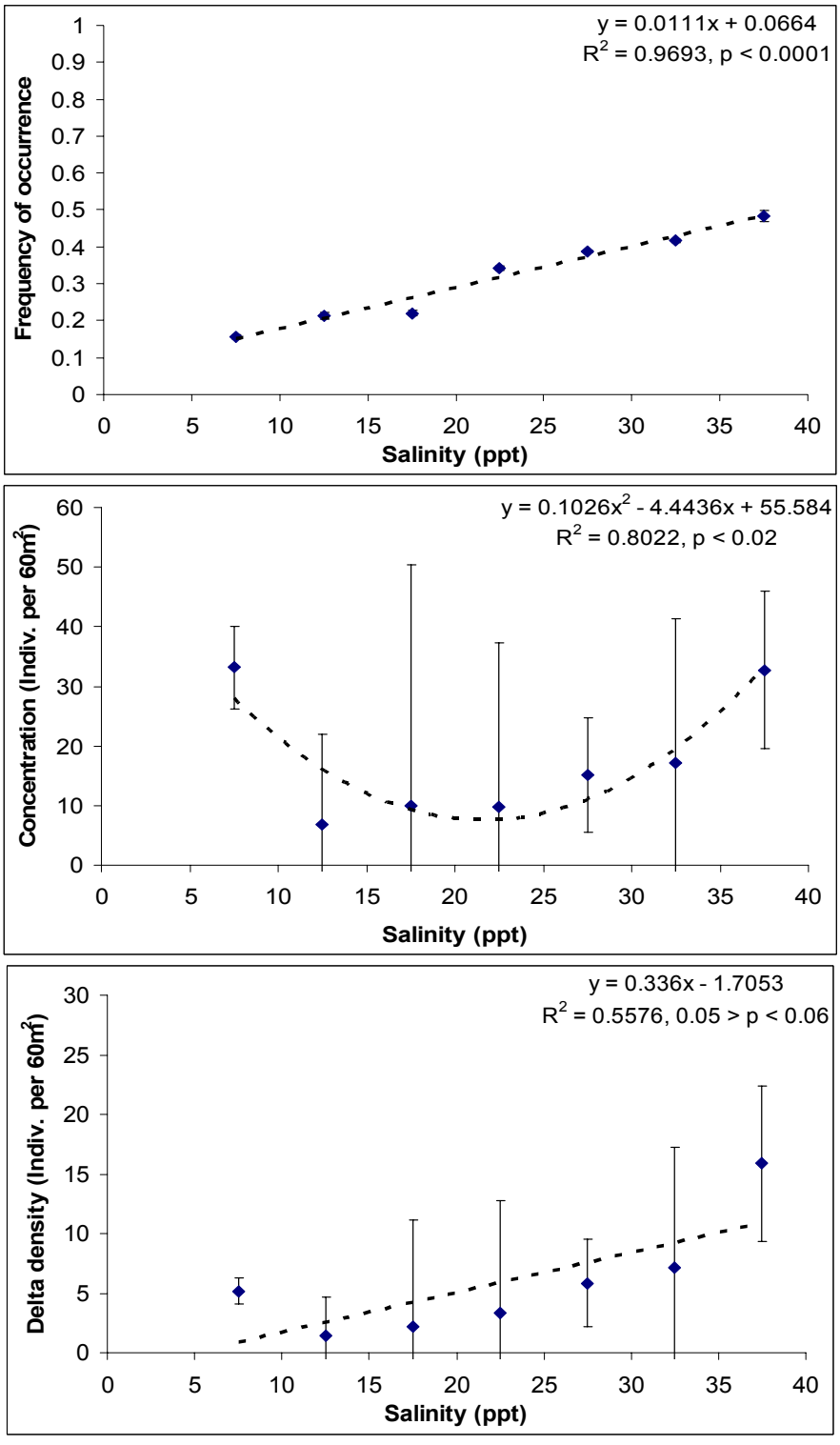

Figure 2.4 (A-C). Abundance metrics for gray snapper *Lutjanus griseus* by salinity level in Biscayne Bay ML shoreline fish surveys during the wet seasons. Regression plots are shown, along with the equation of the relationship, strength of the trendline (R^2) and p-value. Error bars are standard errors of the mean values.

CHAPTER 3. PHYSIOLOGICAL RESPONSES OF GRAY SNAPPER *LUTJANUS GRISEUS* TO CHANGES IN SALINITY

SYNOPSIS

The immediate physiological responses in plasma osmolality and blood haematocrit after abrupt changes in salinity levels were investigated in the gray snapper. Fish were challenged with six different salinity treatments, including a control (0, 5, 30, 50, 60 and 70ppt) for 192 consecutive hours and blood samples were collected at different time points. Results indicate that physiological stress to salinity changes is unlikely to occur at a salinity range of 5 to 50 ppt. At extreme salinities of 0 and 60 ppt transient significant changes in plasma osmolality and/or blood haematocrit are observed, but are corrected after an initial adjustment period of approximately 96 hours. However, at the highest salinity treatment (70 ppt) a constant osmolality cannot be maintained, resulting in death for all fish within 48 hours of exposure. Overall, these findings demonstrate the strong euryhalinity and extraordinary tolerance of this species to both extreme hypo- and hypersaline environments. Further, laboratory results were consistent with those obtained in the field, suggesting that osmoregulatory processes occurred in the same manner in both settings.

BACKGROUND

The physical properties of the aquatic environment are important to fish because they are likely to modify their bioenergetics. Both temperature and salinity have been shown to affect fish feeding and growth rates (Fry, 1971; Lankford and Targett, 1994; Gaumet et al. 1994; Buckel et al. 1995; Secor et al. 2000), metabolism (Fry, 1971; Haney and Walsh, 2003; Wuenschel et al. 2004; Wuenschel et al. 2005), activity level (Swanson, 1998), and survival (Hurst and Conover, 2002). Physiological responses to salinity are usually temperature-dependent, with greater salinity effects seen at higher temperatures (Lankford and Targett, 1994; Wuenschel et al. 2004; Wuenschel et al. 2005). Within estuaries, seasonal salinity fluctuations can be a primary factor for inducing changes in fish distribution patterns (Ley et al. 1999). In addition, estuaries are generally characterized by wide salinity fluctuations over shorter time scales that may vary with rainfall and mainland run-off as well as tidal fluctuations (Tabb and Manning, 1961). As a result, estuarine species rarely face constant salinity levels and often must cope with large fluctuations in this parameter. These changes can represent a significant stress factor depending on osmoregulatory capacity and/or behavioral response of the species in question (Serafy et al. 1997). Osmoregulation is energy consuming and relatively few species have a broad capacity to maintain constant body fluid and ion composition in both dilute and concentrated environments. Therefore, estuaries tend to be dominated by euryhaline residents, able to tolerate a wide range of salinities (Gunter, 1961; Weinstein, 1979; Day et al. 1989; Sheridan, 1992; Mullin, 1995; Ley et al. 1999). Overall, salt and water balance in euryhaline species are considered to be unaffected by

salinity changes, displaying either a transient or no apparent osmoregulatory disturbance (Eckerd and Randall, 2002).

Salinity adaptation 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). In dilute environments, fish must produce large volumes of urine to cope with the diffusive ion loss and osmotic water gain. However, in ion-concentrated environments, patterns of osmoregulation reverse and fish must constantly drink to maintain osmotic balance and to combat diffusive water loss (Marshall and Grosell, 2005). Osmoregulatory responses associated with changes in ambient salinity have received much attention for euryhaline fish species that either encounter different salinity levels in their habitat or move among habitats throughout their life history. A majority of the investigations have measured osmoregulatory abilities as well as gross growth, survival, or feeding rates. Plasma osmolality has been a focus of interest because it is an integrative measure of the ionic concentration (primarily Na^+ and Cl^-) of the blood and water balance thus serving as a physiological indicator of fish health (Denson et al. 2003). For some of the euryhaline species examined, two different phases in the osmoregulatory response following a change in salinity are usually observed: (1) a "crisis" period characterized by an increase or decrease in plasma osmolality; and (2) a regulatory phase as ions reach stable levels (Ferraris et al. 1988; Mancera et al. 1993; Varsamos, 2002; Arjona et al. 2007). This process is usually completed within 2 weeks (Ferraris et al. 1988).

Haematocrit is a measure of the ratio of blood volume that is occupied by red blood cells. It is considered a useful indicator of anemia, stress and overall health status

of fish, and also reflects changes of water content in the blood (Plaut, 1998). Haematocrit has been shown to be affected by environmental salinity (Leray et al. 1981; Brown et al. 2001; Denson et al. 2003). However, there appears to be considerable variation within and among species and little information on the dynamics of response to changes in salinity (Thompson and Withers, 1992). An increase in haematocrit was the overall response of at least 13 studied marine teleosts to hypo-osmotic stress (Woo and Wu, 1982). Likewise, a decrease in haematocrit has been showed for several freshwater teleosts after exposure to hyper-saline environments (Leray et al. 1981; Peterson, 1988; Susanto and Peterson, 1996). Reduced haematocrits after exposure to high salinities suggest cell shrinkage and dehydration (Leray et al. 1981; Susanto and Peterson, 1996) and are usually accompanied by increased haemoglobin and possible decrease in haemoglobin oxygen affinity (Lykkeboe and Weber, 1978; Woo and Wu, 1981; Jensen, 1990; Jensen et al. 2002). The reverse would be expected for increased haematocrits after exposure to low salinities.

There is a range of salinity tolerance below and above which osmoregulation fails to maintain homeostasis, resulting in death (Thompson and Withers, 1992). This range varies greatly for species and has been defined by the concentrations where a constant plasma osmolality can no longer be maintained (Martin, 1990; Foss et al. 2001). Some species spend their entire life cycle in a single habitat, where salinity can be stable or variable, whereas others migrate to different habitats, exposing their successive stages to different salinity regimes. 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). 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 have been long considered estuarine transients (Ley et al. 1999). Juvenile gray snapper have been observed in a variety of nearshore habitats with relatively low salinities (down to freshwater) including mangroves and seagrass beds, while adults are predominantly marine (Springer and Woodburn, 1960; Tabb and Manning, 1961; Thayer et al. 1987; Rutherford et al. 1989ab; Chester and Thayer, 1990; Continental Shelf Associates, 1995; Serafy et al. 2003; Wuenschel et al. 2004; Wuenschel et al. 2005). As the gray snapper migrates from fresh to seawater (and *vice versa*) throughout its life span, the change in external salinity results in physiological (osmotic) stress. The main objective of this chapter is then to assess the immediate physiological responses observed in the gray snapper as a result of abrupt changes in salinity levels as reflected in plasma osmolality and blood haematocrit. This work also aims at determining the gray snapper's upper lethal salinity. Overall, this research serves as a first step towards understanding the basis and limits of the euryhalinity of this species. The information obtained would help to answer the following questions: (1) what are the specific responses of plasma osmolality and blood haematocrit after sudden transfers from seawater to various salinities?, (2) what is the influence of size class on these responses?, (3) does plasma osmolality and/or blood haematocrit of gray snapper

abruptly transferred to different salinities eventually return to baseline or second steady state values? and if so, (4) how long does it take for these parameters to stabilize?

Additionally, blood samples from a specific number of field specimens were collected along the study area to obtain an osmoregulatory profile for the gray snapper with regards to salinity at capture. Here, the specific goal is to address the following questions: (1) how do plasma osmolalities and blood haematocrit in freshwater and marine habitats differ for gray snapper?, and (2) how do plasma osmolalities and blood haematocrit obtained in the laboratory compare to values from animals after field after capture at comparable salinities?

METHODS

Experimental animals

Subadult and adult gray snappers ranging from 13.5 to 24.5cm total length (TL) were collected at full-strength seawater (~30 ppt) from mangrove habitats in Biscayne and/or Florida Bay using hook and line during the period June through November, 2007. A collection permit was obtained from the Florida Fish and Wildlife Conservation Commission under a Special Activity License (SAL) #07SR-1015, valid from February 2, 2007 through February 1, 2009. Upon collection, gray snapper were transported to the University of Miami, Rosenstiel School of Marine and Atmospheric Science (RSMAS) and held in outdoor tanks with flowing, aerated seawater (water temperature and salinity of averaged 27.8^{0}C and 31.5 ppt) for a period of 2-3 weeks prior to experiments, then transferred to the laboratory for experiments. Feeding consisted of live juvenile pink

shrimp (*Farfantepenaeus duorarum)* provided three times a week (M, W and F schedule, ~3% body weight per feeding).

Experimental protocol

Five different salinity treatments were chosen because they reflected the widest known range reported for this species (where either juveniles, subadults or adults have been reported to occur). These 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 determine the upper lethal 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 tap water to the desired salinity. In all cases, transfer of each fish was instantaneous and transfer of all fish to various salinities was completed within 10 minutes. To avoid crowding stress, diseases 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 hours before and after transfers after which feeding was resumed according to the schedule described above. Debris was siphoned from tanks one day after feeding and a 25% water change was performed at 48, 96 and 144 hours. 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 as described below.

Sample collection from abrupt transfers

Fish where lightly anaesthetized with a 0.1g/L MS-222 (3-aminoobenzoic acid ethyl ester, Argent Labs) prior to blood sampling. One fish from each salinity treatment was sampled at 6, 24, 48, 96 and 192 hours post-treatment by caudal puncture using a 1 mL heparinized syringe fitted with a 21 gauge needle. Approximately 200-400 µL of blood was obtained from each fish, a portion of which was extracted into 75µL capillary tubes for haematocrit determination. The capillary tubes were centrifuged for 3 minutes and the volume of red blood cells was then measured as a percentage. The rest of the sample was then centrifuged at 16000 X g to separate plasma and stored at -20^0C until analysis. Plasma osmolality was measured using a Wescor Vapro 5520 vapor pressure osmometer (Wescor Inc., Logan, UT). Osmolalities were measured in water from experimental salinity treatments and were used as reference values (Table 3.1).

Sample collection in the field

Using the same approach described above, additional fish were sampled in the field within 15 minutes after being captured by hook-and-line. Salinity at each site of capture was recorded using a calibrated refractometer. This approach was used to compare plasma osmolalities and blood haematocrits obtained in the laboratory after abrupt transferences with values observed in fish in their natural environment.

Data analysis

Data are reported as means ± Standard Error Mean (SEM). All examined data showed normal distribution and the significance of differences between salinities was determined using a one-way ANOVA, with salinity as the main factor. When statistical

significance was revealed (i.e., $P<0.05$), a Dunnet's post hoc comparison test was used for multiple comparisons.

RESULTS

Abrupt transfers

No mortalities occurred in fish from salinity treatments ranging from 0 to 60 ppt. However, all fish exposed to 70 ppt died within 48 hrs after transfer. Time course changes in plasma osmolality according to experimental salinity are displayed in Figure 3.1. Teleosts normally maintain blood osmolalities within the range of ~260 to 400 mOsm/L, tightly regulated in a species-dependent range of salinities (Jobling, 1995; Varsamos et al. 2005). In this study, mean osmolalities ranged from 269 to 475 mOsm/L for fish transferred to salinities from 0 to 60 ppt. Fish exposed to 70 ppt, however, displayed a mean plasma osmolality that ranged from 437 to 561 mOsm/L, before all fish died. In control fish (30 ppt), osmolality was maintained at 367 (±1.32) mOsm/L (N=35). During transfers to either 5 or 50 ppt, plasma osmolality was maintained at levels very similar to controls throughout the duration of the experiment [349±0.50mOsm/L (N=28) and 388±2.30 mOsm/L (N=26), respectively] and no significant change was observed. Transfers to 0 ppt however, significantly decreased plasma osmolality to 310±7.53 mOsm/L (N=7) at 6hr post-transfer, a value that kept decreasing at 24hr to 269±12.75 mOsm/L (N=7), then started to increase at 48 hr to 271±11.46 mOsm/L (N=5), and became no longer different than the controls at 192 hr with a value of 359±13.73mOsm/L (N=5). On the other hand, transfers to 60 ppt significantly increased plasma osmolality to 445±13.39mOsm/L (N=5) at 24hr post-transfer, a value that increased at 48hr to

475±5.45mOsm/L (N=5), decreased at 96hr to 436±10.09mOsm/L (N=6), and was no longer different than the controls at 192 hr [439±7.71mOsm/L (N=5)], despite water osmolality being around 1850 mOsm/L (Table 3.1). In addition, during the initial 96 hr post-transfer from seawater to both 0 and 60 ppt salinities, fish tended to swim much less, decrease feeding rates, and increase ventilation rates. For fish at 60 ppt surface breathing also seem to occur. Finally, fish transferred to 70 ppt significantly increased plasma osmolality to 437±15.64 (N=5) at 6 hrs post-transfer, a value that increased at 24hr to 561±41.29 (N=5) and resulted in death before 48 hrs. These fish stopped feeding completely, highly increased both ventilation rates and surface breathing and around 24 hrs started displaying erratic rapid swimming movements.

Time course changes in blood haematocrit according to experimental salinity are displayed in Figure 3.2. Normal ranges for blood haematocrit in many species fall between 32-43%. In this study, mean haematocrit measurements ranged from 31 to 43% for fish transferred to salinities from 0 to 60 ppt. Fish exposed to 70 ppt, however, displayed a lower mean blood haematocrit that ranged from 27 to 35%. In control fish (30 ppt), haematocrit was maintained at 35±1.32% (N=35). In transfers to 5, 50 or 60 ppt, blood haematocrit was maintained at levels very similar to controls throughout the duration of the experiment [35±0.76% (N=35); 37±1.20% (N=28); and 35±0.88% (N=27), respectively] and no significant change was observed. Although transfers to 0 ppt significantly increased blood haematocrit to 43±4.1% (N=6) at 6hr post-transfer, values quickly returned to control levels within 24hr [41±1.53% (N=6)]. The opposite occurred in transfers to 70 ppt, which significantly reduced blood haematocrit to

27±4.5% (N=5) at 6hr post-transfer, then values quickly returned to control levels within 24hr [35±1.16% (N=5)], although fish died within 48 hrs.

Field collections

Overall, 98 gray snapper were collected in the field and sampled in a range of salinities from 0 to 38 ppt at many locations within Biscayne and Florida Bays. A salinity binning approach was used to plot results, dividing these in salinity intervals of 5ppt each (see Serafy and Valle 2006). Our goal was to compare osmoregulatory profiles of fish from fresh- and marine habitats. Therefore, no data points are shown for mid-range salinities from 16-30 ppt.

Plasma osmolalities at capture according to salinity measured in the field are displayed in Figure 3.3. Fish collected in the range of salinities from 31-35 ppt were considered to be the "control" group, because these salinities most resembled the salinity used as control in the abrupt transfer experiments. Fish displayed plasma osmolality values not significantly different from those observed in the laboratory at these salinities [411±4.96mOsm/L (N=29)]. As expected, fish collected at salinities ranging from 0 to 5ppt displayed a plasma osmolality significantly different from the "control" group and were quite variable (279±21.92 mOsm/L (N=11), possibly indicating high variation in the "salinity histories" of the fish captured. Fish collected at salinities from 6-10 ppt displayed a plasma osmolality higher than fish collected at salinities from 0 to 5ppt and still significantly lower than the "control" group [354±29.70mOsm/L (N=6)]. On the other hand, plasma osmolalities of fish collected at salinity intervals from 11 to 15ppt [422±10.48mOsm/L (N=10)] and 36-40 ppt [391±4.89mOsm/L (N=42)] were not significantly different from the "control" group.

Mean blood haematocrit values at capture averaged according to salinity measured in the field are displayed in Figure 3.4. In this particular case, no data are shown for the salinity interval 6-10 ppt due to a lack of sample availability. Fish collected in the range of salinities from 31-35ppt were again considered to be the "control" group. These fish displayed haematocrit values that were not significantly different to laboratory measurements at the corresponding salinities [37±1.25% (N=26)]. Overall, no significant changes from the "control" group were observed in blood haematocrits of fish collected at any of the capture salinity intervals used [34±3.18% (N=9) for 0-5ppt; 30±2.32% (N=8) for 11-15 ppt; and 37±1.73% (N=36) for 36-40 ppt].

DISCUSSION

In the present study, gray snapper were subjected to different salinity level changes in a laboratory environment. The main goal was to monitor and assess physiological responses as a first step towards understanding the basis and limits of their ability to adjust to fluctuating salinities. Results demonstrate the strong euryhalinity and extraordinary tolerance to both extreme hypo- and hypersaline environments of both subadult and adult gray snappers. Results also indicate the salinity level at which osmoregulation seems to fail for this species (upper lethal limit). In general, laboratory results were consistent with those obtained in the field, suggesting that osmoregulatory processes occurred in the same manner in both settings. These findings support the notion that larger size classes may be equipped with the same efficient osmoregulatory capabilities that juveniles possess as suggested in the field-based literature. Furthermore, gray snapper have shown remarkable osmoregulatory capacities far outweighing other

resident species in South Florida (e.g., rainwater killifish), perhaps serving as a model for the study of osmoregulation in an exploited tropical marine teleost. Studying the precise mechanisms for the successful osmoregulation of this species is warranted.

Abrupt transfers

After sudden transfers from seawater to various salinities, one of two different physiological responses was expected for an estuary-inhabiting fish such as the gray snapper: a transient or a no apparent osmoregulatory disturbance. Also, plasma osmolality was expected to increase with salinity level and blood haematocrit was expected to increase with decreasing salinity. For successful acclimation to the new salinity level, both parameters were expected to return to baseline values within the experimental time frame. The basic assumption made was that gray snapper was fully adapted when plasma osmolality and blood haematocrit were no longer different from the control group (30 ppt), or reached a constant steady state level. Our results indicate that significant physiological stress to salinity changes is unlikely to occur at a salinity range of 5 to 50 ppt. At extreme salinities of 0 and 60 ppt transient changes in plasma osmolality and/or blood haematocrit are observed, but are corrected after an initial adjustment period of approximately 96 hours. Moreover, both plasma osmolality and blood haematocrit showed no significant differences from control values at 192 hours post-transfer, suggesting a successful adaptation to these new salinity levels despite the large changes in environmental salinity. Only after abrupt transfers to 70 ppt fish lost completely their ability to maintain a constant plasma osmolality, but this was expected, given that this species has never been observed or reported at salinities larger than 66.6ppt.

Limited data exist on the effect of size class on the physiological responses to abrupt salinity changes. Gray snapper has been viewed in the literature as a euryhaline fish with osmoregulatory capacities that change with life-history. Specifically, juveniles are the most capable of tolerating the wide salinity fluctuations that occur to varying degree in Biscayne and Florida Bays. Larger gray snappers were then expected to show less tolerance to the extreme salinities assessed. However, subadult and adult gray snapper responses did not sort by size, as the range of sizes varied greatly in every treatment (average length in each ranged from 14.1cm to 23cm). In addition, a minimum of 25 fish were sampled per treatment (n=5 per sampling period). Hence, fish size did not seem to affect the euryhaline capacity of this species. Our observations seem to challenge Yokel's (1966) contention that young individuals tend to be more tolerant of low salinities, and adults tend to be more tolerant of high salinities. Furthermore, in the few studies that have examined the effect of size in euryhalinity, size appears to have a different effect depending upon the species being studied (Ferraris et al. 1988; Jensen et al. 1998).

The strong euryhalinity of the gray snapper can be compared with the euryhaline European sea bass *Dicentrarchus labrax*. Jensen et al. (1998) showed that this particular species can tolerate abrupt salinity changes and osmoregulate well over the same wide range of salinities used in our study (freshwater to 60 ppt). Yet, 60 ppt seemed to be near the upper tolerance level when directly transferred from 15 ppt. When transferred from seawater instead, this species showed great tolerance to salinities up to 70 ppt as shown by Varsamos (2002). The upper salinity tolerance was estimated at 90 ppt, which increased the osmolality to 640 mOsm/L and resulted in the death of all fish within 3hrs

(Varsamos, 2002). The study on the European sea bass suggested that salinity tolerance is related to ontogenetic stage and the gradual acquisition and progressive development of the osmoregulatory capacities/mechanisms. Additionally, the recovery time appeared to be related to the amplitude of the salinity variation, (osmolality in sea bass transferred to 50 ppt was restored 48 hr post-transfer, whereas osmolality in fish transferred to 70 ppt was restored 96 hr post-transfer). In our study, recoveries from extreme salinity treatments (0 and 60 ppt) occurred after 96 hours post-transfer. In addition, the upper salinity tolerance was estimated at 70 ppt for gray snapper, which increased the mean osmolality to 561 mOsm/L and resulted in the death of all fish within 48 hrs.

Overall, similar physiological adaptive responses in blood haematocrit and plasma osmolality have been reported for other marine teleosts after sudden transfers to different salinity treatments, in particular from fresh (FW) or brackish (isosmotic) water (BW) to seawater (SW) and *vice versa*. Some of these species include: red grouper *Epinephelus akaara* and black sea bream *Mylio macrocephalus* (Woo and Wu, 1982), *Fundulus heteroclitus* (Jacob and Taylor, 1983), juvenile red drum *Sciaenops ocellatus* (Crocker et al. 1983), milkfish *Chanos chanos* (Ferraris et al. 1988), flounders *Platichthys flesus* and *Paralichthys orbignyan* (Nonnotte and Truchot, 1990; Jensen et al. 2002; Sampaio and Bianchini 2002) and gilthead sea bream *Sparus aurata* (Mancera et al. 1993; Tort et al. 1994). On the other hand, some species have been shown to be unable to cope with extreme sudden changes in salinity. For example, Serafy et al. (1997) exposed 10 common fish from Biscayne Bay (including gray snapper) from SW to FW and quantified mortality elicited in these species after 24 hr. Pinfish *Lagodon Rhomboides*, rainwater killifish *Lucania parva*, spotted seatrout *Cynoscion nebulosus* and white grunt

Haemulon plumieri all exhibited mortality rates that ranged from 12.5 to 100% (silver jenny *Eucinostomus gula*, bluestriped grunt *Haemulon sciurus*, gulf toadfish *Opsanus beta*, gray snapper *Lutjanus griseus* and sailors choice *Haemulon parra* appeared highly tolerant with 0% mortality).

Field collections

The objective of collecting gray snapper blood samples directly in the field was to assess differences between plasma osmolalities of individuals in fresh water and marine habitats and compare results with those obtained in the laboratory after abrupt transfers. Fish collected in freshwater and low salinity habitats ranging from 0 to 10 ppt displayed a plasma osmolality significantly different from fish collected in habitats ranging 11-40 ppt. Lower variation in osmolality values with increasing salinities is consistent with the fact that these are areas with minimum expected fluctuation in salinity levels (e.g., reefs). Furthermore, fish osmolalities measured in the field were very similar to those measured in the laboratory after abrupt transfer. Significant differences in plasma osmolality were expected for fish collected at salinities from 0 to 5ppt. No significant differences were expected for field-collected fish at 6 to 10 ppt, given that plasma osmolality of fish abruptly transferred to 5 ppt in the laboratory was maintained at values very similar to control fish in SW. This issue may be explained by considering the following factors: (1) fish collected at salinities ranging from 0-10 ppt in the field were all from riverine or freshwater canal-dominated areas, whereas fish collected at higher salinities were from marine lagoons or offshore; (2) osmolalities from these fish may reflect differences in the time each one spent at the particular salinity of capture (indicative of migration among habitats); and (3) potential osmoregulatory dysfunction followed by physical stress after

capture and handling/sampling. Osmolality has been shown to be affected in different species after handling or transporting (Redding and Schreck, 1983; Denson et al. 2003). This finding may explain why most osmoregulatory studies have an acclimation period after fish capture and/or rearing. In the case of blood haematocrit, even though increased values for fish collected at salinities from 0 to 5ppt were expected (as observed in the laboratory), significant differences were not uncovered among these fish or any of the other fish captured at other salinities, perhaps indicating efficient prevention of cell dilution or dehydration.

Ecological implications

The success of many euryhaline species like the gray snapper that enter estuaries and freshwater habitats as juveniles may depend on the degree of tissue resistance to changes in body fluid concentrations (Crocker et al. 1983; Jensen et al. 1998) and the species-specific capacity to osmoregulate (Serafy et al. 1997). The ecological performance of these species ultimately depends on the physiological suitability of the habitat (Huey, 1991; Serafy et al. 1997). In South Florida, alteration of freshwater flow has changed the salinity natural patterns 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 CERP. Gray snapper and other species that are subjected to pulses of freshwater flow can either remain in this area if physiologically capable, or leave and risk predation and/or food scarcity while seeking a better habitat (Serafy et al. 1997). The present study suggests that even though freshwater pulses may represent a significant source of osmoregulatory stress to the gray snapper, it in itself will not lead to death. Therefore, it is proposed that gray snapper faced with a

freshwater pulse in its natural habitat will probably remain in this area, rather than risk predation or food scarcity. Furthermore, adults could benefit from feeding in near-shore habitats with abundant food supply and relative scarcity of larger predators physiologically capable of tolerating such low salinities.

Salinity (ppt)	*Water osmolality (mOsm/L)*
0	25
5	143
Control (30)	934
50	1522
60	1857
70	2150

Table 3.1. Osmolalities measured in water from different experimental salinities in laboratory experiments and used as reference values.

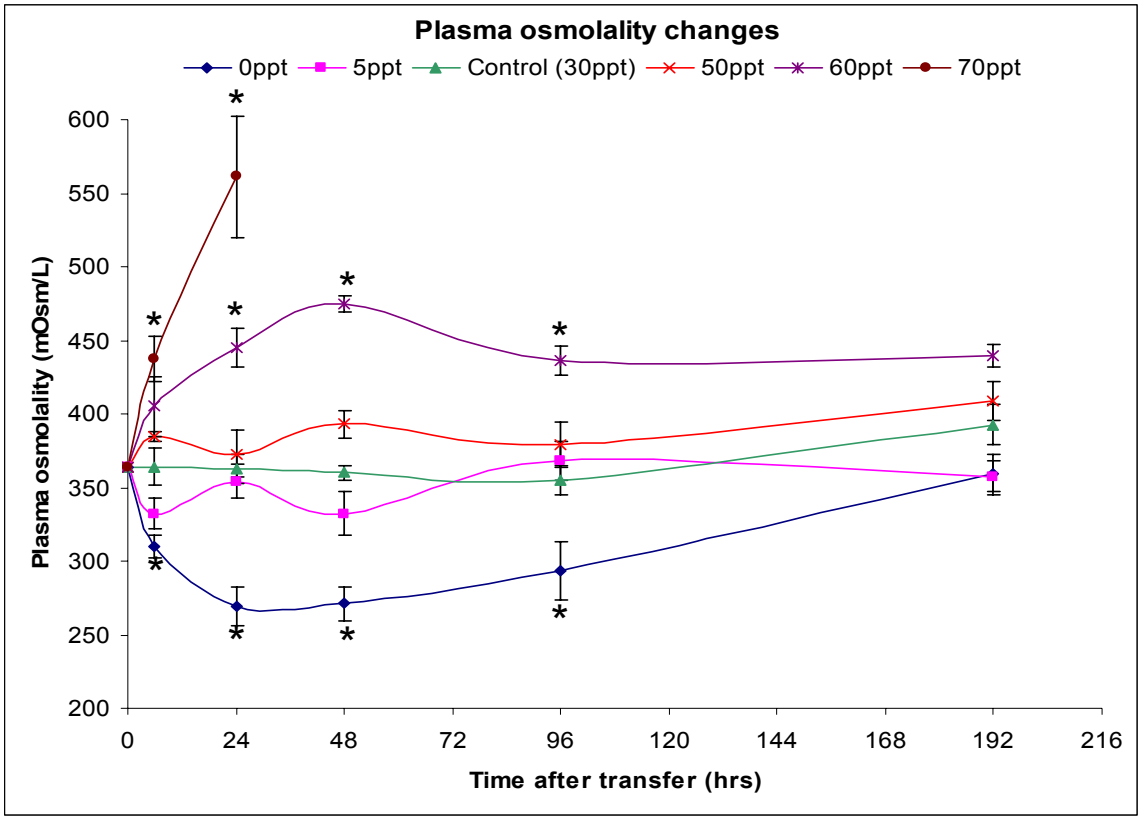

Figure 3.1. Changes in plasma osmolality for gray snapper *Lutjanus griseus* following abrupt transfers to different experimental salinities. All fish exposed to 70 ppt died after 24 hrs post-transfer. Asterisks correspond to significant statistical differences with respect to controls ($P<0.05$; Analysis of variance and Dunnet's post hoc comparison test).

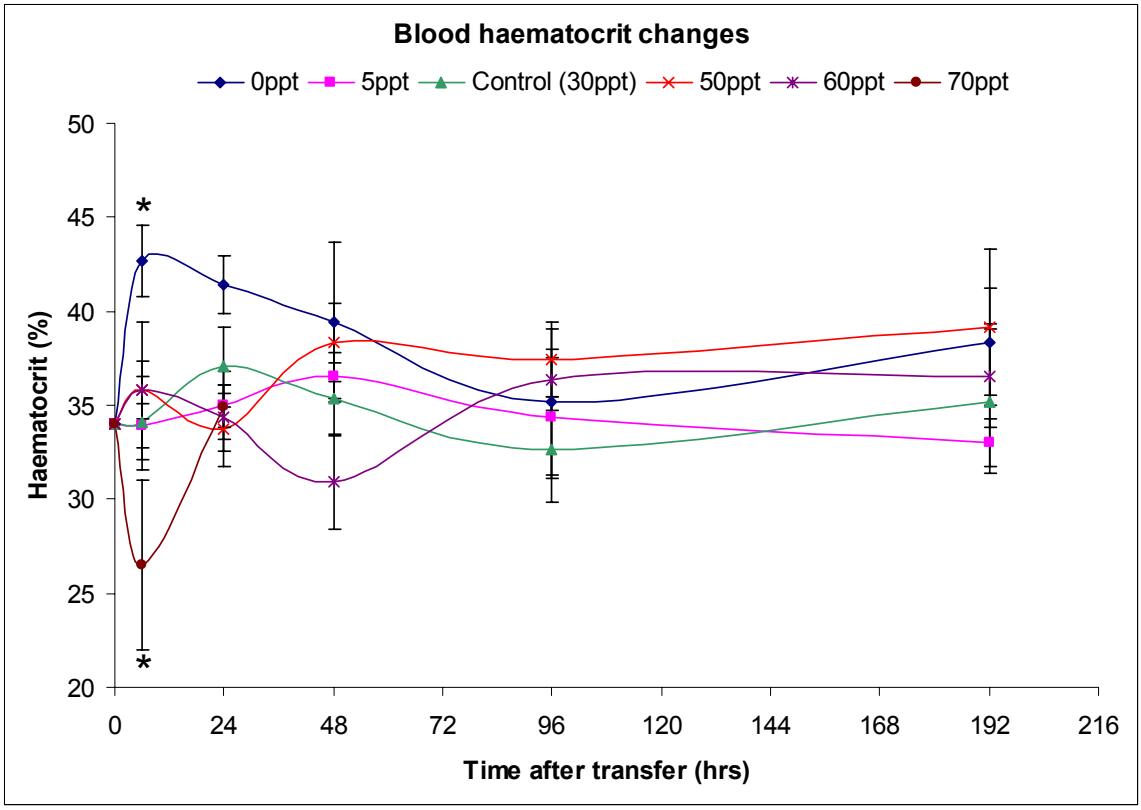

Figure 3.2. Changes in blood haematocrit for the gray snapper *Lutjanus griseus* following abrupt transfers to different experimental salinities. All fish exposed to 70 ppt died after 24 hrs post-transfer. Asterisks correspond to significant statistical differences with respect to controls ($P<0.05$; Analysis of variance and Dunnet's post hoc comparison test).

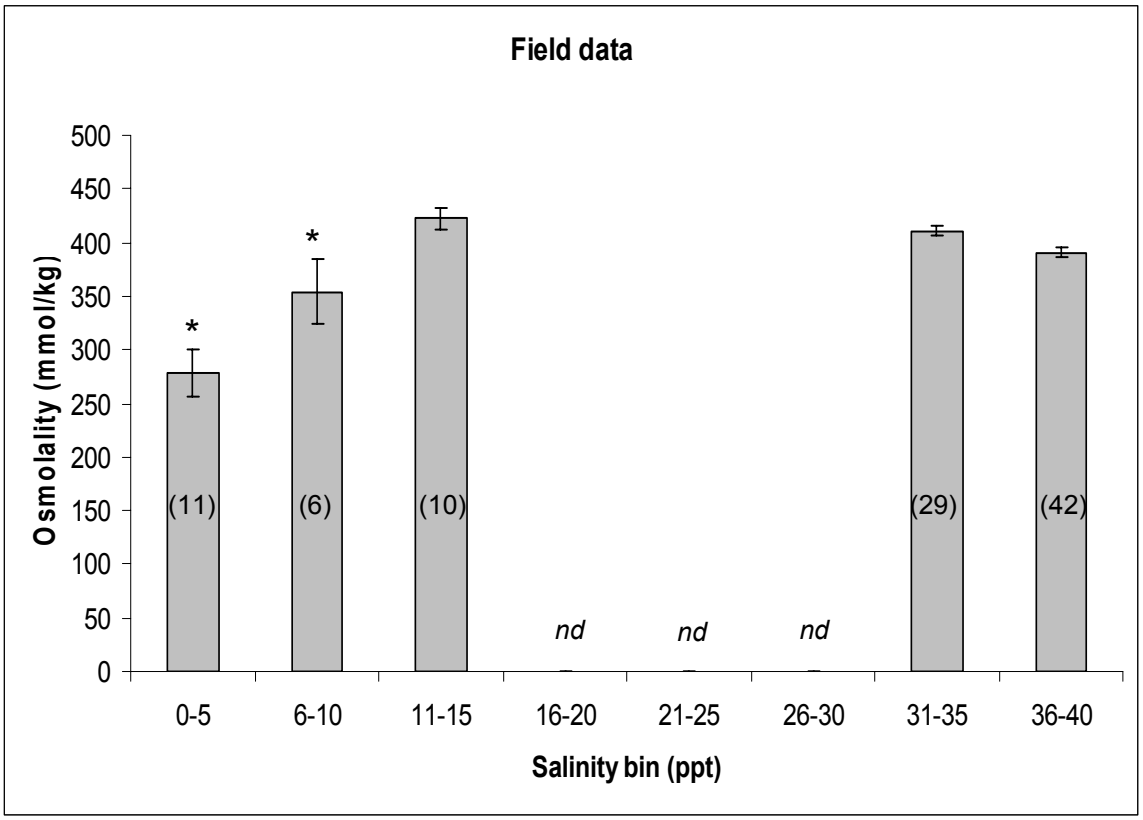

Figure 3.3. Plasma osmolalities for gray snapper *Lutjanus griseus* after capture in the field. No data (*nd*) are shown for salinities ranging from 16-30 ppt. Numbers in parenthesis represent the amount of fish sampled at each salinity bin. Asterisks correspond to significant statistical differences with respect to the "control group", defined as salinities from 31-35 ppt ($P<0.05$; Analysis of variance and Dunnet's post hoc comparison test).

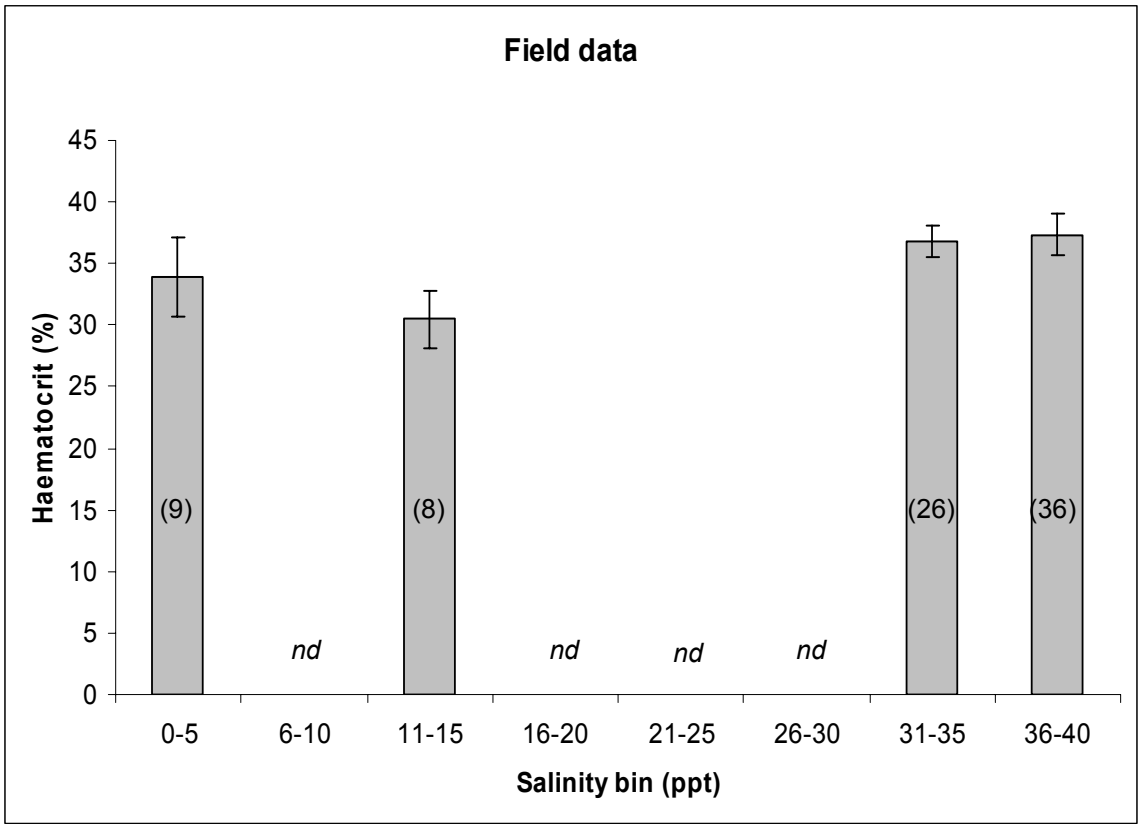

Figure 3.4. Blood haematocrit of gray snapper *Lutjanus griseus* after capture in the field. No data (*nd*) are shown for salinities in the range of 6-10 ppt and 16-30 ppt. Numbers in parenthesis represent the amount of fish sampled at each salinity bin. Asterisks correspond to significant statistical differences with respect to the "control group", defined as salinities from 31-35ppt ($P<0.05$; Analysis of variance and Dunnet's post hoc comparison test).

CHAPTER 4. BEHAVIORAL SALINITY PREFERENCE OF GRAY SNAPPER *LUTJANUS GRISEUS* AND EFFECTS ON SWIMMING BEHAVIOR

SYNOPSIS

The salinity preference and effects on swimming behavior of the gray snapper were investigated in an automated salinity choice shuttlebox via 48-hr trials. Subadult and adult gray snapper of sizes ranging from 18-23 cm (overall mean of 19.8 ± 0.49 cm) were collected and acclimated at full-strength seawater (~30 ppt) for a period of 2 weeks before being tested using this system. Results indicate that the 11 gray snapper tested displayed either one of two distinctively different salinity preferences. Half of gray snappers displayed a salinity preference in the range of 9-15 ppt with an overall mean preferred salinity of 12.9 ± 0.99 ppt (N=5), whereas the other half displayed a salinity preference in the range of 19-23 ppt with an overall mean preferred salinity of 21.3 ± 0.53 ppt (N=5). Recorded swimming speeds in all fish tested reflected a significant but weak negative linear relationship with salinity during both time periods of the day (light and dark); however, gray snapper were usually most active during the dark period across all salinities. Overall, our findings reveal that gray snapper prefer slightly hyperosmotic salinities that may minimize the physiological costs of osmoregulation compared to extreme salinities. Thus, reduced swimming speeds observed at high salinities could be the result of compensation for higher osmoregulatory costs.

BACKGROUND

For fishes, physiological adaptation is perhaps the primary strategy for coping with environmental variation, allowing them to successfully occupy diverse habitats and/or tolerate wide fluctuations (Neill and Magnuson, 1974). However, fishes are not randomly distributed within habitats, tending to be concentrated in some areas and scarce or absent in others (Neill and Magnuson, 1974). Fish are mobile organisms potentially capable of regulating the conditions they experience by spending more time in particular environments (Neill and Magnuson, 1974). Experiments investigating the behavioral regulation (or environmental preference) have normally been conducted in three types of chambers: those producing a horizontal or vertical gradient (Javaid and Anderson 1967; Hesthagen, 1979; Garside et al. 1977; Kwain and McCauley, 1978; Kellog and Gift 1983) and electronic shuttleboxes that produce a horizontal gradient controlled by the spatial movements of the organism (McCauley et al. 1977; Reynolds and Casterlin, 1979; Neill et al. 1972; Schurmann et al. 1991). Most studies have focused on the behavioral thermoregulation or temperature preference (Neill et al. 1972; Neill and Magnuson, 1974; Garside and Morrison, 1977; McCauley and Huggins, 1979; Hesthagen, 1979; Reynolds and Casterlin, 1979; Schurmann et al. 1991; Stauffer et al. 1984, Stauffer et al. 1985; Stauffer, 1986; Stauffer and Boltz, 1994; Myrick et al. 2004). These studies have assumed that when presented a choice of ambient temperature (as in a thermal gradient), organisms would tend to congregate or spend the most time within a relatively narrow temperature range (Reynolds and Casterlin, 1979). Usually, this “preferred” zone consists of a range of temperatures bounded by upper and lower “avoidance” temperatures, therefore being characterized by some measure of central tendency (i.e., mode, median),

dispersion (i.e., range, standard deviation) and skewness (Pitt et al. 1956; DeWitt, 1967; Reynolds and Casterlin, 1976; Reynolds, 1977; Reynolds and Casterlin, 1979; Schurmann et al. 1991). For example, Schurmann et al. (1991) examined the preferred temperature of rainbow trout (*Oncorhynchus mykiss)* using an electronic shuttlebox very similar to the one employed in the present study, which allowed fish to regulate the temperature in experimental tanks with their spatial movements. The authors proposed that fish would initially select an "acute" preferred temperature, dependent upon the acclimation temperature; however, after 24 hours, fish would select a species-specific "final" preferred temperature that was independent of acclimation history, and thus independent of seasonal changes in ambient temperature (Schurmann et al. 1991).

Salinity preference has been studied in many invertebrate species and several fishes (McInerney, 1964; Fivizzani and Meier, 1977; Iwata et al. 1990; McGaw and Naylor, 1992; Damgaard and Davenport; 1994; Lankford and Targett, 1994; Bell and Brown, 1995; Chung, 2001; Parkyn et al. 2002; Edeline et al. 2005; Webster and Dill, 2007), but not, as yet, in species like the gray snapper, which spawn in the vicinity of coral reefs. Experiments assume that when organisms are offered with a continuous salinity gradient, some salinities will be more frequented, and others will be avoided. When ambient salinities differ from the internal concentration of fish, this incurs an energetic regulatory cost for osmoregulation (Swanson, 1998). The assumption made is that salinities with osmolalities near the osmolality of the fish's blood (isosmotic) minimize the physiological cost of osmoregulation, allowing more energy for other processes, such as growth and reproduction (Lankford and Targett 1994; Cardona 2000; Hurst and Conover, 2002). Further, many euryhaline teleosts have been reported to have

minimal metabolism costs at isosmotic salinities (Farmer and Beamish, 1969; Rao, 1971; Potts et al. 1973; Frame, 1973; Woo and Wu, 1982; Furspan et al. 1984; Febry and Lutz, 1987; Morgan and Iwama, 1991; Nordlie et al. 1991; Swanson, 1998). We hypothesized that given a choice of salinities gray snappers would tend to select salinities that would minimize osmoregulatory cost thus providing optimal conditions for growth, aerobic metabolism and/or locomotion.

Although the energetics of fishes has been addressed in some freshwater and marine species, little is known about the physiological cost (energetic response) of habitat selection by coral reef fishes, such as the gray snapper, that use different nearshore and estuarine habitats as juveniles (Jones and McCormick, 2002; Wuenschel et al. 2005). Previous studies on fish species that migrate through water of different salinities (e.g., sea bass *Dicentrarchus labrax*) have shown a direct link between their ability to perform aerobic exercise and their capacity to osmoregulate (Brauner et al. 1992; Brauner et al. 1994; McKenzie et al. 2001ab; Chatelier et al. 2005). Salinity has also been found to influence the maximum sustained or critical swimming speed (U_{crit}) of euryhaline fish. However, the response appears to vary by species (Kolok and Sharkey, 1997). Overall, the general expectation has been that a reduced metabolic scope in less than optimal conditions would result in decreased swimming performance (Fry, 1947; Wakeman and Wohlschlag, 1977).

Over their life span, gray snapper migrate and forage among waters of distinctly different salinities (Springer and Woodburn, 1960; Tabb and Manning, 1961; Thayer et al. 1987; Rutherford et al. 1989ab; Chester and Thayer, 1990; Continental Shelf Associates, 1995; Serafy et al. 2003; Wuenschel et al. 2004; Wuenschel et al. 2005). In a

previous study (Serrano et al., unpublished data), we demonstrated the strong euryhalinity and extraordinary tolerance of this species to both extreme hypo- and hypersaline challenges. The main goal of the present study was to address the following questions: (1) do gray snapper have a particular salinity preference given a choice of salinities in a salinity gradient?, if so, (2) is there a physiological basis for this salinity preference?, and (3) what are the effects of salinity on swimming behavior?

METHODS

Experimental animals

Subadult and adult gray snapper of sizes ranging from 18-23 cm (overall mean of 19.8 ± 0.49 cm) were collected, transported, fed and held at full-strength seawater (~30 ppt) in outdoor tanks for a period of 2-3 weeks prior to experiments as described previously (Serrano et al., unpublished data). Fish selected for behavioral experiments were starved for 48 hours and transported to the laboratory housing the shuttlebox system.

Equipment

An electronic shuttlebox system developed in collaboration with Loligo Systems (Denmark) was employed for the automated testing of salinity preference for gray snapper (Figure 4.1). This system was a modification of the system described by Schurmann et al. (1991) used for the automatic determination of temperature preference of the Atlantic cod (*Gadus morhua*). It consists of 2 tanks 72 cm in diameter each, connected by a 20 cm clear tube with an approximate diameter of 14 cm. The water depth in the chambers is approximately 52 cm. The system includes 2 recirculation pumps, 2

shunt pumps, 2 dosage pumps (for fresh- and seawater inflow), 4 infrared floodlights, an infrared sensitive video camera, 2 conductivity meters, an instrument to control the activity of the three sets of pumps (RELAY 3) and a computer with a video software and a program for data acquisition/pump control. The video camera mounted over the shuttlebox records the position of the fish continuously (based on a principle of contrast) and exports X and Y coordinates to the computer at a 1 Hz frequency (LoliTRACK Lite version 1.1, Loligo Systems, Denmark), which then controls the activity of the three sets of pumps (via LABTECH NOTEBOOK software), depending on the position of the fish and the difference in salinity between the two tanks (see Figure 4.1, upper panel). Passage of the fish into the higher salinity tank prompts dosage pumps to increase the salinity in both tanks continuously; the reverse happens if the fish swims into the low salinity tank. When the fish is stationary in the connecting tube, all pumps turn off and salinity is maintained at a constant value until the fish moves again into one of the experimental tanks (see Figure 4.2, bottom panel). Finally, the salinity difference between tanks was always kept at 5 ppt regardless of overall salinity in the system, creating a smooth salinity gradient in the connecting tube.

Our system is unique in that it allows individual gray snapper to regulate ambient salinities by moving between tanks through the connecting tube. The fish's swimming direction controls the salinity change, whereas the swimming speed controls the rate of the salinity change in experimental tanks. The system obviates the need for manual maintenance of salinity gradients in large volumes of water, data is collected continuously and automatically even in dark conditions, and its output form is appropriate for direct processing using a computer statistical package.

Initial troubleshooting

First, the system was originally constructed with an opaque connecting tube. Experimental fish tended to "park themselves" inside this darkened tube hidden from the camera for the duration of the experiment. Consequently, without camera-tracking, no data was generated. This behavior is consistent with the natural behavior of gray snapper, which commonly shelter in the shaded interstices of mangrove prop-roots (Starck, 1970). Second, the light generated from the infrared floodlights was too strong, preventing a distinctive contrast between the tanks and the fish being tested while tracking at night. Therefore, we attached two small pieces of diffusive light panels to the top of the infrared lights in order to create a uniform tank background.

Experimental protocol

After the acclimation period in holding tanks, individual gray snapper were introduced into the shuttlebox containing air-saturated water with similar conditions to acclimation tanks (average temperature and salinity of 27.8^{0}C and 31.5 ppt). The approximate 16h:8h light/dark cycle of the outdoor holding tanks was also maintained throughout the duration of the experiment. Next, each fish was allowed to regulate the salinity in the electronic shuttlebox for the duration of 48 hours. After the experiment, total length of fish was measured.

DATA ANALYSES

Data sampling

Gray snappers in the shuttlebox were usually most active during the dark period, which is consistent with their natural behavior (Starck and Davis, 1966; Starck, 1970).

Therefore, data from this period were collected and used for the analysis of salinity preference.

Data handling

Following the rationale of Schurmann et al. (1991), the mean of the median preferred salinities was used as a measure of the final salinity preference. In our study, the mode and range were also calculated to provide a complete analysis. In addition, routine swimming speeds were calculated by measuring the distance traveled by the fish every second (in cm) while being tested in the shuttlebox system and then using the formula shown below.

Statistics

The medians of salinities from both high and low saline tanks were generated at intervals of 10 minutes each during the 48 hrs of individual experiments. Then, the salinity preference was obtained for every fish, along with the mode and the occupied range of salinities. Differences in mean salinity preferences were tested with a one-way ANOVA and a Student's t post hoc comparison test was used if significance was revealed. Swimming speeds (*expressed as body lengths per second*) were calculated using the following equation: Speed= (SQRT [{($Xpos_1$-$Xpos_2$)^2}+{($Ypos_1$-$Ypos_2$)^2}])/1. Then, swimming speeds of fish were averaged by salinity and regressed against a salinity gradient (ranging from 0 to 33 ppt) by light and dark periods. Significant differences between the regressions were then assessed using an ANCOVA. For all our analysis, statistical significance level was considered at the 95%.

RESULTS

Salinity preference

Most fish "learned" to swim through the connecting tube within 3 hours. Two fish out of 13 apparently did not "learn" how to use the system and thus were omitted from data analysis. Thus, the results of 11 fish tested for salinity preferences are shown in Table 4.1. From these 11 fish, roughly half (N=5) displayed a salinity preference in the range of 9-15 ppt, whereas the other half (N=5) displayed a salinity preference in the range of 19-23 ppt. Curiously, we were unable to obtain a matching preference with the salinity distributions for one of the fish (#6) to allow its proper grouping into either preference group. Therefore, we decided to omit this fish's results from the rest of the preference analysis. Overall, for most fish the mode tended to correlate well with actual preferences, while the occupied salinity range seemed to be quite different and variable for fish tested.

In general, gray snapper tested exhibited either one of two distinctively different salinity preferences. A time course of the mean salinity preferences for all fish during a 48-hr period is shown in Figure 4.2. Results from the one-way ANOVA showed that the means of these salinity preferences [21.3 ± 0.53 (N=5) for the high preference and 12.9 ± 0.99 (N=5) for the low preference] are significantly different from each other. In addition, salinity fluctuations seemed to be less variable during the dark periods than those during the light periods (especially during the first 24 hrs). Gray snapper's overall salinity distributions by percent of time spent at each is shown in Figure 4.3. An example of the salinity distributions for two individual fish showing a low (upper panel) or a high preference (bottom panel) is given in Figure 4.4.

Swimming speeds

The effect of salinity on the swimming behavior of gray snapper was measured by comparing the regressions of routine swimming speeds (*in body lengths/s*) between time periods (light *vs.* dark) by salinity level. Figure 4.5 shows the scatter plot of the mean swimming speeds recorded under dark and light conditions for all fish by salinity, in an interval ranging from 0 to 33 ppt. The typical nocturnal behavior of gray snapper was observed in the system as fish were usually most active during the dark period across all salinities. Further, results from the ANCOVA showed that while the slopes of both regressions (light and dark) against salinity level were similar, the Y-intercepts were statistically different. In addition, swimming speeds reflected a significant, but weak negative linear relationship with salinity for both time periods (R^2=0.36 and R^2=0.41, for light and dark periods, respectively).

DISCUSSION

Salinity preference

Even though it can be argued that the mode best fits the definition of final preference because it represents the salinity most occupied by the fish (Fry, 1947), the mode may be altered if the salinity distribution shows a broad distribution (Schurmann et al. 1991). Since the distribution of preferred salinities in our study tended to be sometimes very broad and/or skewed, the median of the salinity values was used as a measure of the final salinity preference following Schurmann et al. (1991) rationale. An advantage of using this parameter is that it tends to be less sensitive to extreme scores than the mean and a better measure for highly skewed distributions than the mode.

Our study appears to be the first to investigate the salinity preference of the gray snapper, which presented two distinctively different salinity preferences either in the range of 9-15 ppt or 19-23 ppt. That half of the gray snapper tested displayed a "low" preference around 12 ppt was consistent with our expectations, as these fish selected isosmotic salinities that would require less energy for osmoregulation. It is uncertain why the remainder of the fish displayed for preference for ~21 ppt, rather than 12 ppt, although this group clearly avoided the salinity extremes, in particular those salinities <10 ppt. Further, only the fish that preferred the lowest salinity recorded (fish #10), exposed itself to salinities lower than 5 ppt for a certain amount of time. The rest of the fish (even those displaying a low preference) appeared to avoid these salinities <5 ppt.

We propose three main reasons to explain why all fish did not select similar salinities as follows: (1) maturity stage, (2) sex differences, and/or (3) differing salinity "histories" of fish tested. Although fish sizes were similar (19.78 ± 0.49), they were close to the maturation point for this species (minimum size of maturity is 20 cm; Claro et al. 2001). Stauffer et al. (1985) found that sexual maturity can affect the temperature preference and tolerance in sailfin molly *Poecilia latipinna*. However, the same conclusion can not be made based on our data because we did not assess fish maturity, nor did we determine fish gender before or after experimentation. Yet, sex differences seem unlikely as it would be expected that the need to minimize costs for osmoregulation is constant regardless of gender. In fact, studies addressing the effect of sex on thermal preferences in the sand goby *Pomatoschistus minutus* and in sailfin molly *Poecilia latipinna* did not find significant differences between males and females (Hesthagen, 1979; Stauffer et al. 1985). Thus, we believe that previous "salinity histories" of fish

tested may have resulted in the two different salinity preferences during our study. We hypothesize that fish that have previously experienced very low salinities in their natural habitat prior capture might have been less reluctant to select low salinities in our system. This assessment is supported by our observations of fish in the shuttlebox system. Overall, we observed that most fish that exposed themselves to low salinities tended to also select lower salinity preferences.

Swimming speeds

Our swimming speed data are unique in the sense that they allowed us to measure routine or "resting" swimming speeds of gray snapper while being tested in the shuttlebox. Most studies have measured the maximum swimming speeds of fish with regards to salinity during continuous exercise, and therefore their data are not comparable with ours. Initially in our experiments we did not expect significant diel differences in routine swimming speeds. However, the fact that active regulatory behavior was increased under darkness probably reflects the natural nocturnal foraging behavior observed for larger size classes of this species along seagrass beds and nearshore shallow habitats (Starck and Davis, 1966; Starck, 1970). Further, the observation that gray snapper would be inclined to move to lower salinities at the beginning of the first dark period is consistent with field observations, as near-shore shallow habitats (which often fluctuate in salinity levels, sometimes reaching salinities as low as freshwater) are usually used as feeding grounds nocturnally by this species. In addition, a recent study (Luo et al., unpublished data) found that gray snapper exhibits a distinct diel migration pattern of movement between mangroves and nearby seagrass beds; whereas mangroves appeared to be daytime "resting" areas, these areas tended to be vacated at night as individuals

searched for food in adjacent seagrass beds (Nagelkerken, 2000; Luo et al., unpublished data). In addition, Serafy et al. (2008) proposed that because gray snappers are known to forage widely by night around seagrass beds, it could be expected that they would experience a wider array of salinities during this time period while searching for food.

Our predictions on the effect of salinity in swimming behavior (regardless of time period of the day) were based upon the rationale that metabolic scope for activity is usually reduced at less than optimal conditions (Fry, 1947). Thus, we expected to obtain the highest routine swimming speeds near isosmotic salinities, which tend to minimize the energetic costs needed for osmoregulation. Consistent with our expectations, results showed an overall pattern of swimming speeds decreasing with increasing salinity. ANCOVA test results suggests that even though the relationship of swimming speeds with salinity was very similar in both light and dark periods of the day (i.e., similar slopes), this relationship could be roughly an order of magnitude higher during the dark period. Increase in swimming speeds at lower salinities may have been further affected by the fact that low salinities were greatly avoided by the majority of fish, especially at night. Therefore, it is possible that this avoidance behavior may have been translated into elevated swimming speeds. Nonetheless, we propose that salinity could have influenced the activity level of gray snapper by causing reductions in maximal swimming performance at high salinities. The reduced activity at high salinities might not only be the result of compensation for higher osmoregulatory costs, but also the minimizing of activity-related costs for osmoregulation. These results are supported by previous studies in the gray snapper. Specifically, Wuenschel et al. (2004, 2005) demonstrated that the gross growth efficiency of small juvenile gray snappers (25-50 mm) was significantly

lower under high salinity conditions (35 and 45ppt). The authors attributed their results to increased energetic costs and higher oxygen consumption rates at these salinities. Thus, we propose that nearly isosmotic salinities would require far less energetic costs associated with osmoregulation (compared to high salinities), which may have translated into fish swimming faster at these salinities.

Ecological implications

Habitats frequented by the gray snapper are generally characterized by wide salinity fluctuations over short time scales (Tabb and Manning, 1961). However, the possible links between salinity preference, swimming behavior, activity level and ultimately habitat selection of the gray snapper are not yet fully understood. While investigating the role of salinity preference in the control of habitat selection in the glass eel *Anguilla anguilla*, Edeline et al. (2005) found a highly significant link between salinity preference and locomotor activity. The authors proposed that preference for fresh water in highly active eels is a behavioral pattern likely to promote the colonization of freshwater habitats, whereas preference for sea water in less active eels is likely to promote precocious settlement in marine and estuarine habitats. Although our data would not specifically support the same conclusions, it suggests that salinity preference and swimming activity level of gray snappers are not independent of each other and may be connected during the behavioral control of their habitat distribution.

Fish	*Size(cm)*	*Preference ± SEM*	*Mode (ppt)*	*Range (ppt)*	
				Minimum	Maximum
1	20.0	22.8 ± 0.3	20	17.7	30.4
2	23.0	13.8 ± 0.1	14	11.0	16.6
3	18.0	20.6 ± 0.3	24	11.7	24.3
4	18.0	14.1 ± 0.3	16	8.7	32.1
5	19.5	18.3 ± 0.8*	33	9.1	33.0
6	18.0	12.7 ± 0.3	11	8.0	22.1
7	20.0	21.2 ± 0.3	23	12.0	26.0
8	19.5	19.5 ± 0.8	10	9.2	32.5
9	20.0	22.2 ± 0.2	23	19.0	27.8
10	20.0	9.1 ± 0.6	5	3.1	30.3
11	21.0	14.6 ± 0.6	8	5.0	26.0

Table 4.1. Measurements obtained for individual fish in the behavioral system: size, salinity preference (given as the mean of median preferred salinities), mode and occupied salinity range. The star denotes a fish that was not further included for analysis of salinity preference (*see Results/Discussion for details*).

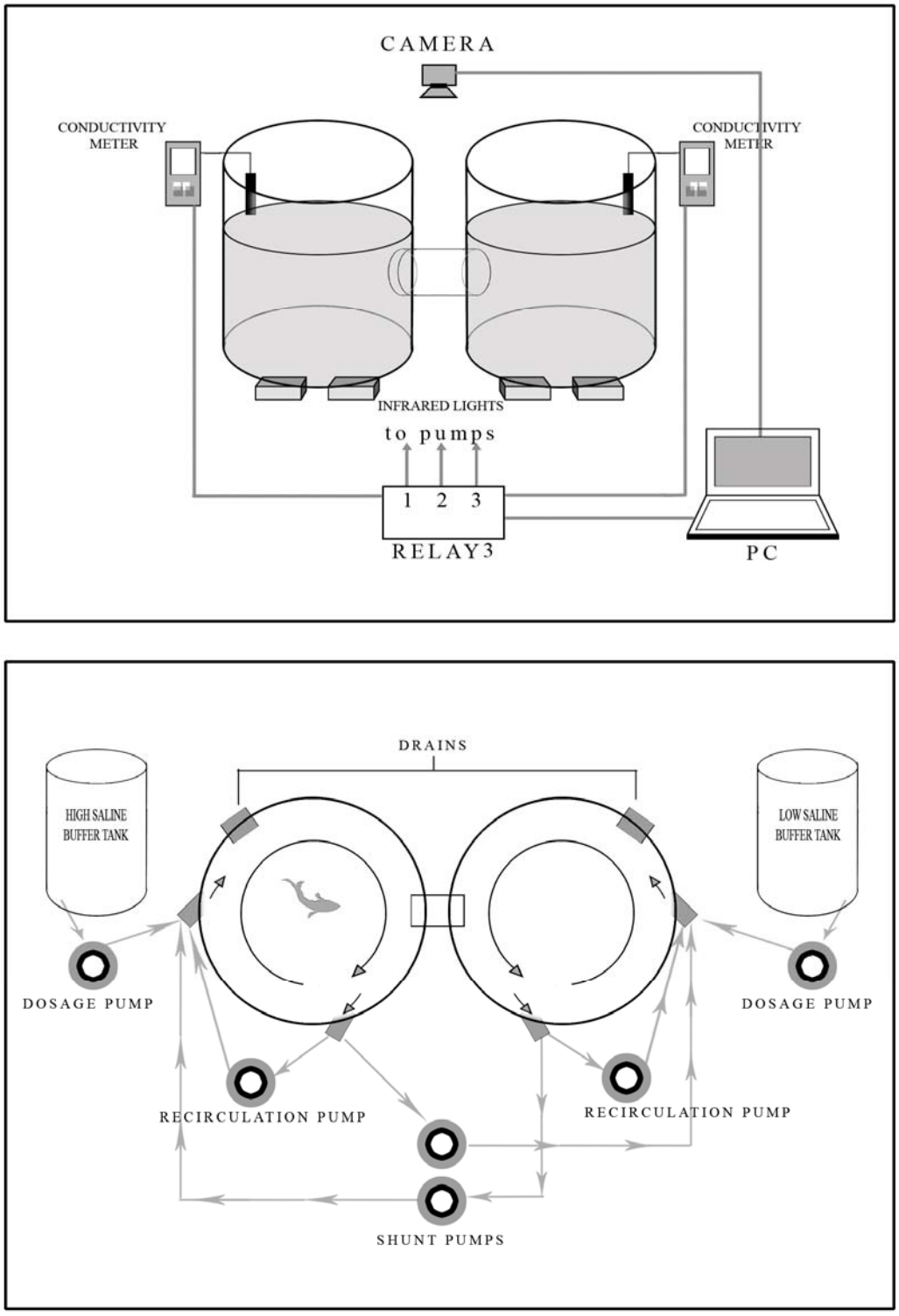

Figure 4.1. Shuttle box for salinity choice depicting all major components. Electronic equipment shown in upper panel (side view), pump system shown in bottom panel (top view). S*ee Methods for details*.

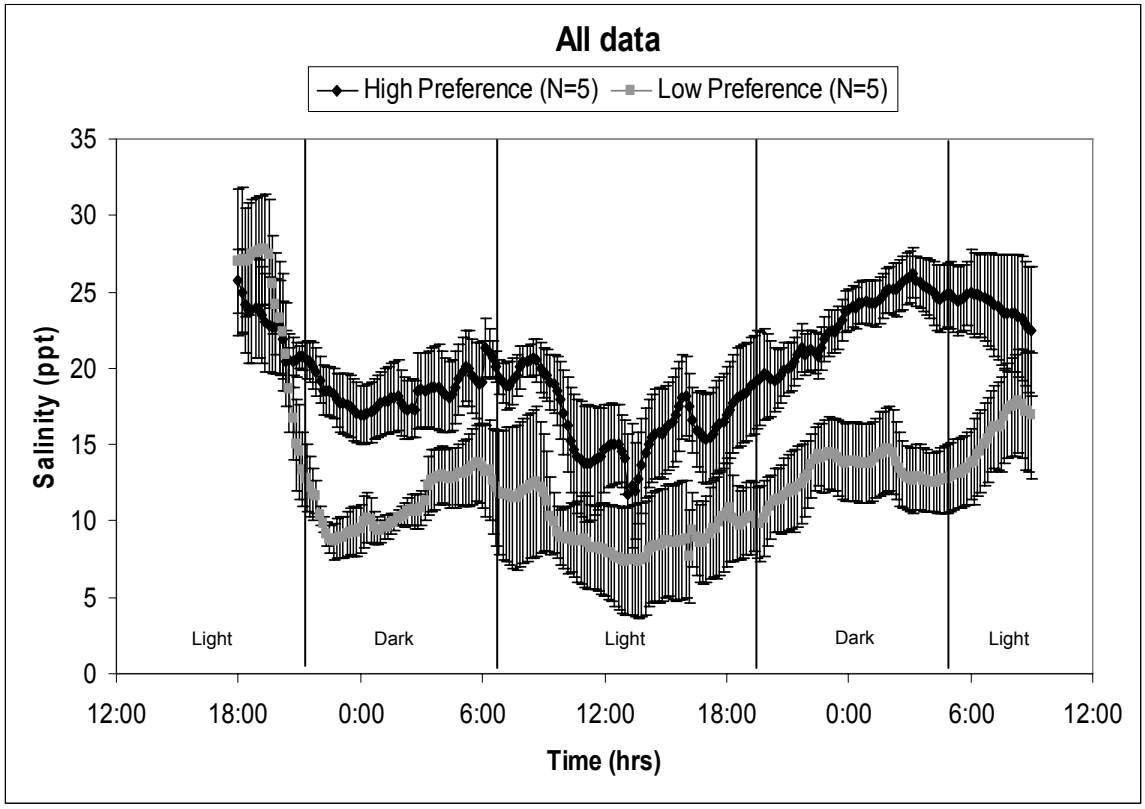

Figure 4.2. Time courses (48 hrs) of mean salinity preferences for gray snapper *Lutjanus griseus* in the shuttlebox system with standard errors shown. Mean preferences [21.3 ± 0.53 (N=5) for the high preference and 12.9 ± 0.99 (N=5) for the low preference] are significantly different from each other ($P<0.05$; Analysis of variance and Student's t post hoc comparison test). Salinity fluctuations seemed to be less variable during the dark periods than those during the light periods (especially during the first 24 hrs).

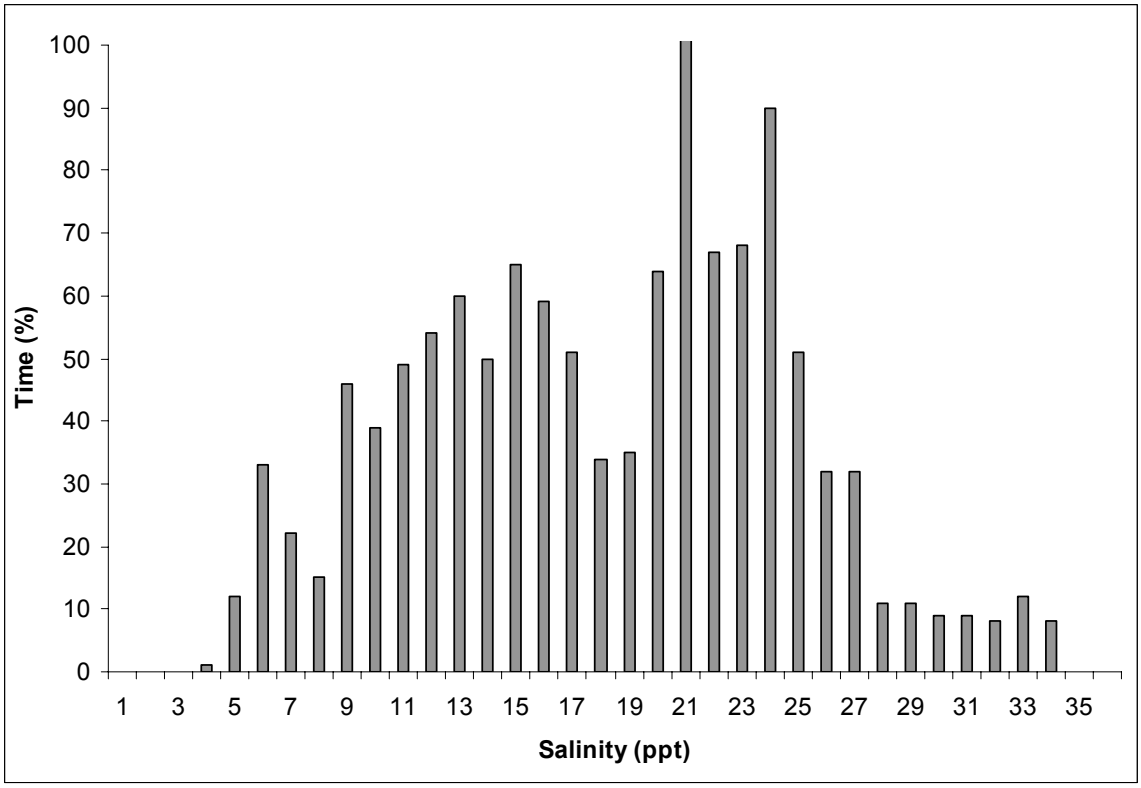

Figure 4.3. Overall salinity distributions by percent of time spent at each for all gray snapper *Lutjanus griseus* tested (N=10, *see Results*). Salinity preferences were established as follows: low salinity preference→ salinities in the range of 9-15 ppt; high salinity preference→ salinities in the range of 19-23 ppt.

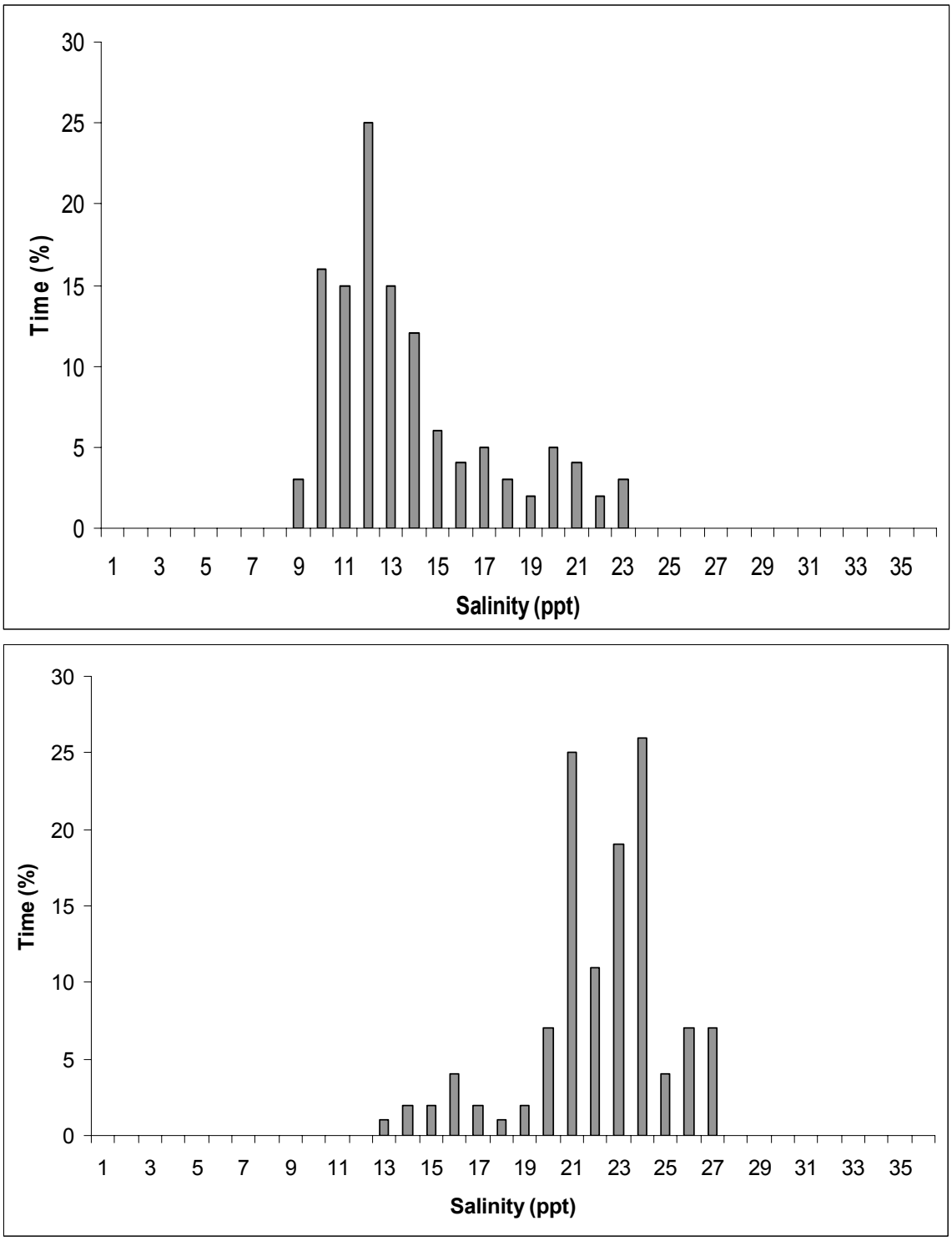

Figure 4.4. An example of the salinity distributions for an individual fish showing: low preference (upper panel) and high preference (bottom panel). S*ee Results for details*.

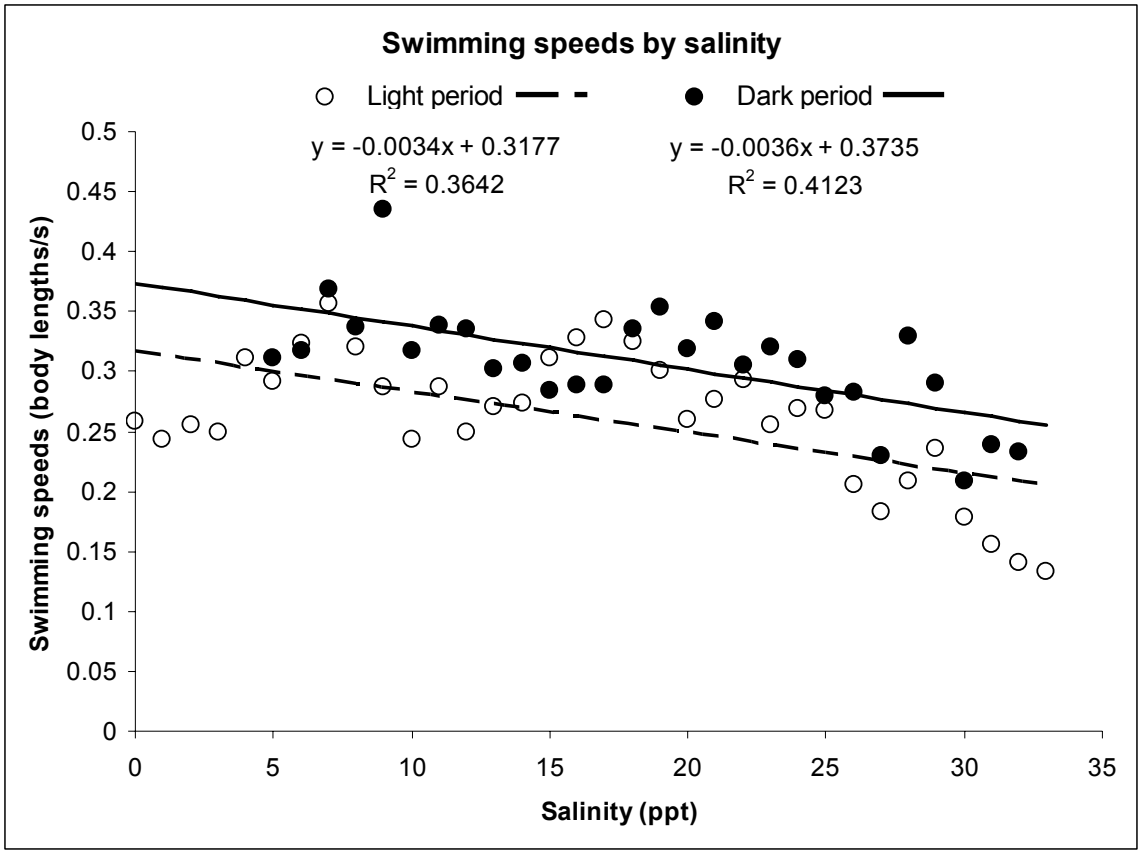

Figure 4.5. Scatter plot of mean swimming speeds recorded under dark and light conditions for all fish by salinity, in an interval ranging from 0 to 33 ppt. The slopes of both regression lines (shown) are similar but the Y-intercepts are significantly different ($P<0.05$; Analysis of Covariance). Regression trendlines are significant for both relationships.

CHAPTER 5: SUMMARY, IMPLICATIONS AND RECOMMENDATIONS

Summary

The main goal of this thesis was to investigate the ecophysiological basis of habitat selection for the gray snapper, a locally abundant and economic/ecologically important species. More specifically, this work aimed to supplement fieldwork with laboratory experiments in an effort to gauge the effects salinity on different aspects of the biology of this species. Three specific objectives were pursued: (1) examine patterns of distribution and abundance across gradients in environmental salinity; (2) measure physiological responses to controlled salinity challenges; and (3) conduct behavioral trials to examine salinity preferenda.

Towards this end, information gaps pertaining to gray snapper and salinity were first identified from a review of the available literature in Chapter 1. Next, in Chapter 2, empirical data on gray snapper collected from Biscayne Bay were examined to test the null hypothesis that gray snapper abundances were evenly distributed along the full salinity range at which samples have been collected. Using the delta approach, three abundance metrics (frequency of occurrence, concentration and delta density) were used as indices of the distribution and abundance of this species. Results indicated that abundance patterns for the smaller gray snapper were consistent with a strategy of reducing osmoregulatory costs by selecting intermediate salinities. However, corresponding abundance patterns for subadult gray snapper were inconsistent with this strategy of minimizing energetic costs, suggesting that this life stage may be indifferent to the range of salinities at which they were observed. These patterns helped development

of further hypotheses regarding ecophysiology of juvenile and subadult gray snapper, and subsequent testing via laboratory experiments as discussed in following chapters.

In Chapter 3, the immediate physiological responses in plasma osmolality and blood haematocrit after abrupt changes in salinity levels were investigated. Fish were challenged with six different salinity treatments, including control (0, 5, 30, 50, 60 and 70 ppt) for 192 consecutive hours and blood samples were collected at different time points. In treatments from 0 to 60 ppt, results did not show significant differences from control values at 192 hours post-transfer, suggesting a successful adaptation to these new salinity levels despite the large changes in environmental salinity. However after abrupt transfers to 70 ppt, the fish lost their ability to maintain a constant plasma osmolality. Nonetheless, this observation was expected, given that this species has never been observed or reported at salinities higher than 66.6 ppt. Overall, these findings demonstrated the strong euryhalinity and osmoregulatory capacity of gray snapper to both extreme hypo- and hypersaline environments.

Finally, in Chapter 4, I examine the salinity preferences and effects on swimming behavior of the gray snapper in an automated salinity choice shuttlebox. This particular study represents the first known attempt to assess the salinity preference of a fish that ultimately resides in reef habitats. Findings revealed that gray snapper prefer a range of salinities that may minimize the physiological costs of osmoregulation. Thus, reduced swimming speeds observed at high salinities high salinities could have been the result of compensation for higher osmoregulatory costs. Overall, the integrative approach used in this study demonstrated the interdependence of osmoregulation costs, behavioral

compensation and physiological constraints in defining the responses of gray snapper to different salinities.

Implications and Recommendations

It has been suggested that the success of many euryhaline species that enter estuaries and freshwater habitats as juveniles depends on the species-specific capacity to osmoregulate. Gray snapper have been long considered estuarine transients; juveniles have been observed in a variety of near-shore habitats with salinities ranging from fresh water to hypersaline. As the gray snapper migrates among brackish and marine waters (and *vice versa*) throughout its life span, changes in external salinity have energetic costs. Rectification of osmotic balance in response to salinity stress thus requires energy expenditure, often at the cost of many other vital processes, such as growth and reproduction.

Certainly one of the most important outcomes of this study was quantifying the exceptional osmoregulatory capacity of subadult and adult gray snapper. This species was shown to successfully acclimate to salinities in the range of 0-60 ppt after abrupt transfers. Fish size did not appear to affect this euryhaline capacity, as both subadult and adult individuals displayed the same responses. Overall, these results challenge past contentions that young individuals of species using estuaries as juveniles are more tolerant of low salinities and salinity fluctuations than adults. However, an important factor to point out in the present study is that early attempts to draw multiple blood samples from the same individuals resulted in excessive mortalities, especially at the extreme salinities. Consequently, individual fish were sampled only once in our laboratory experiments. This particular finding suggests that, when combined with other

stressors (e.g., capture on hook-and-line) lesser salinity challenges than I tested may be lethal. Further, environmental extremes are often coupled; for example, high salinities usually occur together with high temperatures. Thus, while my results suggest gray snapper will survive if exposed to very low or high salinities, this may not be the case if other stressors are present. Therefore, it is imperative that the effect of multiple stressors on the osmoregulatory capacities of this species, and others, be further studied.

Ultimately, the ecological performance of any aquatic species depends on the physiological suitability of the habitat. In South Florida, alteration of freshwater flow has changed natural salinity patterns and degraded estuarine and near-shore habitats currently and historically occupied by gray snapper. Salinity regimes are expected to undergo more significant changes with the implementation of CERP. Gray snapper subjected to pulses of freshwater flow can either remain in this area, if physiologically capable, or leave at the risk of entering a new habitat. The present study suggests that although freshwater pulses and/or moderately hypersaline conditions represent a source of osmoregulatory stress, these alone will not lead to death. CERP implementation, aiming at restoring more natural salinity patterns in South Florida's estuaries, should result in both increased freshwater water delivery as well as decreased salinity variability. Based on the physiological results observed within this thesis, it can be expected that CERP will have positive consequences in term of the gray snapper habitat.

Finally, my findings suggest that the distribution patterns observed for larger fish sizes in the field with respect to salinity (Chapter 2) do not correlate with the observed salinity preferences in Chapter 4. The likely reason is that the reproductive imperative to move towards offshore (high-salinity) spawning sites overrides most salinity

considerations at this life stage. Another important consideration is that in the natural habitat, numerous other factors (e.g., temperature, depth, mangrove density) may combine with salinity to define and influence the distribution and abundance patterns of the gray snapper. Therefore, a future research challenge is to reconstruct the salinity history of individual prior to their capture. This would help not only in understanding why I found different abundance patterns for smaller and larger individuals, but also why I found a bimodal distribution of salinity preferences. Two alternative methods that require development for achieving this goal include: (1) deploying electronic tags with salinity sensors on fish; and (2) using of fish otoliths as natural tags for reconstructing the environmental salinity history prior field capture. At this time, neither approach is proving entirely feasible, but this situation could rapidly change with appropriate technological breakthroughs.

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