

THE RESPONSES OF SUBTROPICAL NEARSHORE FISHES TO CLIMATE CHANGE

BY

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## **Abstract**

Global climate change is expected to alter seawater conditions and marine ecosystems. The Intergovernmental Panel on Climate Change (IPCC) predicts that sea surface pH will decrease by 0.06 - 0.32 pH units and temperature will increase by 0.6-2.0°C over the next 100 years, and extreme weather events, such as major storms (e.g., tropical cyclones), floods, heat waves, and cold spells, are expected to increase in intensity and frequency. Nearshore ecosystems serve as critical habitat for juvenile fishes (e.g., schoolmaster snapper, yellowfin mojarra) and function as foraging grounds for adults (e.g., bonefish, checkered puffer), yet it is unclear how nearshore fishes will respond to forecasted increases in temperature and pCO<sub>2</sub>. To fill this knowledge gap, I assessed the physiology, behavior, and thermal plasticity of nearshore species challenged by climate change stressors, and determined how the presence of a predator will influence habitat choice under forecasted conditions.

Climate change research on marine organisms has primarily focused on species that inhabit the open ocean or coral reef environments. Elevated temperature, salinity, and acidity (reduced pH) have all been shown to result in varying degrees of physiological disturbance in organisms across ecosystems, yet little effort has been devoted to understanding the magnitude of these disturbances in nearshore fishes across seasons. Moreover, few studies have investigated the synergistic (or canceling) effects of combined climate-related challenges. Based on this background, the purpose of this aspect of the study was to assess the relative impacts of climate-related challenges across seasons on fishes that inhabit the nearshore ecosystem. To do this, blood-based physiological responses to elevated temperature, salinity, acidity, and temperature + acidity of adult bonefish, adult checkered puffer, and juvenile yellowfin mojarra were compared across seasons (i.e., summer and winter). Bonefish exposed to climate-related challenges experienced elevated Cl<sup>-</sup>, Ca<sup>2+</sup>, lactate, and glucose levels in the summer relative to winter, and elevated

temperature resulted in glucose and ionic disturbances in both seasons. Similarly, independent of season, checkered puffer exhibited elevated hematocrit, glucose, and cortisol levels when exposed to elevated temperature relative to ambient conditions. Yellowfin mojarra experienced minimal physiological disturbance to the climate-related challenges, but independent of season, elevated temperature increased hematocrit values relative to ambient conditions. Interestingly, the combined stressor, temperature + acidity, did not result in a synergistic or additive physiological disturbance in nearshore fishes. Results indicate that adult bonefish exposed to climate-related challenges experience the greatest physiological disturbances in the summer relative to the winter, with temperature being the most challenging stressor. Collectively, temperature change will be the most challenging climate-related stressor for nearshore fishes, particularly in the summer, and may result in more energy being expended to maintain homeostasis, which could translate into altered behavior, performance, and/or habitat choice under forecasted conditions.

Global climate change scenarios forecasted by the Intergovernmental Panel on Climate Change (IPCC) are expected to alter the performance of marine fishes and potentially disrupt ecosystems. Indeed, climate-related stressors have been shown to reduce metabolic performance in fish from coral reef environments, which may have consequences for growth, reproduction, and distribution of these fishes. Unfortunately, very few studies focus on more than one species or expose fish to several scenarios, which hinders our ability to predict how fish communities and ecosystems will respond to future climate change. The purpose of this aspect of the study was to define the performance of subtropical nearshore fishes to projected climate change scenarios and relate these findings to the ecology of nearshore ecosystem in the future. Adult bonefish, adult checkered puffer, juvenile yellowfin mojarra and juvenile schoolmaster snapper, were acclimated to ambient conditions, along with a range of elevated pCO<sub>2</sub>, salinity, and temperatures that

coincide with, or exceed, the worst-case scenario predicted by the IPCC in 100 years. Post acclimation, fish performance was quantified by measuring standard metabolic rate (SMR) with intermittent-flow, static respirometry, and by chasing the fish to exhaustion (i.e., swimming performance (SP)). Bonefish and yellowfin mojarra experienced an increase in SMR of more than 65% when acclimated to seawater at 33°C, and all species experienced an increase in SMR and a decrease in SP at 34°C. Nearshore fishes acclimated to elevated pCO<sub>2</sub> and salinity experienced minimal disturbances in SMR or SP. Results indicate that elevated temperatures that coincide with (33°C), or exceed (34°C), the worst-case scenario predicted by the IPCC increased standard metabolic rates and reduced swimming performance for a number of nearshore fishes, with elevated pCO<sub>2</sub> and salinity resulting in minimal disturbances in performance. To avoid costs associated with elevated temperatures, nearshore fishes may choose to migrate to more thermally stable environments, allocate energy differently, or increase feeding rates to meet energetic demands. The capacity of fishes to offset these costs will determine ecological “winners” and “losers” in the future and potentially result in a slow restructuring of the nearshore ecosystem.

The proximity of fishes to their thermal limits, coupled with their potential to acclimatize to future environmental conditions, will be additional factors influencing the structure of marine ecosystems as the climate changes. Species that have relatively high thermal maxima are expected to have a limited capacity to acclimatize to new conditions, but this assumption has not been tested on fishes in variable thermal environments in subtropical nearshore ecosystems. The goal of this aspect of the study was to assess the vulnerability of nearshore fishes to climate change and to evaluate the ability of these fishes to adjust their physiological limits across seasons (i.e., phenotypic plasticity). To do this, the critical thermal maximum (CTMax) and minimum (CTMin) of adult bonefish, adult checkered puffer, juvenile yellowfin mojarra, and juvenile schoolmaster

snapper were determined across seasons. Acclimatization response ratios (AZRR;  $\Delta CT_{Max} \Delta T^{-1}$  and  $\Delta CT_{Min} \Delta T^{-1}$ ) were typically greater than 0.60 for all species, a value greater than most previously reported for fish species from variable thermal environments. Present day maximum and minimum temperatures in the nearshore environment are approximately equal to or exceed the thermal tolerance limits of the fish in this study, making thermal safety margins (TSM; i.e., the difference between thermal tolerance limit and extreme environmental temperature) very small or even negative for nearshore fishes (TSM upper = -4.9-0.5; lower = -0.2-0.4). The thermal landscape in the nearshore ecosystem in the future will likely benefit species with positive thermal safety margins that are capable of acclimatizing (e.g., schoolmaster snapper) while relatively intolerant species (e.g., bonefish) may select habitats in this ecosystem less frequently or will be absent from this ecosystem in the future.

Habitat selection in fish is typically governed by the tradeoff between the benefit and cost of acquiring food. Nearshore fishes reside in shallow environments to reduce the threat of predation and/or take advantage of feeding opportunities and routinely experience challenging abiotic conditions. Little is known about the tradeoff between accepting the physiological costs of maintaining homeostasis in novel environments (e.g., elevated temperature associated with climate change) or risking predation by moving to new, less physiologically demanding habitats. The purpose of this aspect of the study was to define the relative cost of habitat selection by measuring temperature and pCO<sub>2</sub> avoidance thresholds of nearshore fishes under altered abiotic conditions in the presence or absence of a predator, (i.e., a juvenile lemon shark). To do this, common subtropical nearshore fishes (i.e., juvenile schoolmaster snapper, juvenile yellowfin mojarra, adult bonefish, and adult checkered puffer) were acclimated to a behavioral choice arena (i.e., two chambers connected by a central corridor). Temperature or CO<sub>2</sub> were manipulated in

one chamber while the other chamber was maintained at ambient conditions. Results show that elevated temperatures and pCO<sub>2</sub> alter habitat choice in all nearshore fishes in this study, and that temperature and pCO<sub>2</sub> avoidance thresholds increase in the presence of a predator. Collectively, elevated temperatures and pCO<sub>2</sub> may alter habitat use and distribution of fishes in nearshore ecosystems, with community structure in predator-rich environments looking very different from environments with low predator burdens.

The outcomes of this research indicate that nearshore fishes exposed to elevated temperatures experience physiological disturbances in the summer, decreased swimming and metabolic performance, some plasticity in thermal tolerance limits, and altered habitat choice in the presence of a predator. Collectively, temperatures that coincide with and exceed future predictions will have species-specific impacts, potentially resulting in shifting community structure in the nearshore ecosystem.

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## TABLE OF CONTENTS

CHAPTER 1: GENERAL INTRODUCTION .....	1
CHAPTER 2: SEASONAL BLOOD CHEMISTRY RESPONSE OF SUBTROPICAL NEARSHORE FISHES TO CLIMATE CHANGE .....	8
CHAPTER 3: STAYING ALIVE IN A CHANGING WORLD: METABOLIC COSTS AND SWIMMING PERFORMANCE OF SUBTROPICAL NEARSHORE FISHES.....	34
CHAPTER 4: ACCLIMATIZATION AND THERMAL SAFETY MARGINS OF NEARSHORE FISHES: IMPLICATIONS FOR MARINE ECOSYSTEMS IN A CHANGING CLIMATE.....	58
CHAPTER 5: OUT OF THE FRYING PAN AND INTO THE FIRE – TRADEOFFS BETWEEN PHYSIOLOGICAL COSTS AND PREDATION IN A CHANGING CLIMATE .	79
CHAPTER 6: GENERAL DISCUSSION .....	104
BIBLIOGRAPHY .....	112



## CHAPTER 1: GENERAL INTRODUCTION

Global climate change is expected to alter abiotic conditions in the world's oceans, impacting a range of different marine characteristics. For example, the worst-case scenario predicted by the Intergovernmental Panel on Climate Change (IPCC) suggests that sea surface temperatures will increase by 2°C, while pCO<sub>2</sub> will increase by approximately 600 µatm, by the year 2100, and may increase by 1500 µatm by the year 2300 (IPCC, 2013). Climate change is also predicted to increase the intensity and frequency of extreme weather events (e.g., major storms, heat waves, cold snaps) (Kerr 2011; Trenberth and Fasullo 2012). An increase in mean and extreme temperatures and pCO<sub>2</sub> are expected as climate changes, which can result in a range of negative effects in marine ecosystems including physiological stress, altered foraging behavior and redistribution of fishes (Munday *et al.*, 2009; Wernberg *et al.*, 2012; Ferrari *et al.*, 2014).

Nearshore marine ecosystems are dynamic environments utilized by both transient and obligate species (Sheaves, 2005). Seawater conditions in nearshore ecosystems can change considerably over a 24 hour period, and over seasons. For example, mean temperature in subtropical nearshore ecosystems can be approximately 9°C lower in the winter (21.2°C) than summer (30.3°C). Extreme temperatures in this environment can be as low as 11°C in the winter and as high as 43 °C in summer (Shultz *et al.*, 2014), which equates to a thermal environment with seasonal fluctuations similar to ecosystems in temperate regions. Due to limited thermal inertia, these ecosystems are predicted to be some of the first to experience the effects of elevated temperatures associated with climate change (Madeira *et al.*, 2012). Similarly, pCO<sub>2</sub> in nearshore environments can fluctuate on a daily basis by as much as 1000 µatm, with extreme conditions reaching 1400 µatm early in the morning due to the respiration of aquatic organisms (Borges *et al.*, 2003; Shaw *et al.*, 2012). A wide range of species use the dynamic nearshore environment,

with juvenile species (e.g., schoolmaster snapper and yellowfin mojarra) typically being confined to the nearshore environment (i.e., obligate) to avoid predation, while adults (e.g., bonefish and checkered puffer) are often transient in this ecosystem. The aforementioned species frequently occur in the nearshore ecosystem throughout the Western Atlantic (Layman *et al.*, 2004; Froese & Pauly, 2011). To cope with these variable abiotic and biotic conditions, fishes may employ several different strategies such as adjusting physiological processes to maintain homeostasis under elevated temperatures (Seebacher *et al.*, 2014), or migrating away from abiotically demanding environments (e.g., seeking deeper/cooler water) (Brown & Thatje, 2015), but potentially increase their risk of predation (Crawshaw & Podrabsky, 2011). Collectively, the dynamic nearshore environment likely requires fish to employ several strategies to cope with these challenges, but it is unclear how these strategies will benefit fish under future conditions associated with climate change.

At the cellular level, challenges associated with climate change (e.g., heat wave) may require fish to mount a stress response and expend energy to maintain homeostasis. The ability of fish to respond to this challenge requires the co-ordination of internal components (e.g., cells, organelles, and tissues) and processes (e.g., intra- and extracellular acid-base chemistry) to maintain homeostasis. For example, elevated pCO<sub>2</sub> results in acid-base imbalances in fish that are typically regulated by uptake/retention of HCO<sub>3</sub><sup>-</sup> and/or excretion of H<sup>+</sup> (Heuer & Grosell, 2014). Energy spent on maintaining homeostasis may reduce the performance of the organism by limiting the energy available for non-maintenance activities such as growth, reproduction, and predator avoidance (Munday *et al.*, 2009), and detriments in performance will be based on the efficiency of the stress response in fish (Pörtner & Farrell, 2008). To date, most physiological research has focused on fish that inhabit relatively stable environments (e.g., coral reefs) (Munday *et al.*,

2012a), with little effort dedicated to how fish from dynamic environments will respond to climate-related stressors. Similarly, work seeking to quantify the impacts of altered abiotic environments often apply stressors in isolation (e.g., an increase in temperature only), with insufficient effort given to synergistic (or canceling) effects of multiple stressors predicted to occur with climate change such as elevated temperatures and pCO<sub>2</sub> (Hofmann *et al.*, 2010). Most importantly, climate change research typically focuses on how a single species will respond to climate-related stressors, with little thought given to how multiple species and life-stages that inhabit an ecosystem (i.e., a fish community) will respond to forecasted conditions (Russell *et al.*, 2012). Together, the magnitude of the physiological response of fishes to climate change stressors is a useful tool in identifying which species will be most at risk, and can indicate which stressor(s) may be responsible for the greatest loss in performance of the whole organism.

Altered abiotic conditions associated with climate change also have the potential to alter performance for an entire organism (Pörtner & Farrell, 2008). Specifically, elevated temperatures have been shown to increase standard metabolic rates and reduce maximal metabolic rates, with net effect of reducing aerobic scope (Nilsson *et al.* 2009; but see Norin *et al.* 2014), which may limit the ability of fish to perform non-maintenance tasks (e.g., growth) (Kordas *et al.*, 2011). Similarly, elevated pCO<sub>2</sub> has been hypothesized to have similar impacts on metabolic rates and aerobic scope due to the extra energy required to maintain acid-base balance in acidified seawater (Pörtner & Farrell, 2008). Support for this hypothesis is limited, however, (Munday *et al.*, 2009), with recent studies indicating no change in performance, or even improved aerobic performance, under elevated pCO<sub>2</sub> (Couturier *et al.*, 2013; Rummer *et al.*, 2013a). Measurements of non-maintenance tasks (e.g., swimming performance) can provide insights into a fish's ability to forage and evade predators in an ecosystem (Castro-Santos, 2011). Elevated temperatures associated with

climate change can reduce swimming performance in coral reef fishes, which might translate into reduced ability to forage or evade predators and result in population-level consequences (Johansen & Jones, 2011). Again, most of this research has been conducted on fish in coral reef environments (Munday *et al.*, 2012a), and these studies use a limited number of climate change scenarios to show performance loss/gain in coral reef fish. Moreover, these studies typically use the worst-case scenario predicted by the IPCC in 100 years, and rarely expose fish to forecasted conditions beyond this benchmark. There is a clear need to address how the performance of species from other marine ecosystems, specifically critical nursery habitats in the nearshore ecosystem, will respond to a wide-range of climate change scenarios. Taken together, the magnitude of the loss/gain in performance under future climate change scenarios will provide some insight into “winners” and “losers” in fish communities in novel climate conditions created by climate change, with fishes that maintain and/or improve their performance being most likely to persist in the future (Somero, 2010).

Plasticity in physiological processes and tolerance limits may buffer species against the negative consequences associated with climate change (Huey *et al.*, 2012). Two prevailing hypotheses drive our understanding of how plastic traits may be, and, by extension, how vulnerable marine fish may be to climate change. The latitudinal hypothesis suggests that species residing near low latitudes/aseasonal environments have a limited ability to acclimate/acclimatize to novel environmental conditions relative to species that experience seasonal fluctuations in temperature (Janzen, 1967). This, in part, explains why tropical biota are expected to be more vulnerable to climate change (Pörtner & Farrell, 2008). Conversely, the trade-off hypothesis predicts that organisms with a relatively high thermal tolerance will have the lowest ability to acclimate/acclimatize to novel environmental conditions (Somero, 2010). This hypothesis stems

from observations that organisms from variable environments have limited abilities to acclimate/acclimatize (Stillman, 2003). Recent work has provided evidence that organisms from variable environments have limited plasticity in thermal tolerance limits and live in close proximity to their thermal extremes (i.e., small thermal safety margins) (Seebacher *et al.*, 2014; Sunday *et al.*, 2014), yet this assumption has not been tested on fish that inhabit variable nearshore environments. Overall, fishes and ectotherms appear to have limited thermal plasticity, which indicates migrating into cooler environments (i.e., behavioural thermoregulation) may play a larger role in buffering these animals against climate change (Gunderson & Stillman, 2015).

Habitat choice is driven by a fish's need to consume food and the costs associated with foraging, with fish that minimize costs while maximizing food consumption benefiting the most (Abrahams, 2011). These costs can come in the form of finding, capturing, and handling prey items, or evading predators (Mittlebach, 2008). There may also be costs associated with foraging in new habitats, especially if these habitats are abiotically challenging (e.g., elevated temperatures, pCO<sub>2</sub>) requiring fish to spend more energy on maintaining homeostasis (Crawshaw & Podrabsky, 2011). Unfortunately, the relative costs of stressors associated with climate change on habitat choice, and how biotic interactions may alter these choices has rarely been evaluated (Kordas *et al.*, 2011), especially for fishes in dynamic environments.

Based on this background, it is clear that our understanding of how climate change will impact fishes has grown considerably in recent years, but major knowledge gaps still exist in how marine fishes, particularly in the variable nearshore environment, will respond to climate-related challenges. My dissertation aims to fill this gap by assessing the physiological and behavioral responses of subtropical nearshore fishes to climate change. Together, the outcome of this work represents a considerable leap forward in our understanding of the factors that will shape nearshore

ecosystems of the future. Specifically, the aim of my dissertation was to evaluate the responses of four common subtropical nearshore fishes (i.e., a community approach) to climate-related stressors across seasons, and assess the role that biotic interactions may have on habitat choice under forecasted conditions.

My dissertation is divided into four chapters that collectively address physiological and behavioral response of subtropical nearshore fishes to climate change. In chapter 2, I quantify the blood-based physiological response of nearshore fishes exposed to climate-related challenges in both the summer and winter, which has allowed me to identify which species are most at risk in each season, which stressor(s) result in the greatest physiological disturbance, and if multiple stressors (e.g., elevated temperature and pCO<sub>2</sub>) result in a synergistic response. In chapter 3, I quantify the swimming and metabolic performance of nearshore fishes acclimated to climate-related challenges that coincide with, or exceed forecasts made by the IPCC, which has allowed me to characterize the performance of nearshore fishes across a range of potential climate change scenarios. In chapter 4, I evaluate the critical thermal maximums and minimums of nearshore fishes in the summer and winter, which has allowed me to determine thermal safety margins (i.e., proximity to extreme environmental temperatures) of nearshore fishes in each season, and the ability of these fish to acclimatize to new thermal environments across seasons. Finally, in chapter 5, I expose nearshore fishes to climate-related stressors in a behavioral choice arena in the presence and absence of a predator, which has allowed me to evaluate the tradeoff between the costs of altered abiotic conditions and the threat of predation.

When all 4 chapters are considered together, my data allow me to suggest which species will be ecological “winners” and “losers” under forecasted conditions. To build on this, I use species-level vulnerability to climate-related stressors to predict how the structure of nearshore

and adjacent ecosystems may change in the future. To add another layer, I highlight how interactions with the biotic community (e.g., predators) can complicate simple predictions based on physiological responses to climate-related stressors. Finally, I indicate when these alterations in the ecosystem might occur by discussing physiological and behavioral tipping points in the context of future climate change scenarios predicted by the IPCC. Collectively, this dissertation provides insight into the structure of the nearshore fish community of the future and suggests how this information can be used to manage nearshore ecosystems.

## CHAPTER 2: SEASONAL BLOOD CHEMISTRY RESPONSE OF SUBTROPICAL NEARSHORE FISHES TO CLIMATE CHANGE<sup>1</sup>

### Abstract

Climate change due to anthropogenic activity will continue to alter the chemistry of the oceans. Future climate scenarios indicate that sub-tropical oceans will become more acidic, and the temperature and salinity will increase relative to current conditions. A large portion of previous work has focused on how future climate scenarios may impact shell-forming organisms and coral reef fish, with little attention given to fish that inhabit nearshore habitats; few studies have examined multiple challenges concurrently. The purpose of this study was to quantify the blood-based physiological response of nearshore fishes to a suite of seawater conditions associated with future climate change. Fish were exposed to an acute (30 min) increase in salinity (50 ppt), acidity (decrease in pH by 0.5 units) or temperature (7–10°C), or temperature and acidity combined, and held in these conditions for 6 h. Their physiological responses were compared across seasons (i.e. summer vs. winter). Bonefish (*Albula vulpes*) exposed to environmental challenges in the summer experienced a suite of blood-based osmotic and ionic disturbances relative to fish held in ambient conditions, with thermal challenges (particularly in the summer) being the most challenging. Conversely, no significant treatment effects were observed for yellowfin mojarra (*Gerres cinereus*) or checkered puffer (*Sphoeroides testudineus*) in either season. Together, results from this study demonstrate that acute climate-induced changes to thermal habitat will be the most challenging for sub-tropical fishes (particularly in the summer) relative to salinity and pH stressors, but significant variation across species exists.

<sup>1</sup>This chapter appeared in its entirety in Conservation Physiology and is referred to later in this dissertation as “Shultz et al., 2014”. Shultz, A.D., Zuckerman, Z.C., Stewart, H.A., and Suski, C. D. 2014. Seasonal blood chemistry responses of sub-tropical fishes to climate change. 2:1-12. This article is reprinted with the permission of the publisher and is available from <http://conphys.oxfordjournals.org/content/2/1/cou028.full>



## Introduction

Since the industrial revolution, anthropogenic disturbances, such as the burning of fossil fuels and deforestation, have resulted in an increase in atmospheric carbon dioxide (CO<sub>2</sub>). Carbon dioxide levels have exceeded historical concentrations over the past 650 000 years and have culminated in changes to global climate (Trenberth *et al.*, 2007). In addition to warmer temperatures, climate change also alters the chemistry of the oceans through changes in the evaporation–precipitation cycle (Stott *et al.*, 2008; Feely *et al.*, 2009). Salinity of the ocean is expected to increase in sub-tropical regions as a result of locally reduced precipitation as the planet warms (Gilman *et al.*, 2008). Future climate change predictions indicate that tropical hurricanes will increase in intensity and frequency, resulting in greater amounts of freshwater runoff into nearshore areas during these storms (Knutson *et al.*, 2010). Recent research has also shown that pH decreases by a mean of ~0.3 units during the rainy season relative to the dry season (Sousa *et al.*, 2013), and this drop in pH is likely to be exacerbated as storms increase in intensity and frequency. Current worst-case scenario predictions by the Intergovernmental Panel on Climate Change (IPCC) indicate that ocean temperatures are expected to increase by 0.3–2.0°C over the next 100 years (Stocker *et al.*, 2013). Taken together, seawater quality parameters (i.e. salinity, pH and temperature) will become more extreme and variable as the climate changes.

Recent research has unequivocally demonstrated tropical marine organisms to be sensitive to future climate change scenarios, with expected negative consequences mainly due to three processes. First, a decrease in pH has been shown to increase mortality in marine zooplankton, disrupt metabolite concentrations in fish and reduce growth rates in invertebrates (Yamada & Ikeda, 1999; Kurihara & Shirayama, 2004; Rodrigues *et al.*, 2013). Second, an increase in temperature has been shown to reduce available dissolved oxygen and concomitantly increase the

rate of metabolic processes, which together can reduce the capacity of organisms to perform aerobically (Pörtner & Farrell, 2008; Munday *et al.*, 2009). Third, organisms in the tropics experience a relatively narrow range of temperatures annually compared with organisms in temperate regions and have adapted to these narrow thermal environments to minimize maintenance costs, resulting in organism-specific thermal niches that can overlap (Pörtner & Farrell, 2008; Huey *et al.*, 2009). At present, a large proportion of recent research examining the impact of future climate-induced changes on tropical marine ecosystems has focused on calcifiers (shell-forming organisms), invertebrates, coral and fish that inhabit either open ocean or coral reef ecosystems, with little effort devoted to other marine ecosystems (Przeslawski *et al.*, 2008; Hofmann *et al.*, 2010). More physiological and ecological research on fish from different habitats in the tropics is needed to improve our understanding of and ability to predict how tropical marine ecosystems will respond to future climate change (Roessig *et al.*, 2005).

Nearshore habitats are characterized by dynamic abiotic conditions that fluctuate over short periods of time, including diurnal periods of seawater inundation and drying. Moreover, fluctuations in the levels of a number of abiotic conditions, such as salinity, temperature, pH, oxygen and carbon dioxide content, vary depending on the time of day, tidal cycle and season (Lam *et al.*, 2006). The ability of fish to respond to this dynamic abiotic environment relies on the co-ordination of internal components (e.g. cells, organelles and tissues) and processes (e.g. intra- and extracellular acid–base chemistry) to maintain homeostasis. A disruption in one of these components or processes sets the physiological limits for the whole organism, and identifying which species demonstrate the greatest whole-organism sensitivity to environmental challenges will be important when evaluating limits to climate change (Somero, 2010). Currently, little information exists on the blood chemistry of species of fish that inhabit nearshore ecosystems,

much less their physiological response to environmental challenges associated with climate change (Lam *et al.*, 2006).

Based on this background, the purpose of this study was to assess the relative impacts of climate change stressors on several fish species in the nearshore ecosystem (i.e. a community approach) and identify the physiological mechanisms that respond to these stressors. To do this, the blood-based physiological response of bonefish (*Albula vulpes*), checkered puffer (*Sphoeroides testudineus*) and yellowfin mojarra (*Gerres cinereus*) was quantified after exposure to seawater conditions associated with future climate change. Specifically, fish were exposed to an acute increase in salinity, acidity or temperature, or temperature and acidity combined, and their responses were compared across seasons (i.e. summer vs. winter). Results from this study will improve our understanding of how nearshore fish will cope with future climate change, indicate which species are more susceptible to changes in environmental conditions and identify which component of future climate change scenarios will be most challenging for nearshore fishes.

## **Methods**

### *Study site*

This study was conducted at a remote field station, The Cape Eleuthera Institute (CEI), in Eleuthera, The Bahamas (24°50'05" N 76°20'32" W). All research conformed to the University of Illinois Institutional Animal Care and Use Committee protocol (Protocol # 09160). Adult bonefish (summer fork length, mean  $\pm$  SEM 382  $\pm$  5 mm, range = 300–452 mm; and winter fork length, mean  $\pm$  SEM 428  $\pm$  6 mm, range = 362–506 mm), checkered puffer (summer total length, mean  $\pm$  SEM 191  $\pm$  3 mm, range = 145–240 mm; and winter total length, mean  $\pm$  SEM 158  $\pm$  11 mm, range = 164–244 mm) and juvenile yellowfin mojarra (summer fork length, mean  $\pm$  SEM 129

$\pm 3$  mm, range = 105–185 mm; and winter fork length, mean  $\pm$  SEM  $158 \pm 4$  mm, range = 87–210 mm) were captured by seining local tidal creeks near CEI and transferred to plastic 76 l totes filled with ambient sea water. Fish were transported by boat to the CEI wetlab in <30 min, and seawater in the totes was exchanged every 5 min (Murchie *et al.*, 2009).

Upon arrival at the CEI aquatic facility, fish were transferred to two large holding tanks (3.7 m diameter  $\times$  1.25 m height; 13 180 l) continuously supplied with fresh seawater ( $1800 \text{ l h}^{-1}$ ) and aerated with a low-pressure pump (Sweetwater model S41; 15 V; 3450 rpm; Aquatic Ecosystems, Apopka, FL, USA). Dissolved oxygen (in milligrams per litre), salinity (in parts per thousand, ppt), acidity (pH) and temperature (in degrees Celsius) were monitored regularly during holding (YSI 55, 85, pH10A, Yellow Springs, OH, USA; Table 1). All fish were acclimated to laboratory conditions for a minimum of 48 h prior to experimentation. During holding, fish were fed frozen sardines (*Sardenella aurita*) to satiation, but were starved for 24 h prior to experimentation.

To quantify ambient water characteristics in nearshore ecosystems, seawater parameters were measured in two tidal creeks in the winter and summer. A single autonomous temperature logger (iButton DS1923; Maxim, Dallas, TX, USA) was deployed in the mouth of each collection creek at a depth of  $\sim 0.5$  m (at low tide), and temperature ( $\pm 0.5^\circ\text{C}$ ) was sampled hourly. Dissolved oxygen, salinity and pH were measured several times per week during both the summer and winter sampling periods (Table 1).

#### *Acute response to climate change stressors*

To quantify the response of nearshore fishes to acute changes in environmental conditions, fish were exposed to one of the following four separate challenges: (i) increase in salinity; (ii) decrease in pH; (iii) increase in temperature; and (iv) temperature increase coupled with a

concurrent decrease in pH (referred to as T + pH). The environmental challenges exceeded the predictions of future oceanic conditions generated by the IPCC, but were still representative of conditions for nearshore ecosystems (Trenberth et al. 2007; Table 2). To accomplish the environmental challenges, fish were transferred from large holding tanks into individual, aerated plastic totes, scaled according to fish size (bonefish, 76 l; checkered puffer and yellowfin mojarra, 14 l) resting in a raceway (3.09 m length × 0.65 m width × 0.17 m height), and allowed to acclimate for a minimum of 12 h prior to experimentation. The individual totes were continuously supplied with recirculating seawater (Eheim pump 1046A; 5 l min<sup>-1</sup>) from a reservoir tank (Igloo cooler 108 l), completing a closed water system (Vanlandeghem *et al.*, 2010). Treatment levels for the environmental challenges were attained by gradually adjusting seawater conditions over a 30 min period to target conditions, and then maintaining these target conditions for 6 h. An acute change in seawater conditions has been used to assess the sensitivity of fish species to climate change stressors (Gräns *et al.*, 2013). Salinity was increased from 36 to 50 ppt by dissolving sea salt (Instant Ocean; Aquatic Ecosystems, Apopka, FL, USA) in seawater and then mixing it into the reservoir tank (Haney & Walsh, 2003); salinity was verified using a hand-held meter (YSI 85). The pH was decreased from  $8.1 \pm 0.06$  (mean  $\pm$  SEM; range = 8.2–7.9) to  $7.54 \pm 0.05$  (mean  $\pm$  SEM; range = 7.7–7.4) by transferring small amounts (1–3 ml) of 31.45% HCl (muriatic acid; Sunnyside Corporation, Wheeling, IL, USA) into the reservoir tank, in a similar manner to Kurihara and Shirayama (2004). These conditions were maintained by transferring HCl into the reservoir tank as needed (HCl was used to decrease the pH of the water instead of CO<sub>2</sub> because cylinders of compressed CO<sub>2</sub> were not available at this remote field station).

Temperature was increased by using immersion heaters in the main reservoir and distributing warmed water to fish in the plastic totes (Vanlandeghem *et al.*, 2010). Previous work

has shown that upper lethal temperature, incipient lethal temperatures and chronic thermal stress for animals can vary seasonally, partly as a result of acclimation/acclimatization (Murchie *et al.*, 2011), which tracks with seasonal increases/decreases in oceanic temperatures. For the present experiment, an absolute thermal maximum treatment for each species was not used; rather, temperature levels for the thermal treatment were 7°C above ambient conditions for bonefish and 10°C above ambient conditions for checkered puffers and yellowfin mojarra. These values go beyond the predictions for sea surface temperatures of oceans projected by the IPCC (Stocker *et al.*, 2013), but are not unrealistic temperature values for nearshore ecosystems (Table 1). The same change in temperature was used for this treatment in the winter (e.g. ambient seawater at 20°C was increased to 27°C for bonefish). For the T + pH treatment, temperature was simultaneously increased by the addition of immersion heaters, while the pH was decreased by the addition of HCl to the reservoir (bonefish, 7°C and 0.5 pH units; checkered puffer and yellowfin mojarra, 10°C and 0.5 pH units). Fish in the control treatment were handled in an identical manner to the experimental fish described above, except that water conditions were not altered.

#### *Blood sampling and analysis*

Briefly, blood samples were drawn from fish using a heparinized 22 gauge needle attached to a 1 ml syringe inserted into the caudal vessel, following the 6 h exposure to an environmental challenge or control conditions. The samples were transported to a laboratory at the University of Illinois, and the following blood parameters were quantified: haematocrit, potassium (K<sup>+</sup>), sodium (Na<sup>+</sup>), chloride (Cl<sup>-</sup>), calcium (Ca<sup>2+</sup>), glucose and cortisol (for details see Shultz *et al.* 2011). These blood parameters have been shown to change in marine fish exposed to temperature, pH and salinity challenges (Ishimatsu *et al.*, 2004; Evans *et al.*, 2005; Nordlie, 2009).

### *Data analysis*

Statistical analyses were performed separately for each fish species, with a focus on intraspecific differences in blood chemistry values between seasons. Blood-based metrics were normally distributed and compared using a two-way analysis of variance (ANOVA) with treatment and season as main effects, and treatment  $\times$  season as an interaction term. Tukey's post hoc test was performed when at least one main effect or the interaction term was deemed significantly different. Data analysis was completed using JMP 7.0.2 (SAS Institute, Cary, NC, USA) with  $\alpha = 0.05$ .

## **Results**

### *Bonefish*

Bonefish exposed to environmental challenges associated with climate change experienced a suite of blood-based physiological disturbances. When compared with control values, plasma  $\text{Cl}^-$  concentrations increased by  $\sim 20\%$  after bonefish were exposed to a 14 ppt increase in salinity (Tables 3 and 4). Likewise, both an increase in temperature by  $7^\circ\text{C}$  and an increase in temperature by  $7^\circ\text{C}$  coupled with a decrease in pH by 0.5 units resulted in nearly a 25% increase in plasma  $\text{Cl}^-$  concentrations relative to control values. Acidified seawater (decrease of 0.5 pH units) resulted in the greatest increase (33%) in  $\text{Cl}^-$  values when compared with control values. Plasma lactate values increased over 4-fold when fish were held in acidified seawater relative to fish held in ambient conditions (Table 3). Bonefish exposed to an increase in temperature experienced a doubling of plasma glucose concentrations relative to control concentrations (Table 3). None of the treatments in the summer caused  $\text{Na}^+$ ,  $\text{K}^+$ , haematocrit or cortisol values to differ significantly

from control values. In contrast, plasma  $\text{Ca}^{2+}$  was the only variable that changed in the winter, and it decreased by nearly 40% when fish were held in water 7°C warmer than ambient (Table 3).

Several physiological disturbances were observed when climate change stressors were compared across seasons. In the summer, bonefish in the acidity and T + pH treatments displayed an increase of nearly 20% in plasma  $\text{Cl}^-$  concentrations relative to values for fish in the same treatments during the winter (Table 3). Likewise, bonefish exposed to acidified seawater during the summer experienced a 20-fold increase in plasma lactate concentrations relative to fish in the winter. Moreover, bonefish exposed to an increase in temperature during the summer exhibited an increase in plasma glucose and  $\text{Ca}^{2+}$  levels by ~60% relative to fish in the same treatment during the winter (Table 3).

#### *Checkered puffers*

No significant interactions between treatment and season were observed when checkered puffers were exposed to climate change stressors in the summer and winter, with significant treatment effects limited to that of season or treatment independently (Tables 5 and 6). Independent of season, haematocrit levels were significantly greater when fish were exposed to an increase in acidity, temperature or T + pH relative to ambient conditions. Glucose and cortisol concentrations were also elevated in fish exposed to an increase in temperature by 10°C when compared with fish held in ambient seawater (Table 6). Independent of treatment, plasma  $\text{Cl}^-$ ,  $\text{Na}^+$  and  $\text{K}^+$  concentrations were significantly greater in the winter than in the summer. Conversely, haematocrit levels and plasma cortisol concentrations were elevated in the summer relative to the winter (Table 6).



### *Yellowfin mojarra*

When qualitatively compared with the other two nearshore species examined in this study, yellowfin mojarra experienced fewer physiological disturbances following the 6 h treatments. No significant interactions between season and treatment were observed when yellowfin mojarra were exposed to climate change stressors in the summer and winter. Independent of season, the temperature and T + pH treatment resulted in a significant increase in haematocrit levels relative to control levels (Tables 7 and 8). Independent of treatment, plasma  $\text{Ca}^{2+}$  and glucose concentrations in the winter were elevated relative to the summer. Conversely, plasma  $\text{K}^{+}$  levels in the summer were significantly greater than values in the winter (Table 7).

### **Discussion**

Of the three species of nearshore fish examined, bonefish displayed the greatest degree of physiological disturbances following exposure to the common environmental challenges, with disturbances in the summer being greater than those in the winter. More specifically, plasma  $\text{Cl}^{-}$  concentrations increased when bonefish were exposed to acidified seawater, salinity and thermal challenges that exceeded the predictions of the IPCC. In addition, bonefish exposed to acidified seawater also experienced an increase in plasma lactate concentrations. An increase in temperature resulted in greater concentrations of glucose in the blood of bonefish, probably to fuel metabolic demands (Wendelaar Bonga, 1997). Overall, salinity, acid, temperature or T + pH applied in the summer resulted in a plasma  $\text{Cl}^{-}$  imbalance that required bonefish to expend energy to return to homeostasis. Moreover, the acid and temperature treatments produced additional imbalances in metabolites, suggesting that these two challenges are most physiologically difficult for bonefish to cope with during the summer.

Independent of season, checkered puffers exhibited several physiological disturbances when exposed to environmental challenges that exceeded future IPCC ocean scenarios. More specifically, haematocrit increased when fish were exposed to an increase in temperature, acidity or T + pH. An increase in temperature results in an increase in metabolic rate, which means that more oxygen must be delivered to cells to maintain aerobic metabolism (Pörtner, 2012). Checkered puffers increased either the number or the size of their red cells in an effort to bind more oxygen. Independent of treatment, plasma ion concentrations were higher in the winter relative to the summer, while haematocrit and cortisol levels were also higher in the summer than the winter. While the exact mechanism for these seasonal differences is not known, it could be related to annual cycles independent of temperature (Evans, 1984) or to differences in the activity rates or number of pumps in the cell membrane related to temperature and salinity (Fiess *et al.*, 2007; Sardella *et al.*, 2008), and should be the subject of future study.

Independent of season, yellowfin mojarra demonstrated the lowest degree of physiological disturbance when exposed to environmental stressors relative to bonefish and puffer. An increase in haematocrit values was observed when these fish were exposed to an increase in temperature or T + pH. Independent of treatment, Ca<sup>2+</sup> and glucose concentrations were higher in the winter, and K<sup>+</sup> concentrations were higher in the summer. Glucose concentrations were higher in the winter relative to the summer.

Previous climate change work has used treatments that represented the worst-case scenario predicted by the IPCC and found considerable physiological disturbances in shell-forming organisms and reef fish. For example, intertidal gastropods exposed to pH and temperature values expected for 2100 (i.e. decrease in pH by 0.3 units and increase in temperature by 5°C) experienced lower shell growth rates and a disruption in metabolic processes (Melatunan *et al.*, 2013).

Likewise, cardinal fish (*Ostorhinchus doederleini* and *Ostorhinchus cyanosoma*) and lemon damselfish (*Pomacentrus moluccensis*) found in relatively stable environmental conditions on reefs demonstrated a reduction in metabolic scope when exposed to future climate change scenarios, which can have negative implications for feeding, growth and reproduction (Munday *et al.*, 2009; Nilsson *et al.*, 2010). In contrast to reef environments, abiotic factors (pH, temperature and salinity) in nearshore ecosystems tend to fluctuate daily, seasonally and with precipitation events (Lam *et al.*, 2006; Rummer *et al.*, 2013b). For example, seasonal variation in temperatures measured in tidal creeks near CEI ranged from 40°C in the summer to 11°C in the winter. Adult bonefish migrate between the dynamic abiotic environment in the nearshore ecosystem to deeper (>2 m), relatively stable waters, such as coral reef habitats, with the flooding and ebbing of the tides (Murchie *et al.*, 2013), probably avoiding extreme conditions in nearshore ecosystems, which may explain why these fish were relatively less tolerant to climate change stressors. Conversely, checkered puffers and yellowfin mojarra reside almost exclusively in nearshore ecosystems (Layman & Silliman, 2002) and are therefore regularly subjected to a wide range of temperatures and pH levels, making them more tolerant to conditions that exceed future climate change scenarios. This differential response to climate change has the potential to alter fish assemblages in the future by excluding intolerant species from nearshore ecosystems (e.g., bonefish) and/or reducing their population size, while tolerant species (e.g., yellowfin mojarra and checkered puffers) may become more dominant in these systems.

Interestingly, none of the species from the present study experienced an additive or synergistic physiological response when exposed to two climate change stressors in the T + pH treatment. Previous work has documented an additional reduction in aerobic scope when coral reef fish were exposed to an increase in temperature coupled with acidified seawater (i.e., a

synergistic effect) compared with the aerobic scope of these fish in ambient seawater and elevated temperatures (Munday *et al.*, 2009). Future research on nearshore fish should focus on the mechanisms that allow them to cope with multiple stressors.

Results from this study indicate that temperature was the most challenging acute stressor associated with future climate change relative to pH, salinity and temperature + pH. Moreover, changes in the summer caused elevated physiological disturbances relative to changes in the winter. Nearshore fish are likely to have a relatively robust ability to regulate osmotic/ionic balances, including pH (Lam *et al.*, 2006). In contrast, elevated temperatures may cause these mechanisms to break down and can cause proteins to denature, which results in physiological disturbances. These problems are most severe in the summer as fishes may be approaching their 'pejus' temperatures (Pörtner & Farrell, 2008), thereby reducing their thermal scope. While these changes may not directly result in mortality for nearshore fishes, there may be other sub-lethal consequences, such as altered habitat selection, which may result in increased likelihood of predation or reduced feeding. Additionally, as reviewed by Boeuf and Payan (2001), osmotic regulation accounts for 20–50% of the resting energy expenditure of several freshwater fishes. The energetic cost to maintain osmotic balance is likely to increase in the future as the climate changes. Increased water temperature will result in a concomitant increase in metabolic rate for nearshore fishes, which increases food demands and foraging, which may alter predator–prey dynamics (Eme *et al.*, 2011; Kordas *et al.*, 2011). Finally, in extreme cases, prolonged exposure to sub-optimal water conditions can result in chronic stress for fishes, which can lead to reduced growth rates, reduced reproductive output and increased susceptibility to disease (Doney *et al.*, 2012). Together, results from the present study indicate that an acute change in temperature will be the most challenging component of future ocean conditions for nearshore fishes, particularly in

the summer, with fish experiencing increased sub-lethal disturbances that could manifest in behavioural or habitat shifts.

## Tables

Table 1. Mean daily water conditions for laboratory holding tanks and two tidal creeks across seasons. Holding tank conditions in the summer were measured from August 5-15, 2009 and in the winter from February 12 – March 13, 2010. Dissolved oxygen, salinity, and acidity in the tidal creeks were measured at the mouth in the summer from June 3 – July 14, 2011 and in the winter from January 27 – February 15, 2011. Temperature was recorded at the mouth in the summer June 1 – August 31, 2011 and winter January 27 – March 27, 2011.

Location	Season	Descriptive Statistic	Dissolved Oxygen (mg L <sup>-1</sup> )	Salinity (ppt)	Acidity (pH)	Temperature (°C)
Holding Tank	Summer	Mean±SE	5.64±0.1	38.7±0.7	8.1±0.1	29.4±0.9
		Range	5.06-6.08	38.1-39.7	7.9-8.2	28.0-30.6
		N	7	7	7	7
	Winter	Mean±SE	5.64±0.1	41.9±0.1	8.1±0.1	20.8±0.6
		Range	4.71-7.27	41.2-42.8	7.9-8.2	16.8-25.6
		N	7	7	7	7
Tidal Creeks	Summer	Mean±SE	5.66±0.1	36.2±0.3	8.3±0.06	30.3±0.06
		Range	2.46-8.71	17.2-40.5	7.3-9.3	23.5-43
		N	118	119	49	2922
	Winter	Mean±SE	3.75±0.2	41.5±0.4	9.2±0.1	21.2±0.08
		Range	2.64-4.64	40.4-43.2	8.9-9.9	11-35.7
		N	11	11	11	2850

Table 2. Water quality conditions for bonefish, yellowfin mojarra, and checkered puffer held for 6 hours in altered seawater conditions in the summer and winter. Water quality values were pooled across species for control, salinity, and acidity treatments.

Season	Species	Treatment	Water Quality Parameter	Mean	Standard Error	
Summer	All Species	Control	Dissolved Oxygen (mg L <sup>-1</sup> )	5.93	0.07	
			Salinity (ppt)	39.7	0.33	
			Acidity (pH)	8.02	0.03	
			Temperature (°C)	28.2	0.47	
	Bonefish	Salinity	(ppt)	49.8	0.19	
			Acidity	(pH)	7.57	0.01
			Temperature	(°C)	35	0.11
			Temperature & Acidity	(°C)	35	0.06
	Checkered Puffer	Temperature	(pH)	7.55	0.02	
			(°C)	37.6	0.03	
			Temperature & Acidity	(°C)	37.7	0.23
			(pH)	7.5	0.09	
	Yellowfin Mojarra	Temperature	(°C)	37.6	0.08	
			Temperature & Acidity	(°C)	38.1	0.11
(pH)			7.37	0.12		
(pH)			7.37	0.12		
Winter	All Species	Control	Dissolved Oxygen (mg L <sup>-1</sup> )	6.8	0.09	
			Salinity (ppt)	42.6	0.04	
			Acidity (pH)	8.71	0.05	
			Temperature (°C)	20.7	0.22	
	Bonefish	Salinity	(ppt)	49.5	0.62	
			Acidity	(pH)	8.0	0.02
			Temperature	(°C)	27.5	0.08
			Temperature & Acidity	(°C)	27	0.14
	Checkered Puffer	Temperature	(pH)	8.02	0.01	
			(°C)	33.2	0.53	
			Temperature & Acidity	(°C)	33.2	0.45
			(pH)	7.8	0.04	

Table 2 cont.

Yellowfin Mojarra	Temperature	(°C)	32.2	0.14
	Temperature & Acidity	(°C)	33.4	0.54
		(pH)	7.97	0.04

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Table 3. Concentrations of different plasma constituents for bonefish following a suite of environmental challenges applied in the summer and the winter. The different challenges applied were control (C), an increase in salinity (S), a decrease in pH (A), an increase in temperature (T) and a combination of increased temperature and reduced pH (T + pH). Bonefish were held in these treatments for 6 h prior to blood sampling. A Tukey's post hoc test was used to compare blood-based stress metrics (sample sizes in parentheses) for bonefish exposed to climate change stressors in the summer and winter. Means with different superscript letters indicate a significant difference between a treatment and the control group within a season. An asterisk denotes a significant difference across seasons for the same treatment. ANOVA results are shown in Table 4.

Plasma variable	Season and treatment									
	Summer					Winter				
	C	S	A	T	T + pH	C	S	A	T	T + pH
Na <sup>+</sup> (mmol/L)	174.3 ±3.5 (11)	174.7 ±4.6 (6)	174.8 ±3.7 (8)	184.0 ±5.5 (7)	165.1 ±8.7 (7)	193.9 ±8.6 (8)	210.7 ±8.1 (8)	211.6 ±9.3 (8)	231.9 ±13.4 (7)	220.4 ±9.1 (8)
K <sup>+</sup> (mmol/L)	5.54± 0.72 (11)	4.63± 0.54 (6)	6.1± 0.65 (8)	5.24± 0.19 (7)	5.55± 0.61 (7)	4.7± 0.46 (8)	4.22± 0.36 (7)	3.99± 0.52 (8)	6.13± 0.25 (7)	4.87± 0.36 (8)

Table 3 cont.

Cl <sup>-</sup> (mmol/L)	<b>154.8</b> <sup>a</sup> <b>±3.6</b> <b>(11)</b>	<b>183.9</b> <sup>b</sup> <b>±4.1</b> <b>(7)</b>	<b>206.3</b> <sup>b*</sup> <b>±8.</b> <b>4(8)</b>	<b>195.4<sup>b</sup></b> <b>±8.9</b> <b>(7)</b>	<b>191.1<sup>b*</sup></b> <b>±3.3</b> <b>(8)</b>	158.8 ±1.7 (8)	179.1± 6.9 (8)	172.3± 9.8 (8)	168.9± 5.1 (8)	160.6± 2.5 (8)
Ca <sup>2+</sup> (mmol/L)	10.8± 0.56 (10)	12.3± 0.35 (6)	10.6± 0.8 (8)	<b>12.4<sup>*</sup>±</b> <b>0.67</b> <b>(4)</b>	11.8± 0.11 (6)	<b>10.8<sup>a</sup>±</b> <b>0.37</b> <b>(8)</b>	<b>10.4<sup>a</sup>±</b> <b>0.39</b> <b>(8)</b>	<b>10.9<sup>a</sup>±</b> <b>0.75</b> <b>(8)</b>	<b>7.8<sup>b</sup>±</b> <b>0.85</b> <b>(7)</b>	<b>9.8<sup>a</sup>±</b> <b>0.45</b> <b>(8)</b>
Cortisol (ng/ml)	25.1± 4.7 (11)	35.8± 5.3 (7)	37.0± 11.1 (8)	50.3± 4.7 (4)	35.4± 5.7 (7)	9.3± 0.9 (8)	17.8± 3.0 (8)	15.5± 2.2 (8)	10.7± 1.8 (7)	13.1± 2.9 (7)
Lactate (mmol/L)	<b>0.29<sup>a</sup></b> <b>±0.08</b> <b>(9)</b>	<b>0.40<sup>a</sup></b> <b>±0.13</b> <b>(6)</b>	<b>1.5<sup>b*</sup></b> <b>±0.3</b> <b>(8)</b>	<b>0.35<sup>a</sup></b> <b>±0.03</b> <b>(3)</b>	<b>0.97<sup>a</sup></b> <b>±0.24</b> <b>(6)</b>	<b>0.21<sup>a</sup>±</b> <b>0.07</b> <b>(8)</b>	<b>0.22<sup>a</sup>±</b> <b>0.11</b> <b>(8)</b>	<b>0.07<sup>a</sup>±</b> <b>0.05</b> <b>(8)</b>	<b>1.4<sup>b</sup>±</b> <b>0.29</b> <b>(7)</b>	<b>0.60<sup>a</sup>±</b> <b>0.18</b> <b>(8)</b>
Glucose (mmol/L)	<b>3.8<sup>a</sup>±</b> <b>0.39</b> <b>(11)</b>	<b>4.0<sup>a</sup></b> <b>±0.21</b> <b>(7)</b>	<b>3.1<sup>a</sup></b> <b>±0.39</b> <b>(8)</b>	<b>7.6<sup>b*</sup></b> <b>±0.68</b> <b>(8)</b>	<b>5.5<sup>a</sup></b> <b>±0.76</b> <b>(8)</b>	3.4± 0.17 (8)	3.2± 0.40 (8)	3.4± 0.30 (8)	4.5± 0.20 (7)	4.40± 0.37 (8)
Hematocrit (PCV)	0.25± 0.01 (11)	0.31± 0.04 (7)	0.36± 0.05 (5)	0.38± 0.02 (8)	0.33± 0.02 (7)	0.22± 0.01 (8)	0.28± 0.02 (8)	0.24± 0.01 (8)	0.27± 0.01 (6)	0.29± 0.03 (8)

Table 4. Results of a two-way ANOVA, with treatment, season, and the treatment  $\times$  season interaction as effects, comparing the physiological response of bonefish to five treatments in two seasons. Data tested by ANOVA are presented in Table 3.

	Treatment			Season			Treatment $\times$ Season		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Plasma concentrations									
Na <sup>+</sup>	2.43	4	0.06	<b>61.96</b>	<b>1</b>	<b>&lt;0.0001</b>	1.61	4	0.18
K <sup>+</sup>	1.24	4	0.30	3.45	1	0.07	1.99	4	0.11
Cl <sup>-</sup>	<b>9.47</b>	<b>4</b>	<b>&lt;0.0001</b>	<b>23.54</b>	<b>1</b>	<b>&lt;0.0001</b>	<b>4.26</b>	<b>4</b>	<b>0.0038</b>
Ca <sup>2+</sup>	0.98	4	0.43	<b>17.93</b>	<b>1</b>	<b>&lt;0.0001</b>	<b>4.53</b>	<b>4</b>	<b>0.0028</b>
Cortisol	1.79	4	0.14	<b>46.92</b>	<b>1</b>	<b>&lt;0.0001</b>	1.23	4	0.30
Lactate	<b>4.99</b>	<b>4</b>	<b>0.0015</b>	2.6	1	0.1114	<b>9.63</b>	<b>4</b>	<b>&lt;0.0001</b>
Glucose	<b>14.10</b>	<b>4</b>	<b>&lt;0.0001</b>	<b>13.72</b>	<b>1</b>	<b>0.0004</b>	<b>4.0</b>	<b>4</b>	<b>0.0056</b>
Hematocrit	<b>5.17</b>	<b>4</b>	<b>0.0011</b>	<b>22.15</b>	<b>1</b>	<b>&lt;0.0001</b>	2.20	4	0.08

Table 5. Concentrations of different plasma constituents for checkered puffer following a suite of environmental challenges applied in the summer and the winter. The different challenges applied were control (C), an increase in salinity (S), a decrease in pH (A), an increase in temperature (T) and a combination of increased temperature and reduced pH (T + pH). Checkered puffer were held in these treatments for 6 h prior to blood sampling. No significant differences were observed between treatments and the control group within a season. Similarly, no significant differences were observed across seasons for the same treatment. ANOVA results are shown in Table 6.

Plasma variable	Season and treatment									
	Summer					Winter				
	C	S	A	T	T + pH	C	S	A	T	T + pH
Na <sup>+</sup> (mmol/L)	165.6 ±5.32 (8)	172.4 ±2.73 (6)	166.0 ±2.92 (6)	180.8 ±2.98 (6)	181.1 ±2.23 (4)	190.8 ±12.99 (7)	191.2 ±9.70 (8)	192.0 ±10.3 (8)	195.0 ±16.0 (6)	178.1 ±10.4 (6)
K <sup>+</sup> (mmol/L)	4.15 ±0.24 (8)	4.02 ±0.46 (6)	3.32 ±0.20 (6)	4.51 ±0.47 (6)	4.56 ±0.32 (4)	5.48 ±0.55 (7)	5.09 ±0.39 (8)	4.50 ±0.32 (8)	5.3 ±0.58 (6)	5.28 ±0.79 (5)
Cl <sup>-</sup> (mmol/L)	138.4 ±5.97 (8)	141.9 ±3.24 (6)	142.6 ±4.8 (7)	151.8 ±6.52 (6)	156.1 ±6.67 (4)	159.8 ±6.04 (6)	178.6 ±11.8 (7)	159.4 ±2.51 (7)	167.3 ±8.17 (6)	168.8 ±8.91 (6)

Table 5 cont.

Ca <sup>2+</sup> (mmol/L)	10.13 ±0.58 (8)	9.82 ±0.99 (7)	10.5 ±1.79 (6)	10.2 ±1.15 (4)	12.5 ±0.70 (3)	11.4 ±0.66 (6)	10.3 ±0.70 (7)	10.3 ±0.31 (7)	11.3 ±0.26 (6)	10.8± 0.56 (6)
Cortisol (ng/ml)	19.9 ±3.49 (6)	5.03 ±1.7 (5)	12.4 ±2.52 (6)	59.3 ±21.1 (3)	27.3 ±9.80 (4)	3.25 ±0.86 (5)	4.82 ±2.17 (3)	2.44 ±0.85 (6)	22.1 ±6.90 (6)	12.0 ±2.14 (5)
Lactate (mmol/L)	0.19 ±0.19 (7)	0.0 ±0.0 (4)	0.0 ±0.0 (6)	0.02 ±0.01 (4)	0.02 ±0.02 (3)	0.08 ±0.05 (6)	0.09 ±0.02 (8)	0.07 ±0.04 (8)	0.19 ±0.04 (6)	0.07 ±0.04 (6)
Glucose (mmol/L)	1.37 ±0.19 (8)	1.93 ±0.46 (7)	1.59 ±0.18 (5)	2.91 ±0.43 (7)	2.27 ±0.21 (6)	1.48 ±0.15 (7)	1.59 ±0.26 (7)	1.30 ±0.18 (5)	2.2 ±0.47 (6)	1.69 ±0.16 (6)
Hematocrit (PCV)	0.22 ±0.01 (8)	0.23 ±0.01 (8)	0.28 ±0.05 (6)	0.31 ±0.02 (8)	0.27 ±0.02 (6)	0.13 ±0.02 (7)	0.17 ±0.01 (7)	0.20 ±0.02 (8)	0.22 ±0.03 (7)	0.28 ±0.02 (6)

Table 6. Results of a two-way ANOVA, with treatment, season, and the treatment  $\times$  season interaction as effects, comparing the physiological response of checkered puffers to five treatments in two seasons. Data tested by ANOVA are presented in Table 5.

	Treatment			Season			Treatment $\times$ Season		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Plasma concentrations									
Na <sup>+</sup>	0.34	4	0.85	<b>7.16</b>	<b>1</b>	<b>0.0098</b>	0.66	4	0.62
K <sup>+</sup>	2.01	4	0.11	<b>13.79</b>	<b>1</b>	<b>0.0005</b>	0.17	4	0.95
Cl <sup>-</sup>	1.42	4	0.24	<b>20.52</b>	<b>1</b>	<b>&lt;0.0001</b>	0.88	4	0.48
Ca <sup>2+</sup>	1.23	4	0.31	0.15	1	0.70	1.08	4	0.37
Cortisol	<b>11.38</b>	<b>4</b>	<b>&lt;0.0001</b>	<b>18.68</b>	<b>1</b>	<b>&lt;0.0001</b>	2.39	4	0.07
Lactate	0.67	4	0.62	1.03	1	0.32	0.78	4	0.54
Glucose	<b>4.62</b>	<b>4</b>	<b>0.0028</b>	3.39	1	0.07	0.57	4	0.69
Hematocrit	<b>8.98</b>	<b>4</b>	<b>&lt;0.0001</b>	<b>23.53</b>	<b>1</b>	<b>&lt;0.0001</b>	1.55	4	0.20

Table 7. Concentrations of different plasma constituents for yellowfin mojarra following a suite of environmental challenges applied in the summer and the winter. The different challenges applied were control (C), an increase in salinity (S), a decrease in pH (A), an increase in temperature (T) and a combination of increased temperature and reduced pH (T + pH). Yellowfin mojarra were held in these treatments for 6 h prior to blood sampling. No significant differences were observed between treatments and the control group within a season. Similarly, no significant differences were observed across seasons for the same treatment. ANOVA results are shown in Table 8.

Plasma variables	Season and treatment									
	Summer					Winter				
	C	S	A	T	T + pH	C	S	A	T	T + pH
Na <sup>+</sup> (mmol/L)	172.4 ±2.27 (8)	178.9 ±5.74 (5)	182.5 ±5.85 (7)	180.6 ±2.27 (7)	191.2 ±3.30 (8)	186.0 ±9.67 (11)	181.2 ±5.75 (6)	189.2 ±5.94 (10)	175.3 ±12.9 (6)	181.0 ±4.17 (7)
K <sup>+</sup> (mmol/L)	5.83 ±0.35 (8)	5.51 ±0.45 (5)	4.25 ±0.32 (7)	6.32 ±0.83 (7)	5.31 ±0.20 (8)	3.19 ±0.68 (10)	3.59 ±0.68 (6)	3.90 ±0.57 (10)	4.24 ±0.33 (6)	4.36 ±0.45 (7)
Cl <sup>-</sup> (mmol/L)	144.8 ±2.63 (8)	171.2 ±8.14 (5)	162.6 ±10.1 (7)	156.5 ±6.64 (7)	150.7 ±2.59 (8)	168.5± 9.07 (11)	153.4± 6.23 (6)	165.3 ±7.12 (10)	163.2 ±8.32 (6)	171.4 ±3.87 (7)

Table 7 cont.

Ca <sup>2+</sup> (mmol/L)	9.0 ±0.96 (5)	8.97 ±0.74 (6)	9.59 ±0.56 (8)	9.65 ±0.31 (6)	11.4 ±0.66 (7)	13.22 ±1.50 (11)	11.48± 0.71 (6)	11.88 ±0.61 (10)	11.9 ±0.91 (6)	12.1 ±0.92 (7)
Cortisol (ng/ml)	43.4 ±31.8 (7)	26.4 ±6.47 (7)	29.3 ±18.1 (6)	417.6 ±56.8 (6)	21.0 ±11.8 (8)	62.75 ±21.4 (11)	33.6 ±13.3 (6)	27.79 ±11.3 (10)	53.83 ±17.94 (6)	37.4 ±8.18 (7)
Lactate (mmol/L)	0.31 ±0.12 (7)	0.06 ±0.04 (5)	0.48 ±0.41 (7)	0.36 ±0.12 (8)	0.22 ±0.13 (7)	0.04 ±0.03 (11)	0.09 ±0.06 (6)	0.20 ±0.11 (10)	0.23 ±0.11 (6)	0.26 ±0.08 (7)
Glucose (mmol/L)	3.35 ±0.21 (8)	2.88 ±0.39 (7)	3.56 ±0.23 (8)	3.68 ±0.25 (8)	3.36 ±0.21 (8)	4.05 ±0.60 (11)	3.77 ±0.63 (6)	3.68 ±0.51 (9)	4.54 ±0.42 (6)	4.5 ±0.26 (7)
Hematocrit (PCV)	0.21 ±0.02 (8)	0.21 ±0.02 (6)	0.20 ±0.02 (8)	0.28 ±0.01 (7)	0.29 ±0.01 (8)	0.24 ±0.02 (11)	0.24 ±0.008 (7)	0.23 ±0.02 (10)	0.25 ±0.02 (6)	0.29 ±0.02 (7)

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Table 8. Results of a two-way ANOVA, with treatment, season, and the treatment  $\times$  season interaction as effects, comparing the physiological response of yellowfin mojarra to five treatments in two seasons. Data tested by ANOVA are presented in Table 7.

	Treatment			Season			Treatment $\times$ Season		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Plasma concentrations									
Na <sup>+</sup>	0.62	4	0.65	0.10	1	0.75	1.0	4	0.41
K <sup>+</sup>	1.31	4	0.28	<b>20.21</b>	<b>1</b>	<b>&lt;0.0001</b>	1.31	4	0.28
Cl <sup>-</sup>	0.33	4	0.86	2.33	1	0.13	2.30	4	0.07
Ca <sup>2+</sup>	0.58	4	0.68	<b>14.08</b>	<b>1</b>	<b>0.0004</b>	0.77	4	0.55
Cortisol	0.78	4	0.54	1.55	1	0.22	0.21	4	0.93
Lactate	0.84	4	0.50	1.53	1	0.22	0.50	4	0.74
Glucose	0.89	4	0.47	<b>7.49</b>	<b>1</b>	<b>0.0079</b>	0.43	4	0.78
Hematocrit	<b>6.58</b>	<b>4</b>	<b>0.0002</b>	1.07	1	0.31	1.02	4	0.40

## CHAPTER 3: STAYING ALIVE IN A CHANGING WORLD: METABOLIC COSTS AND SWIMMING PERFORMANCE OF SUBTROPICAL NEARSHORE FISHES

### Abstract

Global climate change projections for the oceans indicate that temperature, CO<sub>2</sub>, and salinity will increase subtropical zones over the next century, yet very little information exists on the performance of nearshore fishes under these altered conditions. The objective of this experiment was to define the performance of fishes from nearshore, subtropical ecosystems acclimated to climate change scenarios, and relate findings to ecology of these ecosystems in the future. To accomplish this task, fishes were acclimated to ambient conditions, and elevated pCO<sub>2</sub>, salinity, and temperatures that coincide with, or exceed predictions made by the Intergovernmental Panel on Climate Change (IPCC) for the next 100 years. Post acclimation, the performance of nearshore fishes was determined by measuring standard metabolic rate (SMR) using intermittent-flow, static respirometry, and by chasing fish until exhaustion (i.e., swimming performance (SP)). Results show that elevated temperature 1 °C above IPCC predictions (i.e., 34 °C) more than doubled SMR and decreased SP by approximately 1 minute for nearshore fishes. Nearshore fishes experienced minimal disturbance in performance at elevated pCO<sub>2</sub> or salinity concentrations. Collectively, conditions that coincide with, or exceed the predicted worst-case scenarios reduced the performance of fishes in the nearshore ecosystem, particularly when fish were exposed to elevated temperatures. To avoid costs associated with elevated temperatures, nearshore fishes may choose to migrate to more thermally stable environments, allocate energy differently, or increase feeding rates to meet energetic demands. The net effect will be a slow restructuring of the fish community in the nearshore environment as the climate changes.

## Introduction

Global climate change is expected to alter the chemistry of the oceans by changing the evaporation/precipitation cycle, increasing pCO<sub>2</sub> (the partial pressure of free CO<sub>2</sub> dissolved in sea water), and increasing sea surface temperatures (Stott *et al.*, 2008; IPCC, 2013). The Intergovernmental Panel on Climate Change (IPCC) predicts that sea surface pH will decrease by 0.06 - 0.32 pH units and temperature will increase by 0.6-2.0°C over the next 100 years (IPCC, 2013). In addition, the salinity of the ocean is expected to increase in subtropical regions as a result of locally reduced precipitation as the planet warms (Gilman *et al.*, 2008). Together, human-induced changes to the global climate are forecasted to alter current abiotic conditions in the ocean with the potential to disrupt biotic systems.

Global climate change is also expected to have negative consequences across multiple levels of biological organization (Helmuth, 2009), with many such changes having been experienced at present. At the ecosystem level, warming seas due to climate change have recently resulted in a number of biotic changes such as the loss of coral reefs and a poleward shift in the distribution of exploited and nonexploited marine fish (Perry *et al.*, 2005; Brierley & Kingsford, 2009). At the individual level, the biogeographic distribution of fish is constricted by species-specific thermal limits and the avoidance of other unfavorable conditions (e.g., hypoxic environments) (Deutsch *et al.*, 2015). As the climate changes, species with narrow thermal limits that inhabit niches close to their thermal maximum, such as polar stenotherms and warmly adapted species in the tropics, will likely be the first species to experience a decrease in performance (e.g., reduced swimming ability, elevated maintenance costs) and possibly fitness (Pörtner & Farrell, 2008).

When juxtaposed to relatively stable coral reef systems (Nilsson *et al.*, 2009), subtropical nearshore ecosystems (< 2m deep) represent dynamic environments that change seasonally, and with ebbing and flooding of the tide. For example, tidal creek systems in The Bahamas show considerable variability in temperature (11 – 43°C), pCO<sub>2</sub> (385 to 750 µatm), and salinity (17.2 – 43.2ppt) (Borges *et al.*, 2003; Shultz *et al.*, 2014). Several studies have demonstrated that fish have developed adaptations to maintain homeostasis in variable environments (e.g., increased bicarbonate levels in the blood under elevated CO<sub>2</sub> conditions (Baker *et al.*, 2009)), yet little information on performance metrics exist for fish that inhabit critical nursery habitats such as nearshore subtropical marine environments, making it challenging to define how fish residing in these dynamic environments will respond to future changes in ocean conditions. Although debated in the literature, recent evidence suggests that fish from dynamic environments may be more susceptible to climate change (Seebacher *et al.*, 2014). Moreover, very few studies focus on more than one species, which hinders our ability to predict how fish communities and ecosystems will respond to future climate change (Pfister *et al.*, 2014).

The objective of this study was to define the performance of fishes from nearshore, subtropical ecosystems acclimated to climate change scenarios, and relate findings to biogeography and ecology of subtropical ecosystems in the future. Specifically, we examined adult checkered puffer, *Sphoeroides testudineus*, juvenile yellowfin mojarra, *Gerres cinereus*, and juvenile schoolmaster snapper, *Lutjanus apodus*, as these three species are confined to the nearshore environment during these life stages. Additionally, we examined adult bonefish, *Albula vulpes*, which migrate with the tides between the nearshore environment and deeper water. Collectively, these species are commonly found throughout the subtropical Western Atlantic and will provide a broad view on how a number of nearshore fishes will respond to multiple climate

change scenarios. Outcomes from this study will have implications for potential climate-driven changes at the individual level (i.e., growth, reproduction, and predator avoidance), and, more importantly, at the ecosystem level (i.e., the structure of marine food webs).

## **Methods**

### *Fish collection*

This study was conducted at the Cape Eleuthera Institute (CEI) on the island of Eleuthera, The Bahamas (24°50'05" N; 76°20'32" W). Bonefish, checkered puffer, juvenile yellowfin mojarra, and juvenile schoolmaster snapper were all collected from a number of tidal creeks by deploying a seine net (13 mm mesh, 46 m long; 32 mm mesh, 76 m long; 70 mm mesh, 61m long) at the mouth of each creek and waiting for the fish to exit the system on an outgoing tide (Table 9). Schoolmaster snapper were also collected by baiting the water and then throwing a cast net over them, or by conventional hook-and-line angling. All fish were then transferred to coolers on a boat for transportation back to the CEI seawater facility. Water changes were performed periodically (approximately every 5 minutes) en route to the facility and fish were immediately transferred to large, aerated holding tanks (3.7 m diameter × 1.25 m height, 13,180 L) supplied with filtered seawater pumped from the ocean at a rate of approximately 1800L/h (Murchie *et al.*, 2009). During holding, fish were fed daily to satiation with both frozen sardines *Sardenella aurita* and pelletized fish feed, but starved for 48 hours prior to experimentation. All research was conducted in accordance with the policies of the University of Illinois Institutional Animal Care and Use Committee (Protocol 09160).

### *Acclimation*

Nearshore fishes were collected from local tidal creeks, transported to the Cape Eleuthera Institute's seawater facility, and acclimated in aerated treatment tanks (1.44 m diameter × 0.86 m height, 1,399 L) supplied with filtered seawater to a range of abiotic conditions that coincide with, or exceed, predictions made by the IPCC for the next 100 years. Conditions were gradually manipulated over a 24 hour period, and fish were held at these novel conditions for 7-11 days – a period of time shown to induce adjustment of physiological systems following exposure to novel aquatic conditions (Munday *et al.*, 2009). Fish in the control group were held in identical aerated tanks supplied with ambient seawater for 7-11 days. Seawater was exchanged daily for fish in the salinity treatments, and flow-through seawater systems were designed for the temperature and CO<sub>2</sub> treatments to control the buildup of waste.

To mimic future ocean acidification scenarios, CO<sub>2</sub> was diffused into the treatment tank's water and regulated continuously by an automated pH controller/monitor connected to an electronic solenoid valve (Milwaukee, SMS122 pH controller, Rocky Mount, NC) (Munday *et al.*, 2009). The pH of seawater was cross-validated with a pH pen (YSI pH10a, Yellow Springs, Ohio; accuracy pH ± 0.01) and both the pH controller/monitor and pen were calibrated on a regular basis (Moran, 2014). Fish were maintained at the following endpoints: pCO<sub>2</sub> =150 (i.e., ambient conditions – controls), 400, 700, 1000, and 2400 µatm, which represent, respectively, the lowest CO<sub>2</sub> level that commonly occurs in the nearshore system over a 24 hour period, the maximum CO<sub>2</sub> level in a nearshore system, the worst case scenario for CO<sub>2</sub> levels in the year 2100 as predicted by the IPCC (increase in pCO<sub>2</sub> of ~250 µatm), and a maximum CO<sub>2</sub> level in a nearshore system plus levels that slightly exceed the worst-case scenario in 2300 (Borges *et al.*, 2003; IPCC, 2013).

For temperature treatments, immersion heaters (Process Tech Heaters #H18T, 1,800 W, 115 v, 15 amps; Process Tech Heater Controller #NA30DX; Aquatic Ecosystems, Apopka, FL, USA) were used to increase water temperature by  $1.0 \pm 0.05^\circ\text{C}$  per hour up to target temperatures of  $33$  and  $34^\circ\text{C} \pm 1^\circ\text{C}$ , which represent the mean summer temperature ( $\sim 31^\circ\text{C}$ ) in the nearshore environment plus the worst case scenario defined by the IPCC ( $\sim 2^\circ\text{C}$ ), and an average summer temperature in the nearshore environment plus an increase that slightly exceeds the worst-case scenario predicted by the IPCC (IPCC, 2013; Shultz *et al.*, 2014).

For the salinity acclimation, salinity was increased by  $1.0 \pm 0.01$  ppt per hour by adding salt (Instant Ocean sea salt, Instant Ocean, Blacksburg, VA, USA) to the holding tank while being monitored with a hand-held salinity meter (YSI 55, Yellow Springs Incorporated, Yellow Springs, OH) up to a target salinity level. Predictions for future salinity concentrations in the subtropics only indicate a qualitative increase in salinity due to an increase in evaporation and a decrease in precipitation (IPCC, 2013), making a specific target salinity difficult to define. Salinity treatment levels of  $40$ ,  $42$ , and  $44$  ppt  $\pm 0.1$  ppt were chosen because these values present-day salinity concentrations in the nearshore environment during the dry season ( $41.5$  ppt) plus slightly saltier conditions that may occur in the future (Shultz *et al.*, 2014).

Fish used as controls were held in a tank supplied with ambient seawater (average =  $8.09$  pH, range  $8.22$ - $7.96$  pH;  $\text{pCO}_2$   $156.1 \pm 14.2$ ; mean  $\pm$  SE; average salinity =  $35.1$  ppt, range  $32.3$ - $38.7$  ppt), handled in a manner identical to animals in other groups, and these controls were used in all experimental treatments. Fish served as controls for the temperature treatment during the summer when ambient seawater temperatures reached their maximum (average =  $29.5^\circ\text{C}$ ; range  $27.7$ - $31.2^\circ\text{C}$ ).

### *Metabolic rate*

The effects of increased temperature, pCO<sub>2</sub> and salinity on standard metabolic rate (SMR) were determined using intermittent-flow, static respirometry. SMR represents a quantitative measure of the total activity of all physiological mechanisms, which includes maintenance costs (ion regulation, heart rate, opercular beats, etc.) when fish are not digesting food and are resting, and stressful conditions (e.g., an increase in temperature and/or CO<sub>2</sub> levels) typically results in an increase in SMR as the animal expends additional energy to maintain homeostasis (Pörtner, 2010 but see Rummer *et al.*, 2013a). Following acclimation, fish were netted from their tank, weighed  $\pm 1.0$  g (Super 6 water-proof counting scale Jinan Jinzhong Electronic Scale Co.) and their volume (ml) measured by water displacement prior to being inserted into a respirometry chamber. Seawater conditions in the respirometry tank matched the conditions of the seawater in the acclimation tanks. The methods for measuring resting O<sub>2</sub> consumption followed the protocols described by Shultz *et al.* (2011). The respirometer consisted of four individual plexiglass/glass chambers resting in a tank (3.09 m length  $\times$  0.65 m width  $\times$  0.17 m height) filled with aerated seawater. The internal diameter and total water volume of the chambers used for each species varied according to the species' size and shape. Each chamber was outfitted with a fiber optic dissolved oxygen probe allowing for simultaneous monitoring of oxygen consumption from four animals (Loligo INC., Hobro, Denmark). Each chamber was connected to two aquarium pumps (Eheim 1046A, 5 L/min), one for re-circulating water within the chamber, and one for flushing oxygenated water into the chamber. Oxygen consumption for the fish was measured in each chamber with cycles that consisted of a measurement phase during the recirculation period, a flush period to replace water in each chamber, and a 1 min wait period following each flushing prior to commencing measurements.



During each measurement period, water from the chambers was continually re-circulated over the fiber optic oxygen probes to ensure adequate mixing and dissolved oxygen concentrations were recorded within the chamber every 2 seconds. The change in oxygen concentration ( $\alpha$ ) for each chamber was calculated as the slope ( $\Delta O_{2\text{saturation}}/\Delta t$ ), and the oxygen consumption rate ( $MO_2$ ,  $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ) for each fish was calculated as

$$MO_2 = \alpha V_{\text{resp}} \beta M_b^{-1}$$

where  $V_{\text{resp}}$  is the volume of each chamber minus the volume of the fish (L),  $\beta$  is oxygen solubility (adjusted daily for both temperature, salinity, and barometric pressure), and  $M_b$  is the fish mass (kg) measured before placing the fish in the respirometer chamber (Steffensen, 1989). The coefficient of determination ( $r^2$ ) for all slope measurements was  $> 0.95$  for each fish, during each trial. All calculated dissolved oxygen values were corrected for background oxygen consumptions generated for each specific fish and chamber, and data were recorded with AutoResp software (Version 1.4, Schurmann & Steffensen, 1997). The sides and top of the respirometry tank were covered to limit visual disturbance to fish in the chambers, however, this cover allowed light penetration for the fish to experience normal light cycles (day and night cycles). The chambers were closed, and  $O_2$  levels were continuously recorded overnight. Standard metabolic rate (SMR) values were calculated as the average of the six lowest values recorded between 21:00 and 6:00 (Schurmann & Steffensen, 1997).

### *Swimming performance*

Fish must expend energy beyond maintenance costs when foraging, reproducing and avoiding predators, and stressful conditions can decrease the ability of fish to perform these activities by affecting their SP (Castro-Santos, 2011). To quantify a fish's SP after being exposed to future climate change scenarios, fish were removed from their respirometry chamber and transferred to a smaller circular tank (1.6 m diameter  $\times$  0.5 m height; 500 L) containing seawater that matched treatment conditions. Approximately 30 seconds after a fish was placed in the tank, a swimming challenge commenced whereby the fish was chased by hand around the tank by tail pinching until they no longer responded (did not attempt to swim away) to two consecutive gentle tail pinches, signifying exhaustion (Mckenzie *et al.*, 1996). This technique has been successful in detecting differences in SP between treatment groups of subtropical fish (Szekeres *et al.*, 2014). The time required for fish to become fatigued was measured using a stopwatch to the nearest second. Following recovery, fish were released back into the ocean.

### *Data analysis*

The partial pressure of CO<sub>2</sub> (pCO<sub>2</sub>,  $\mu$ atm) in seawater for ocean acidification treatments was calculated in CO<sub>2</sub>calc using mean seawater conditions measured over the duration of the experiment (Robbins *et al.*, 2010), with constants from Dickson (1990) and Lueker *et al.* (2000) (Feidantsis *et al.*, 2013) (Table 10). Total alkalinity ( $1217.5 \pm 20.9 \mu\text{mol kg}^{-1}$  SW; mean  $\pm$  SE) showed little variation and was considered to be constant across sampling periods when calculating pCO<sub>2</sub> ( $\mu$ atm) (Barry *et al.*, 2010). A one-way analysis of variance (ANOVA) was used to define the effects of an increase in salinity, pCO<sub>2</sub>, or temperature on SMR and SP of each species, with each of these response variables treated as separate statistical hypotheses. Prior to running each

ANOVA, a Hartely F Max test was used to verify equal variances across treatment groups (Hartley, 1950). A Shapiro-Wilk's test was used to assess if data were normally distributed; non-normal data were log transformed to meet this assumption. When significance was identified, a Tukey's post hoc test was used to identify differences between treatment levels (Sokal & Rohlf, 1995). All means are reported  $\pm$  standard error (SE) where appropriate, all significance was assessed at  $\alpha=0.05$ , and all statistical tests were run using JMP 7.0.2 (SAS Institute, Cary, NC, USA).

## Results

### *pCO<sub>2</sub>*

Yellowfin mojarra and bonefish acclimated to elevated pCO<sub>2</sub> did not exhibit a significant change in SMR relative to fish held in ambient seawater (Figure 1a and 1d; Table 11). However, checkered puffer acclimated to 700, 1000 and 2400  $\mu\text{atm}$  pCO<sub>2</sub> for at least seven days experienced an increase of more than 45 % in SMR compared to fish maintained at ambient conditions (Figure 1c; Table 11). The SMR of schoolmaster snapper exposed to 2400  $\mu\text{atm}$  increased by 75 % relative to fish acclimated to 400  $\mu\text{atm}$ , but these SMR values were not significantly different relative to fish held in ambient conditions (Figure 1b).

The SP of yellowfin mojarra and bonefish was not affected by an increase in pCO<sub>2</sub> (Figure 1a and 1d; Table 12). Checkered puffers acclimated to 1000  $\mu\text{atm}$  experienced improved SP by more than a 1 minute relative to fish held in ambient conditions (Figure 1c). Schoolmaster snapper held in ambient seawater (140  $\mu\text{atm}$  pCO<sub>2</sub>) exercised for more than 4 minutes before exhaustion. Time until exhaustion decreased by approximately 2 minutes when schoolmaster snapper were maintained at a higher pCO<sub>2</sub> (400, 700, and 1000  $\mu\text{atm}$ ). Surprisingly, this trend did not continue for schoolmaster snapper that were acclimated to a pCO<sub>2</sub> of 2400  $\mu\text{atm}$  (Figure 1b).

### *Temperature*

SMR for yellowfin mojarra, checkered puffer, schoolmaster snapper, and bonefish increased when acclimated to higher temperatures. Specifically, yellowfin mojarra and bonefish all exhibited an increase in metabolic rate by more than 65 % when acclimated to seawater maintained at 33° C compared to ambient conditions (29.5° C) (Figure 2a and 2d; Table 11). Metabolic rates remained elevated for both species at 34° C relative to ambient conditions. Similarly, checkered puffer also experienced an increase in SMR by more than 50 % when maintained at 5° C above ambient seawater temperature in the summer (29.5°C) (Figure 2c). Schoolmaster snapper more than doubled their SMR when acclimated to 34°C relative to fish maintained at ambient conditions (Figure 2b).

The SP of yellowfin mojarra, schoolmaster snapper, checkered puffer, and bonefish all decreased following acclimation to elevated water temperatures (Figure 2a, 2b, 2c, and 2d). Yellowfin mojarra held in ambient conditions (29.5° C) swam for more than two minutes before exhaustion. Exhaustion occurred after approximately 1 minute when yellowfin mojarra were acclimated to 34° C (Figure 2a). Schoolmaster snapper held in ambient conditions (29.5° C) became exhausted after approximately 5 minutes of exercise. When acclimated to 33° C and 34°C, schoolmaster snapper became exhausted after a little more than 2 minutes of continuous exercise (Figure 2b). Checkered puffer maintained in ambient seawater (29.5° C) swam for than 4 minutes before becoming exhausted. The time until exhaustion decreased by more than 1 minute when checkered puffers were acclimated to 34°C (Figure 2c). Bonefish held in ambient seawater (29.5° C) exercised for more than 2 minutes whereas fish acclimated to 34° C exercised for approximately 1 minute (Figure 2d; Table 12).

## *Salinity*

The SMR of yellowfin mojarra acclimated to 40 and 44 ppt salinity was 40 % greater than fish held in ambient conditions (Figure 3a; Table 11). Conversely, schoolmaster snapper, checkered puffer, and bonefish did not experience a change in SMR relative to control conditions despite exposure of 7-11 days to elevated salinity (Figure 3b, 3c, and 3d; Table 11).

The SP of schoolmaster snapper decreased more than 40 % when exposed to 40 ppt relative to fish held in ambient conditions (35.1 ppt); SP returned to control levels at 42 and 44 ppt (Figure 3b). The SP of checkered puffers was reduced by more than 60 % when exposed to 44 ppt salinity relative to fish held in ambient conditions (Figure 3c). Bonefish in the 42 ppt treatment swam more than 50 % longer than fish in ambient conditions, but this trend did not continue for fish 40 ppt or 44 ppt treatments (Figure 3d). The SP of yellowfin mojarra was not affected by elevated salinity relative to controls (Figure 3a; Table 12).

## **Discussion**

The IPCC predicts future sea surface temperatures in low latitudes will increase by a maximum of 2°C, salinity will increase due to an increase in evaporation and a decrease in precipitation, and excess CO<sub>2</sub> will decrease the pH of the oceans by a maximum of 0.32 pH units (an increase in pCO<sub>2</sub> of approximately 250 µatm) in the next 100 years (IPCC, 2013). Numerous studies have indicated direct negative consequences of altered seawater conditions on the physiology and behavior of fish from abiotically stable coral reef environments (Munday *et al.*, 2009; Nilsson *et al.*, 2009; but see Rummer *et al.*, 2013a). In contrast, subtropical nearshore ecosystems, such as tidal flats and creeks, are characterized by variable conditions driven by wind,

precipitation, tides, water depth, and biotic community (Nagelkerken *et al.*, 2008). This variability in conditions for the nearshore environment makes predicting the response of nearshore organisms to climate change difficult (Pfister *et al.*, 2014), and relative to coral reef ecosystems, the physiological response and performance of nearshore fishes to future climate change scenarios is understudied.

Elevated temperatures that coincide with, or exceed, the worse-case scenario predicted by the IPCC in 100 years resulted in an increase in SMR and a reduction in SP for a number of nearshore fishes. Specifically, SMR increased by 65 % for bonefish and yellowfin mojarra at approximately 3° C above ambient conditions, and increased by more than 50 % for all nearshore fishes in this study at approximately 4° C above ambient conditions. Fishes are poikilothermic, and elevations in temperature result in a concomitant increase in metabolic rate due to an increase in oxygen demand, enzyme activity, and production of heat shock proteins (Hofmann & Todgham, 2010). This physiological response, measured as an increase in SMR, directs energy towards maintenance of bodily function at a cost to somatic growth and reproduction, which can have negative consequences at the population level (Donelson *et al.*, 2011). Coral reef fishes exposed to elevated temperatures 1-4° C above ambient experienced an increase in SMR by as much as 50 %, potentially having negative consequences on growth, reproduction, and development if this additional energetic demand is not met (Munday *et al.*, 2009; Nilsson *et al.*, 2009). Moreover, Munday *et al.* (2009) held cardinal fish (*Ostorhinchus doederleini* and *O. cyanosoma*) at 33° C (3-4° C above ambient) for 7 days, which resulted in a mortality rate of more than 33 %. In contrast, all of the mangrove fishes in the current study that were held for 7-11 at 33° C and 34° C (3-4° C above ambient) survived, indicating that fish in the nearshore environment are likely more tolerant to increases in temperature than fish from the coral reef ecosystem (Shultz *et al.*, 2014). The ability

of fish to swim is influenced by a number of factors such as the coordination of neural, muscular, and skeletal components, as well as temperature, with elevated temperature causing declines in SP due to muscle kinetics and oxygen transport capacities (Bennett, 1990). The IPCC worst-case scenario for temperature decreased SP for most, but not all nearshore fishes, and just 1° C beyond the worst case scenario predicted by the IPCC resulted in decreased SP for all species in the current study. A decrease in SP was observed in damselfish when exposed to higher temperatures of similar magnitude, which may reduce the ability of these fish to swim against the tide and wave action as well as perform ecological functions such as foraging (Johansen & Jones, 2011). Taken together, elevated seawater temperatures predicted to occur with future climate change will increase physiological costs and decrease SP in nearshore subtropical fishes.

An increase in pCO<sub>2</sub> levels that coincides with, or exceed, IPCC predictions resulted in minimal disturbances for nearshore fishes. Checkered puffers experienced an increase in SMR of 60 % when exposed to pCO<sub>2</sub> that coincide with and exceed future conditions in the nearshore environment (approximately 700, 1000, and 2400 μatm). Schoolmaster snapper also experienced an increase in SMR when exposed to levels that exceed future predictions (2400 μatm) relative to low pCO<sub>2</sub> (~400 μatm). Similar work conducted over a range of temperatures on cardinal fish, *Ostorhinchus doederleini* and *O. cyanosoma*, from the coral reef environment also noted a ~45-60 % increase in SMR when fish were exposed to a pH of 7.8 (approximately 400 μatm), and this increase was attributed to the cost associated with maintaining acid-base balance in acidified seawater (Munday *et al.*, 2009). Compensation for this acid-base imbalance is expected to redirect energy to maintaining homeostasis, reduce growth, and possibly translate to additional negative consequences on fitness (Heuer & Grosell, 2014). Only two of the four nearshore species in this study experienced these negative consequences at pCO<sub>2</sub> that are equal to or greater than projected

levels in 100 years. Interestingly, SP increased for checkered puffers acclimated to 1000  $\mu\text{atm}$  and decreased for schoolmaster snapper held at 400  $\mu\text{atm}$  and 1000  $\mu\text{atm}$ , but returned to control levels at 2400  $\mu\text{atm}$ . Elevated  $\text{pCO}_2$  may trigger biochemical changes such as an increase in extracellular buffering capacity through the uptake of bicarbonate and/or the excretion of  $\text{H}^+$  that allows for compensation for higher  $\text{pCO}_2$  (Heuer & Grosell, 2014). This compensation at the cellular level may allow for whole-organism performance metrics to remain at baseline levels under elevated  $\text{pCO}_2$ . It is not surprising that a relatively small increase in  $\text{pCO}_2$  did not have a strong impact on metabolic rate and SP of these fish given the variability of  $\text{pCO}_2$  (diel 385-750  $\mu\text{atm}$ ) in the subtropical nearshore ecosystem (Borges *et al.*, 2003), with environmental  $\text{pCO}_2$  during the rainy season likely exceeding the treatments used in this study (Chidambaram *et al.*, 2014). Together, fish in the nearshore ecosystem demonstrated a species-specific response to elevated  $\text{pCO}_2$ , with bonefish and yellowfin mojarra having a neutral SMR and SP response, and schoolmaster snapper and checkered puffers demonstrated minimal disturbance with some potential to compensate for elevated  $\text{pCO}_2$ . On the whole, elevated  $\text{pCO}_2$  due to climate change will likely have minimal influence on the performance of nearshore fishes.

Elevated salinity levels resulted in minimal disturbances in SMR and SP for fish in this study. Yellowfin mojarra was the only species that demonstrated a significant increase in SMR when exposed to higher salinities (40 and 44ppt). Saltwater fishes continually take in seawater and excrete salts (NaCl) to maintain osmotic balance (Hwang *et al.*, 2011). Yellowfin mojarra likely increased the activity levels of ion excreting pumps to cope with higher salt concentrations in seawater and, therefore, consumed more oxygen as ion pumps activity levels increased to maintain homeostasis. Maintaining homeostasis at higher salinity concentrations has been linked to higher energetic costs and slower growth rates in gray snapper *Lutjanus griseus* (Wuenschel *et*



*al.*, 2005), and may have similar consequences for yellowfin mojarra, but not the other species in this study. Bonefish was the only species that experienced an increase in SP at 42ppt, a salinity concentration slightly below present-day extremes, but this trend did not continue at 44ppt. In contrast, schoolmaster snapper and checkered puffer experienced a decrease in SP at 40 and 44 ppt, respectively, but did not experience a decrease in SP in the other salinity treatment levels, making trends difficult to interpret. Overall, a moderate increase in salinity concentrations in subtropical environments in the future will have a limited impact on the performance of nearshore fishes.

In general, the ecological response of the nearshore fish community to future climate change will be an interplay between the physiological costs associated with altered abiotic conditions and interactions with the biotic community. Results from the current series of experiments suggests that elevated temperatures will result in the greatest challenges for nearshore fishes relative to other climate-related challenges, with costs related to the maintenance of homeostasis and reductions in SP predicted to occur. As a result of these costs, there are a number of potential outcomes for nearshore subtropical ecosystems such as animals electing to change habitats or compensate behaviorally for these disturbances. For example, following an increase of 2 °C (worst-case scenario predicted by the IPCC), yellowfin mojarra and bonefish may be forced to vacate unfavorable nearshore habitats to avoid increases in metabolic rates (i.e., Bogert effect). If IPCC worst-case scenario is conservative by just 1 °C (i.e., temperature increases by 3 °C by the year 2100), then all of the nearshore fishes in this study may seek alternative habitats to avoid increases in metabolic rates and decreases SP. These alternatives include migrating to elevated latitudes (Burrows *et al.*, 2011), deep/cooler environments (Perry *et al.*, 2005), microhabitats within the nearshore ecosystem (e.g., under the canopy of mangroves) (Huey & Tewksbury, 2009),

or a combination of these options. Conversely, fishes may reside in nearshore environments, but, due to unfavorable conditions, they may need to increase feeding rates to meet elevated metabolic rates (Nowicki *et al.*, 2012), and may be more susceptible to predation (Allan *et al.*, 2015), or they may be forced to allocate energy differently, possibly away from reproduction (Donelson *et al.*, 2010). In either case, the tradeoff between the physiological costs associated with an altered abiotic environment and food web dynamics will determine ecological “winners” and “losers” under future climate change scenarios, resulting in a slow restructuring of the nearshore environment and adjacent ecosystems.

## Tables and Figures

Table 9. Length (mean  $\pm$  SE) of juvenile yellowfin mojarra, bonefish, checkered puffer, and schoolmaster snapper used in this study. Fish anatomy dictated the choice of metric used to measure each species: a fork length was generated for species that had a forked or furcate caudal fin, and a total length was taken for species with a truncate or rounded caudal fin.

Species	Total Length (mm)	Fork Length (mm)	Range (mm)
Yellowfin mojarra	NA	135 $\pm$ 2	103-175
Bonefish	NA	385 $\pm$ 3	306-454
Checkered puffer	182 $\pm$ 2	NA	136-242
Schoolmaster snapper	NA	135 $\pm$ 2	103-175

Table 10. Mean  $\pm$  SE water quality conditions used to calculate the pCO<sub>2</sub> ( $\mu$ atm) of future ocean acidification scenarios.

Target	N	pH	Temperature (°C)	Salinity (ppt)	Alkalinity ( $\mu$ mol kgSW <sup>-1</sup> )	pCO <sub>2</sub> ( $\mu$ atm)
150	39	8.15 $\pm$ 0.03	27.1 $\pm$ 0.4	37.3 $\pm$ 0.5	1217.5 $\pm$ 20.9	156.1 $\pm$ 14.2
400	15	7.78 $\pm$ 0.02	24.5 $\pm$ 0.9	42.2 $\pm$ 0.8	1217.5 $\pm$ 20.9	393.0 $\pm$ 20.6
700	9	7.56 $\pm$ 0.02	24.2 $\pm$ 1.1	41.7 $\pm$ 1.2	1217.5 $\pm$ 20.9	704.2 $\pm$ 32.5
1000	10	7.41 $\pm$ 0.03	26.0 $\pm$ 0.6	40.6 $\pm$ 1.4	1217.5 $\pm$ 20.9	1068.9 $\pm$ 63.5
2400	8	7.08 $\pm$ 0.05	25.6 $\pm$ 1.6	34.8 $\pm$ 0.2	1217.5 $\pm$ 20.9	2433.8 $\pm$ 231.0

Table 11. Results of one-way analyses of variance (ANOVAs) examining the effects of climate change stressors on the standard metabolic rate (SMR) of yellowfin mojarra, checkered puffer, schoolmaster snapper, and bonefish after exposure to the stressor for at least 7-11 days.

Variable	Source	Yellowfin Mojarra			Checkered Puffer			Schoolmaster Snapper			Bonefish		
		df	F	P	df	F	P	df	F	P	df	F	P
pCO <sub>2</sub>	Whole model	4	1.26	0.3	4	4.25	<b>0.0066</b>	3	5.49	<b>0.0051</b>	4	0.83	0.51
	Error	38			35			24			30		
	Total	42			39			26			34		
Temperature	Whole model	2	8.52	<b>0.002</b>	2	4.2	<b>0.03</b>	2	25.8	<b>0.0001</b>	2	7.16	<b>0.0040</b>
	Error	22			20			17			21		
	Total	24			22			19			23		
Salinity	Whole model	3	4.8	<b>0.009</b>	3	2.29	0.10	3	0.77	0.52	3	0.86	0.47
	Error	25			31			27			30		
	Total	28			34			30			33		

Table 12. Results of one-way analyses of variance (ANOVAs) examining the effects of climate change stressors on the swimming performance of yellowfin mojarra, checkered puffer, schoolmaster snapper, and bonefish after exposure to the stressor for at least 7-11 days.

Variable	Source	Yellowfin Mojarra			Checkered Puffer			Schoolmaster Snapper			Bonefish		
		df	F	P	df	F	P	df	F	P	df	F	P
pCO <sub>2</sub>	Whole model	4	2.4	0.07	4	3.80	<b>0.0115</b>	3	14.57	<b>&lt;0.0001</b>	4	1.18	0.3404
	Error	38			35			26			30		
	Total	42			39			29			34		
Temperature	Whole model	2	4.53	<b>0.0219</b>	2	9.37	<b>0.0013</b>	2	10.17	<b>0.0012</b>	2	5.19	<b>0.0148</b>
	Error	23			20			17			21		
	Total	25			22			19			23		
Salinity	Whole model	3	1.64	0.2	3	4.14	<b>0.0144</b>	3	5.76	<b>0.0034</b>	3	6.49	<b>0.0016</b>
	Error	25			30			28			30		
	Total	28			33			31			33		

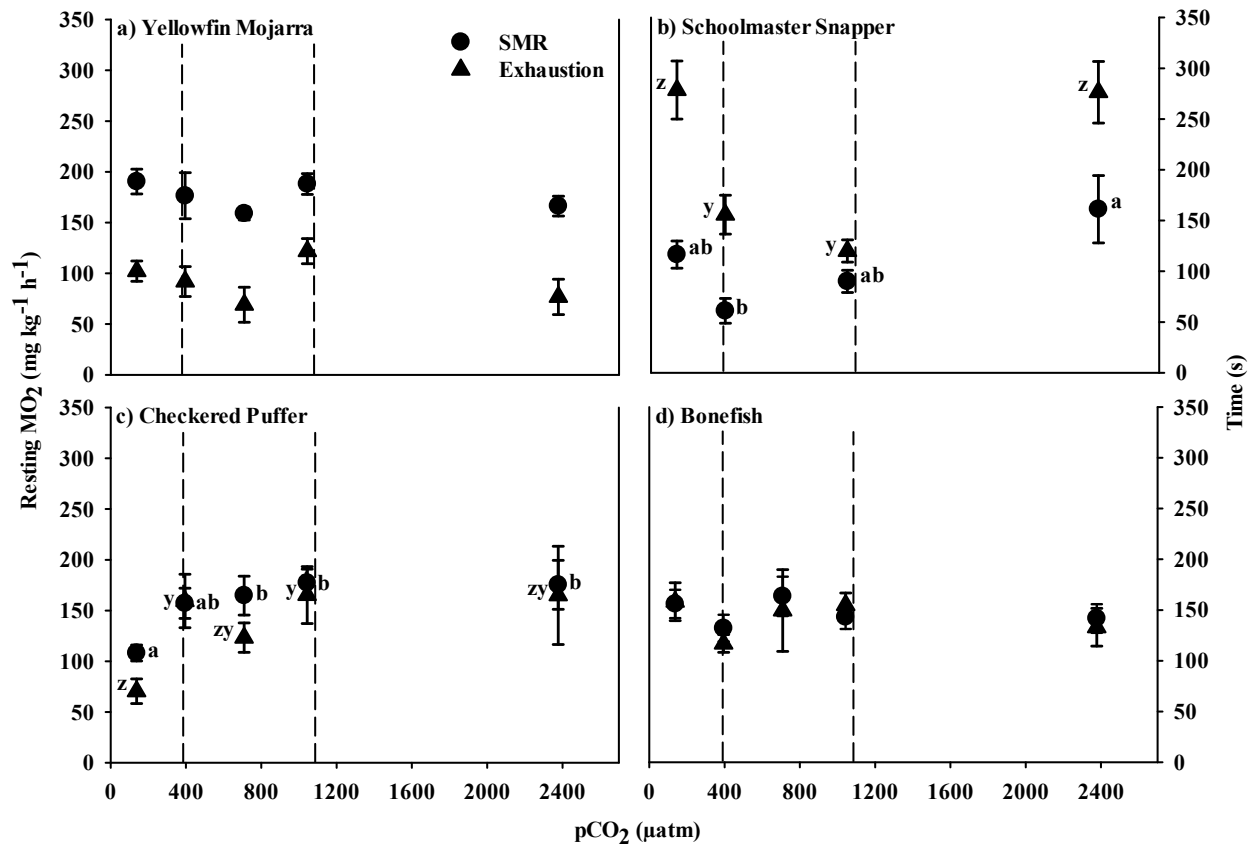


Figure 1. Resting metabolic rates and time until exhaustion of A) yellowfin mojarra (n=7-10), B) schoolmaster snapper (n=5-9), C) checkered puffer (n=6-10), and D) bonefish (n=4-9) acclimated for 7-11 days to elevated pCO<sub>2</sub> values (400, 700, 1000, and 2400 μatm). Fish maintained in ambient seawater (150 μatm pCO<sub>2</sub>) served as controls. Vertical dashed lines indicate the lowest pCO<sub>2</sub> that commonly occurs in the nearshore system over a 24 hour period and the maximum pCO<sub>2</sub> in a tidal creek system + worst case scenario predicted by the IPCC in 100 years. Symbols are shown as means ± SE. A Tukey's post hoc test was used to determine significant difference between control and treatment values. Dissimilar letters denote significant differences.

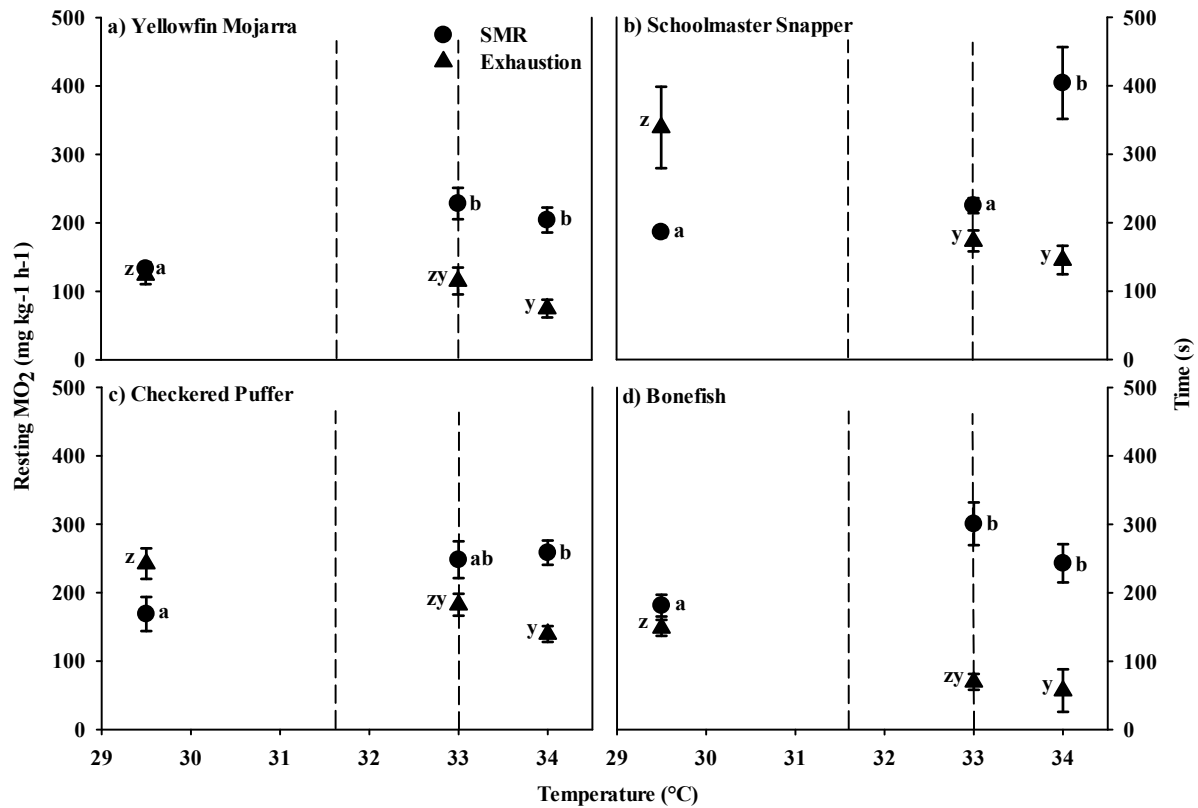


Figure 2. Resting metabolic rates and time until exhaustion of A) yellowfin mojarra (n=8-9), B) schoolmaster snapper (n=6-7), C) checkered puffer (n=8), and D) bonefish (n=6-10) acclimated for 7-11 days to higher temperatures (33 and 34°C). Fish maintained in ambient seawater (29.5°C) served as controls. Vertical dashed lines indicate the mean tidal creek temperature in the summer plus the lower and upper predictions for seawater temperatures in 100 years as predicted by the IPCC. Values represent means  $\pm$  SE. A Tukey's post hoc test was used to determine significance difference between control and treatment values. Dissimilar letters denote significant differences.



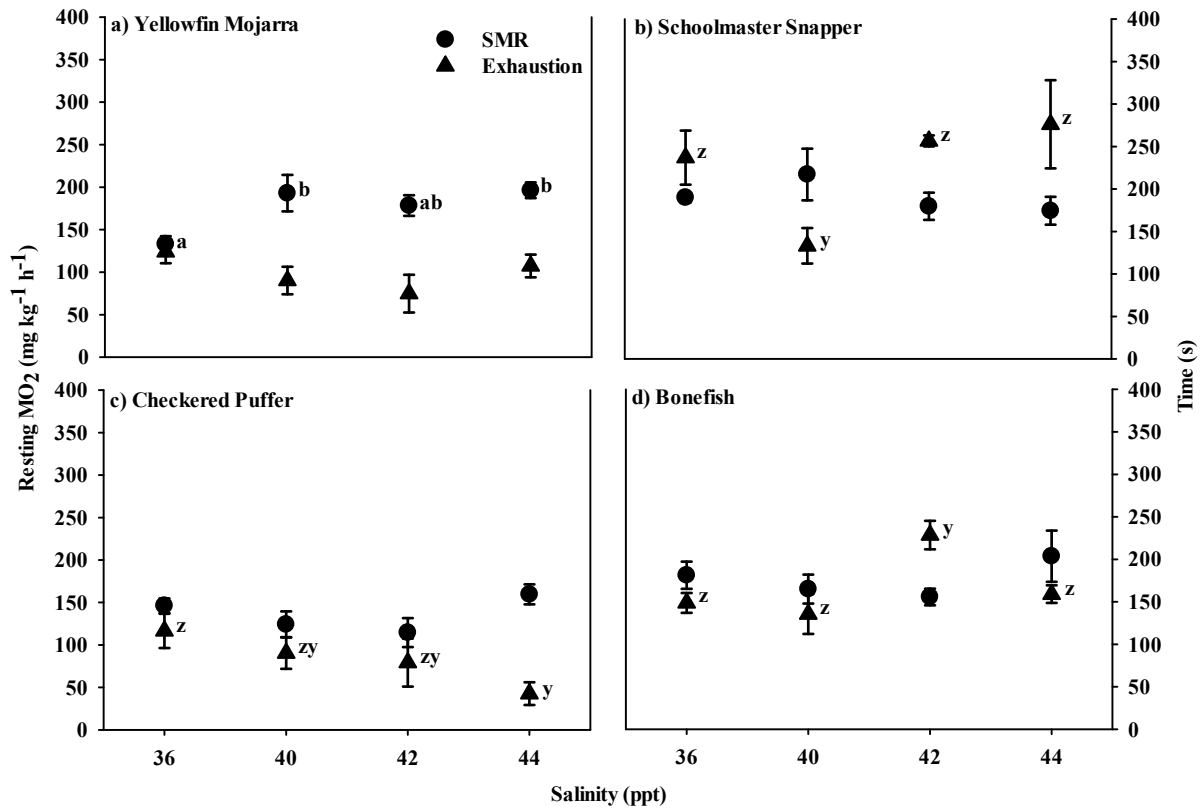


Figure 3. Resting metabolic rates and time until exhaustion of A) yellowfin mojarra (n=4-12), B) schoolmaster snapper (n=8), C) checkered puffer (n=8-11), and D) bonefish (n=8-10) acclimated for 7-11 days to higher salinity concentrations (40, 42, and 44 ppt). Fish maintained in ambient seawater (36 ppt) served as controls. Values represent means  $\pm$  SE. Dissimilar letters denote significant differences.

## CHAPTER 4: ACCLIMATIZATION AND THERMAL SAFETY MARGINS OF NEARSHORE FISHES: IMPLICATIONS FOR MARINE ECOSYSTEMS IN A CHANGING CLIMATE

### Abstract

Global climate change is predicted to increase the variability in weather patterns with more extreme weather conditions occurring on a more frequent basis. Little information exists on thermal limits of fishes from highly variable environments. This study evaluated the thermal maximum and minimum of checkered puffers, yellowfin mojarra, schoolmaster snapper, and bonefish across seasons. Thermal scope (i.e., CTMax – CTMin) of nearshore fishes ranged from 24-28.6°C across seasons, with thermal scopes typically being larger in the winter (January 1<sup>st</sup>, 2012 – March 22<sup>nd</sup>, 2012) than in the summer (June 26<sup>th</sup>, 2012 – November 9<sup>th</sup>, 2012). Acclimatization response ratios (AZRR;  $\Delta\text{CTMax } \Delta T^{-1}$  and  $\Delta\text{CTMin } \Delta T^{-1}$ ) were typically greater than 0.60 for all species, a value greater than most previously reported for fish species from variable thermal environments. Present day maximum and minimum temperatures in the nearshore environment are approximately equal to or exceed the thermal tolerance limits of the fish in this study, making thermal safety margins (TSM; i.e., the difference between thermal tolerance limit and extreme environmental temperature) very small or negative for nearshore fishes (TSM upper = -4.9-0.5; lower = -0.2-0.4). The IPCC's worse case scenario will push maximum temperatures beyond the TSM of all nearshore fish in this study. Distribution of fishes in the nearshore environment in the future will depend on available thermal refuge, cost of migrating, and foodweb interactions. Overall, the thermal landscape in the nearshore environment in the future will likely benefit species with positive thermal safety margins that are capable of

acclimatizing (e.g., schoolmaster snapper) while relatively intolerant species (e.g., bonefish) may inhabit these systems less frequently or will be absent in the future.

## **Introduction**

Global climate change due to anthropogenic sources has altered weather patterns, the physical characteristics of the oceans, and the distribution of species (Roessig *et al.*, 2005). In the next 100 years, marine temperatures are expected to increase by as much as 2°C (IPCC, 2013). Moreover, extreme weather events, such as major storms (i.e., tropical cyclones), floods, heat waves, and cold spells, are expected to increase in both intensity and frequency as the climate changes (Knutson *et al.*, 2010; Kerr, 2011). Temperature is one of the main drivers behind the distribution of ectothermic species (e.g., fish) in the ocean (Somero, 2010), and recent evidence indicates that climate change has altered the distribution and community interactions of some marine species (Perry *et al.*, 2005; Poloczanska *et al.*, 2013). For example, an extreme weather event increased seawater temperatures 3-5°C above normal for more than ten weeks along the West Coast of Australia, altering the distribution and abundance of demersal fish, sessile invertebrates, and seaweeds in habitats throughout this region (Wernberg *et al.*, 2012). On the whole, environmental temperature may exceed the physiological limits of species in the future thereby affecting their biogeographical distributions.

The proximity of animals to their thermal limits, coupled with their potential to acclimatize to future environmental conditions, will both be factors influencing the reshaping of ecosystems as the climate changes (Stillman, 2003; Somero, 2012). Ectotherms in the tropics are expected to be adapted to a relatively narrow range of temperatures due to relatively small seasonal variation in temperature, and therefore may not have the capacity to acclimatize to warming seas

(Ghalambor *et al.*, 2006). However, a recent meta-analysis indicates that the capacity to acclimatize to thermal environments in marine ectotherms is not dependent on latitude or thermal seasonality (Gunderson & Stillman, 2015). Tropical ectotherms from thermally stable environments are also suspected to be vulnerable to climate change because they live closer to their thermal limits relative to organisms in temperate regions (i.e., smaller thermal safety margins) (Pörtner and Farrell 2008; Nilsson *et al.* 2009; Pörtner and Peck 2010). However, recent evidence indicates that organisms from variable environments (e.g., intertidal zones) may be in closer proximity to their thermal limits relative to organisms from stable environments (e.g. coral reefs), making them vulnerable to temperature fluctuations associated with climate change (Madeira *et al.*, 2012; Norin *et al.*, 2014; Seebacher *et al.*, 2014). Defining which species currently live near their upper thermal limit can provide a basis for evaluating how marine ecosystems will change in the future, especially during extreme weather events, and which species will be most vulnerable to local extinction (Somero, 2010). Similarly, the ability of organisms to acclimatize (i.e., seasonal or long-term phenotypic alterations to new abiotic conditions) will play a role in buffering species against climate change (Hofmann *et al.*, 2010). Previous research on fish acclimatized to the same temperature across different seasons has demonstrated differences in metabolic rates, indicating that seasonal shifts in physiological traits can occur (Evans, 1984; Chipps *et al.*, 2000). In addition, the upper thermal tolerance of several subtropical species of fish have been determined to be significantly higher during summer compared to winter, demonstrating a seasonal component to tolerance limits for these species (Fangue & Bennett, 2003; Murchie *et al.*, 2011; Gunderson & Stillman, 2015). Species that have relatively high thermal maxima are expected to have a limited capacity to acclimatize to new conditions (Stillman, 2003; Magozzi & Calosi, 2014), but this

assumption has not been tested on species found in variable thermal environments in nearshore subtropical ecosystems.

Nearshore ecosystems provide a number of important ecosystem services such as protecting coasts, sequestering carbon, and acting as nursery areas, yet they are some of the most anthropogenically disturbed ecosystems on the planet (Valiela *et al.*, 2001; Barbier *et al.*, 2011). Nearshore habitats are characterized by dynamic abiotic conditions, such as temperature, pH, and pCO<sub>2</sub>, that fluctuate over diurnal, tidal, and seasonal scales (Lam *et al.*, 2006). Though many species of nearshore fishes demonstrate an ability to cope with these dynamic conditions (Lam *et al.*, 2006; Shultz *et al.*, 2014), it is unknown if they have evolved similar physiological limits that will allow them to cope with extreme weather patterns predicted to occur in the future due to climate change. The duration and number of heat waves and cold snaps are expected to increase as the climate changes (Kerr, 2011). For example, a record breaking sea surface temperature anomaly in the Caribbean of 29.5°C was recorded in the month of September 2010 (Trenberth & Fasullo, 2012), and a 12-day cold snap in January of 2010, Florida, USA, decreased nearshore water temperatures by 11.2°C in Butternut Key, Florida from 19.3°C on January 1<sup>st</sup> to 8.1°C on January 12<sup>th</sup> (NOAA, 2010). Considering the proximity of fish to their thermal limits across seasons, coupled with understanding the physiological plasticity of fish to abiotic variables when acclimatized to seasonal conditions, will be important tools for evaluating the response of fish to the future oceanic conditions and extreme weather events associated with climate change (Pörtner, 2002). Moreover, fish from highly variable environments, such as nearshore marine ecosystems and latitudes between 20 and 35 (i.e., subtropical regions), are underrepresented in the thermal tolerance literature (Sunday *et al.*, 2011).

Based on this background, the objective of this study was twofold: 1) determine the vulnerability of nearshore fishes to climate change, 2) evaluate the ability of these fishes to adjust their physiological limits across seasons (i.e., phenotypic plasticity). To do this, we defined the critical thermal maxima (CTMax) and minima (CTMin), and estimated thermal scope (CTMax-CTMin), acclimatization response ratios (AZRR), and thermal safety margins (TSM) of four common nearshore fishes across summer and winter seasons. Juvenile and adult life stages of different fishes coexist in this ecosystem, and therefore, both life stages were included when we evaluated vulnerability and phenotypic plasticity of the nearshore fish community to climate change. Collectively, the outcomes of this research will help improve predictions of how species, fish communities, and ultimately ecosystems, will respond to climate change.

## **Methods**

This study was conducted at The Cape Eleuthera Institute (CEI) in Eleuthera, The Bahamas (N 24°50'05" W 76°20'32"). All research conformed to the University of Illinois Institutional Animal Care and Use Committee protocol (Protocol # 09160). Fish (adult checkered puffer, *Sphoeroides testudineus* (Linnaeus, 1758), adult bonefish, *Albula vulpes* (Linnaeus, 1758), juvenile yellowfin mojarra, *Gerres cinereus* (Walbaum, 1792), and juvenile schoolmaster snapper, *Lutjanus apodus* (Walbaum, 1792)) were collected from tidal creeks in the winter (January 1<sup>st</sup>, 2012 – March 22<sup>nd</sup>, 2012) and summer (June 26<sup>th</sup>, 2012 – November 9<sup>th</sup>, 2012) by seining on an outgoing tide, transported to aerated holding tanks (3.7 m diameter × 1.25 m height, 13,180 L) supplied with fresh seawater (1800 L h<sup>-1</sup>) drawn directly from the nearshore environment at CEI, and given 48 hours to recover (Murchie et al. 2009). Fish held in the wetlab facility at CEI experienced typical day/night cycles in each season (Murchie *et al.*, 2011), and fish were not fed

during recovery or prior to experimentation. Seawater temperatures during recovery in the wetlab fluctuated daily (YSI 85, Yellow Springs Incorporated, Yellow Springs, OH) in both the winter ( $23.5 \pm 0.16^{\circ}\text{C}$ ; mean  $\pm$  SE,  $22.3 - 24.7^{\circ}\text{C}$ ; range) and summer ( $29.5 \pm 0.18^{\circ}\text{C}$ ; mean  $\pm$  SE,  $26.4 - 32^{\circ}\text{C}$ ; range) and represent typical temperatures in the nearshore environment in each season (winter:  $21.2 \pm 0.08^{\circ}\text{C}$ ; mean  $\pm$  SE,  $11 - 35.7^{\circ}\text{C}$ ; range, summer:  $30.3 \pm 0.06^{\circ}\text{C}$ ; mean  $\pm$  SE,  $23.5 - 43^{\circ}\text{C}$ ; range reported in Shultz et al. 2014). Investigating thermal tolerance of fishes exposed to fluctuating water temperatures yields ecologically relevant data for species that inhabit thermally dynamic environments (Currie *et al.*, 2004).

Following recovery, eight fish of the same species and of similar size were placed into aerated, opaque, individual plastic chambers resting in a raceway (3.09 m length  $\times$  0.65 m width  $\times$  0.17 m height) continuously supplied with recirculating seawater (Eheim pump 1046A,  $5 \text{ L min}^{-1}$ ) from a common reservoir (Igloo cooler 108 L), completing a closed water system (Vanlandeghem et al. 2010; Table 13). This system allowed for the critical tolerance limits of eight fish to be evaluated simultaneously, and species were tested sequentially in each season. The temperature of seawater in the closed water system prior to experimentation was considered the acclimatization temperature for each species in each season (Table 14). Critical thermal tolerance limits were attained by gradually increasing/decreasing the temperature until a fish experienced a loss of equilibrium for one minute (Murchie *et al.*, 2011). Changes in seawater temperature (measured with a multiparameter meter, YSI 85, Yellow Springs Incorporated, Yellow Springs, OH) were achieved using either an immersion heater (Process Tech Heaters #H18T, 1,800W, 115 volts, 15 amps; Controller #NA30DX; Aquatic Ecosystems, Apopka, FL, USA) or heat exchanger in the common reservoir at a consistent rate of  $0.18 \pm 0.02^{\circ}\text{C min}^{-1}$ ; mean  $\pm$  SE (Beitinger & Bennett, 2000; Fangue & Bennett, 2003; Murchie *et al.*, 2011).

### *Statistical analyses*

A t-test was used to quantify differences in critical maxima/minima and thermal scope (i.e., CTMax – CTMin) for each species across seasons (Sokal & Rohlf, 1995). Additionally, a t-test was used to compare breadth in tolerance for each species across seasons (i.e., the absolute value of acclimatization temperature – critical thermal tolerance limit) (Sokal & Rohlf, 1995; Duarte *et al.*, 2012), to determine which season fishes would be most at risk of exceeding their critical thermal limits. A small breadth in tolerance indicates that a species may be at risk to warmer or cooler temperatures in a season. Prior to running each t-test, a Hartely F Max test was used to verify equal variances across treatment groups (Hartley, 1950), and a Shapiro-Wilk's test was used to determine normality of data (Sokal & Rohlf, 1995). The magnitude of a species' ability to acclimatize to new thermal environments can be expressed as an acclimatization response ratio (AZRR;  $\Delta\text{CTMax } \Delta T^{-1}$  and  $\Delta\text{CTMin } \Delta T^{-1}$ ) (Claussen, 1977). These ratios were calculated across seasons to determine the relative acclimatization response of each species (Hopkin *et al.*, 2006; Reyes *et al.*, 2011), and a total AZRR score was calculated to evaluate the relative thermal plasticity of fishes within the nearshore ecosystem. Present-day thermal safety margins (TSM; summer CTMax – maximum environmental temperature and minimum environmental temperature – winter CTMin) (Deutsch *et al.*, 2008; Sunday *et al.*, 2014) were calculated for each species using extreme temperatures in the nearshore environment that these fish inhabit (summer - 43°C; winter - 11°C reported in Shultz *et al.* 2014). Positive TSM values indicate that environmental temperatures do not exceed tolerance limits. Negative values indicate present-day temperatures currently exceed tolerance limits, requiring fish to spend less time in this ecosystem and more time in thermal refugia (e.g., deeper/adjacent ecosystems) (Sunday *et al.*, 2014). All statistical analyses were performed using JMP 7.0.1 (SAS Institute Inc., 2005), all means are



reported  $\pm$  standard error (SE) where appropriate, and the level of significance for all tests ( $\alpha$ ) was 0.05.

## Results

### *Yellowfin mojarra*

The critical thermal maxima and minima of yellowfin mojarra differed significantly between seasons, and CTMax at which fish lost equilibrium was almost 5°C greater in the summer than in winter (Figure 4a; Table 15). Moreover, the upper breadth in tolerance (i.e., CTMax – acclimatization temperature) of yellowfin mojarra in summer (11°C) was approximately 2°C smaller than in winter (13°C). Conversely, CTMin for yellowfin mojarra was approximately 4°C lower in winter than in summer, and the lower breadth in tolerance (i.e., acclimatization temperature – CTMin) of fish in winter (11.2°C) was almost 4°C smaller relative to summer (15.5°C). The thermal scope (summer = 26.2°C; winter = 25.4°C) did not differ significantly between seasons for yellowfin mojarra. The maximum environmental temperature of 43°C in the nearshore environment exceeded the summer CTMax of yellowfin mojarra by 2°C resulting in a negative upper TSM. A minimum environmental temperature of 11°C was 0.2°C cooler than the winter CTMin of yellowfin mojarra resulting in a negative lower TSM. Yellowfin mojarra had an upper and lower AZRRs intermediate to other species in this study (Table 16).

### *Checkered Puffer*

The thermal scope of checkered puffers was smaller in summer (24.9°C) compared to winter (28.3°C) (Table 15). CTMax for checkered puffers was 3°C higher in summer compared to winter (Figure 4b), and the upper breadth in tolerance of checkered puffers in summer (11.2°C) was 3.5°C

smaller relative to winter (14.7°C). CTMin was 6°C lower in winter than in summer, and the lower breadth in tolerance in the summer (11°C) was 1°C smaller relative to the winter (12°C). The maximum environmental temperature of 43°C in the nearshore environment exceeded the summer CTMax of checkered puffers by more than 1°C resulting in a negative upper TSM. Checkered puffers demonstrated a winter CTMin that was 0.8°C cooler than the minimum environmental temperature of 11°C giving checkered puffer the most positive lower TSM relative to other species. Checkered puffers had the smallest upper AZRR and the largest lower AZRR relative to all species (Table 16).

#### *Schoolmaster snapper*

The thermal maxima and minima of schoolmaster snapper differed significantly across seasons, with CTMax approximately 5°C higher in summer relative to winter (Figure 4c). Thermal scope for schoolmaster snapper was greater in the summer (28.6°C) compared to the winter (27.1°C) (Table 15). The upper breadth in tolerance of schoolmaster snapper was not significantly different across seasons (~12°C). CTMin values were over 4°C lower in the winter relative to the summer, and the lower breadth in tolerance in winter (11.2°C) was almost 3°C smaller compared to summer (14°C). The summer CTMax of schoolmaster snapper exceeded the maximum environmental temperature of 43°C by half a degree resulting in the only positive upper TSM. The winter CTMin was higher than the minimum temperature (11°C) observed in the nearshore environment, resulting in a negative lower TSM. Schoolmaster snapper had the largest upper AZRR and an intermediate lower AZRR relative to all species (Table 16).

## *Bonefish*

The thermal scope of bonefish was lower in the summer (24°C) relative to the winter (24.7°C), and CTMax was almost 3°C higher in summer compared to winter (Figure 4d; Table 15). Moreover, the upper breadth in tolerance of bonefish in summer (8.8°C) was almost 3°C smaller relative to winter (11.6°C). Additionally, the lower breadth in tolerance of bonefish in winter (13.1°C) was more than 2°C smaller relative to summer (15.3°C). The maximum environmental temperature of 43°C in the nearshore environment exceeded the summer CTMax of bonefish by almost 5°C resulting in the largest negative upper TSM. The CTMin of bonefish was almost half a degree lower than the coolest water temperature of 11°C in the nearshore environment resulting in a positive lower TSM. Bonefish had an intermediate upper AZRR and the smallest lower AZRR relative to all species (Table 16).

## **Discussion**

Quantifying the thermal tolerance of marine fishes, and identifying how those tolerances change across seasons, is important for predicting the physiological vulnerability of fish to the more extreme climates of the future (Gunderson & Stillman, 2015). Relative to other tropical/sub-tropical marine species, the adult and juvenile nearshore fishes in this study had some of the highest critical thermal maximums reported; the critical thermal maximum for checkered puffer, yellowfin mojarra, and schoolmaster snapper in the summer acclimatized to 30.4°C, 30°C, and 31°C was 41.6°C, 41.0°C, and 43.5°C, respectively. In comparison, pink cardinalfish, *Apogon pacifici*, that typically inhabit thermally stable coral reef environments exhibit a critical thermal maximum of approximately 35 °C when acclimated to a temperature of 26.5°C (Mora & Ospina, 2001), but this estimate may be low due to a lower heating rate of 1°C h<sup>-1</sup> relative to the 0.18°C min<sup>-1</sup> used in this

study. Alternatively, bullseye pufferfish, *Sphoeroides annulatus*, typically inhabit thermally dynamic nearshore ecosystems and demonstrated a critical thermal maximum that exceeded 40°C when acclimated to a temperature of 28°C (Reyes *et al.*, 2011), a thermal maximum that more closely matched that of the species in the current study. Again, this estimate of CTMax maybe low due to a lower heating rate of 1°C min<sup>-1</sup> relative to the 0.18°C min<sup>-1</sup> used in this study. The eurythermal sheepshead minnow, *Cyprinodon variegatus*, is commonly found in tidal pools, an environment that experiences extreme temperatures, and has a higher thermal tolerance of 45.1°C when acclimatized to 37-42°C at heating rate 0.1°C min<sup>-1</sup> (similar to the rate used in this study) (Bennett & Beitinger, 1997). Species-specific differences and life stage likely play a role thermal tolerance limits as well, but it is clear that the critical tolerance limits of fishes that primarily reside in the thermally dynamic nearshore environment more closely resemble the limits of fish from extreme environments (e.g., tidal rockpools) than from more thermally stable environments (e.g., coral reefs).

Thermal scope is defined as the range of temperatures in which an organism can persist, including passive anaerobic existence (Pörtner & Farrell, 2008). Tropical species were previously thought to have a narrow thermal scope that will make them less tolerant of future climate change relative to species from temperate environments (Pörtner & Farrell, 2008; but see Seebacher *et al.*, 2014). Recent data, however, suggests that there is little difference in thermal scope between temperate (28.3°C) and tropical fish species (25.9°C) (Sunday *et al.* 2011). In the current study, thermal scope for all species ranged from 24°C - 28.6°C, indicating that these species can cope with a wide range of environmental temperatures. When all nearshore fishes are considered together, summer CTMax increased by 3-5°C relative to winter, and winter CTMin decreased by 3-6°C relative to the summer, demonstrating plasticity in thermal scope across seasons, with

thermal scope typically being narrower in the summer relative to the winter. Indeed, temperatures are variable across seasons in the nearshore environment, with the mean temperature in the winter of 21.2°C increasing by almost 10°C to a summer temperature of 30.3°C (Shultz *et al.*, 2014). The range of thermal scopes observed in nearshore fishes in this study can be found in both tropical and temperate environments (Sunday *et al.*, 2011), indicating that nearshore fishes may be more tolerant to climate change than previously expected (i.e., a mean increase of 2°C in the next 100 years above mean environmental temperatures is within the thermal scope of nearshore fishes).

The acclimatization responses (i.e., the magnitude of a species' ability to acclimatize to new thermal environments), as well as breadth in tolerance, is valuable when evaluating which species across and within ecosystems will be most at risk to climate change. Based on acclimation temperatures and CTMax data, marine Crustacea (maximum = 0.35) found in the intertidal zone in temperate climates, and the Mexican bullseye puffer fish, *Sphoeroides annulatus* (maximum = 0.38) found in the nearshore environments in subtropical climates have some of the highest AZRR scores reported in the literature (Hopkin *et al.*, 2006; Reyes *et al.*, 2011). Surprisingly, all of the upper and lower AZRR scores for nearshore fishes in this study were typically greater than 0.60, a value greater than many recently reported AZRR scores for fish (Gunderson & Stillman, 2015), suggesting that these fish have a relatively high capacity to acclimatize to thermal conditions. This enhanced ability to acclimatize to new thermal environments (i.e., phenotypic plasticity) has previously been attributed to mechanisms such as an increase in heat shock proteins, stimulation of the cellular stress response, or improved cardiorespiratory function (Fader *et al.*, 1994; Eliason *et al.*, 2011; Feidantsis *et al.*, 2013; Jayasundara & Somero, 2013). Within the nearshore ecosystem, adult bonefish had the lowest total AZRR score (combined upper and lower AZRR scores) of 1.12 relative to other fish species (1.46-1.72), indicating a limited ability to acclimatize

to variable thermal landscapes. Similarly, adult bonefish demonstrate a lower breadth in tolerance limit in the summer (8.8°C) relative to adult checkered puffer, juvenile schoolmaster snapper, and juvenile yellowfin mojarra (11-12°C) which suggests this fish is more at risk to mean and extreme increases in temperature in the next 100 years relative to other species in this study. Overall, nearshore fishes may have a high capacity to acclimatize to a variable thermal landscape in the future relative to fish species studied to date (Gunderson & Stillman, 2015), but the capacity to acclimatize is life stage and/or species specific in the nearshore ecosystem with bonefish having the smallest capacity.

Fish that live near their thermal maximum/minimum (i.e., small TSM) are likely to be more susceptible to a variable climate in the future (e.g., cold snaps and heat waves) if animals should experience temperatures outside critical limits (Somero, 2010). Even relatively short-lived extremes in temperature can result in a restructuring of fish communities and biogeographical distributions (Wernberg *et al.*, 2012; Smale & Wernberg, 2013). For example, damselfish, *Acanthochromis polyacanthus*, found in the tropical coral reef environment exposed to an elevated temperature of 34°C, just 3°C above ambient conditions, for a relatively short period of time (maximum 14 days) were pushed beyond their physiological limits, resulting in 100 % mortality (Rummer *et al.*, 2013b). Similarly, an extended cold snap in 2010 resulted in high mortality rates for nearshore fish around Florida and has been attributed to the decline in bonefish populations in this area (Szekeres *et al.*, 2014). In this study, present day maximum and minimum temperatures in the nearshore environment are approximately equal to or exceed the thermal tolerance limits of the fish in this study, making thermal safety margins very small or negative. For example, seawater in the nearshore environment during the summer exceeds 40°C (Shultz *et al.*, 2014), a temperature that surpasses the CTMax value for bonefish (38.1°C) in the summer. Bonefish move into the

nearshore environment on an incoming tide and often migrate to deeper cooler waters on the outgoing tide, potentially using these areas as thermal refuge (Murchie *et al.*, 2013). Unfortunately, sea surface temperatures are expected to increase by 0.3 - 2°C over the next 100 years due to climate change (IPCC, 2013), and heat waves are expected to increase in both frequency and intensity (Coumou & Rahmstorf, 2012). This increase in both mean and extreme temperatures has potential to restrict available thermal habitat for bonefish, forcing these fish to spend less time in the nearshore ecosystem and more time in cool refugia. While cool refugia have the potential to alleviate thermal stress, inhabiting these environments may result in missed feeding opportunities and/or elevated mortality due to predation, possibly leading to negative impacts at the population level (Crawshaw & Podrabsky, 2011). The extremes in temperature may have as much, if not more, influence on the distribution and persistence of individual species than mean temperatures (Parmesan *et al.*, 2000; Sunday *et al.*, 2014). On the whole, extreme temperatures due to climate change have potential to influence the survival and/or distribution of nearshore species, and a gradual increase in temperature of 2°C in the next 100 years will restrict some of the available thermal habitats of these species.

Current mean summer temperatures in the nearshore environment (30.3°C) fall below the critical thermal maximum of the most sensitive species, bonefish (CTMax = 38.1°C). The IPCC's worst-case thermal scenario predicts that sea surface temperatures will increase by 2°C in the next 100 years, and push maximum temperatures beyond the thermal safety margins of all nearshore fish in this study, including the schoolmaster snapper that has the largest present-day upper thermal safety margin of 0.5°C. Moreover, nearly all species in this study had relatively large negative upper TSM values in summer relative to the winter, indicating that extreme temperatures associated with heat waves in the summer will likely exceed thermal limits of these

fish more frequently than cold snaps in the winter. This study, however, did not evaluate the phenotypic plasticity of fish to thermal limits within a season which may be an interesting research path to follow in the future now that it has been established that nearshore fish can acclimatize to thermal environments across seasons. This research will be particularly fruitful in the summer when nearshore fishes are most at risk due to small upper breadth in tolerance and TSM relative to the winter.

Altered thermal regimes have the potential to increase mortality, alter habitat choice, constrict the range of species, and alter foodweb dynamics (Pörtner & Farrell, 2008). The possibility of species in the nearshore environment being able to adapt genetically to elevated temperatures is likely small due to long generation times (e.g., bonefish maximum age 20 years) and delayed maturation (e.g., bonefish mature after ~3years), although most basic life history traits of many species in this ecosystem have not been described. Alternatively, the ability to acclimatize to new thermal environments and proximity to their thermal limits will likely play a larger role in structuring nearshore ecosystems the future. Fishes with a limited ability to acclimatize and that inhabit thermal environments near their limits will likely be most at risk as the oceans warm (Madeira *et al.*, 2012). Specifically, nearshore fishes may be forced to migrate to cooler water (e.g., deeper water or cooler micro-habitats such as under the canopy of mangroves) (Huey & Tewksbury, 2009), adapt to an increase in temperature (Hofmann & Todgham, 2010), or a combination of these options (Pörtner & Farrell, 2008). Overall, perturbations in thermal conditions because of climate change will alter fish communities in the nearshore environment, a critical nursery habitat for many marine fishes. Species with positive thermal safety margins that are capable of acclimatizing to new thermal environments (e.g., schoolmaster snapper) will likely



persist in these systems while relatively intolerant species (e.g., bonefish) may inhabit these systems less frequently or will be absent in the future.

### Tables and Figures

Table 13. Length (mean  $\pm$  SE) of yellowfin mojarra, checkered puffer, schoolmaster snapper, and bonefish used in this study. Fish anatomy dictated the choice of metric used to measure each species: a fork length was generated for species that had a forked or furcate caudal fin, and a total length was taken for species with a truncate or rounded caudal fin. Each species in each season had a sample size of eight fish.

Species	Season	Total Length (mm)	Fork Length (mm)	Range (mm)
Yellowfin Mojarra	Summer	NA	133 $\pm$ 3	110-160
	Winter	NA	131 $\pm$ 5	85-168
Checkered Puffer	Summer	173 $\pm$ 6	NA	107-202
	Winter	176 $\pm$ 3	NA	136-195
Schoolmaster Snapper	Summer	147 $\pm$ 5	NA	115-190
	Winter	108 $\pm$ 3	NA	93-155
Bonefish	Summer	NA	389 $\pm$ 6	350-445
	Winter	NA	400 $\pm$ 5	355-469

Table 14. Final seasonal temperature (°C) prior to thermal tolerance assays of yellowfin mojarra, checkered puffer, schoolmaster snapper, and bonefish in the winter and summer.

Season	Treatment	Acclimitization Temperature (°C)			
		Yellowfin	Checkered	Schoolmaster	Bonefish
		Mojarra	Puffer	Snapper	
Winter	CTMax	24.2	23.8	24.6	23.8
	CTMin	23.6	22.5	23.4	23.7
Summer	CTMax	30	30.4	31	29.3
	CTMin	28.7	27.8	29.1	29.4

Table 15. Results of t-tests comparing the seasonal variation in thermal scope (CTMax – CTMin), CTMax, upper breadth in tolerance (CTMax – acclimation temperature), CTMin, and lower breadth in tolerance (acclimation temperature – CTMin) of yellowfin mojarra, checkered puffer, schoolmaster snapper and bonefish (summer vs winter). Each treatment in each season had a sample size of eight fish and DF =14 for each test.

Variable	Yellowfin		Checkered		Schoolmaster		Bonefish	
	mojarra		puffer		snapper			
	T	P	T	P	T	P	T	P
Thermal Scope	0.96	0.37	5.33	<b>0.0002</b>	6.33	<b>&lt;0.0001</b>	2.43	<b>0.0355</b>
CTMax	8.68	<b>&lt;0.0001</b>	9.20	<b>&lt;0.0001</b>	22.28	<b>&lt;0.0001</b>	12.86	<b>&lt;0.0001</b>
Upper Breadth in Tolerance	5.19	<b>0.0014</b>	10.62	<b>&lt;0.0001</b>	0.37	0.72	12.15	<b>&lt;0.0001</b>
CTMin	15.29	<b>&lt;0.0001</b>	11.24	<b>&lt;0.0001</b>	17.53	<b>&lt;0.0001</b>	11.8	<b>&lt;0.0001</b>
Lower Breadth in Tolerance	4.78	<b>0.0004</b>	2.13	0.05	9.30	<b>&lt;0.0001</b>	7.80	<b>&lt;0.0001</b>

Table 16. Seasonal change in CTMax, CTMin, acclimatization temperature, and acclimatization response ratios (AZRR) and thermal safety margin (TSM) for yellowfin mojarra, schoolmaster snapper, checkered puffer, and bonefish. The change in temperature = acclimatization temperature in the summer – acclimatization temperature in the winter. Change in CTMax = CTMax in the summer – CTMax in the winter. Change in CTMin = CTMin in the summer – CTMin in the winter. Each treatment in each season had a sample size of eight fish.

Variable	Yellowfin Mojarra	Schoolmaster Snapper	Checkered Puffer	Bonefish
$\Delta$ CTMax (°C)	3.6	5.3	3.1	2.7
$\Delta$ T (°C)	5.8	5.2	6.6	5.3
AZRR (upper)	0.62	1.02	0.47	0.51
TSM (upper)	-2.0	0.5	-1.4	-4.9
$\Delta$ CTMin (°C)	4.3	3.6	6.6	3.5
$\Delta$ T (°C)	5.1	5.7	5.3	5.7
AZRR (lower)	0.84	0.63	1.25	0.61
TSM (lower)	-0.2	-0.1	0.8	0.4
Total AZRR	1.46	1.65	1.72	1.12

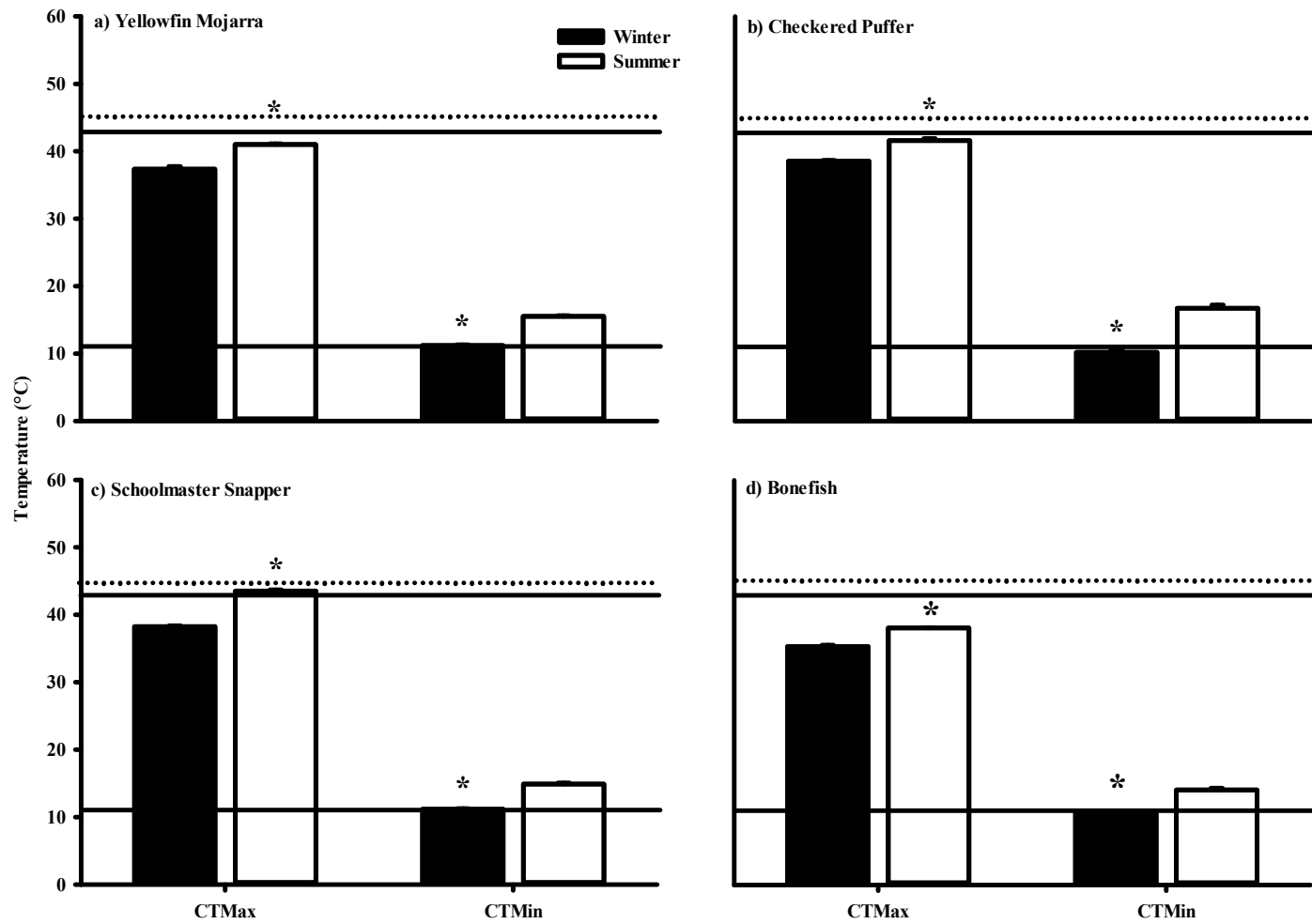


Figure 4. Critical thermal limits of a) yellowfin mojarra, b) schoolmaster snapper, c) checkered puffer, and d) bonefish recovered in laboratory conditions in the winter (22.3-24.7°C) and summer (26.4-32°C). An asterisk denotes significant differences in CTMax and

CTMin across seasons. Each treatment in each season had a sample size of eight fish. Error bars represent  $\pm$  SE and are challenging to discern for CT data due to low variation across individuals. Horizontal solid lines indicate extreme minimum temperature in the winter and extreme maximum temperature in the summer in the nearshore ecosystem reported by Shultz et al. (2014). Horizontal dotted line signifies extreme maximum temperature in the summer plus the Intergovernmental Panel on Climate Change worst-case scenario for sea surface temperatures in 100 years.

## **CHAPTER 5: OUT OF THE FRYING PAN AND INTO THE FIRE – TRADEOFFS BETWEEN PHYSIOLOGICAL COSTS AND PREDATION IN A CHANGING CLIMATE**

### **Abstract**

Habitat selection in fish is typically governed by the tradeoff between the benefit and cost of acquiring food, although other factors such as competition and reproduction may influence this choice. Global climate change is expected to create elevated temperature and pCO<sub>2</sub> in the oceans over the next century, which can increase the cost of maintaining homeostasis. Nearshore fishes reside in shallow environments to reduce the threat of predation, but as a result routinely experience challenging abiotic conditions. Little is known about the tradeoff between accepting the physiological costs of maintaining homeostasis in novel environments or risking predation by moving to new, less physiologically demanding habitats. Here we show that elevated temperature and pCO<sub>2</sub> alters habitat choice in four common nearshore fishes, and that some, but not all fish increase temperature avoidance thresholds (i.e., cope with unfavorable conditions) to avoid a predator. Collectively, altered abiotic conditions associated with climate change will likely result in an asymmetrical redistribution of species, resulting in novel biotic interactions, with predators playing a key role in determining community structure in the future.

### **Introduction**

Habitat selection in fish is heavily influenced by the tradeoff between the benefit and cost of acquiring food, although other factors such as competition and reproduction may influence this choice. Costs related to food acquisition can come in many forms, such as the energy needed to locate, handle, and consume prey items (Mittlebach, 2008). Predators also impose costs, through injury and mortality, and can also alter fish behaviors through intimidation (i.e., risk of predation)

(Lima & Dill, 1990). The magnitude of behavioral changes owing to the risk of predation range from no reaction, to complete avoidance of a habitat, depending on the degree of risk and morphological characteristics of the prey such as body size relative to the predator, the presence of armour or spines, and chemical deterrents (Edmunds, 1974; Lima & Dill, 1990; Abrahams, 1995). No reaction to a predator may increase mortality rates, while complete avoidance of a habitat (e.g., hiding, evasion) has both direct energetic costs and indirect costs such as missed foraging opportunities (Lima & Dill, 1990). When locating food or avoiding predators, fish may also encounter potentially challenging abiotic conditions (e.g., elevated temperature or pCO<sub>2</sub>) that require them to expend additional energy on maintaining homeostasis (Crawshaw & Podrabsky, 2011). If challenging conditions are extreme (e.g., lack of food), fishes may be forced to occupy novel habitats to avoid stressful conditions, and these novel habitats may potentially result in unintended risks, such as exposure to predators (Abrahams & Dill, 1989). Fish that maximize food intake while minimizing risks associated with predators and challenging abiotic conditions will obtain the energy needed for growth and reproduction (Abrahams, 2011).

Climate change is predicted to generate a number of widespread changes to marine environments that will impact fish habitat. For example, worst-case scenarios for future climate conditions suggest that sea surface temperatures may increase by 2°C (IPCC, 2013), and extreme climatic events (e.g., heat waves) are expected to increase in both intensity and frequency (Coumou & Rahmstorf, 2012; Trenberth & Fasullo, 2012). Similarly, although nearshore environments currently experience fluctuations in the partial pressure of carbon dioxide in the water (pCO<sub>2</sub>) between 450 and 750 µatm daily (Borges *et al.*, 2003), climate change may push values over 1000 µatm by the year 2100 (IPCC, 2013). For coral reef fishes, these changes to ocean conditions have been documented to increase metabolic costs associated with maintaining homeostasis (Munday



*et al.*, 2009), alter the distribution of species locally and regionally (Poloczanska *et al.*, 2013; Brown & Thatje, 2015), increase bold/risky behaviors (Munday *et al.*, 2012b) or reduce the ability of prey species to detect the odor of predators (Munday *et al.*, 2012b). Together, climate-related stressors are expected to alter habitat use and predator-prey interactions in marine ecosystems, which may have profound implications for individuals, populations, and communities (Kordas *et al.*, 2011; Russell *et al.*, 2012).

Fish communities in highly variable nearshore environments (e.g., mangroves) may be more vulnerable to climate-related stressors than fishes from more stable, low seasonality habitats (e.g., tropical coral reefs) due to limited plasticity in physiological systems/limits and small thermal safety margins (Madeira *et al.*, 2012; Seebacher *et al.*, 2014; Gunderson & Stillman, 2015). Many subtropical fishes forage in the mangroves during high tide and have evolved different strategies to avoid predators and/or adverse abiotic conditions (e.g., high temperatures, low oxygen) by migrating to other habitats (e.g., seagrass beds) or hiding in the mangroves at low tide (Sheaves, 2005). As abiotic conditions in nearshore environments become more challenging because of climate change, fish will be confronted with a tradeoff: accept the physiological costs of maintaining homeostasis in this challenging environment, or risk predation by moving to new, less physiologically demanding habitats. Unfortunately, in marine fishes, there is a dearth of information on how biotic factors such as predation can influence habitat choice at a local scale, and distribution at a global scale, particularly for a community assemblage across environmental gradients (Gilman *et al.*, 2010; Blois *et al.*, 2013; Wisz *et al.*, 2013). Defining how biotic and abiotic factors interact to drive habitat selection will provide valuable insights into the changing structure and make-up of nearshore fish communities under future climate scenarios (Kordas *et al.*, 2011; Russell *et al.*, 2012).

In the current study, we quantify the relative cost of habitat selection by measuring temperature and pCO<sub>2</sub> avoidance thresholds of nearshore fishes under altered abiotic conditions, both in the presence or absence of a predator, a juvenile lemon shark *Negaprion brevirostris*, commonly found in the nearshore environment. To do this, four common subtropical nearshore fishes (i.e., juvenile schoolmaster snapper *Lutjanus apodus*, juvenile yellowfin mojarra *Gerres cinereus*, adult bonefish *Albula vulpes*, and adult checkered puffer *Sphoeroides testudineus*) in the western atlantic were acclimated to a behavioral choice arena (i.e., two chambers connected by a central corridor). Temperature or CO<sub>2</sub> were manipulated in one chamber while the other chamber was maintained at ambient conditions. Following this initial assay, a predator was added to the chamber held at ambient conditions to assess the risk of predation. Our objective was to determine which climate-related stressor has the strongest influence on habitat choice of nearshore fishes, the relative cost of the threat of predation, and use that information to understand how these abiotic and biotic factors could shape nearshore fish communities in the future. We predict that temperature will have the strongest influence on habitat choice, small bodied fishes (e.g., schoolmaster snapper and mojarra) with limited antipredator defenses will perceive a greater threat of predation, and a species-specific response to abiotic and biotic challenges will alter fish communities.

## **Methods**

This study was conducted at The Cape Eleuthera Institute (CEI) in Eleuthera, The Bahamas (N 24°50'05" W 76°20'32"). All research conformed to University of Illinois Institutional Animal Care and Use Committee protocol (Protocol # 09160). Juvenile yellowfin mojarra, checkered puffer, bonefish, juvenile schoolmaster snapper, and juvenile lemon sharks were captured by

seining local tidal creeks near CEI and transferred to plastic totes (76 L) filled with ambient seawater (Table 17). Fishes were transported by boat to the CEI wetlab, and seawater in the totes was exchanged every five min (Murchie *et al.*, 2009). Upon arrival at the CEI aquatic facility, fish were transferred to two large holding tanks (3.7 m diameter × 1.25 m height, 13,180 l) that were continuously supplied with fresh seawater (1800 l/h). Each tank was aerated with a low-pressure pump (Sweetwater model S41; 15 V; 3450 rpm; Aquatic Ecosystems, Apopka, FL, USA) and seawater quality parameters (dissolved oxygen ( $\text{mg l}^{-1}$ ), acidity (pH), and temperature ( $^{\circ}\text{C}$ ); YSI multimeter, Yellow Springs, Ohio) were monitored regularly during holding. Total alkalinity was measured with a commonly available titration test kit (LaMotte #4533-DR-01, Chestertown, Maryland, USA). Total alkalinity ( $1218 \pm 21 \mu\text{mol kg}^{-1} \text{ SW}$ ; mean  $\pm$  standard error, SE) and salinity ( $36.9 \pm 0.06 \text{ ppt}$ ; mean  $\pm$  SE) were considered to be constant across time periods (Barry *et al.*, 2010). All fish were acclimated to laboratory conditions for a minimum of 48 hours prior to experimentation. During holding fishes were fed frozen sardines (*Sardenella aurita*) to satiation, but food was withheld for 24 hours prior to experimentation.

Avoidance behaviors were quantified in a dynamic choice arena. Briefly, the system consisted of two chambers (diameter 123 cm, height 73 cm, water depth 25 cm), each with an independent water supply and regulation capacity, connected by a central corridor (width 29 cm, height 73 cm, water depth 25 cm) (similar to Kates *et al.*, 2012). Individual fish were randomly placed in one of the two chambers. After a one-hour acclimation period, the abiotic conditions in the chamber where the fish was located were altered in a linear fashion until fish were motivated to move through the narrow corridor to the opposite chamber (maintained at ambient sea water). An increase in the temperature of seawater was achieved using an immersion heater (Process Tech Heaters #H18T, 1,800W, 115v, 15amps; Controller #NA30DX; Aquatic Ecosystems, Apopka, FL,

USA), and an increase in pCO<sub>2</sub> was accomplished by the common method of bubbling CO<sub>2</sub> into the reservoir (Munday *et al.*, 2009). The rate of temperature change was 0.2°C per min (0.15 ± 0.003°C; mean ± SE) (Murchie *et al.*, 2011), and real-time temperature values were taken with an iButton (iButton DS1922L; Maxim, Dallas, TX, USA) placed at the bottom of each chamber and set to record the temperature (± 0.5°C) every two mins. Similarly, pH (a commonly-used proxy for CO<sub>2</sub> in seawater) was changed at a rate of 0.07 ± 0.005 pH units per min (mean ± SE) to mimic the rapid diurnal increase/decrease in pH observed in shallow nearshore environments (Borges *et al.*, 2003; Shaw *et al.*, 2012). Real-time pH values were recorded by sampling the outflow from each chamber every two mins (YSI pH10a, Yellow Springs, Ohio; accuracy ± 0.01 pH units). The pH of seawater was frequently cross-validated with a second pH pen (YSI pH10a, Yellow Springs, Ohio; accuracy ± 0.01 pH units), and all pH instruments were regularly calibrated to ensure accuracy and precision (Moran, 2014). Fish position was monitored with an overhead video camera during each trial, and conditions in each chamber were noted when the fish began to display erratic behaviors (elevated swimming activity, twitching, and surface ventilations), moved from chamber A to chamber B, or lost equilibrium (Kates *et al.*, 2012). Once fish moved to Chamber B, the process was repeated to refine the avoidance temperature or pH for an individual fish and generate replicate measurements per fish (Petersen & Steffensen, 2003). A trial ended and abiotic conditions were noted when the fish moved to chamber B and remained there for at least four mins (i.e., avoidance threshold), or if the fish lost equilibrium. Once this point was reached, fish were removed from the choice arena allowed to recover for 2-4 hours, and then released into the ocean once they were swimming confidently. Together, this experiment defined the abiotic conditions that fish (n=10-12 per species) avoided without the presence of a predator, and this level served as the control for the next experiment.

Following this initial experiment, a new individual fish was acclimated to the dynamic choice arena for one hour, and then a predator (juvenile lemon shark) was added to the second chamber (ambient sea water conditions). The predator was restricted to the second chamber, while the fish could move freely between both chambers. Seawater conditions were again manipulated in a manner identical to that described above, and the same response variables were recorded as outlined above. Together, this second series of experiments quantified the avoidance behavior of fish (n=10-11 per species) in relation to seawater conditions under the threat of predation.

### *Analysis*

Temperature (°C), alkalinity and pH of seawater were first combined in CO2Calc (Robbins *et al.*, 2010), using constants from Lueker *et al.* (2000) and Dickson (1990), to express CO<sub>2</sub> data as pCO<sub>2</sub> (µatm). A repeated measures ANOVA was used to define significant differences in avoidance temperature or pCO<sub>2</sub> with species and treatment (presence or absence of a predator) as fixed effects, and individual ID entered as a random effect. The use of a random effect accounted for multiple, and potentially autocorrelated, measurements taken for each fish during a trial (e.g., multiple avoidance temperatures were recorded for an individual fish in the absence of a predator) (Laird & Ware, 1982; Lindstrom & Bates, 1990). A Hartley F-Max test was used to assess homogeneity of variances prior to analysis (Hartley, 1950), and a visual analysis of a normal probability plot of fitted residuals was used to assess normality (Anscombe & Tukey, 1963). Data were log transformed if they did not meet the assumptions of normality and homogeneity of variances (Sokal & Rohlf, 1995). A Tukey-Kramer HSD post hoc test was used to identify significant differences between the interaction term and main effects. Erratic behaviors were also analyzed with a repeated measures ANOVA with treatment, and temperature or CO<sub>2</sub> as fixed

effects, and fish ID as a random effect. Proportion of erratic behaviors were arcsine transformed to meet assumptions of the analysis (Sokal & Rohlf, 1995). Again, a Tukey-Kramer HSD post hoc test was used to separate means. Temperature and pCO<sub>2</sub> values were grouped into bins for the analysis with behavioral measurements grouped by every 4°C or 1000 µatm. All statistical analyses were performed using JMP 7.0.1 (SAS Institute Inc., 2005), all means are reported ± SE where appropriate, and the level of significance for all tests ( $\alpha$ ) was 0.05.

## Results

In the absence of a predator, all species of nearshore fishes in this study displayed a temperature avoidance threshold above 33.5°C (Figure 5). Across species, yellowfin mojarra had a threshold of 36.0°C, approximately 2.5°C above that of bonefish (Table 18; Figure 5). In the presence of a predator, however, the avoidance threshold of yellowfin mojarra increased by almost 4°C. Moreover, the increase in avoidance threshold experienced by yellowfin mojarra was approximately 3°C higher than bonefish exposed to a predator (Table 18; Figure 5).

All nearshore species in this study exhibited a pCO<sub>2</sub> avoidance threshold above 1000 µatm when a predator was absent (Figure 6). Again, considering all nearshore species together, the presence of a predator increased pCO<sub>2</sub> avoidance thresholds by approximately two-fold (Table 1; Figure 6). Regardless of the threat of predation, the increase in pCO<sub>2</sub> avoidance thresholds of yellowfin mojarra and schoolmaster snapper were more than twice that observed for bonefish and checkered puffer (Table 18; Figure 6).

All four species of nearshore fishes in this study experienced an increase in erratic behaviors (e.g., burst-swimming) at elevated temperatures, regardless of the presence or absence of a predator, with the difference being greatest in the bonefish and least in the checkered puffer

(Figure 7). Only in the schoolmaster snapper, however, did the presence of a predator cause any increase in the proportion of erratic behaviors across all temperatures (Table 19; Figure 7a).

Surprisingly, elevated pCO<sub>2</sub> had little effect on erratic behavior, with schoolmaster snapper being the only species that exhibited a decrease in erratic behaviors at elevated pCO<sub>2</sub> regardless of the threat of predation (Table 19; Figure 8). Independent of pCO<sub>2</sub>, yellowfin mojarra exhibited a decrease in erratic behaviors when exposed to a predator relative to the absence of a predator (Figure 8b). Additionally, a decrease in erratic behaviors was observed for checkered puffers in the presence of a predator at 3000 μatm pCO<sub>2</sub> (Table 19; Figure 8d). Bonefish did not display a change in erratic behaviors in the presence of a predator or at elevated pCO<sub>2</sub> concentrations (Figure 8c).

## **Discussion**

This study sought to quantify the relative risk of increasing temperature and pCO<sub>2</sub> in the presence or absence of a predator, and link these risks to habitat choice decisions in the natural environment. Without the threat of predation, altered abiotic conditions (e.g., elevated temperatures) can increase physiological costs, resulting in fish choosing new, less abiotically challenging habitats in an effort to maximize energetic gains (Crawshaw & Podrabsky, 2011). Mean temperature in subtropical nearshore environments in the summer can reach 30.4°C, and maximum temperatures can exceed 43°C (Shultz *et al.*, 2014). As a result, present-day temperatures in nearshore environments can exceed avoidance thresholds (33.5°C - 35.9°C) and increase erratic behaviors of the fish in this study, which indicates these fish likely alter habitat choices due to high temperatures, potentially seeking thermal refuges (e.g., deeper/cooler environments) (Brown & Thatje, 2015) or incur physiological costs associated with thermal stress.

Similarly, the increase in erratic behaviors observed at elevated temperatures indicates that all species experienced stress below environmental extremes. Interestingly, in the absence of a predator, fish in the current study did not alter habitat choice until pCO<sub>2</sub> values exceeded 10,000 μatm (with the exception of checkered puffer who altered habitat choice at approximately 1,300 μatm). The nearshore environment currently experiences daily fluctuations in pCO<sub>2</sub> between 450 μatm and 750 μatm (Borges *et al.*, 2003), and can exceed 1000 μatm during periods of high aquatic respiration (Shaw *et al.*, 2012). As a result, elevated temperatures seem to have a greater impact on habitat choice, behavior, and energetic costs than ecologically relevant, but elevated, pCO<sub>2</sub> values.

In this study, the threat of predation has a pronounced influence on habitat choice when fish are exposed to challenging abiotic conditions. Biotic interactions are expected to be important factors dictating the distribution, abundance, and loss of species (local and global) as the climate changes (Blois *et al.*, 2013). In particular, novel conditions that induce increased interactions with a predator can result in direct (e.g., injury or mortality) and indirect costs (e.g., energy spent avoiding predators, missed foraging opportunities) (Lima & Dill, 1990), which might result in population-level consequences such as a decrease in abundance of prey, altered biogeographical distributions, and unexpected community structures (Harley, 2011). Specifically, yellowfin mojarra increased their avoidance threshold to 39.8°C in the presence of a predator, almost a 4°C higher relative to when a predator was absent, and all nearshore fishes increased pCO<sub>2</sub> avoidance threshold in the presence of a predator. This temperature is considerably higher than the mean temperature for subtropical nearshore environments of (30.4°C) and is approaching the most extreme temperatures (e.g., 43°C) (Shultz *et al.*, 2014). This indicates that, as climate change progresses, nearshore obligate fishes may choose to continue to reside in shallow and thermally



challenging habitats to avoid predation, requiring them to cope with physiological costs associated with warmer temperatures (Shultz *et al.*, 2014). Extreme present-day pCO<sub>2</sub> in nearshore environments can exceed 1000 µatm (Shaw *et al.*, 2012), but in the presence of a predator the avoidance thresholds of nearshore obligate and transient fishes were above 5000 µatm. Again, this indicates that nearshore fishes may choose to continue to reside in shallow, more abiotically challenging conditions (e.g., elevated pCO<sub>2</sub>) over risking predation. Interestingly, erratic behaviors under elevated pCO<sub>2</sub> decreased under the threat of predation for some nearshore species. Sensory systems and behavior under elevated pCO<sub>2</sub> may be altered due to a disruption in normal neurological function associated with GABA-A receptors in the brain (Nilsson *et al.*, 2012) and/or the anesthetic effects of elevated pCO<sub>2</sub> (Yoshikawa *et al.*, 1991). The outcome of these alterations often equate to either a reduced ability to recognize predators, or in some instances prey are attracted to predators (Dixson *et al.*, 2010; Lönnstedt *et al.*, 2013). Collectively, the presence of a predator resulted in nearshore fishes increasing avoidance thresholds at elevated temperatures and pCO<sub>2</sub>, indicating that nearshore fishes may be constrained to habitats with fewer predators (e.g., shallow environments) that are more abiotically challenging and physiologically costly as the climate changes.

The species examined in the present study all responded differently both to alterations in abiotic conditions and to the presence of a predator. Specifically, adult bonefish, a species that migrates into nearshore habitats with incoming tides and to deeper ecosystems on the outgoing tides (Murchie *et al.*, 2013), had some of the lowest temperature and pCO<sub>2</sub> avoidance thresholds in this study. Moreover, habitat choice in bonefish was not influenced by the threat of predation. This species likely encounters predators routinely, and, being large relative to the other species examined, has a better chance of avoiding predators in all nearshore habitats (Abrahams, 2011).

Similarly, adult checkered puffer also experienced low avoidance thresholds for both temperature and pCO<sub>2</sub>, and the presence of a predator did not influence avoidance thresholds. Several anti-predator defenses likely influence the behavioral response of checkered puffer to the threat of predation: they avoid being eaten by inflating their body, which is covered in small spines, to nearly double its size, making the fish difficult to consume (Abrahams, 2011). Moreover, this species is also known to produce tetrodotoxin, a potent neurotoxin that likely deters predators from eating it (Bane *et al.*, 2014). In contrast, juvenile yellowfin mojarra and juvenile schoolmaster snapper, nearshore-obligate species, exhibited some of the highest avoidance thresholds in this study, and for yellowfin mojarra the presence of a predator drove its avoidance threshold even higher. These fishes inhabit warm, shallow, and structurally complex habitats (e.g., mangrove roots) that allow them to hide from predators and water depth limits predator access to nearshore ecosystems (Rypel *et al.*, 2007; Abrahams, 2011). Taken together, in predator rich nearshore environments, species that have adapted multiple strategies to evade predators (e.g., large body size, toxic, difficult to consume) will likely choose habitats that minimize physiological costs associated with climate change, while species that rely on structure/shallow water to avoid predators will likely be confined to shallow water and may incur higher physiological costs in the future.

Based on the existence of species-specific responses to altered abiotic conditions and the threat of predation, the structure of nearshore fish communities may shift following modifications driven by climate change. Present-day extreme temperatures regularly exceed avoidance thresholds of nearshore fishes. The worst-case scenario projected by the IPCC indicates a 2°C increase in mean sea surface temperatures and extreme weather events are expected to increase in intensity and frequency (IPCC, 2013; Trenberth *et al.*, 2015). In localized areas with low predator

burdens, thermal avoidance thresholds will likely drive the composition of the fish community, with adult fish exiting nearshore ecosystems more frequently as temperatures increase. Juvenile fish will likely reside in nearshore ecosystems at temperatures above the thresholds of adults, but may be confined to relatively small areas. Under these conditions, fishes may experience elevated metabolic costs associated maintaining homeostasis in warmer environments (Nilsson *et al.*, 2009), as well as issues associated with confinement such as an increase in disease, parasitism, and a decrease in food and oxygen availability (Crawshaw & Podrabsky, 2011). If environmental temperatures rise, even temporarily, above the thresholds of juvenile fishes (e.g., in a heat wave), then these fish may also be forced to move away from the protection of shallow water into predator rich environments, which may increase predator/prey interactions and, therefore, predation rates (Rypel *et al.*, 2007). Elevated temperatures associated with climate change may also accelerate the loss in performance (e.g., the ability to evade predators, perform aerobically) and potentially decrease the abundance fish (Munday *et al.*, 2009). To offset additional costs of maintaining homeostasis and/or avoiding predators under altered abiotic conditions, fish could increase the amount of time spent foraging or increase the quality of food items, but these options may be limited to habitats with low predator burdens. Similarly, local refuges (e.g., deeper habitats) may be important in minimizing the impact of elevated temperature or pCO<sub>2</sub> in nearshore ecosystems, but again these habitats may be limited to areas with low predator burdens. Collectively, elevated temperatures that coincide with and exceed future predictions in nearshore ecosystems will have species-specific physiological and behavioral impacts, with biotic interactions with predators playing a key role in shaping community structure.

This study adds a new dimension to understanding the distribution of fishes in a changing climate by evaluating fine-grain habitat choice under the threat of predation at a local scale. Global

climate change will continue to alter the biogeographical distribution of organisms, with a general trend of species migrating poleward at the cold-edge (i.e., range expansion) and typically lagging behind the warm-edge (i.e., range constriction) (Cahill *et al.*, 2012). A host of studies have sought to predict future ranges of organisms by coupling thermal tolerance data with projected water temperature in correlative models, species distribution models (SDMs), or climate envelope models that relate field/lab tolerance observations or presence/absence data to environmental/spatial predictor variables (Guisan & Thuiller, 2005; Elith & Leathwick, 2009; Elith *et al.*, 2010; Wisz *et al.*, 2013). Recently, studies have documented that factors beyond abiotic habitat variables can drive future species distributions, including dispersal, competition, evolution and predation (Pearson & Dawson, 2003; Wisz *et al.*, 2013), and that the complexity of relationships between climate, biotic interactions, and community dynamics will make individual species-climate relationships insufficient to predict the distribution of species (Gilman *et al.*, 2010; Wisz *et al.*, 2013). Predation has long been a factor known to drive species distributions (Holt & Barfield, 2009), but the impact of predation on future distributions, or how to quantify this interaction, has been a challenge for climate modelers (Wisz *et al.*, 2013). More importantly, when attempting to integrate predator-prey interactions into distribution models, it is challenging to differentiate from ‘true’ cause-and-effect relationships between predator and prey and simple overlap in species ranges due to other biotic or abiotic factors (Wisz *et al.*, 2013). Data from this study indicate that the threat of predation can play a large role in thermal habitat selection for nearshore fishes, indicating that future studies should quantify interactions with predators and incorporate outcomes into models to make predictions more accurate. For example, Hein *et al.*, (2012) showed that the addition of a predator into an SDM helped explain future range distribution of arctic char. Similarly, Öhlund *et al.*, (2015) showed that temperature had differing impacts on

the attack speed of predators and escape speed of prey in an experimental setting, highlighting the impacts of temperature on predator/prey dynamics in the future. Clearly, we need to consider thermal impacts on predator-prey dynamics, particularly as they relate to species distributions on both a local and global scale, to better predict the structure of marine ecosystems of the future.

## Tables and Figures

Table 17. Length (mean  $\pm$  SE) of yellowfin mojarra, checkered puffer, schoolmaster snapper, and bonefish used in this study. Fish anatomy dictated the choice of metric used to measure each species: a fork length was generated for species that had a forked or furcate caudal fin, and a total length was taken for species with a truncate or rounded caudal fin.

Species	Total Length (mm)	S.E.	Range (mm)
Yellowfin Mojarra	154	2.02	124-209
Checkered Puffer	173	2.57	140-225
Schoolmaster Snapper	158	2.14	126-194
Bonefish	422	10.6	310-550
Lemon Shark	685	14.7	648-720

Table 18. Results of a repeated measures ANOVA that assessed the difference in avoidance temperature and pCO<sub>2</sub> of nearshore fishes in presence and absence of a predator. Juvenile lemon sharks were used as the predator (i.e., treatment), and schoolmaster snapper (n=10-11), yellowfin mojarra (n=10-12), bonefish (n=10-11), and checkered puffer (n=10-11) were the species used in these models.

Variable	Treatment			Species			Treatment × Species		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Temperature	8.69	1	0.0039	20.11	3	<0.0001	106.9	3	0.0027
pCO <sub>2</sub>	7.63	1	0.0066	23.26	3	<0.0001	1.51	3	0.22

Table 19. Results of a repeated measures ANOVA that assessed difference in erratic behaviors of nearshore fishes exposed to linear increases in temperature or pCO<sub>2</sub> in presence and absence of a predator. Juvenile lemon sharks were used as the predator (i.e., treatment). Temperature and pCO<sub>2</sub> values were grouped into 4°C or 1000 µatm bins in the presence or absence of a predator (i.e., treatment) for the behavioral analysis.

Variable	Species	Treatment			Temperature/pCO <sub>2</sub> Bin			Treatment × Bin		
		<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Temperature	Schoolmaster snapper	5.27	1	<b>0.0327</b>	30.82	2	<b>&lt;0.0001</b>	2.50	2	0.08
	Yellowfin mojarra	0.94	1	0.34	28.24	2	<b>&lt;0.0001</b>	1.91	2	0.15
	Bonefish	1.07	1	0.31	34.58	2	<b>&lt;0.001</b>	0.77	2	0.46
	Checkered puffer	1.00	1	0.34	3.49	2	<b>0.0310</b>	0.40	2	0.67
pCO <sub>2</sub>	Schoolmaster snapper	0.48	1	0.50	2.74	4	<b>0.0283</b>	1.59	4	0.18
	Yellowfin mojarra	7.14	1	<b>0.0122</b>	1.73	4	0.14	1.24	4	0.29
	Bonefish	1.05	1	0.32	0.57	4	0.69	1.14	4	0.34
	Checkered puffer	13.79	1	<b>0.0005</b>	5.47	4	<b>0.0003</b>	2.40	4	<b>0.0495</b>



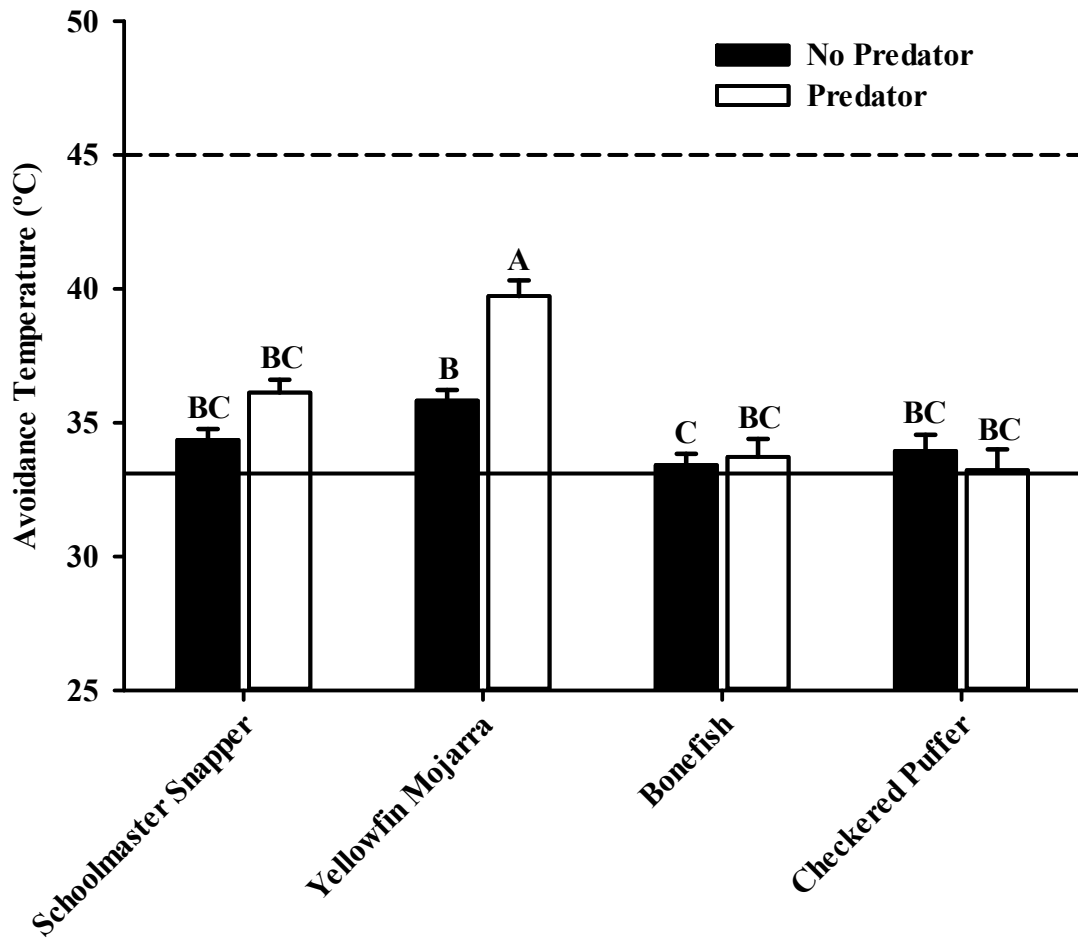


Figure 5. Avoidance temperature for nearshore fishes exposed to a gradual increase in temperature in a behavioral choice experiment. A juvenile lemon shark served as the predator for all trials. Each species in each treatment had a sample size of 10-11 fish. Dissimilar letters signify a significant difference in avoidance temperature within and between nearshore fishes in the presence and absence of predator. Horizontal solid line indicates the worst-case scenario predicted by Intergovernmental Panel on Climate Change for sea surface temperatures in 100 years plus extreme temperature during the summer in nearshore ecosystems. Horizontal dashed line indicates worst-case scenario predicted by Intergovernmental Panel on Climate Change for sea surface

temperatures in 100 years plus the average temperature in nearshore ecosystems in the summer.

Error bars represent  $\pm$  S.E.

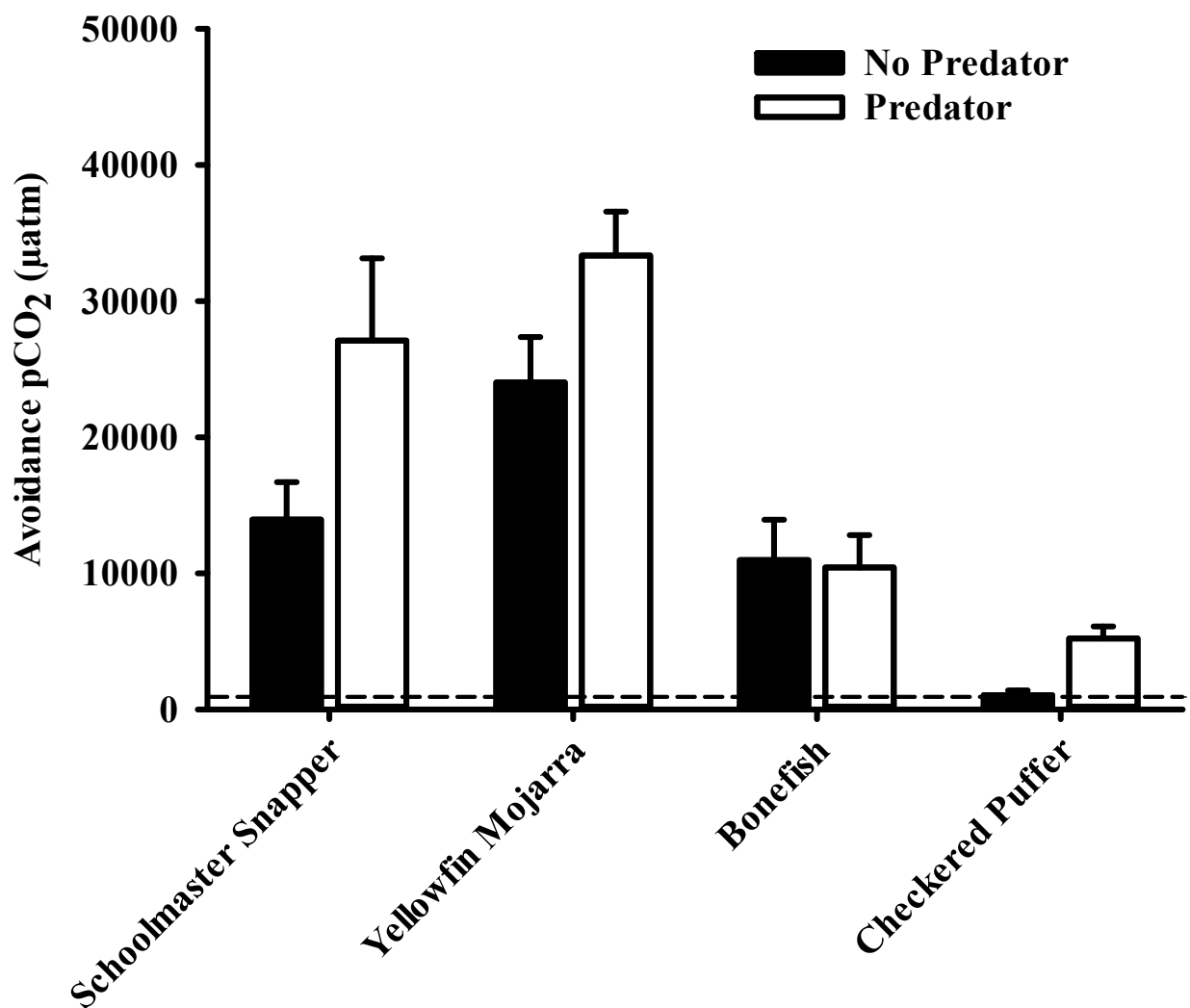


Figure 6. Avoidance pCO<sub>2</sub> (µatm) for nearshore fishes exposed to a gradual increase in CO<sub>2</sub> levels in a behavioral choice arena. A juvenile lemon shark served as the predator for all trials. Each species in each treatment had a sample size of 10-12 fish. Ambient pCO<sub>2</sub> was ~ 380 (µatm) at the beginning of each trial. Horizontal short dashed line indicates the Intergovernmental Panel on Climate Change worst-case scenario for sea surface pCO<sub>2</sub> (µatm) in 100 years (i.e., 1000 µatm). Error bars represent ± S.E.

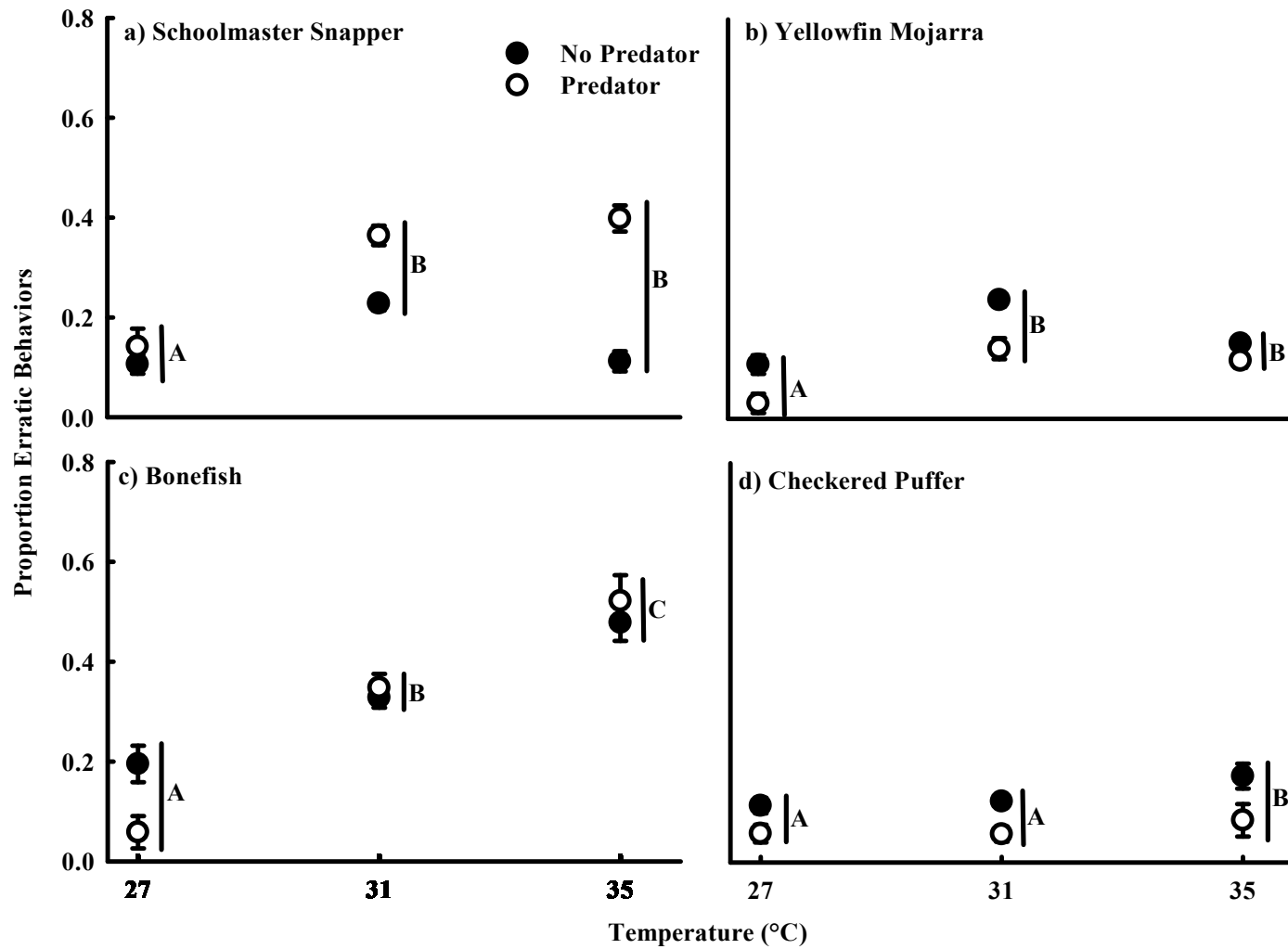


Figure 7. Proportion of erratic behaviors displayed by nearshore fishes exposed to a gradual increase in temperature in the presence and absence of a predator. A juvenile lemon shark served as the predator for all trials. Each species in each treatment had a sample size of

10-11 fish. Dissimilar letters signify a significant difference in the proportion of erratic behaviors between temperatures regardless of the presence or absence of a predator. Independent of temperature, only the schoolmaster snapper displayed an increase in erratic behaviors when a predator was present relative to when it was absent. Error bars represent  $\pm$  S.E.

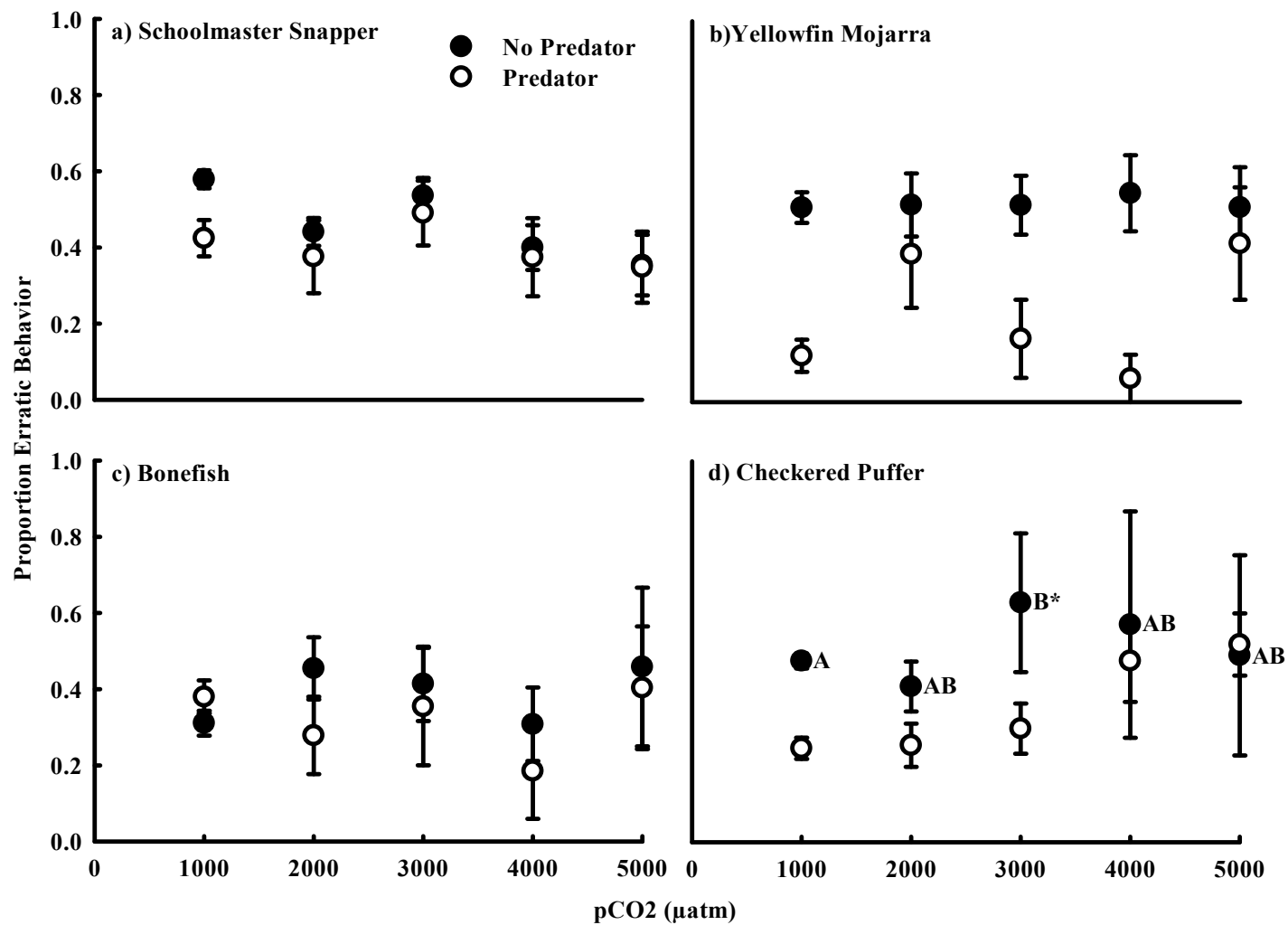


Figure 8. Proportion of erratic behaviors displayed by nearshore fishes exposed to a gradual increase in pCO<sub>2</sub> (μatm) in the presence and absence of a predator. A juvenile lemon shark served as the predator for all trials. Each species in each treatment had a sample size

of 10-12 fish. Dissimilar letters signify a significant difference in the proportion of erratic behaviors between pCO<sub>2</sub> within a treatment . An asterisk denotes significant differences in the proportion of erratic behaviors across treatments at the same pCO<sub>2</sub>. Error bars represent  $\pm$  S.E.

## CHAPTER 6: GENERAL DISCUSSION

The limited knowledge surrounding the physiology and behavior of nearshore fishes to climate-related stressors is an impediment to understanding how the abundance and distribution of these fish may change in the future. Keeping this in mind, the overall goal of this dissertation was to evaluate the responses of four common subtropical nearshore fishes (i.e., a community approach) to climate-related stressors across seasons, and assess the role that biotic interactions will have on habitat choice under forecasted conditions. The dissertation has provided insight into the physiology, behavior, and thermal plasticity of several nearshore species challenged by climate-related stressors, as well as identified how the presence of a predator will influence habitat choice under forecasted conditions. Collectively, the findings of this dissertation can be used to identify vulnerable species, predict the structure of nearshore ecosystems of the future, highlight potential climatic tipping points for fishes that inhabit nearshore ecosystems, and provide direction for future research.

### **Temperature**

Climate-related stressors influenced the physiological and behavioral responses of nearshore fishes examined in this dissertation. Specifically, elevated temperatures resulted in the most physiological disturbance in nearshore fishes, compared to pH, salinity, and temperature + pH challenges (Chapter 2). As a consequence, it is not surprising that elevated temperatures that coincide with (33°C), or exceed (34 °C), the worst-case scenario predicted by the IPCC over the next 100 years, resulted in decreased swimming and metabolic performance for a number of species, again suggesting that elevated temperatures will likely be the most challenging relative to other climate-related stressors (Chapter 4). Unfortunately, these fish inhabit dynamic thermal



environments, with present-day maximum temperatures (e.g., 43°C) in the nearshore environment approximately equal to or exceeding the thermal tolerance limits of the species examined, making thermal safety margins very small or even negative (Chapter 5). Finally, considering forecasted conditions in 100 years (i.e., worst-case scenario increase of 2°C and ~600  $\mu\text{atm}$  above present-day conditions), elevated temperatures had the greatest influence on avoidance thresholds of nearshore fishes relative to elevated  $\text{pCO}_2$  values (Chapter 6). Taken together, temperature has traditionally been considered the “master factor” governing a wide-range of physiological and biological processes (Brett, 1971), and appears to also be the “master factor” that will guide the responses of four common nearshore fishes to climate change.

### **Vulnerable Species**

Results suggest that nearshore fishes in this dissertation experience a suite of physiological disturbances in response to climate-related challenges. Of the species examined, bonefish displayed the greatest degree of physiological disturbance following exposure to the common environmental challenges, with disturbances in the summer being greater than those in the winter (Chapter 2). Similarly, standard metabolic rates increased by 65% for bonefish and yellowfin mojarra at approximately 3°C above ambient conditions, and increased by more than 50% for all nearshore fishes examined at approximately 4°C above ambient conditions in the summer (29.5°C) (Chapter 3). Swimming performance decreased for all nearshores fishes examined at approximately 4°C above ambient conditions (Chapter 3). Bonefish had the lowest total acclimatization response ratio relative to other fish species, indicating a limited ability to acclimatize to variable thermal landscapes (Chapter 4). Moreover, bonefish exhibited the most negative upper thermal safety margin (i.e.,  $\text{CT}_{\text{Max}} - \text{maximum environmental temperature}$ )

relative to all other nearshore fishes. On the whole, physiological disturbances in bonefish are comparable, and in many instances, exceed the response of yellowfin mojarra, schoolmaster snapper, and checkered puffer, suggesting that bonefish are the most vulnerable species examined in this dissertation.

### **Acclimatization**

Results indicated that some, but not all, nearshore fishes in this dissertation have a high capacity to acclimatize to new thermal environments. Acclimatization is one potential mechanism for fish to cope with elevated temperatures associated with climate change (Somero, 2010). A recent meta-analysis on plasticity in thermal tolerance limits suggests that only partial compensation will occur through acclimatization, with most acclimatization response ratios (AZRR) below 0.5 (50% compensation) and none reaching 1.0 (100% compensation), indicating that physiological plasticity will likely play only a small role in buffering fish against warming oceans (Gunderson & Stillman, 2015). Elevated temperatures in the summer were identified as the most physiologically challenging for nearshore fishes (Chapter 2), suggesting that upper AZRR may be more important to evaluate relative to lower AZRR. Unfortunately, the upper AZRR of bonefish (0.51) and checkered puffer (0.47) were small relative to other species examined (Chapter 4), indicating these species may only partially acclimatize to warming oceans. Conversely, AZRR of schoolmaster snapper (1.02) was high relative to other species examined in this dissertation, likely resulting in complete acclimatization to warmer temperatures in the future. On the whole, species-specific differences in acclimatization capacity may result in some ecological winners (e.g., schoolmaster snapper) and losers (e.g., checkered puffer and bonefish) as the oceans warm.

## **Biotic Interactions**

Findings indicate that biotic interactions (e.g., threat of predation) have the potential to alter habitat choice in some, but not all, species examined in this dissertation. Specifically, yellowfin mojarra exhibited higher thermal avoidance thresholds in the presence of a predator relative to its absence (Chapter 5). If environmental temperatures rise, even temporarily, above the thresholds of juvenile fishes (e.g., in a heat wave), then these fish may be forced to move away from the protection of shallow water into predator rich environments, which may increase predator/prey interactions and, therefore, predation rates (Rypel *et al.*, 2007). Conversely, thermal avoidance thresholds of bonefish were not influenced by the threat of predation (Chapter 5), indicating that these fish will exit nearshore habitats when temperatures exceed  $\sim 33.5^{\circ}\text{C}$ . Collectively, elevated temperatures that coincide with and exceed future predictions in nearshore ecosystems appear to have species-specific physiological and behavioral impacts, with biotic interactions with predators playing a key role in shaping community structure.

## **Potential Changes in Abundance and Distribution**

Climate change has the potential to alter the distribution, abundance, and community interactions at a local and global scale (Albouy *et al.*, 2014). At a local scale, the structure of the nearshore environment, a nursery for many ecologically and economically important fishes, may be considerably altered in the future. Juvenile fish use this shallow ecosystem to avoid predators and find food, while adult fish primarily access this system as the tide rises to take advantage of the food resources in these productive nearshore environments (Sheaves, 2005). Predicting how the distribution and abundance of species will change is a difficult task because of the variation in species' physiological responses and biotic interactions (Blois *et al.*, 2013). Nevertheless, I

highlight two scenarios based on the findings in this dissertation that may alter the distribution and abundance in the nearshore ecosystem under forecasted conditions.

Elevated temperatures, particularly in the summer, appear to have the greatest impact on bonefish because of its associated considerable physiological disturbance (Chapter 2) and loss of swimming and metabolic performance (Chapter 3). This species has a relatively low capacity to adjust upper thermal limits to new thermal environments (Chapter 4), indicating that only partial compensation to elevated temperatures will likely occur. Based on these physiological responses, bonefish in the nearshore environment are predicted to expend additional energy on maintaining homeostasis, experience higher mortality rates due to a limited ability to avoid predators, and slower growth rates due to reduced capacity to capture prey, ultimately resulting in a decrease in abundance. Fortunately, bonefish avoid temperatures above 33.5°C regardless of the threat of predation (Chapter 5), which indicates these fish will likely exit the nearshore environment at or below temperatures that result in loss of performance. It remains unclear, however, how biotic interactions in adjacent ecosystems may influence the distribution and abundance of bonefish.

Physiological disturbances and biotic interactions associated with elevated temperatures are also predicted to influence the distribution and abundance of yellowfin mojarra in the nearshore ecosystem but in a slightly different way. Juvenile yellowfin mojarra experience minimal physiological disturbances at elevated temperatures (Chapter 2), but a loss in swimming and metabolic performance at 34°C (Chapter 3), an intermediate capacity to acclimatize thermal environments, with extreme present-day temperatures surpassing upper tolerance limits of this species (Chapter 4). Behavioral thermoregulation (i.e., move to more favorable thermal environments) may not buffer yellowfin mojarra against the negative physiological consequences associated with elevated temperatures because avoidance thresholds in the absence (36°C) and

presence of a predator (~39°C) surpass temperatures that result in a loss of performance (34°C) (Chapter 3, Chapter 5). Small-bodied fish often seek the shelter of shallow, structurally complex habitats to hide or evade predators (Rypel *et al.*, 2007; Abrahams, 2011). Yellowfin mojarra will most likely be confined to the nearshore environment as temperatures increase (Chapter 5), and may be restricted to relatively small microhabitats (e.g., mangrove roots) in an effort to hide from predators, particularly at low tide when inundated areas are limited. Fish crowded into a small area may quickly deplete food resources and available oxygen (Crawshaw & Podrabsky, 2011), resulting in an inability to meet increasing energetic demands at elevated temperatures. To exacerbate this situation, yellowfin mojarra also experience a reduction in swimming performance at elevated temperatures (Chapter 3), which will likely reduce the ability of this fish to capture prey. Interspecific variation in performance species at elevated temperatures can result in superior competitors becoming more dominant in the community (Kordas *et al.*, 2011). Schoolmaster snapper demonstrated superior swimming performance across a gradient of temperatures relative to yellowfin mojarra (Chapter 3), suggesting that schoolmaster snapper may become a dominant competitor in the nearshore environment. Collectively, physiological disturbance and undesirable biotic interactions (e.g., threat of predation limiting available thermal habitats) may have negative consequences on the distribution and abundance of yellowfin mojarra in the future.

### **Tipping Points**

The expected worse-case scenario for temperature over the next 100 years will be a tipping point (i.e., unacceptable change) for most nearshore fishes examined in this dissertation. At 33°C, bonefish, yellowfin mojarra, and schoolmaster snapper experience a loss in performance (Chapter 3). If the IPCC predictions are conservative by just 1°C, then all nearshore fishes examined will

experience an increase in the cost to maintain bodily functions and a decrease in swimming performance (Chapter 3). At a tipping point of 34°C, checkered puffer and bonefish will likely choose cooler habitats (e.g., deeper environments) regardless of the threat of predation (Chapter 5), indicating that these species will inhabit nearshore ecosystems less frequently.

Extreme weather (e.g., heat wave) is expected to occur more frequently and in greater intensity as the climate changes (Trenberth & Fasullo, 2012), and may be an important driver of ecosystem change (Wernberg *et al.*, 2012). Extreme present-day temperatures of 43°C exceed thermal avoidance thresholds (or tipping points) of all nearshore fishes examined regardless of the threat of predation and considerably surpass the physiological tipping point (34°C) of these species. More frequent extreme temperatures for longer durations will likely limit available thermal habitats for these species. Overall, the worst-case scenario predicted by the IPCC (i.e., 2°C increase), in conjunction with more extreme weather, will likely push nearshore fishes examined in this dissertation beyond their tipping points, most likely resulting in considerable alterations in the distribution and abundance of these species at a local and global scales.

### **Future Research**

Findings of this dissertation highlight several areas for future research. First, short-term extreme climatic events may be important drivers of abundance and distribution of species (Wernberg *et al.*, 2012). Evaluating the capacity for thermal acclimatization over relatively short periods of time (14-30 days) may provide insight into the capacity of nearshore fishes to acclimatization to these short-lived extremes in temperature. Second, even though no synergistic or canceling effects were observed in Chapter 2, experimental designs should still manipulate both temperature and pCO<sub>2</sub> in an effort to mimic conditions projected by the IPCC. Manipulated conditions should account for daily and seasonal fluctuations in abiotic variables (e.g., pCO<sub>2</sub>) in

the ecosystem. For example, pCO<sub>2</sub> in nearshore environments can range from 70-1325 μatm on a daily basis (Shaw *et al.*, 2012), but best practices for ocean acidification research suggest a treatment level of 1000 μatm to simulate conditions in the next 100 years (Barry *et al.*, 2010), which is clearly lower than present-day conditions in this environment. Third, certain life stages are hypothesized to be more vulnerable to climate-related stressors due to a narrow aerobic scope (Pörtner & Farrell, 2008). Identifying which stage in the life cycle is most vulnerable to climate-related stressors can help focus conservation efforts (e.g., designing marine reserves to protect this vulnerable life stage). Lastly, accurately predicting how the distribution and abundance of species will change under forecasted conditions will require models that incorporate both abiotic conditions and biotic interactions.

In closing, the insight gained from these studies will improve our predictions of the structure of nearshore ecosystems in the future, and highlights the need to incorporate biotic interactions into these predictions. Managing for climate change is a difficult task because it is a world-wide problem that spans many geo-political boundaries, but at a local-scale, management strategies that minimize additional stressors (e.g., habitat degradation) and maintain or enhance ecological resilience is our best chance at buffering species against climate-related stressors (Hoegh-guldberg & Bruno, 2010). The most promising conservation measure is to delineate a network of marine sanctuaries/national parks that incorporate current and projected changes in the distribution of organisms, particularly for highly vulnerable species (e.g., bonefish, yellowfin mojarra) that inhabit the nearshore ecosystem.

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