Physiological ecology and behaviour of bonefish (*Albula vulpes*) in tropical tidal flats ecosystems

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Dedication

To my parents, Rosie and Bill Murchie, for their never-ending encouragement in my pursuit to learn about fishes, their environments, and myself along the way. To my supervisors, Steve Cooke and Andy Danylchuk, for the opportunity to get my hands wet in the most beautiful environment I have ever seen and to further develop my skills as a fisheries biologist. To my friends, literally across the world, who have been there for me to talk about science, my journey, and things that make us smile. To the bonefish on which this thesis was built, I am forever indebted.

Abstract

Knowledge of the physiological ecology and behaviour of fishes is particularly lacking for subtropical and tropical tidal flats when compared to temperate systems. The goal of this thesis was to use bonefish (*Albula vulpes*) as a model to determine and describe the environmental relations of fish in tropical tidal flats, and examine how energy is transported through these ecosystems. Prior to beginning experimentation, I developed strategies to facilitate the acclimation of wild bonefish to captivity, a prerequisite for laboratory studies that followed. A combined field and laboratory study examining the thermal biology of bonefish revealed that bonefish occupied habitats approaching their laboratory-determined thermal tolerance and can apparently do so without significant physiological consequences or mortality, except when exposed to additional stressors (e.g., catch and release angling). The strategy of how bonefish allocate their food energy was revealed through an examination of proximate body composition. Bonefish were composed of 72% water, 21% protein, 4% ash, and 3% lipid, which is consistent with the wet weight values of the majority of fishes studied to date. The lack of seasonal differences in whole body and liver lipid content suggested a sufficient food supply year round. Energetics and behaviour were also investigated using acoustic telemetry in the wild, with both traditional (position only) and acceleration transmitters, accompanied with ethograms in a mesocosm environment and laboratory respirometry experiments. Bonefish exhibited periods of site fidelity interspersed with transient behaviour, ultimately demonstrating connectivity between tidal creeks and nearshore coastal areas. Acceleration data revealed that bonefish typically operate between 40-60% of their estimated metabolic scope, and use swim-then-drift behaviours to maximize their energy efficiency. Collectively, this body of work revealed that bonefish use strategies that make them capable of handling the stochastic environment of subtropical and tropical tidal flats. Such information should be useful for management of the bonefish fishery, bioenergetics modeling, determining boundaries for marine protected areas, and assessing resiliency in tidal flats systems to future disturbances (e.g., climate change, habitat alteration, pollution, etc.).

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Co-authorship

Chapter 2: Strategies for the capture and transport of bonefish, *Albula vulpes*, from tidal creeks to a marine research laboratory for long-term holding. K.J. Murchie, S.E. Danylchuk, C.E. Pullen, E. Brooks, A.D. Shultz, C.D. Suski, A.J. Danylchuk, and S.J. Cooke.

While this study is my own, the work is a collaborative effort and required the valuable assistance of my co-authors. Specifically, the project was conceived by Murchie, Danylchuk (both S.E. and A.J.), Suski, and Cooke. The field work was performed by all co-authors. All data analysis was conducted by Murchie. Data were interpreted by Murchie, Danylchuk (both S.E. and A.J.), Suski, and Cooke. All writing was conducted by Murchie. All co-authors provided feedback on the manuscript. The manuscript has been published with the following citation:

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Chapter 3: Thermal biology of bonefish (*Albula vulpes*) in Bahamian coastal waters and tidal creeks: an integrated laboratory and field study. K.J. Murchie, S.J. Cooke, A.J. Danylchuk, S.E. Danylchuk, T.L. Goldberg, C.D., Suski, and D.P. Philipp.

While this study is my own, the work is a collaborative effort and required the valuable assistance of my co-authors. Specifically, the project was conceived by all co-authors. Telemetry field work was performed by all co-authors. Critical thermal maximum experiments were performed by Murchie. All data analysis was conducted by Murchie. Data were interpreted by Murchie, Danylchuk (A.J.), and Cooke. All writing was conducted by Murchie. All co-authors provided feedback on the manuscript. The manuscript has been submitted for publication to the Journal of Experimental Marine Biology and Ecology.

Chapter 4: Seasonal energetics and condition of bonefish from different subtropical tidal creeks in Eleuthera, The Bahamas. K.J. Murchie, S.J. Cooke, and A.J. Danylchuk.

While this study is my own, the work is a collaborative effort and required the valuable assistance of my co-authors. Specifically, the project was conceived by all co-authors. All field and laboratory work was conducted by Murchie. All data analysis was conducted by Murchie. Data were interpreted by Murchie, Cooke, and Danylchuk. All writing was conducted by Murchie. All co-authors provided feedback on the manuscript. The manuscript is in press with the journal Marine and Coastal Fisheries.

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Chapter 5: Bonefish (*Albula vulpes*) movement patterns in tidal creeks and coastal waters of Eleuthera, The Bahamas. K.J. Murchie, S.J. Cooke, A.J. Danylchuk, S.E. Danylchuk, T.L. Goldberg, C.D. Suski, and D.P. Philipp.

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Chapter 6: Estimates of field activity and metabolic rates of bonefish (*Albula vulpes*) in coastal marine habitats using acoustic tri-axial accelerometer transmitters and intermittent-flow respirometry. K.J. Murchie, S.J. Cooke, A.J. Danylchuk, and C.D. Suski.

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Chapter 1: General introduction

State of coastal ecosystems

Coastal ecosystems are both very productive and very vulnerable (Mann, 2000). High productivity and visual aesthetics have attracted 60% the world's population along the coastline, with half of these residents in developing countries (Barnabé and Barnabé-Quet, 2000). As a focal point for human settlement and marine resources, coastal areas have faced overexploitation, habitat degradation, and pollution (Lotze et al., 2006). In the past two decades, 35% of the world's mangrove forests have been lost (Valiela et al., 2001), and we have entered a global crisis for seagrass habitats (Orth et al., 2006). Consequences of such coastal transformations have included the collapse of fisheries (Jackson et al., 2001; Worm et al., 2006; Andalecio, 2010) and increased loss of human life following natural disasters such as the 2004 Asian tsunami (Danielsen et al., 2005) and Hurricane Katrina in 2005 (Stokstad, 2005). With a current interest in managing marine ecosystems for resilience (Hughes et al., 2005; Hofmann and Gaines, 2008), there is a need to understand more about coastal ecosystems and their service providers (Kremen, 2005).

The stochastic tidal flats environment

Tidal flats extend seaward from mangrove forests along many subtropical and tropical coastlines (Dittman, 2002). At depths of less than 4 m, tidal flats ecosystems encompass a myriad of habitat types including mangrove creeks, sand and algal plains, seagrass meadows, and patches of coral reef (Fig. 1.1). Semidiurnal tidal cycles restrict the exclusive use of many of the habitats by fish, causing them to use alternate habitats such as seagrass meadows when mangroves are inaccessible (Sheaves, 2005). The movement of biota coupled with the physiochemical processes associated with tides inherently connects the habitat mosaic comprising tidal flats systems (Moberg and Folke, 1999; Semeniuk, 2005; Mumby, 2006).

Dynamic environments can present physiological challenges for resident species, and distributional limits can be dictated by various abiotic factors (Reise, 1985). The tropical ocean coast offers abiotic conditions as inimical to life as the supposedly more adverse, higher latitudes due to frequent and intense disturbances (Alongi, 1998). In aquatic systems, water temperature is often referred to as the master factor among environmental variables affecting fish (Fry, 1967; Brett 1971; Beitinger and Fitzpatrick, 1979). Daily fluctuations in water temperature within the flats environment can be dramatic (Alongi, 1998). Salinity and oxygen concentration are also subject to variation in tidal flats environments. The amount of oxygen that can be held in seawater is a function of temperature, whereas salinity can be influenced by rainfall events, and also through evaporation under hot tropical conditions or windy conditions (Nybakken and Bertness, 2005). Physiological diversity is responsible for how an organism will respond to various disturbances in the abiotic environment, and is a result of genetic, developmental or environmental influences (or some combination of these factors) (Spicer and Gaston, 1999). Although acclimatization to these conditions may lead to shifts in an individual’s tolerance to changes, these shifts can also constitute costly elements of an energy budget (Somero, 2002).

Energy flow in animals

As energy is the currency of natural economies, understanding how energy flows through an ecosystem is one of the most basic principles of ecology (Smith, 1992). Because ecosystems are composed of many populations of producers, consumers, and decomposers, the transfer of energy may follow several pathways but always must follow physical principles such as the law of thermodynamics (Nybakken and Bertness, 2005). Animals function as closed systems resulting in all energy acquired by an organism being used in metabolic processes, lost as waste, or used in growth or reproduction (Winemiller and Polis, 1996; Diana, 2004). However, ecosystems are not closed, and the connectivity resulting from animal migrations and physicochemical processes translates into substantial energy movement (Mann, 1982; Valiela, 1991; Deegan, 1993). An appreciation of the tactics used to deal with a fluctuating environment and the trophic relationships that occur is fundamental to understanding the ecology of these coastal environments (Odum, 1976), yet little is known about this topic in tropical and subtropical flats. Such a dearth in scientific literature on basic aspects of flats ecology is concerning as it is imperative for the effective management of coastal environments.

Model species

A model species that moves and feeds among the mosaic of habitats characteristic of tropical flats could be used to attain a greater understanding of the ecology of these dynamic systems. Bonefish (*Albula* spp.) are a group of such fishes that not only demonstrate the interconnectedness of habitats within the flats, but also the connectivity of the flats with other marine ecosystems. Although aspects of the life cycle of bonefish are not fully understood, it is thought that adult bonefish spawn in deeper water roughly between October and May (Mojica et al., 1995), where eggs hatch into leptocephali, (ribbon-like planktonic larvae) (Fig. 1.2). The leptocephalus larval stage lasts between 1.5 to 7 months depending on location (see Mojica et al., 1995; Pfeiler et al., 1988). Metamorphosis into the juvenile stage may be delayed until environmental conditions favour onshore settlement (Mojica et al., 1995). The location of juvenile bonefish habitats overall remains an enigma, but they have been captured on occasion in sandy beaches in tidal flats (Ault et al., 2008; Murchie et al., unpublished data). The sub-adult and adult phases inhabit nearshore coastal areas, and are the life stage in which the majority of studies have focused on to date. Throughout their circumtropical distribution, sub-adult and adult bonefish move into shallow flats to feed on benthic invertebrates and small fish during high tide, and then move into deeper water on the ebb tide (Colton and Alevizon, 1983a,b; Humston et al., 2005) (Fig 1.3). Because of their predictable movements and benthic lifestyle, sub-adult and adult bonefish may provide a number of fundamental ecosystem services (see Holmulund and Hammer, 1999 for definition) (e.g., nutrient cycling, regulation of sediment processes through bioturbation) to tidal flats ecosystems and beyond.

The notion that bonefish may play a critical role in flats ecology is significant as bonefish are a highly prized sport fish throughout their worldwide distribution (Pfeiler et al., 2000) (Fig 1.4). The appeal of stalking bonefish on the flats and the thrill of the fight when hooked have culminated in a billion dollar per year industry in the Florida Keys alone (Humston, 2001). Indeed, estimates in Florida suggest that each bonefish has a lifetime value of $75 000 (Ault et al., 2006), although this fails to consider the ecological services that they may provide (Costanza et al., 1997; Holmlund and Hammer, 1999).

Recreational fishing for this species could possibly support the economy of coastal communities in small island nations such as The Bahamas, where tourism is responsible for 50% of the gross domestic product (Buchan, 2000; Danylchuk et al., 2008). Indeed, recent estimates suggest that recreational bonefishing generates $141 million in total economic benefits to the Bahamian economy annually (BFFA, 2010). Given that tourism is commonly concentrated in coastal areas, shoreline development could have a significant impact on bonefish stocks and entire flats ecosystems if bonefish indeed provide key ecosystem services, and if habitat is destroyed. In addition, angling practices that do not maximize the post-release survival of bonefish (see Cooke and Philipp, 2004; 2007) could reduce adult population numbers while also threatening the integrity of the flats ecosystems.

Goals

Despite their known economic value and possibility as a key ecosystem service provider, there are considerable gaps in the scientific literature surrounding the biology of bonefish (Ault, 2008). Thus given the lack of understanding on tropical and subtropical flats and one of their main vertebrate inhabitants, the goal of this research is to use assessments of physiological ecology and behaviour of bonefish to 1) determine and describe the environmental relations of fish in tropical tidal flats, and 2) examine how energy is transported through these ecosystems. Because of the dearth of information on tidal flats inhabitants, Chapter 2 deals with strategies to successfully capture, transport, and retain bonefish in holding facilities so that laboratory experiments can be conducted on them. Such laboratory work would complement field-based research and offers the precision associated with a controlled environment. Specifically, detailed observations of bonefish behaviour, physical appearance, and physiological assessments of blood chemistry were made given the importance of understanding the stress response of fish to such handling and holding procedures (Waring et al., 1996). Since water temperature can be an overriding factor influencing nearly all physiological and life history activities of fish (Fry, 1967; Brett, 1971), Chapter 3 examines the thermal biology of bonefish through a combined laboratory and field study. The maximum thermal tolerance of bonefish was determined at two different seasonal acclimation temperatures and was combined with an examination of stress physiology associated with reaching their critical thermal maxima. Because stressors rarely act on their own in the natural environment, the combined effects of thermal stress and capture/holding stress were examined. Lastly, the spatial ecology of bonefish was linked to thermal regimes experienced in tropical tidal flats habitats. Because the fluctuating nature of various environmental variables in tidal flats habitats can influence how individuals partition energy between growth, reproduction, and survival (Claireaux and Lefrançois, 2007), the strategy of seasonal energy allocation in bonefish was investigated through an assessment of proximate body composition and fish health indices in Chapter 4. Specifically, energy partitioning between tissues in relation to abiotic (e.g., season, location) and biotic (e.g., sex, size) factors were examined, as well as baseline values of bonefish health. A further appreciation energy dynamics within tidal flats can be gained by assessing the spatial ecology of an organism. In Chapter 5, acoustic telemetry is used to investigate patterns of habitat use, relative activity space of individuals, schooling behaviour, and the influence of tidal cycles. Diel and seasonal trends in movement patterns are also investigated. Ultimately, this chapter allows an assessment of a critical aspect of bonefish ecology, as well as providing a basis for understanding how energy moves through tidal flats systems. Chapter 6 examines energy expenditure of free-swimming bonefish in the wild at an even finer resolution through the use of acoustic acceleration transmitters. Field data were linked to laboratory calibration of transmitters, behavioural observations (ethograms) in a mesocosm environment, and laboratory assessments of respiration. Field activity and metabolic rates of bonefish were determined. Finally, Chapter 7 provides an assessment of what has been learned about how animals make a living in tropical tidal flats environments, the limitations of current research, methods to overcome these limitations, and future directions for research in this field.

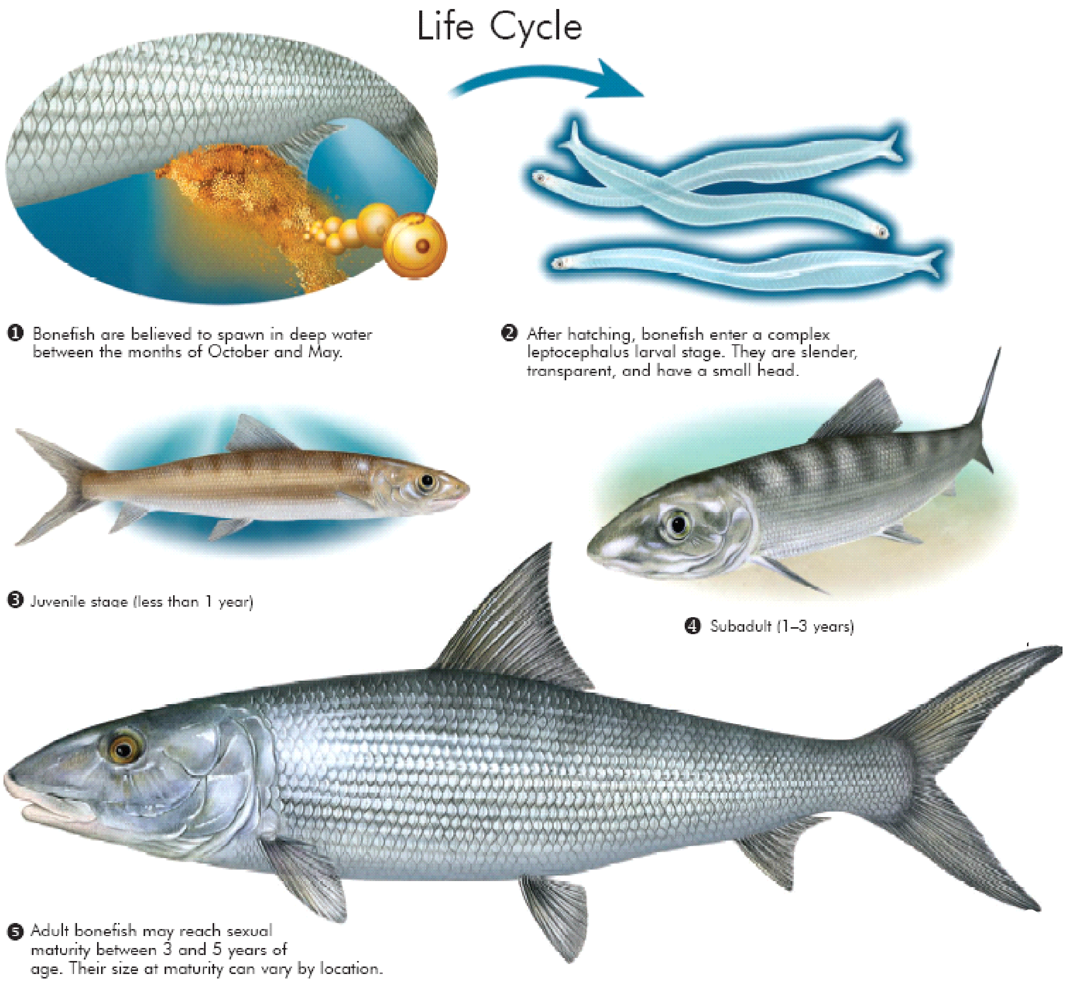
Study location

The Bahamas are a prime location for investigations of flats habitats as the archipelago contains the largest tropical shallow water area in the Western Atlantic (Buchan, 2000). Approximately 40% of the total area of The Bahamas is shallow water banks (BEST, 2002). Bonefish are common inhabitants within the Bahamian Archipelago and play an important role in supporting the regional economy (Danylchuk et al., 2008). To date, the majority of investigations into bonefish biology have occurred in the Florida Keys in flats habitats that have been heavily degraded and where extensive exploitation of bonefish populations have occurred (Bruger and Haddad, 1986; Ault et al., 2002). Even in the Pacific, where recreational fisheries resources are shared with subsistence and small-scale commercial fisheries, it is difficult to conduct baseline studies on bonefish due to overexploitation (Friedlander et al., 2008). Currently, the island of Eleuthera in The Bahamas has not undergone extensive coastal development, and angling pressure has been minimal, but pressures are forthcoming for this, and other ‘family islands’ of The Bahamas (Buchan, 2000; BEST, 2002; Gruber and Parks, 2002; Danylchuk et al., 2007a). Another benefit of conducting research around Eleuthera is that there is only one species of bonefish present (i.e., *A. vulpes*) (Danylchuk et al., unpublished data), which is in contrast to many locations in Hawaii, Florida, and Brazil (Bowen et al., 2008). The lack of additional cryptic species of bonefish facilitates the assessment of the physiological ecology and behaviour of *A. vulpes*. The research contained in this dissertation will provide critically needed baseline data for the area which will be useful for directing future conservation and management strategies for coastline development, proposed marine protected areas and fisheries regulations, not only in The Bahamas, but globally.

Figures

Figure 1.1: Photographs of the habitat mosaic which make up subtropical and tropical tidal flats ecosystems. The top photograph shows a mangrove lined tidal creek. The bottom left photograph shows a seagrass meadow. The bottom right photograph is an underwater shot of mangrove prop roots.

Figure 1.2: Bonefish life cycle schematic (adapted from the Loxahatchee River District Poster Series No. 2, funded by The Nature Conservancy).



Life Cycle

❶ Bonefish are believed to spawn in deep water between the months of October and May.

❷ After hatching, bonefish enter a complex leptocephalus larval stage. They are slender, transparent, and have a small head.

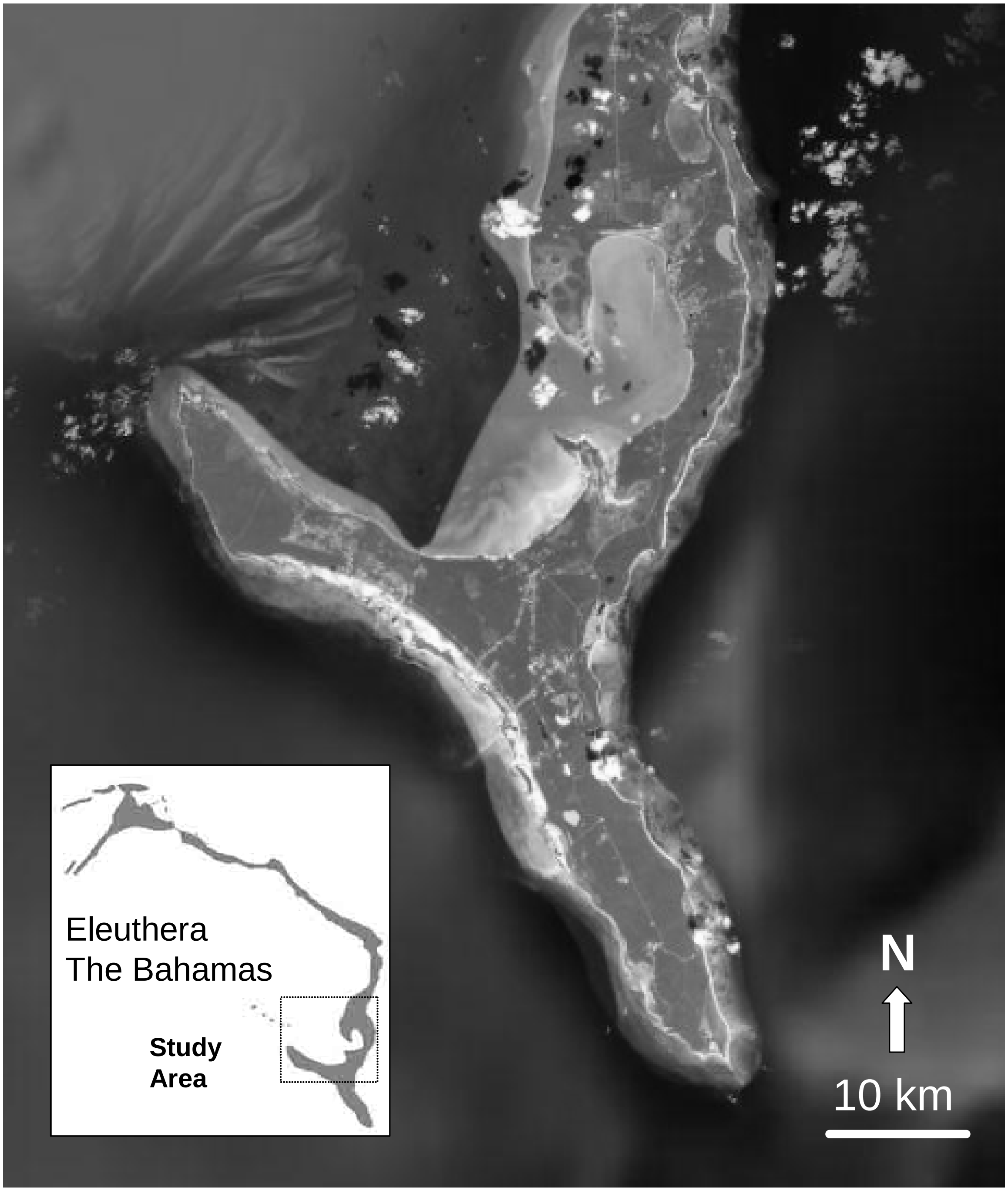
❸ Juvenile stage (less than 1 year)

❹ Subadult (1–3 years)

❺ Adult bonefish may reach sexual maturity between 3 and 5 years of age. Their size at maturity can vary by location.

Figure 1.3: The upper panel shows a school of bonefish swimming and feeding in a tidal flat in Eleuthera, The Bahamas. The bottom panel shows an angler fly-fishing for bonefish in a tidal creek, with an inset picture of a bonefish post-capture.

Figure 1.4: Study area in south Eleuthera, The Bahamas. The inset map displays the entire island of Eleuthera with the study area highlighted.



Chapter 2: Strategies for the capture and transport of bonefish, *Albula vulpes*, from tidal creeks to a marine research laboratory for long-term holding

Abstract

Throughout their circumtropical distribution, bonefish (*Albula* spp.) play a vital role in local economies as a highly prized sport fish. Recent interest in stock enhancement to sustain bonefish fisheries has led to the recognition that there currently are no data on how to live capture large numbers of adults (potential broodstock), transport them to captive facilities, and how to handle them to ensure high survival. The objective of this study was to develop strategies for the capture and relocation of wild bonefish to a marine research holding facility to enable basic research and explore the potential for culturing bonefish for stock enhancement. Bonefish *Albula vulpes* (Linnaeus, 1758) were captured as they entered or left tidal creeks on Eleuthera, The Bahamas using seine nets and then transported by boat or truck to the laboratory. The relocation process evoked secondary stress responses at the metabolic, osmoregulatory and hematological levels as indicated by changes in blood glucose, lactate, hematocrit and ion values, relative to control fish. Physical and behavioural disturbances were also observed in bonefish that were unable to acclimate to laboratory conditions. Successful laboratory acclimation and long-term holding of wild bonefish was achieved through an adaptive learning process, whereby we identified a series of strategies and handling techniques to facilitate the acclimation of wild adult bonefish to captivity. This

knowledge will enable future laboratory research on bonefish and is a prerequisite to the culture of this highly prized sport fish, and other subtropical and tropical marine species.

Introduction

In recent years, the apparent world-wide decline in marine fish populations (e.g., Pauly et al., 2003; Pauly et al., 2005; Worm et al., 2006) has renewed interest in the development of techniques for holding fish in captivity to enable culture for wild stock enhancement (e.g., Blankenship and Leber, 1995; Leber 2004; Bell et al., 2006; True et al., 1997), captive food production (i.e., mariculture; De Silva, 1998; Naylor et al., 2000), or for scientific investigations related to basic biology, conservation and management. Activities such as the capture and transport of fish are routine in the aquaculture sector (e.g., Robertson et al., 1987; Garcia et al., 2000), and are necessary for experiments in which wild fish are brought into the laboratory. Handling and transport, however, can have negative consequences on the physiology and survival of fish (Portz et al., 2006; Hur et al., 2007). Indeed, not all fish transferred from the wild to the laboratory acclimate to captivity and survive. To minimize the detrimental effects associated with the relocation and holding process and facilitate rapid acclimation to captivity, researchers have studied the stress response associated with different handling practices (e.g., capture, transport, handling). However, most of the studies to date have focused on salmonids (e.g., Ackerman et al., 2000; Barton, 2000), and a range of temperate, non-salmonid freshwater species (e.g., Pankhurst et al., 1992; Waring et al., 1996), with proportionately fewer data on tropical and sub-tropical fish (De Silva, 1998; Grutter and Pankhurst, 2000; Biswas et al., 2006). The lack of information on species from tropical and subtropical areas is concerning as fisheries are more crucial to the sustainability of livelihoods in tropical as opposed to temperate regions (Baras et al., 2002). Furthermore, marine stock enhancement and mariculture are considered challenging and knowledge is not as advanced as for freshwater taxa (De Silva, 1998; Leber et al., 2004).

An example of a marine fishery that is economically important but where large gaps in scientific knowledge exist is that of the bonefish (*Albula* spp.). Throughout their circumtropical distribution, bonefish play a vital role in local economies as a highly prized sport fish (Colton and Alevizon, 1983a; Pfeiler et al., 2000). Estimates suggest that recreational angling for bonefish is a billion dollar per year industry in the Florida Keys alone (Humston, 2001). Bonefishing could possibly support the economy of coastal communities in small island nations such as The Bahamas, where tourism is responsible for 50% of the gross domestic product (Buchan, 2000; Danylchuk et al., 2008). Despite their recognized economic value, very little is known about the ecology, physiology, or population dynamics of bonefish (Ault et al., 2008). Although recreational fishing for bonefish is primarily catch-and-release (Humston, 2001), mortality rates can be high (up to 39%) when fish are released in areas with high predator densities (Cooke and Philipp, 2004). Angling related mortalities coupled with habitat degradation in coastal areas where bonefish occur may be responsible for observed decreases in some local bonefish populations, along with shifts in size structure (see Bruger and Haddad, 1986; Ault et al., 2008). Locals, anglers, guides, fisheries managers, and scientists are interested in conservation strategies that will ultimately lead to the sustainability of bonefish stocks.

Recent interest in stock enhancement for bonefish (see comments in Ault, 2008) has led to the recognition that there currently are no data on how to live capture large numbers of adults (potential brood stock), transport them to captive facilities, and how to handle them to ensure high survival. Holding bonefish in captivity would also enable basic research on bonefish biology as well as better understanding how they respond to variable environments and other relevant stressors. Such laboratory work would complement field-based research and offer the precision associated with being able to control both animals and their environments experimentally (Goldstein and Pinshow, 2002; Costa and Sinervo, 2004). To our knowledge, few previous studies have attempted laboratory-based experiments on bonefish, or have held large number of individuals for long-periods. A study by Crabtree et al. (1998b) involved holding eleven adult bonefish in an outdoor pond and repeatedly angling them over a one year period to evaluate hooking mortality related to recreational fishing but they provide no information on field capture, handling, transport, and laboratory care. Thus, the objective of this study was to use a combination of detailed observations, adaptive learning, and physiological assessments to develop optimal strategies for the capture, transport and holding of bonefish in captivity to facilitate future laboratory studies and culture.

Materials and methods

This study took place in south Eleuthera, The Bahamas (18364035 E, 2747609 N) in a number of tidal creek and tidal flats systems, as well as at the Cape Eleuthera Institute (CEI) research facility (Fig. 2.1). Preliminary genetic analyses on bonefish from this area indicated that all bonefish specimens were *Albula vulpes* (Danylchuk et al., 2007a). Research was conducted in two phases: the first phase consisted of an assessment of the transportation and lab acclimation processes (February 17 to April 17, 2007). The second phase was an assessment of handling and long-term holding of bonefish (April 20 to September 14, 2007). This study was conducted in accordance with the policies of the Canadian Council on Animal Care as administered by the Carleton University Animal Care Committee (Protocol B07-03, 04) and with approval of the CEI Research Advisory Committee.

Phase 1 – Assessment of Transportation and Lab Acclimation

Fish Capture Techniques

Based on our interaction with anglers and locals, it became apparent that most bonefish are captured by rod and reel and catch-per-unit-effort can be low making this an unsuitable technique for capturing large numbers of individuals. Some artisanal fishers employ gill nets but all fish tend to be dead or moribund even if used for short sets. Therefore, study fish were captured from tidal creeks and tidal flats using various seine nets (0.6 cm mesh, 46 m long; 1.3 cm mesh, 46 m long; 3.2 cm mesh, 76 m long; 7.0 cm mesh, 61 m long) deployed at creek mouths to intercept bonefish on incoming or outgoing tides. When a school of bonefish approached, the net was moved quickly to encircle the fish. Upon capture, individual fish were dip netted or passed by bare hand into flow-through holding pens (1.3 m x 0.8 m x 1.25 m tall, 3.1 cm extruded plastic mesh) submerged in a minimum of 0.6 m of water, where they remained until ready for transport to CEI. Only in one case, at Plum Creek, were coolers (108 L) used to hold captured fish; frequent water changes were made while holding these fish.

Transportation of Fish

Fish were transported from the field back to the research facility either by flatbed truck or by boat, depending on road access to the location, distance to CEI, and ease of hauling sampling equipment and personnel. A 1068 L (1.0 m length × 1.1 m width × 1.0 m diameter) square tank was secured on the deck of the truck along with a 11.5 hp generator (6000 watt) and a 1 hp aeration pump (Sweetwater model S41; 15 volt; 3450 RPM; Aquatic ecosystems Inc., Apopka, Florida). The boat used was a 19 ft Carolina Skiff equipped with a 60 hp engine. When using the boat, fish were transported in 108 L coolers. The coolers were not supplied with aeration, but instead had frequent water changes during the transport process (approximately every 5 minutes).

Holding Tanks at the Cape Eleuthera Institute

Upon arrival to CEI, bonefish were transferred to small (1.6 m diameter x 0.85 m height; 1400 L) or large (3.7 m diameter x 1.25 m height; 13 180 L) circular holding tanks that were aerated and continuously supplied with fresh sea water (1800 L/hr) at ambient temperatures. The sea water intake for the facility is located approximately 200 m offshore at a minimum depth of 4 m at low tide. A 15 mm mesh intake screen and 4 mm mesh strainer basket prior to the pump reduced the amount of particulate matter entering the tanks. Fish were fed a diet of queen conch (*Strombus gigas*) (Linneaus, 1758) offal provided by local artisanal fishers within 48 hr of arrival. Tanks were housed in a covered open-sided outdoor facility with natural photoperiod but the tanks themselves were not covered.

Physiological Disturbances Associated with Transport

In addition to observing fish for changes in physical appearance (coloration), behaviour (swimming patterns, schooling), and survivorship, the physiological disturbances associated with capturing, transporting, and holding bonefish were quantified. Physiological disturbances were quantified by non-lethally sampling blood from a sub-set of bonefish at various stages of the relocation process (see Table 2.1 for details). In addition, a sample (n = 7) of bonefish were held in individual sensory deprivation chambers (approximately 100 L volume) for 24 hr to generate control (resting) physiological values for comparison. Secondary stress response parameters (glucose, lactate, sodium and potassium concentrations, and hematocrit) were examined for each blood sample. To live sample bonefish for blood, individuals were restrained by hand in supine position (without the use of anesthetic) in a foam-lined trough filled with sea-water at a depth to completely submerge their gills. Using a 21 gauge needle, approximately 1.5 mL of blood was drawn from the caudal vessel into a 3 mL lithium heparinized vacutainer (BD vacutainer blood collection tube; Becton, Dickinson and Company; Franklin Lakes, NJ). After the blood was drawn (typically less than 45 seconds), it was held in an ice-water slurry until analysis. Total length (to the closest mm) was also recorded on live sampled bonefish.

All blood chemistry parameters were measured on whole blood using field physiology tools (Costa and Sinervo, 2004). Glucose and lactate levels were measured by adding 10 µl of blood to handheld glucose (ACCU-CHEK glucose meter, Roche diagnostics Corp., Indianapolis, IN) and lactate (Lactate Pro LT-1710 portable lactate analyzer, Arkray Inc., Kyoto, Japan) meters. Sodium, potassium and hematocrit concentrations were measured using the i-STAT point of care devise (Heska Corporation, Fort Collins, CO, USA). After a 25% dilution with distilled water, 60 µl of blood were dispensed into an i-STAT E3+ cartridge for analysis. Such portable devices have been previously validated as a reliable tool for fish field physiology (Venn Beecham et al., 2006; Mandelman and Farrington, 2007) and specifically for bonefish (Cooke et al., 2008).

Data Analysis

Differences in blood chemistry were compared between the different stages of the relocation process using a one-way analysis of variance (ANOVA) followed by a Tukey-Kramer HSD test (Day and Quinn, 1989). All analyses were performed using JMP 6.0.2 (SAS Institute, Cary, NC, USA) and the level of significance (α) for all tests was 0.05.

Phase 2 – Assessment of handling and long-term holding

Handling experiment

Based on preliminary observations of bonefish post-transport, it became apparent that handling of fish with dip nets was resulting in the splitting of fins, as has been observed for other fish species (e.g., bluegill [*Lepomis macrochirus*; Rafinesque, 1819]; Barthel et al., 2003). It was also noted anecdotally that most fish suffering mortality had experienced some isolated dermal discoloration (i.e., deviation from whole body colour in localized areas) and abnormal swimming behaviour. As such, an experiment was designed to determine handling methods that would minimize fin damage to bonefish. On April 20, 2007, wild bonefish that had been originally captured during the first phase of this study and retained in captivity were individually dip netted from the holding tank and placed into an aerated cooler (108 L) for experimental handling. Once in the cooler, bonefish were first carefully observed to ensure that no fish exhibited any degree of dermal or fin damage. Following this initial assessment, fish were subjected to 90 seconds of handling with one of three treatment groups: bare hands, gloved hands, or cradle (n = 6 fish per treatment group). Bare hands were treated with sunscreen to replicate handling conditions in the field in tropical environments. Commercially available sun-gloves (Dr. ShadeTM, Reno, Nevada) were chosen as they are common sun protection for field researchers and recreational anglers. A fish cradle, manufactured on site using a non-stretch 5 mm knotless mesh material between two PVC pipes, was also used as it is a popular method of restraint for sport fish used by researchers and anglers (Larson, 1995; Casselman, 2005). Fish were handled in the cooler and kept in the water to reduce air exposure. When bare or gloved hands were used, fish were held with one hand posterior to the pectoral fins and one hand around the caudal peduncle. Bonefish were inserted in the cradle by sliding the cradle under the fish and scooping them into the device. Due to a limited number of fish for this portion of the study, there was no control group. Following handling, fish were measured for total length (mm) and were tagged with a unique colored T-Bar anchor tag corresponding to treatment group and returned to one of three 13 180 L holding tanks such that there were two fish from each treatment group in each tank. Experimental fish were held for 21 d and fed a diet of queen conch offal.

Following return to the holding tank, fish were first observed for 1 minute to note any loss of equilibrium following handling. The loss of equilibrium has been shown to increase the susceptibility of bonefish to predation following catch-and-release angling

(Danylchuk et al., 2007b) however no study has yet to confirm whether the loss of equilibrium results in short-term sub-lethal effects on fish health. The presence of slime on the handling device was noted. Bonefish were also monitored for physical appearance (including isolated discoloration, fin erosion, and fin splitting) and behaviour (feeding and schooling) by a presence or absence score. All observations were made behind a screen next to the tank to avoid startling the fish and disrupting their behaviour. Monitoring lasted three weeks (April 20 to May 11, 2007), with daily observations during week one, and every other day for weeks two and three.

Data analysis

Differences in fish length were compared between the treatment groups using a one-way analysis of variance (ANOVA) followed by a Tukey-Kramer HSD test (Day and Quinn, 1989). All analyses were performed using JMP 6.0.2 (SAS Institute, Cary, NC, USA) and the level of significance (α) for all tests was 0.05. Occurrences of physical abnormalities of fish from each of the three treatment group were pooled over the 21 d observation period and divided by the number of possible observations to give a frequency of occurrence and were compared for differences via Chi-square analysis (Sokal and Rohlf, 1995).

Long-term holding

Water quality measurements (salinity, temperature, and dissolved oxygen) were recorded daily for the duration of the entire study. Upon completion of the handling experiment (May 11, 2007) fish were weaned off a diet of queen conch offal and switched to a commercially available sinking pellet (6 mm, Skretting, Canada) until June 24, 2007, then switched to a larger sinking pellet (13 mm Zeigler, USA) for the remainder of the study. Observations of fish behaviour, physical abnormalities, and mortality were recorded.

Results

Phase 1 – Assessment of the Transportation and Lab Acclimation

A total of 195 wild adult bonefish (436 ± 42 mm total length; mean ± SD) were captured from the various tidal creeks and relocated to the CEI seawater research facility (Table 2.2). Ambient water temperatures ranged from 21 – 24 °C during the collection.

Fish Capture Techniques

The use of seine nets with mesh sizes of 3.2 cm or smaller were most effective at capturing bonefish without injury. Seining with a 7 cm mesh net resulted in entanglement and/or gilling of 95% of the bonefish capture at Starved Creek on February 18, 2007. Although only one bonefish suffered immediate mortality as a result of seine capture (i.e., suffocation) (Table 2.2), the remaining fish from Starved Creek captured that day exhibited substantial scale loss posterior to the head. The use of the 7 cm mesh seine net was discontinued for the duration of the study. Flow-through net pens were used to hold bonefish after capture until they were ready to be transported back to CEI, except in the case of Plum Creek sampling. Coolers were used to hold the five captured bonefish at Plum Creek due to the logistics of the site; a flow-through cage would have to be located far from shore to ensure a minimum depth of 0.6 m on an outgoing tide. The duration of holding prior to transport ranged from 45 – 170 minutes, depending on a variety of factors including physiological sampling, insertion of transmitters for an alternate study, and site logistics. An effort was made to place the flow-through holding cage in a deep area of water outside the main channel to reduce swimming efforts associated with strong tidal flow. Inclement weather on February 18, 2007 at Starved Creek resulted in the fish being subjected to strong storm surges for the last 30 minutes of holding.

Transportation of Fish

Transport of fish was greatly dependent on site logistics and the ability to mobilize field personnel and sampling gear. Most locations required that transportation of captured bonefish occur via truck, whereas a boat was utilized at sampling locations closest to CEI. Transport densities were dependent on the number of fish captured and the method of transportation, and ranged from 3 – 40 kg m-3 (Table 2.3). We were able to maintain oxygen levels above 5 mg L-1 using aeration. The duration of the transport of bonefish to the laboratory ranged from 15 – 95 minutes depending on the sampling site. Trail and road conditions resulted in rough transport of the fish by truck from Starved Creek and Half Sound. The generator which supplied power for the tank aeration system had to be checked frequently due to less-than-ideal terrain. Frequent water changes were more easily achieved by boat transport as compared to truck, however efforts were made to replace at least some of the water when moving the fish via truck by stopping at water access points and hand-bucketing in fresh seawater. During the transport process, two bonefish from Starved Creek (February 18, 2007 sampling) died (Table 2.3). All other fish were placed in holding tanks at CEI.

Lab acclimation and holding

A total of 39 bonefish died within the first 24 hr of holding at CEI following transport (Table 2.4). The majority (n = 33) of bonefish were from the first sampling trip at Starved Creek. All other fish (n = 153) were either terminally sampled for other experiments in the first week of holding (n = 85), or attempted to be acclimated to the lab for protracted holding and experiments (n = 68).

Observations of fish physical appearance and behaviour were documented during the first few days of holding in the laboratory. The bonefish from the first sampling event at Starved Creek exhibited numerous physical and behavioural disturbances. Whole body coloration changed dramatically from a normal silver-white colour to dark olive. Within 12 hr post-capture fish demonstrated fin erosion and hemorrhaging of the pectoral and caudal fins. Additionally there were hand-shaped patterns directly posterior to the head as a result of slime loss. As fish condition deteriorated over the course of a few days, eyes became yellow, and whole body colour further darkened to black. Behavioural changes went through two stages. The first stage involved rigid movements around the tank, often with the dorsal fin protruding out of the water, and lack of schooling with conspecifics. The second stage of behavioural changes included sitting on the bottom of the tank and lack of feeding. Autopsies performed on mortalities revealed that the majority of captured fish were either maturing, ripe and in spawning condition, or were spent. Fish that were handled minimally and not captured using the large-mesh seine, had no significant scale loss or fin fraying, kept at low densities (<30 bonefish/tank), and were minimally disturbed by human observation quickly resumed schooling behaviour and silver-white coloration.

Physiological Disturbances Associated with Transport

A sub-set of fish (n=41) were live sampled for blood to examine associated physiological responses at capture, post-transport, and in various stages of holding (Fig. 2.2). Blood glucose concentrations varied among treatments (ANOVA, $F_{4,36}$=3.37, P=0.019). Specifically, the fish that died during post-transport holding (moribund) had significantly higher glucose levels than all other treatments (Tukey's, P<0.05). Blood lactate levels were significantly different between treatments (ANOVA, $F_{4,36}$=51.55, P<0.001) with lactate levels highest for the fish post-transport and for those that died during post-transport holding (moribund). Blood Na+ values varied significantly among treatments (ANOVA, $F_{4,36}$=8.70, P<0.001) with the moribund fish having the highest levels (Tukey's, P<0.05). Blood K+ levels were significantly different among treatments (ANOVA, F=15.32, df=4,36, P<0.001). In particular, K+ levels were significantly higher for the fish post transport and for those that died during holding than for the other treatments (P's<0.05). Hematocrit values varied among treatments (ANOVA, F=6.24, df=4,35, P<0.05). In general, hematocrit levels were elevated in the capture, post transport, and moribund fish relative to the fish held in tanks or in sensory deprivation chambers.

Phase 2 – Assessment of handling and long-term holding

Handling experiment

A total of 18 bonefish (439 ± 35 mm total length; mean ± SD) were monitored in the handling experiment. There was no significant difference in the size of the bonefish used in each treatment group (P=0.768). Immediately after the 90 second handling treatment and fish tagging, observations for loss of equilibrium upon release was noted. One fish from the gloved hands treatment group lost equilibrium, but quickly regained it upon swimming in the tank. The presence or absence of slime on the handling device was also noted after the 90 second treatment. In 100% of the handling events, both gloved hands and bare hands removed slime. The cradle removed slime in 50% of the fish handled. Over the course of the three week experiment, no behavioural abnormalities were observed as all fish ate and typically schooled with conspecifics. In general, bonefish from the cradle treatment group had fewer occurrences of physical abnormalities (i.e., fin erosion and isolated discoloration) than the bare hand and gloved hand treatments (Table 2.5), however Chi-square analysis revealed no significant differences (P’s>0.05) between the treatment groups.

Long-term holding

Bonefish acclimated well to laboratory conditions when held in densities of 2 kg m-3 or less, with ambient seawater temperatures, and dissolved oxygen levels between 5.08 – 6.01 mg L^{-1}. Tank maintenance was performed on a routine basis by lowering water levels to scrub algae, and by using a pool vacuum to clean waste food and excretion. Approximately once per month, bonefish were removed from their tank and relocated to a clean empty tank to allow for deep cleaning. To minimize handling and stress to the fish during the capture process, half of the tank was blocked off with two of the flow-through pens used for holding fish during the field capture process, and fish were easily netted with long-handled dip nets, by two or more personnel.

Discussion

Each aspect of the relocation process had the potential to influence the survivorship of captured bonefish, and was evaluated through observations of physiology, physical appearance and behaviour. Although our study demonstrated that capturing wild bonefish from the field and relocating them to a holding facility can be challenging for the fish (altering homeostasis and in some cases causing death), we also showed that these challenges can be overcome and that bonefish can be successfully held in captivity.

An understanding of the stress response of marine teleosts to various aquaculture-related practices is invaluable from a fish husbandry perspective (Waring et al., 1996). In our study, blood glucose levels were significantly elevated in bonefish immediately prior to (moribund) or post-death compared to control values. Increase in blood glucose levels are one of the most common indicators of metabolic effects due to stress (Wedemeyer, 1996; Iwama et al., 2006). The level of hyperglycemia detected in moribund bonefish is below measured values for exercised bonefish (Suski et al., 2007). Control values for glucose in this study (4.2 mmol l^{-1}) were similar to those reported for bonefish by Friedlander et al. (2008) (4 mmol l^{-1}). Lactate was significantly higher in moribund fish in all treatment groups except for those immediately post-transport, indicating that persistent stress post-capture may have resulted in shifts in liver gluconeogenesis and build up of lactic acid causing metabolic acidosis and respiratory distress (Wedemeyer, 1996). Considering that lactate is a by-product of anaerobic consumption of energy stores during burst exercise (Wood, 1991), it is not surprising that fish sampled after seine capture and post-transport had elevated values relative to the control. Bonefish captured in seine nets typically swim around the perimeter of the net until being captured by dip net or hand, or they try to force their way out by swimming intensely at the net. Vigorous swimming activity is also known to occur during transportation processes of fish as indicated by electromyogram telemetry (see Chandroo et al., 2005). With increased swimming activity comes increased oxygen consumption. To increase the supply of oxygen to major organs during stress, hematocrit levels are often elevated (Ruane et al., 1999). In this study, hematocrit values were significantly higher in the capture, post transport and moribund tank, relative to control fish levels. Elevations in hematocrit can be caused by decreased plasma volume, swelling of erythrocytes, and/or release of additional red blood cells into the blood (Witters et al., 1990; Pearson and Stevens, 1991). Frisch and Anderson (2000) found similar increases in hematocrit values for coral trout, *Plectropomus leopardus* (Lacepéde, 1802), exposed to capture, handling and transport stress. Ionic concentrations of Na+ were significantly higher in moribund fish relative to control fish values, whereas other treatment groups did not differ significantly. Plasma K+ values were significantly elevated in post-transport and moribund fish relative to all other treatment groups, including the control. Changes in ionic concentrations likely were a result of gill morphology alterations that occurred as part of the secondary stress response to facilitate oxygen uptake (Wendelaar Bonga, 1997) required by the energetic swimming of transported fish, and last efforts to regain homeostasis in the moribund fish. Increases in plasma K+ could also reflect potassium extrusion from muscle cells in response to intracellular acidosis caused by lactate build up (Wood et al., 1983).

Fish exposed to stress commonly exhibit changes in physical appearance (e.g., True et al., 1997) and behaviour (Huntingford et al., 2006). Changes in physical appearance and behaviour were noted for bonefish that experienced entanglement in the large mesh seine net, and those that could not recover from relocation stress. Furthermore, we observed that fish that were handled with a dip net in the field exhibited noticeably more fin fraying. In a controlled laboratory experiment, several alternative handling methods were contrasted. Use of a cradle for moving fish was determined to be the least deleterious method for handling bonefish in the field and in captivity. Fish handled by bare or gloved hands lost slime 100% of the time, whereas fish handled by cradle lost slime 50% of the time. The mucus layer of slime serves as a physical and chemical barrier to infection, blocking bacteria from entering the body (Wedemeyer, 1996). Although no significant difference in physical disturbances were noted between the handling treatment groups, there was still less frequent occurrences of fin erosion and isolated discoloration in fish handled by the cradle.

Collectively, the stressors associated with the capture, handling, and transport of wild bonefish to holding tanks results in the manifestation of physical, behavioural, metabolic, osmoregulatory and hematological changes. The duration of the effects appears to be less than 72 hr as evidenced by no significant difference in any of the secondary stress response variables between fish in the holding tank and control values. Mitigation of physical, behavioural, and physiological disturbances and thus successful laboratory acclimation of bonefish can be achieved by ideal capture, transport and holding methodologies as demonstrated by this study (Box 2.I). Of particular importance is to focus on ensuring that the fish that are introduced to holding tanks for long term acclimation are ones with minimal physical injury. Also, because wild bonefish are quite skitterish in response to human activity (including shadows and noise), it is important to minimize disturbance and human contact during the early phases of laboratory acclimation to enable them to resume feeding, engage in schooling behaviour, and habituate to captivity. Even fish in good condition (i.e., minimal fin fraying or slime/scale loss), failed to habituate to laboratory conditions when they were held in small tanks with frequent human contact during the first several days of holding.

It is important to note that the current study occurred in the winter and spring, when water temperatures were relatively cool (e.g., 21 to 24 °C). It is well known that the metabolic rates of fish (Brett, 1995) and their response to stress (Wilkie et al., 1997) are higher at warmer temperatures. In salmonid aquaculture, it is recommended that fish transport and handling should be done when water temperatures are low (Barton, 2000). Presumably this is also the case for tropical species, although there are few explicit tests of that idea. Garcia et al. (2000) found that cool temperatures alone may be sufficient to ensure low mortality of handled and transported milkfish, *Chanos chanos* (Forsskål, 1775). As such, we would caution the collection, transportation, and attempted acclimation of bonefish during warmer summer months as mortality would be presumed to be higher. Furthermore, there is no information on the oxygen requirements of bonefish. In this study, we attempted to maintain oxygen levels in tanks (transport and holding) at levels that mimicked the ambient environment. Our minimal target during transport was 5 mg L^{-1}. At times during transport when the generator failed for several minutes, dissolved oxygen dipped to around 4 mg L^{-1} and in those instances bonefish began to gulp at the surface of the water. Future research is needed to document the oxygen requirements of bonefish to facilitate transportation and holding.

In summary, this study was the first to document strategies for the successful capture and relocation of wild bonefish for long-term holding in a marine research facility. Benefits from this study extend not only into the opportunity for scientific research on this highly prized sport fish, but also increase our understanding of the stress response for sub-tropical fish. Future studies of tropical and subtropical marine fish husbandry will further enhance our capacity for marine stock enhancement and mariculture which will become increasingly important as the demand for fish protein rises, and wild fish stocks decline.

Tables

Box 2.1: Ideal strategies for the capture and transport of wild bonefish to the laboratory for long-term holding

1. Capture: Use seine nets with a mesh size of 3.2 cm or smaller to avoid gilling or entanglement of bonefish. Hold fish in a flow-through mesh pen in a minimum of 0.6 m water until ready for transport. Avoid placing the flow-through pen in areas of high velocities to minimize unnecessary exercise of the fish.
2. Transport: Transportation of the fish by boat is preferred because frequent water changes can be made which has been found beneficial by other studies (see Maule et al., 1988). When truck transport is necessary, adjust tank density based on distance of travel (<15 minutes of travel, ≤ 30 kg m^{-3}; >15 minutes of travel, ≤ 15 kg m^{-3}).
3. Holding: Bonefish should be held in large circular tanks at densities of 2 kg m^{-3} or less with other conspecifics to promote schooling. Disturbance to the tank should be limited to tank maintenance, feeding and monitoring of water quality. Acclimation to tank conditions is facilitated by tank water temperatures at ambient conditions to the location of capture. Feeding of fish with commercially available sinking pellets should be initiated within 24 hr of holding.
4. Handling: At any point in the capture, transport or holding process when bonefish have to be handled, they should be handled carefully to minimize

slime and scale loss. Although no significant differences were found between the use of bare hands, gloved hands, or a fish cradle, the cradle was the easiest method to hold fish and resulted in the least amount of slime loss.

Table 2.1: Description of treatment groups for assessing physiological disturbances of wild bonefish at various stages in the relocation process from the field to the Cape Eleuthera Institute in The Bahamas

Treatment group	Description
Control	Fish held in sensory deprivation chambers for 24 hr to obtain control values. Fish were not introduced into the chambers until 48 to 72 hr post transport. All fish were from Kemps Creek (n = 7)
Capture	Fish were sampled within 5 minutes of being captured by seine in the field. Fish were captured in a number of creek systems. Blood chemistry was derived from bonefish from Plum Creek (n = 2) and Starved Creek (February 18, 2007) (n = 5)
Post Transport	Fish were sampled immediately following a 50 minute transport (approximately 150 minutes post capture). All fish were from Half Sound (n = 7)
Moribund	Fish were removed from holding tanks at time of death or when they were swimming upside down and ventilations were either slow or non-existent. All fish were from Starved Creek (February 18, 2007) (n = 12)
Holding Tank	Fish sampled from holding tanks via dip net between 48 to 72 hr post transport. Sample fish were from mixed populations (n = 8)

Table 2.2: Summary of the capture details for relocating wild bonefish from the field to the Cape Eleuthera Institute in The Bahamas

Date (2007)	Location	Water temperature (°C)	Seine nets used	Number of bonefish captured	Number of mortalities at capture	Method of holding prior to transport	Duration of holding prior to transport (minutes)
Feb 17	Plum Creek	24	3.2 cm mesh	5	0	coolers	60
Feb 18	Starved Creek	22	0.6 cm, 1.3 cm, 3.2 cm, and 7 cm mesh used but all fish captured in 7 cm mesh	41	1[1]	flow-through cage	150[2]
Feb 19	Starved Creek	23	3.2 cm and 0.6 cm	8	0	flow-through cage	60
Feb 20	Kemps Creek	21	3.2 cm and 0.6 cm	70	0	flow-through cage	45
Feb 23	Broad Creek	21	3.2 cm and 0.6 cm	3	0	flow-through cage	45
Feb 23	Half Sound	22.5	3.2 cm and 0.6 cm	47	0	flow-through cage	100[3]
Mar 16	Broad Creek	22.5	3.2 cm and 0.6 cm	21	0	flow-through cage	120[4]

[1] only 1 fish died directly from gilling, but 39 of the 41 fish captured were gilled or entangled in the net

[2] due to strong tidal flow and storm surge fish were exercised in the flow for the duration of holding

[3] approximately 650 m from seining location to truck

[4] longer duration due to inserting transmitters in 10 bonefish

Table 2.3: Summary of the transportation details for relocating wild bonefish from the field to the Cape Eleuthera Institute in The Bahamas

Date (2007)	Location	Transport method	Transport densities^1 (kg m^{-3})	Duration of Trip (minutes)	Number of mortalities during transport	Comments
Feb 17	Plum Creek	Truck	3	25	0	Half of the trip on un-paved roads, half of the trip on poorly maintained paved roads
Feb 18	Starved Creek	Truck	27	65	2	40 minutes of the trip down very rough, bush trail, and 25 minutes on poorly maintained paved roads. After 20 minutes into the trip, approximately 100 L of water was exchanged in the tank.
Feb 19	Starved Creek	Truck	5	65	0	40 minutes of the trip down very rough, bush trail, and 25 minutes on poorly maintained paved roads. After 20 minutes into the trip, approximately 150 L of water was exchanged in the tank.
Feb 20	Kemps Creek	Truck	40	15	0	15 minutes on poorly maintained paved roads
		Boat	33	15	0	Frequent water changes in the coolers on the way
Feb 23	Broad Creek	Boat	20	20	0	Frequent water changes in the coolers on the way
Feb 23	Half Sound	Truck	31	50	0	15 minutes on unpaved roads, 25 minutes on paved roads, 10 minutes on poorly maintained paved roads. Large amount of foam build-up (protein skimmate) noticed in the tank when stopped half way back to the laboratory to change ¼ of the tank of water with fresh seawater.
Mar 16	Broad Creek	Boat	33	20	0	Frequent water changes in the coolers on the way

[1] density calculation based on average weight of bonefish from the study (0.711 kg) with transport tank volume of 1.068 m^3, and cooler volume (for boat transport) of 0.108 m^3 (assuming maximum five fish per cooler).

Table 2.4: Summary of 24 hr mortality of wild bonefish held in captivity at the Cape Eleuthera Institute in The Bahamas. Note that all fish that succumbed to death were fully analyzed for genetic sampling, length, weight, ageing (otoliths and scales removed), health indices, gut content analysis, stable isotope analysis, and proximate body composition.

Date (2007)	Location	Number of bonefish captured	Number of mortalities after 24 hr holding in tanks	Comments
Feb 17	Plum Creek	5	0	Fish used for other physiological experiments and euthanized within 5 d of capture
Feb 18	Starved Creek	41	33	Remaining fish held
Feb 19	Starved Creek	8	0	Three fish used for other physiological experiments and euthanized within 5 d of capture. Remaining fish held
Feb 20	Kemps Creek	70	0	Fish used for used for other physiological experiments and euthanized within 5 d of capture. Remaining fish held
Feb 23	Broad Creek	3	0	Remaining fish held
Feb 23	Half Sound	47	6	Fish used for other physiological experiments and euthanized within 5 d of capture. Remaining fish held
Mar 16	Broad Creek	21	0	Fish used for handling experiment included in this study

Table 2.5: Summary of the frequency of physical disturbances of wild bonefish handled by bare hands, gloved hands, or a fish cradle during a 21 d observation period at the Cape Eleuthera Institute in The Bahamas. Note that Chi-square analysis found no significant differences in the frequency of physical disturbances between the three handling methods (P's>0.05).

	Handling treatment group		
Physical disturbance	Bare hands	Gloved hands	Cradle
Fin erosion	17.89 %	22.92 %	11.58 %
Fin splitting	76.84 %	61.46 %	65.26 %
Isolated discoloration	34.74 %	34.38 %	29.47 %

Figures

Figure 2.1: Map (developed using Google Earth) of study sites on Eleuthera, The Bahamas. Laboratory holding facilities were located at the Cape Eleuthera Institute. The various creeks represent locations where fish were sampled from (see Table 2.2 for details).

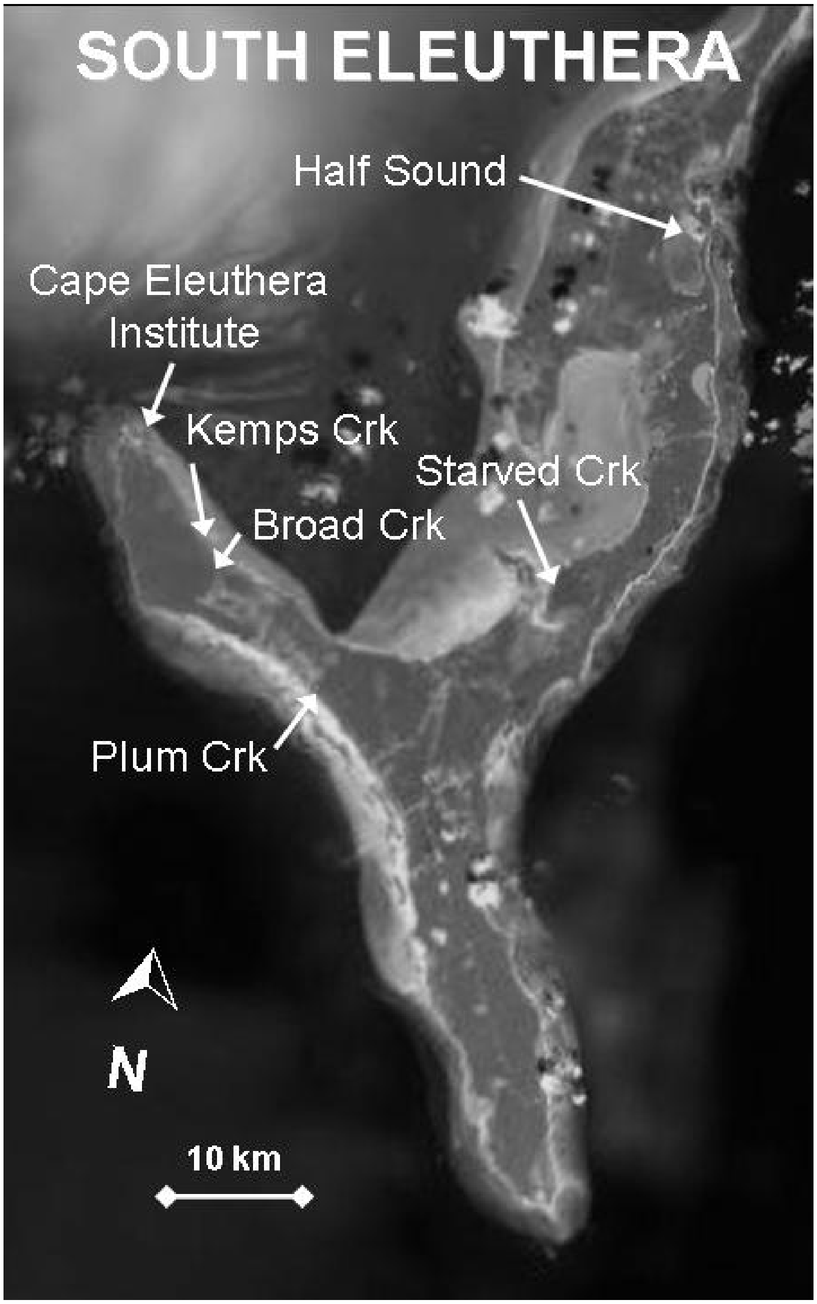
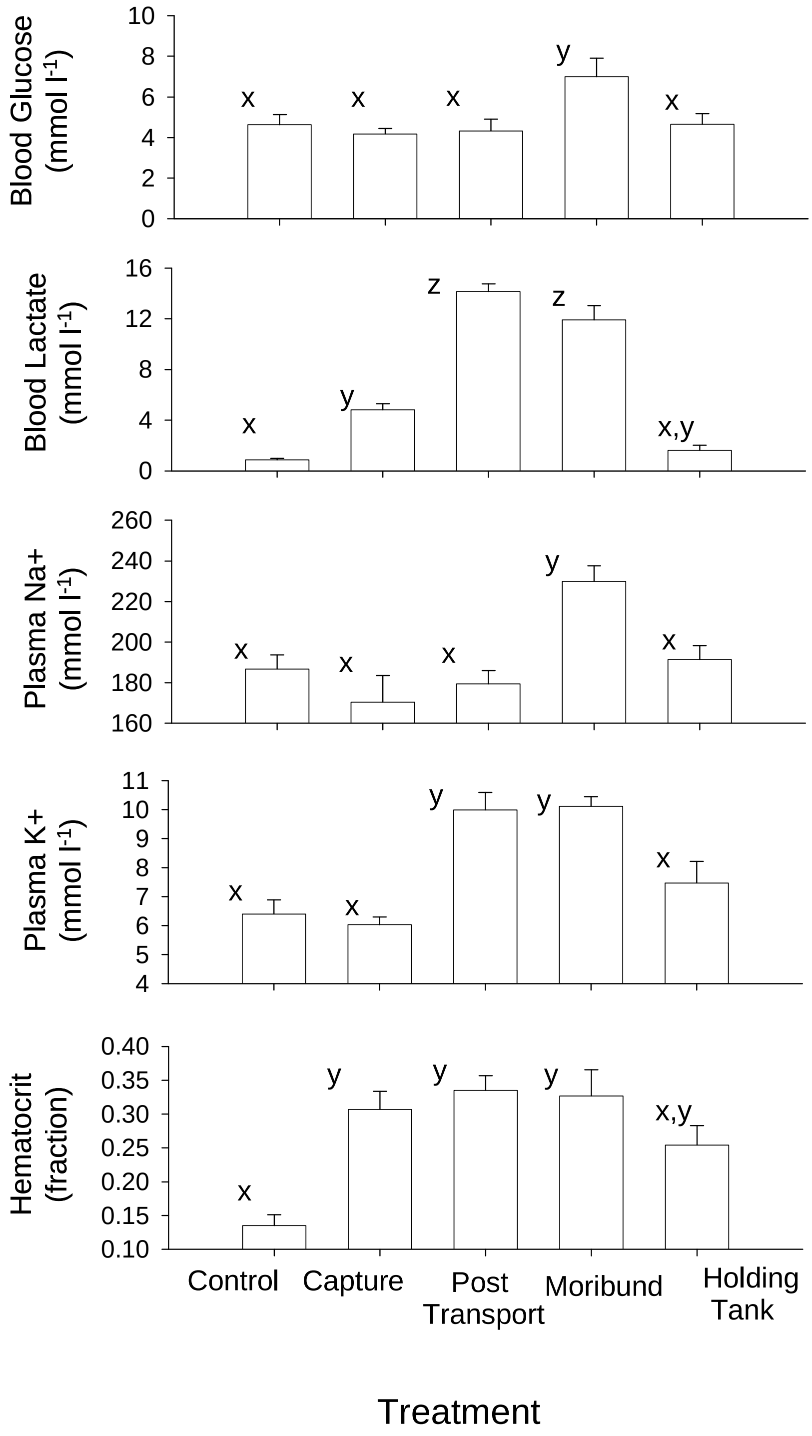


Figure 2.2: Physiological responses of bonefish to various handling, transport, and holding conditions. Dissimilar letters indicated significant differences (Tukey's Post-Hoc Test, P<0.05). Sample sizes for each treatment group are indicated in Table 2.1.



Chapter 3: Thermal biology of bonefish (*Albula vulpes*) in Bahamian coastal waters and tidal creeks: an integrated laboratory and field study

Abstract

Little is known about the thermal tolerances of fish that occupy tropical intertidal habitats or how their distribution, condition, and survival are influenced by water temperature. We used a combination of laboratory and field approaches to study the thermal biology of bonefish, *Albula vulpes*, a fish species that relies on nearshore intertidal habitats throughout the Caribbean. The critical thermal maximum (CTMax) for bonefish was determined to be 36.4 ± 0.5°C and 37.9 ± 0.5°C for fish acclimated to 27.3 ± 1.3°C and 30.2 ± 1.4°C, respectively, and these tolerances are below maximal temperatures recorded in the tropical tidal habitats where bonefish frequently reside (i.e., up to 40.6ºC). In addition, daily temperatures can fluctuate up to 11.4°C over a 24-hr period emphasizing the dramatic range of temperatures that could be experienced by bonefish on a diel basis. Use of an acoustic telemetry array to monitor bonefish movements coupled with hourly temperature data collected within tidal creeks revealed a significant positive relationship between the amount of time bonefish spent in the upper portions of the creeks with increasing maximal water temperature. This behavior is likely in response to feeding requirements necessary to fuel elevated metabolic demands when water temperatures generally warm, and also to avoid predators. For fish held in the laboratory, reaching CTMax temperatures elicited a secondary stress response that included an increase in blood lactate, glucose, and potassium levels. A field study that involved exposing fish to a standardized handling stressor at temperatures approaching

their CTMax generated severe physiological disturbances relative to fish exposed to the same stressor at cooler temperatures. In addition, evaluation of the short-term survival of bonefish after surgical implantation of telemetry tags revealed that there was a positive relationship between water temperature at time of tagging and mortality. Collectively, the data from these laboratory and field studies suggest that bonefish occupy habitats that approach their laboratory-determined CTMax and can apparently do so without significant sub-lethal physiological consequences or mortality, except when exposed to additional stressors.

Introduction

Water temperature exerts more control over fish than any other single abiotic factor (Beitinger and Fitzpatrick, 1979; Magnuson et al., 1979), acting as a regulator of nearly all biochemical, physiological, and life history activities of fish (Fry, 1967; Brett, 1971) as well as a potential source of disturbance (Beyers and Rice, 2002). All fish species have a temperature range within which individuals do not exhibit any signs of stress and/or abhorrent behavior (Portz et al., 2006). The ability of fish to respond to thermal change is dependent on a number of factors (see Hutchison, 1976), among which include thermal history or acclimation temperature (Chung, 2001). Each species will exhibit different capacities for acclimation based on how close they are currently living to their thermal tolerance limits (Somero, 2005). Fish inhabiting water bodies that warm gradually in spring/summer and cool in fall/winter may use thermal changes to coordinate seasonal activities, whereas fish that migrate between thermally distinct habitats have to adapt to these thermal changes to exploit the new environment (Guderley et al., 2001).

Thermal tolerance data are limited for fish inhabiting tropical marine areas, particularly when compared to those inhabiting temperate regions (Ospina and Mora, 2004). Given the ecological and economic importance of coastal areas and especially coral reefs (see Holmlund and Hammer, 1999; Moberg and Folke, 1999), coupled with the influence of thermal phenomena such as El Niño and the impact of global warming in these areas (Mora and Ospina, 2001), the lack of information on thermal tolerances demands a broader investigation into the thermal physiology and ecology of fish from the tropics (Roessig et al., 2004). Bonefish (*Albula* spp.) are a group of fishes that occupy subtropical and tropical nearshore areas around the world (Pfeiler et al., 2000). Bonefish are common benthivorous fish in many tropical areas, moving into shallow water habitats (e.g., tidal creeks and 'flats') to feed on invertebrates and small fish during high tide, and then moving into deeper water at low tide (Humston et al. 2005). During these daily movements, bonefish potentially face large shifts in ambient water temperatures as they may occupy waters less than 0.1 m deep (Colton and Alevizon, 1983b). In addition, bonefish are the object of a popular sport fishery (Pfeiler et al., 2000), making them an interesting model for investigating the thermal tolerances of an organism subjected to diurnal and seasonal fluctuations in tropical waters and exposure to multiple additional stressors (e.g., the stress associated with capture and handling during recreational angling (see Suski et al., 2007; Danylchuk et al., 2007b).

Temperature tolerances of species can either be estimated from field observations or quantified by laboratory studies (Beitinger et al., 2000). Field observations of fish kills resulting from exposure to extreme high or low temperatures or the examination of minimum and maximum water temperatures within a species' natural distribution both provide estimates of a species' thermal tolerance. These approaches, however, are not precise nor do they rule out other potential abiotic or biotic factors that may contribute to the fish's behavior (Beitinger et al., 2000). Because of the limitations of purely empirical studies, a widely accepted laboratory method has been developed to quantify the temperature tolerances of fish (i.e., the critical thermal method (CTM); Bennett and Judd, 1992; Currie et al., 2004). The CTM, i.e., the mean temperature at which individual fish display signs of stress (e.g., loss of equilibrium) after being exposed to a constant linear temperature change, is the most common index (Mora and Ospina, 2002; Cook et al., 2006). With the CTM approach, lethal temperatures are estimated without actually killing fish (Beitinger et al., 2000). Because the upper tolerance limits of a species increases with acclimation temperature (Beitinger and Bennett, 2000), thermal tolerances are typically determined at a number of acclimation temperatures. An issue of growing concern exists, however, in the applicability of laboratory-determined thermal tolerance ranges to fish in natural settings, because diel temperature fluctuations are common in various fish habitats (Wehrly et al., 2007). Vast amounts of literature determining the thermal tolerance of various fish species have been generated (see Beitinger et al., 2000), not only as a result of interest in understanding this critical aspect of fish ecology, but also by the current need to predict the biological effects of climate change (Cook et al., 2006; Mora and Maya, 2006). Unfortunately, there are few studies that link laboratory research on thermal biology with field studies of behavior, or studies that further extend this work to consider the potential impacts of climate change on wild fish.

The purpose of this study was to 1) determine the critical thermal maximum (CTMax) of bonefish at two different seasonal acclimation temperatures; 2) examine the stress physiology associated with bonefish reaching CTMax; 3) examine the combined effects of thermal stress and capture/holding stress, and; 4) link the spatial ecology of bonefish with the thermal regimes experienced in tidal creeks and coastal areas.

Materials and methods

This study took place on the island of Eleuthera, The Bahamas (N 24 50 05 and W 76 20 32) in the laboratory facilities at the Cape Eleuthera Institute (CEI), as well as in a number of tidal creek and tidal flats systems adjacent to Cape Eleuthera (Fig. 3.1). Genetic analyses of bonefish from this area indicated that all bonefish specimens analyzed were *Albula vulpes* (Danylchuk et al., 2007a; J Koppelman, Missouri Department of Conservation, unpublished data). All experiments were conducted in accordance with the policies of the Canadian Council on Animal Care as administered by the Carleton University Animal Care Committee (Protocol B07-03, 04).

Laboratory determination of CTMax and associated stress physiology

Study fish were captured from local tidal creeks using seine nets deployed at creek mouths to intercept bonefish on incoming or outgoing tides between February 17, 2007 and March 16, 2007. Fish were transported to the research facility using the methods outlined in Murchie et al. (2009) and held in large (3.7 m diameter x 1.25 m height; 13180 l) circular tanks housed in a covered, open-sided outdoor facility. Tanks were continuously supplied with fresh sea water (1800 l hr^{-1}) at ambient temperatures, and were maintained under a natural photoperiod. Fish were fed a diet of queen conch (*Eustrombus gigas*) offal and/or commercially available sinking pellets (6 mm, Skretting, Canada; 13 mm, Zeigler, USA). Test fish, however, were not fed 24 hours prior to or during experimental trials.

CTMax experiments were conducted at CEI between May 2, 2007 and August 23, 2007. The CTMax of bonefish was determined at two different seasonal acclimation temperatures, one representative of late spring (27.3 ± 1.3°C; mean ± SD) and one of summer (30.2 ± 1.4°C; mean ± SD) temperatures. The use of fluctuating acclimation temperatures representative of natural conditions, rather than constant acclimation temperatures, is an alternative approach to investigating thermal tolerances of fishes (see Currie et al., 2004). At 24 hours prior to experimentation, bonefish were netted from the large circular holding tanks and placed in individual darkened flow-through tanks (approximately 100 l) to minimize exercise and stress associated with tank capture (Suski et al., 2007). Individual flow-through tanks were also continuously supplied with fresh sea water at ambient conditions. A maximum of three bonefish were placed in individual flow-through tanks at a time in preparation for CTMax determinations the next day. Temperature data loggers (Hobo-H8 temperature logger, Onset Computer Corporation, ±0.7°C accuracy, range of -20°C to 70°C) encased in waterproof housings were used to measure acclimation temperatures within the tanks. During experimentation, an individual was removed from its flow-through tank and quickly transferred to an aerated thermal bath (142 l insulated cooler). Starting temperatures within the thermal bath were consistent with conditions in the individual's flow-through tank immediately prior to transfer. Water temperature within the bath was raised by 0.2°C min^{-1} using two submersible heaters (Model 306, Heet-O-matic, Cole Parmer, Newark, NJ), until the fish lost equilibrium for one minute (Beitinger et al., 2000; Fangue and Bennett 2003).

Following sustained equilibrium loss, the water temperature was recorded and the fish was removed for blood sampling and subsequent determination of secondary indicators of stress. Individuals were restrained by hand in a supine position (without the use of anesthetic) in a foam-lined trough filled with sea-water, at a depth to completely submerge their gills. Using a 21 gauge needle, approximately 1.5 ml of blood was drawn from the caudal vasculature into a 3 ml lithium heparinized vacutainer (BD vacutainer blood collection tube; Becton, Dickinson and Company; Franklin Lakes, NJ). After phlebotomy (typically less than 45 seconds), the blood sample was held in an ice-water slurry until sample processing, storage and analysis as described below. The total length of the bonefish was recorded to the nearest mm, and fish were returned to original holding tanks with conspecifics. To generate control (resting) physiological values for comparative purposes, the above process was repeated without the thermal trial for at least eight bonefish at each acclimation temperature. Trials were conducted between 0700 hr and 2200 hr dependent on when fish were placed in individual flow-through tanks, and the number of bonefish prepared for experimentation that day. CTMax was calculated as the arithmetic mean of the collective equilibrium loss endpoint temperatures (Beitinger et al., 2000).

Effects of multiple stressors

Because stressors rarely act independently in the natural environment, we conducted an experiment to examine the combined effects of thermal stress and capture/holding stress. Bonefish were seined in tidal creeks on an outgoing tide and sampled for blood after being held in a pen (1.3 m x 0.8 m x 1.25 m tall, 3.1 cm extruded plastic mesh) for either a short (6 min or less) or long (7-15 min) duration. The experiment was conducted at two distinct water temperatures; 22°C (February 17-23, 2007) and 32°C (August 28, 2008). Following blood sample collection, glucose and lactate levels were quantified on whole blood using commercially available handheld devices (ACCU-CHECK glucose meter, Roche Diagnostics, Basel, Switzerland and Lactate Pro LT-1710 portable lactate analyzer, Arkray Inc., Kyoto, Japan) previously validated for use on bonefish (see Cooke et al., 2008). An i-STAT point of care device (Heska Corporation, Fort Collins, CO, USA) was used to measure Na+, K+, and Cl- values for fish in the CTMax trials, whereas plasma samples from the field assessment of multiple stressors were analyzed by an accredited animal science diagnostics lab (Vita-Tech, Markham, Ontario, Canada) using a Roche-Hitachi Analyzer (Roche Diagnostics, Basel, Switzerland). Additionally, hematocrit values were assessed by spinning whole blood samples in a centrifuge (Clay Adams Compact II Centrifuge) at 10 000 x g for 5 min to separate plasma from red cells, and by then measuring the ratio with a ruler. To allow for comparison of ion values determined for fish in the CTMax trials with those determined for fish in the field assessment of multiple stressors, values obtained via the i-STAT were converted to 'gold standard' laboratory values using predictive equations derived from Cooke et al. (2008).

Linking spatial ecology with thermal regimes in tidal creeks and coastal areas

Collection of ambient water temperature data

A total of eight temperature data loggers were deployed in the mouths (1.2 m deep) and upper reaches (0.3 m deep) of three tidal creeks (Page Creek, Kemps Creek, and Broad Creek, which has two mouths), plus one open coastline location just off CEI (2.5 m deep) (Fig. 3.1). Temperature loggers were deployed in Broad and Kemps Creek in October 2004, and in Page Creek and off CEI in May 2006 by affixing them to a cinder block with twine. Hourly temperatures were recorded at the water-substrate interface. For the purpose of this study, data collected from the temperature loggers between the date of deployment and December 31, 2007 was used.

Spatial ecology of bonefish

Between November 1, 2005 and March 16, 2007, 47 adult bonefish (495 ± 45 mm total length; mean ± SD) were captured with seine nets (as described above) and were implanted with acoustic transmitters (model V13 coded tags, 13 mm diameter, 36 mm long, 6 g, 700 day battery life, Vemco Inc., Shad Bay, NS.). Bonefish were anesthetized with MS-222 prior to surgery (approx. 100 ppm) and then placed on a surgery table where fish gills were supplied with a maintenance dose of MS 222 (approx. 50 ppm) in fresh seawater. To implant the transmitter, a small incision (2 – 3 cm) was made to one side of the ventral midline, posterior to the pectoral fins. The transmitter was inserted and gently guided towards the pectoral fins. The incision was closed with 3-4 simple interrupted sutures (Ethicon 3-0 PDS II monofilament absorbable suture material, Johnson and Johnson, New Jersey). The total length of the fish (mm) was measured, and sex was determined whenever possible. The entire procedure took less than 5 min. Prior to release, bonefish were held for up to one hour in flow-through holding pens (1.3 m x 0.8 m x 1.25 m tall, 3.1 cm extruded plastic mesh).

Movements of tagged bonefish were monitored through the use of a 27 hydroacoustic receiver array (VR2 and VR2W receivers, Vemco Inc., Shad Bay, NS). The array covered points along a 23 km stretch of coastline, with receivers strategically deployed at choke points (e.g., creek mouths) or as curtains extending up to 1.5 km perpendicular to the shoreline (Fig. 3.1). The location of seven of the receivers corresponded closely to the locations of temperature loggers within the three tidal creeks (Page Creek, Kemps Creek, and Broad Creek) (Fig. 3.1). Individual receivers were secured to a short piece of rebar anchored into a concrete block. Receivers were positioned vertically in the water column at depths greater than 1 m deep at low tide, and were positioned either horizontally or 5-10 degrees above horizontal in water less than 1 m deep at low tide. Range tests determined that receivers located in water greater than 1 m had a radius coverage of 250 m, whereas receivers in shallow water (<1 m) had a radius coverage of as little as 30 m, due to shoreline confinement. Although the range of coverage for receivers in shallow water or positioned horizontally was considerably less, they did provide the necessary coverage to monitor choke points (e.g., creek mouths), such that data correction for receiver range was not required. Wind and wave conditions as well as water depth and tidal cycles influenced the detection range of individual receivers (Heupel et al., 2006). Even at slack low tides, all of the receivers were covered by at least 20 cm of water and had the potential to be accessed by the tagged fish. The hydrophones were deployed between November 2, 2005 and May 19, 2007 and were visited regularly for downloading and cleaning. For the purpose of this study, the last download period included data up to December 31, 2007.

Data analysis

All statistical analyses on collected and derived data were completed using JMP 7.0.2 (SAS Institute, Cary, NC). Normality and homogeneity of variance assumptions were evaluated using Shapiro-Wilk or Kolmogorov Smirnov goodness-of-fit tests and Levene's test, respectively (Sokal and Rohlf, 1995). For laboratory determination of CTMax and associated stress physiology, a one-way analysis of variance (ANOVA) was used to determine if there were any size (total length) differences in bonefish within and between treatment groups (i.e., CTMax or control) and acclimation temperatures (i.e., 27.3°C or 30.2°C). A two-sample t-test was used to compare laboratory determined CTMax values between the two acclimation temperatures. A two-way ANOVA of acclimation temperature and treatment were used to compare blood chemistry values to determine the level of stress associated with reaching the CTMax. The effects of temperature and duration of holding stress from the field trial were also evaluated using a two-way ANOVA.

To link the spatial ecology of bonefish to the thermal regimes in the three tidal creeks where temperature data loggers were deployed, detections recorded within the hydrophone array between June and December 2007 were examined. This time period was selected to 1) exclude periods where movements included forays to offshore locales, which we hypothesize indicate spawning activity (Murchie and Danylchuk, CEI, unpublished data); 2) maximize the number of possible bonefish at large; and, 3) maximize the number of receivers for which bonefish could be detected at, since all 27 receivers had been deployed by May 19, 2007. Receivers were classified as being in one of three habitat types; creek backwaters (receivers #6, #12, and #19), creek mouths (receivers #5, #11, #16, #18, and #27), and open coastline (remaining receivers) (Fig. 3.1). The total number of detections of each bonefish picked up in the array was broken down into months (i.e., June to December) and habitat types. The total number of detections for each fish in each habitat type was divided by the total number of detections of the fish for that month and multiplied by 100 to give a percentage. Percentages were tallied across each habitat type for each month and divided by the total number of bonefish at large during the June to December 2007 period and further divided by the number of receivers in each habitat (due to the uneven number of receivers per habitat). A repeated measures two-way ANOVA followed by a Tukey-Kramer HSD test (Day and Quinn, 1989) was used to determine whether month or habitat influenced the percentage of detections of bonefish. Within the June to December 2007 time period, all detections within creek backwaters were further examined. For each individual bonefish detected in creek backwaters their proportion of detections was calculated for each maximum water temperature. The relationship between the proportion of detections of bonefish in the backwaters of the tidal creeks and the maximum daily water temperature recorded was evaluated with a simple linear regression model. Proportional data were arcsine root transformed and plotted against maximum water temperatures. When bonefish were detected on days when water temperatures approached or exceeded CTMax values, further examination of the data was conducted to determine what the exact water temperature was at the time the bonefish was in the backwater of the creek.

Results

Laboratory determination of CTMax and associated stress physiology

A total of 39 wild adult bonefish (438 ± 34 mm total length; mean ± SD) were captured from local tidal creeks and relocated to the CEI seawater research facility for acclimation and subsequent thermal tolerance testing or use as controls (Table 3.1). There was no significant difference between the size of bonefish within and between treatment groups from the two acclimation temperatures ($F = 2.65$, $p = 0.064$). The CTMax values of 36.4 ± 0.5°C, and 37.9 ± 0.5°C for bonefish acclimated to 27.3°C and 30.2°C, respectively (Table 3.1), were significantly different between acclimation temperatures ($t = 7.22$, $p < 0.0001$). Blood chemistry values after reaching CTMax were significantly different between controls and CTMax fish for all parameters except chloride (Table 3.2). Acclimation temperature was a significant main effect for all parameters, and no interaction between treatment and acclimation temperature was detected (Table 3.2). More specifically, concentrations of $K+$, lactate, and glucose increased relative to control values following CTMax trials for both acclimation temperatures. In addition, acclimation of bonefish to either 27.3°C or 30.2°C had significant impact on the level of change among physiological parameters following CTMax trials. The accumulation of lactate as well as plasma glucose for bonefish acclimated to 30.2°C was significantly higher than that for bonefish acclimated to 27.3°C.

Effects of multiple stressors

When bonefish were exposed to an additional stressor (i.e., handling), fish tended to exhibit the most severe physiological stress response at the highest water temperature (Table 3.3). Indeed, for all parameters except potassium, temperature was a significant main effect (Table 3.4). Lactate values ranged from as low as 3.7 mM/L to as high as 14 mM/L. Recorded values for glucose ranged from 3.4 to 10.8 mM/L. Hematocrit values ranged from 0.21 to 0.64. Ion values ranged from 63.83 - 238.00 mM/L, 1.42 - 11.37 mM/L, and 149.00 - 226.00 mM/L for sodium, potassium, and chloride, respectively. The duration of holding was significant for lactate, glucose, hematocrit, and sodium, but not for potassium or chloride (Table 3.4). An interaction between temperature and the duration of stressor existed for lactate (Table 3.4).

Linking spatial ecology with thermal regimes in tidal creeks and coastal areas

Hourly water temperatures collected within the tidal creeks peaked at 40.6°C in the backwaters of Kemps Creek in June 2007 and reached a low of 9.03°C in January of 2005 in the same location. Daily fluctuations of up to 11.40°C were recorded at the mouth of Kemps Creek on May 28, 2006. Temperatures varied greatest within the backwaters and mouths of tidal creeks, but were much more stable at offshore locations (Fig. 3.2).

Bonefish implanted with transmitters in August of 2006 suffered a high percentage of mortality compared to fish implanted in February or March of 2007. During the two days in which surgeries were conducted in August 2006, the mean water temperature at the mouth of the creek was 29°C. Out of the 21 fish tagged at 29°C, 14% were suspected to be dead within 48 hours, and 57% within one week, as evidenced by the lack of detection at any receivers as of December 31, 2007. Presumptive mortality rates were much lower when bonefish were implanted in cooler (20-23°C) waters during February and March of 2007, with a 4% and 12% mortality after 48 hours and one week, respectively. Of the surviving bonefish, ten were picked up frequently within the hydrophone array between June and December 2007, logging 92,671 detections. Only these fish were analyzed for habitat preferences by month.

The highest percentage of detections between June and December 2007 were at receivers located in the creek mouths, followed by those in the open ocean, and backwater portions of creeks ($F = 26.46$, $p < 0.0001$ (Fig. 3.3). Month was not a significant main effect in the model ($F = 0.18$, $p = 0.981$), and no significant interaction between month and habitat type occurred ($F = 0.92$, $p = 0.531$). A significant positive relationship was determined between the arcsine root transformed proportion of detections in the backwaters of the creeks and water temperatures ($r^2 = 0.09$, $F = 33.19$, $p < 0.0001$) (Fig. 3.4). When maximum daily temperatures exceeded the conservative CTMax of 36.4°C, all detections of bonefish in the backwaters of tidal creeks were examined further to determine the exact times at which the fish were detected at the receiver and the hourly maximum temperature recorded. Although one bonefish was detected on June 25, 2007 when the daily maximum temperature in the backwaters of Kemps Creek reached 37.88°C, the maximum temperature recorded while the bonefish was detected 23 times between 6:16 and 8:03 was 29.5°C. Maximum water temperatures approached the CTMax on June 21, 2007 at 36.13°C. On this day, two bonefish were detected in the backwaters of Broad Creek, between 13:10 and 14:33, when hourly water temperatures of 33.17°C and 34.01°C were recorded at 13:00 and 14:00, respectively. Additionally, one bonefish was detected in the backwaters of Kemps Creek on June 21 between the hours of 14:16 and 14:27 and 18:00 to 18:15, with hourly temperatures of 32.32°C and 32.76°C, respectively.

To further examine the range of temperatures to which bonefish are exposed to on a daily basis, a thermal trace for a 72 hr period in August and January was constructed for one individual bonefish by matching the hourly temperature recorded by the temperature logger at the corresponding receiver (Fig. 3.5). For all receivers outside of the creeks, the temperature recorded by the logger on the coastline off of CEI was used. The traces constructed are representative of other bonefish activity as another tagged bonefish was found schooling with this individual during the same August time period the temperature trace was constructed. The range of water temperatures measured within the 72 hr trace was 5°C in the summer and 3°C in the winter.

Discussion

This study represents the first effort to quantify the thermal tolerance and environmental relations of bonefish. The laboratory-determined CTMax of bonefish, 36.4 ± 0.5°C, and 37.9 ± 0.5°C for individuals acclimated to 27.3 ± 1.3°C and 30.2 ± 1.4°C, respectively, typically exceeds daily maximum temperatures observed in the tidal creeks. Observed thermal tolerances for bonefish are not remarkable when compared to 22 species of freshwater fish found in North America that exhibit CTMax values of 40°C or higher (see Beitinger et al., 2000). For example, largemouth bass (*Micropterus salmoides*) acclimated at 28°C obtained a CTMax of 40.1°C (Smith and Scott, 1975). Although limited data exist for tropical marine species (Kimball et al., 2004), there are a few studies in which comparisons can be made. For 15 species of reef fish acclimated to 26.5 ± 0.5°C, CTMax values ranged between 34.7°C to 40.8°C, with the least tolerant species exhibiting a CTMax 8°C above mean sea temperatures in the tropical eastern

Pacific (Mora and Ospina, 2001). Atlantic stingrays (*Dasyatis sabina*), which inhabit shallow bays prone to rapid temperature changes, had CTMax values of 39.3°C and 43.2°C when acclimated to 20.5°C or 35.1°C, respectively (Fangue and Bennett, 2003). A combination of physiological tolerance and behavioral adaptation may be responsible for allowing bonefish to exploit the backwaters of tidal creeks when temperatures peak. Indeed such a combination has been suggested as the mechanism allowing fish species to occupy hyperthermal rockpools in the Dry Tortugas, even though water temperatures often meet or exceed thermal tolerances (Fangue et al., 2001).

Reaching the CTMax for bonefish induced physiological changes as evidenced by observed changes in blood chemistry. Elevations in blood lactate, glucose, Na^+, and K^+ were observed for bonefish exposed to temperatures at their CTMax relative to controls. Exposure to multiple stressors (i.e., capture/holding stress combined with increased ambient water temperatures) further exacerbated the response in blood lactate, glucose, hematocrit, and several ions (Na^+ and Cl^-). In fact, recorded values of lactate, glucose, and hematocrit for bonefish in field trials at the highest water temperature exceeded those documented in moribund fish following transport and handling at 21 - 24°C (Murchie et al., 2009), suggesting that fish in these experiments underwent severe physiological disturbance. Results documented in this study are consistent with the response of bonefish exposed to acute stressors such as exercise (Suski et al., 2007) and confinement stress (Cooke et al., 2008), and recorded values are within the realm of those determined for other marine species (e.g., coral trout, *Plectropomus leopardus*; Frish and Anderson, 2000; coho salmon, *Onchorhynchus kisutch*, Farrell et al., 2001; sockeye salmon, *O. nerka*, Cooke et al., 2006a; ling cod, *Ophiodon longatus*, Milston et al., 2006). Lactate production occurs in white muscle in response to conditions of strenuous exercise and hypoxia (Driedzic and Kiceniuk, 1976). In this study, activity in the thermal bath increased as bonefish approached their CTMax. Individuals attempted to escape confinement by increasing their swimming activity, and many thrashed against the lid. Despite aerating the CTMax apparatus, dissolved oxygen levels did decrease slightly with rising water temperatures and oxygen consumption by the bonefish. Near the highest water temperatures, dissolved oxygen did approach hypoxic levels (i.e., < 5 mg/l; Suski, University of Illinois, unpublished data). Our field observations suggest that dissolved oxygen in the wild also decreases as waters in tidal creeks approach their maxima (Cooke, Carleton University, unpublished data). As such, this decrease in dissolved oxygen that parallels the increase in water temperature during the CTMax studies is representative of what fish would likely experience in the wild. Increased glucose levels (i.e., hyperglycemia) indicate the release of glucose into the bloodstream to fuel increased muscular activity, and the increase in passive ion influxes is consistent with the loss of water in marine fish during a stress response (Wendelaar Bonga, 1997).

Observations of increased stress in captured bonefish at high water temperatures have implications for activities such as catch-and-release angling. Elevated water temperature has repeatedly been identified as a contributing factor to mortality associated with angling (see Muoneke and Childress, 1994; Thorstad et al., 2003). Danylchuk et al. (2007a) found that although the susceptibility of bonefish to post-release predation was not directly related to water temperature, bonefish spent more time resting following release at higher water temperatures. It is likely that the higher water temperatures associated with our tagging efforts in August played a role in the loss of some transmitter-implanted fish. Surgical error associated with the implantation procedure itself is rare and unlikely, so most surgery-related mortality is likely to be a result of the cumulative stress of handling and post-operative stress rather than surgical injury *per se* (Wagner and Cooke, 2005).

Linkages between the spatial ecology of bonefish and its thermal habitat were accomplished through the use of telemetry coupled with temperature loggers along the coast and in the mouths and upper portions of tidal creeks. Bonefish were found to spend more time at creek mouths than coastal open ocean or backwater habitats. This can be expected as creek mouths are linkages between the ocean and backwater habitats. As water temperatures in backwater areas increased, the proportion of detections in the backwater stretches of creeks increased. Such a response is consistent with the need for more food energy to fuel increase metabolic costs associated with higher water temperature (Hochachka and Somero, 1973). Bonefish were not found in tidal creeks when maximum temperatures exceeded their determined CTMax, but they were still using the creeks at 34.01°C. During a post-release behavior experiment conducted in Broad and Kemps Creek, Danylchuk et al. (2007a) captured bonefish in water with temperatures exceeding 35°C. This is the highest known water temperature recorded when bonefish were present in tidal creeks.

Only two other known studies have attempted to relate bonefish movement to water temperatures. Colton and Alvezion (1983) monitored three individual bonefish for 32 hr total, spread over a 100 day period. During each manual tracking event, water temperature was recorded at approximately 30 minute intervals. The maximum range of water temperature measured during a single tracking event was 8°C (24°C to 32°C). In this study, a range of 5°C was found for a bonefish over a 72 hr period in the summer and 3°C over a 72 hr period in the winter. In the study by Humston et al. (2005), bonefish movements, as determined by an acoustic telemetry array, were related to daily mean and/or maximum air temperatures. Only two of their study fish were detected for a sufficient period (40 to 61 d) for analyses, and only one of the fish demonstrated some variation in movements that could be related to air temperature. The authors observed that the individual fish retreated to deeper waters with abrupt increases in air temperature and back to shallower waters when air temperatures decreased. Both studies inferred the use of deep channels as refugia when water temperatures increased. The use of behavioral thermoregulation may explain how bonefish in this study were able to withstand water temperatures approaching their CTMax. Future work using physiological telemetry where the tags carried by the fish transmit water temperature information in addition to fish identification, date, and time is suggested for achieving the most accurate assessment of thermal habitat selection by bonefish. This will also provide insight into the temperatures in which individuals are acclimatized to prior to making forays into warmer waters. Additional studies, which should include an assessment of maximum and minimum acclimation temperatures as well as CTMax and critical thermal minimum across the acclimation range, would be beneficial so that a thermal tolerance polygon (see Fangue and Bennett, 2003) can be constructed for bonefish and their optimal temperature determined, since aerobic scope, cardiovascular function and growth peak at optimal temperatures (Wood and McDonald, 1997).

Given the importance of tidal creeks as bonefish habitat, it is worth considering potential changes in thermal habitat availability under future climate change scenarios.

The Intergovernmental Panel on Climate Change (IPCC) predicts an increase in water temperature of 1.8°C or 3.4°C per 100 years for atmospheric carbon dioxide concentrations of 550 ppm and 800 ppm by the year 2100, respectively (IPCC, 2001). Given that the future impacts of climate change for the Bahamas are based on IPCC predictions (BEST, 2002), and that IPCC predictions are in-line with historical data for the region (BEST, 2001), examination of tidal creek temperatures under present conditions and predicted scenarios can be compared to determine the percentage of time water temperatures exceed the CTMax of bonefish. Similar approaches have been used for other ecosystems in the tropics (e.g., coral reefs; Hoegh-Guldberg et al., 2007) to predict the effects of global warming. By using water temperature data collected from January 1 to December 31, 2006 from the upper portion of Kemps Creek, where the majority of bonefish detections were logged for backwater stretches, current conditions found water temperatures exceeded the conservative CTMax of 36.4°C 0.27% of the time. Under Scenario 1, an increase in 1.8°C, water temperatures are predicted to exceed CTMax 4.66% of the time. Under Scenario 2, an increase of 3.4°C, temperatures are predicted to exceed CTMax 18.90% of the time. Although there is capacity for individual bonefish to acclimate to changes in seawater temperatures over time, it is possible that populations as a whole will be less tolerant. For example, Pörtner and Knust (2007) observed a strong negative correlation between estimated eelpout (*Zoarces viviparous*) population sizes and summer water temperatures, suggesting that the temperatures causing population declines are lower than critical tolerances. Physiological limitations of cardiac function in waters with elevated temperatures, and thus diminished dissolved oxygen levels, are likely to limit the aerobic scope decreasing the efficiency of foraging and making individuals more prone to predation (Wang and Overgaard 2007; Pörtner and Farrell 2008). In turn, the overall energy budget of the fish is affected, potentially influencing immune function, reproduction, and growth (Barton and Iwama 1991; Somero 2002). A more accurate assessment of the potential performance of bonefish in light of climate change can only be possible, however, with more experimental studies and long-term monitoring of populations.

Collectively, the data from our laboratory and field studies suggest that bonefish occupy habitats that approach their laboratory-determined CTMax and can apparently do so without significant sub-lethal physiological consequences or mortality, except when exposed to additional stressors. Given that human interactions with fish continue to increase in coastal and estuarine habitats (Turner et al., 1996), the potential for more frequent interaction between fish and humans (e.g., general disturbance, Ellison and Farnsworth, 1996; recreational fishing; Cooke et al., 2006b), as well as general changes in habitat quality (Ellison and Farnsworth, 1996; Turner et al., 1996) could make fish that use tropical tidal creeks particularly vulnerable to climate change. Moreover, climate change has the potential to reduce the availability of upper creek habitats that appear to be important for feeding and predator avoidance (Colton and Alevizon, 1983b). Additional research is required to understand the energetic tradeoffs associated with occupying dynamic thermal habitats and how climate change will influence the distribution and condition of a variety of marine fish species that reside in tropical tidal flats (Perry et al., 2005). It is also important to understand how temperature influences the response of fish to hypoxia and salinity fluctuations, two critical environmental variables that will undoubtedly change with warming temperatures (Harley et al., 2006).

We advocate for future studies that integrate laboratory and field data to understand how animals adapt to dynamic environments and to understand how they will be influenced by human activity and environmental change (Wikelski and Cooke, 2006; Pörtner and Farrell, 2008).

Tables

Table 3.1: Summary statistics of bonefish size, CTMax, and blood chemistry results from the CTMax trials at two acclimation temperatures. Values are means ± SD for CTMax and means ± SE for blood chemistry.

Acclimation temperature (°C)	Treatment	n	Total length (mm)	CTMax (°C)	Lactate (mM/L)	Glucose (mM/L)	Na^+ (mM/L)	K^+ (mM/L)	Cl^- (mM/L)
27.3 ± 1.3°C	CTMax	10	428±30	36.4±0.5	9.2±0.9*	8.1±0.8*	171.0±1.6*	11.4±0.9*	168.8±1.0*
	Control	11	422±31		2.9±1.0	5.2±0.4	178.8±3.5	7.9±0.7	167.5±1.0†
30.2 ± 1.4°C	CTMax	10	455±29	37.9±0.5	9.9±0.8	11.7±1.7	182.2±2.7*	9.7±0.6*	173.8±1.0*
	Control	8	453±40		5.9±0.8	6.5±0.5	194.4±8.7‡	6.6±0.6‡	180.9±7.6‡

*n=9, †n=8, ‡n=7

Table 3.2: Two-way ANOVA results for blood chemistry values following the laboratory determination of CTMax for bonefish. All significant values are designated with an asterisk.

Response variable	Factor	n	SS	F	P
Lactate	Treatment	38	251.368	31.975	< 0.0001 *
	Acclimation temperature		34.002	4.325	0.045 *
	Treatment x Acclimation Temperature		12.718	1.618	0.212
Glucose	Treatment	38	155.341	15.548	0.0004 *
	Acclimation temperature		57.254	5.731	0.022 *
	Treatment x Acclimation Temperature		11.703	1.171	0.287
Na^+	Treatment	36	879.508	5.341	0.027 *
	Acclimation temperature		1580.562	9.599	0.004 *
	Treatment x Acclimation Temperature		43.134	0.262	0.612
K^+	Treatment	36	95.535	20.405	< 0.0001 *
	Acclimation temperature		20.540	4.387	0.044 *
	Treatment x Acclimation Temperature		0.580	0.124	0.727
Cl^-	Treatment	33	68.477	0.766	0.389
	Acclimation temperature		686.416	7.680	0.010 *
	Treatment x Acclimation Temperature		147.055	1.645	0.210

Table 3.3: Summary statistics of blood chemistry results from the field trials examining the effects of multiple stressors (temperature and handling stress) on bonefish. Values are means ± SE.

Temperature (°C)	Duration of stressor	n	Lactate (mM/L)	Glucose (mM/L)	Hematocrit (fraction)	Na^+ (mM/L)	K^+ (mM/L)	Cl^- (mM/L)
22	short	9	5.6 ± 0.6	4.4 ± 0.3	0.32 ±0.03	161.0 ± 13.7	5.9 ± 0.6	164.6 ± 3.8
	long	8	10.0 ± 0.9	6.3 ± 0.5	0.39 ± 0.02	189.2 ± 6.2	7.9 ± 0.8	169.0 ± 4.6
32	short	9	11.6 ± 0.3	7.9 ± 0.5	0.54 ± 0.02	224.6 ± 1.6	6.5 ± 0.3	190.6 ± 1.2
	long	12	11.1 ± 0.6	8.4 ± 0.4	0.56 ± 0.02	228.0 ± 4.4	6.3 ± 0.5	192.8 ± 5.0

Table 3.4: Two-way ANOVA results for blood chemistry values following field trials examining the effects of multiple stressors (temperature and handling stress) on bonefish. All significant values are designated with an asterisk.

Response variable	Factor	n	SS	F	P
Lactate	Temperature	38	117.402	28.930	<0.0001*
	Duration of stressor		35.755	8.811	0.006*
	Temperature x Duration of stressor		55.040	13.563	0.0008*
Glucose	Temperature	38	72.259	41.720	<0.0001*
	Duration of stressor		13.009	7.511	0.010*
	Temperature x Duration of stressor		3.993	2.305	0.138
Hematocrit	Temperature	37	0.344	83.553	<0.0001*
	Duration of stressor		0.017	4.241	0.047*
	Temperature x Duration of stressor		0.009	2.152	0.152
Na^+	Temperature	38	24316.498	44.634	<0.0001*
	Duration of stressor		2329.408	4.276	0.046*
	Temperature x Duration of stressor		1426.215	2.618	0.115
K^+	Temperature	38	2.579	0.850	0.363
	Duration of stressor		6.879	2.267	0.141
	Temperature x Duration of stressor		10.123	3.336	0.077
Cl^-	Temperature	28	3175.319	20.910	0.0001*
	Duration of stressor		57.143	0.376	0.545
	Temperature x Duration of stressor		6.671	0.044	0.836

Figures

Figure 3.1: Study area along the north coast of Cape Eleuthera, Eleuthera, The Bahamas, showing the locations of the 27 hydrophone receivers (black squares), the various tidal creeks, and the location of the Cape Eleuthera Institute (CEI). Receivers were roughly numbered sequentially from west to east. Hydrophone receivers with associated temperature loggers are #5 and #6 (Page Creek mouth and backwaters, respectively), #11 and #12 (Kemps Creek mouth and backwaters, respectively), and #16, #18, and #19 (the two mouths of Broad Creek and the backwater, respectively). An additional temperature logger, deployed along an open stretch of coastline off of CEI, is denoted by a star. The inset map displays the entire island of Eleuthera with the study area highlighted.

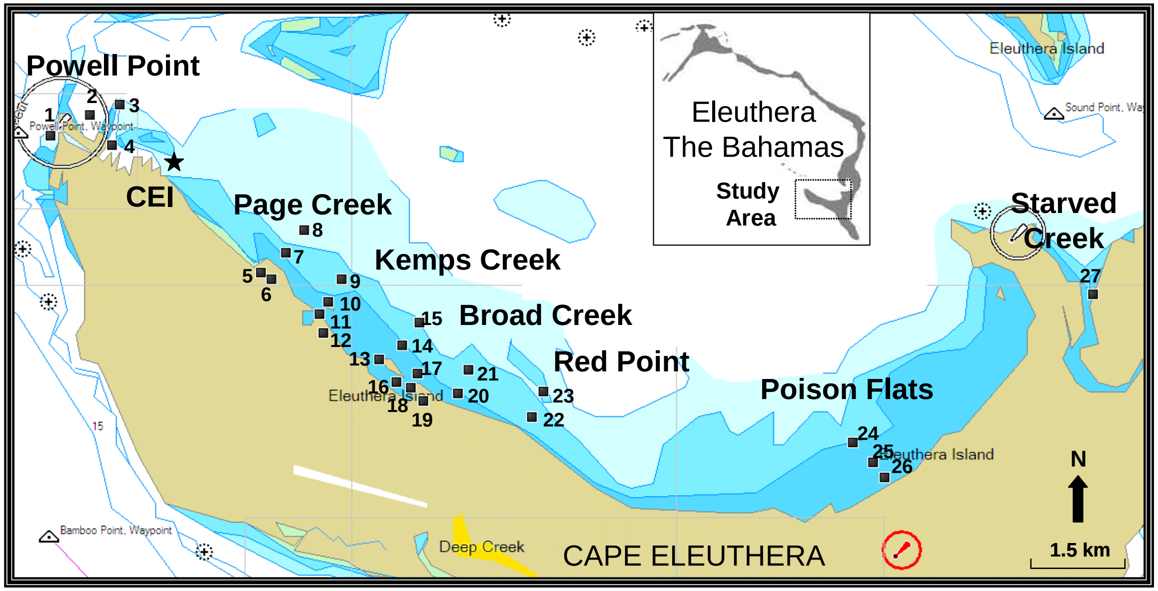


Figure 3.2: A representative sample of hourly temperature data collected from the backwaters and mouths of tidal creeks, along with data from an offshore data logger. The upper panel is data from the backwaters of Kemps Creek, the middle panel is data from the mouth of Kemps Creek, and lower panel is data from offshore of CEI, between January 1 and December 31, 2006. The solid horizontal line provides a point of reference of the lowest determined CTMax of bonefish (i.e., 36.4°C), and the dashed lines represent the upper and lower SD.

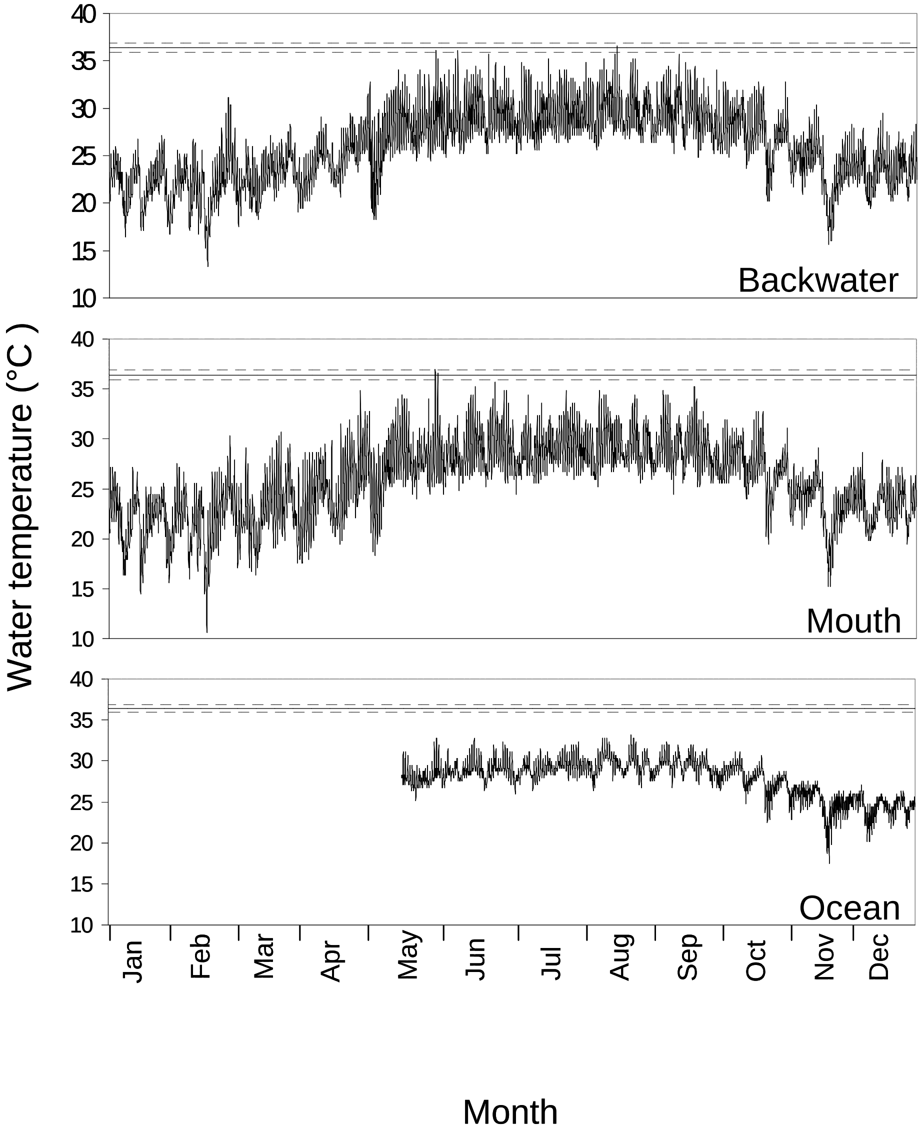


Figure 3.3: Weighted percentage of detections of tagged bonefish in various habitat types between June and December 2007. A description of the weighting procedure is provided in the methods.

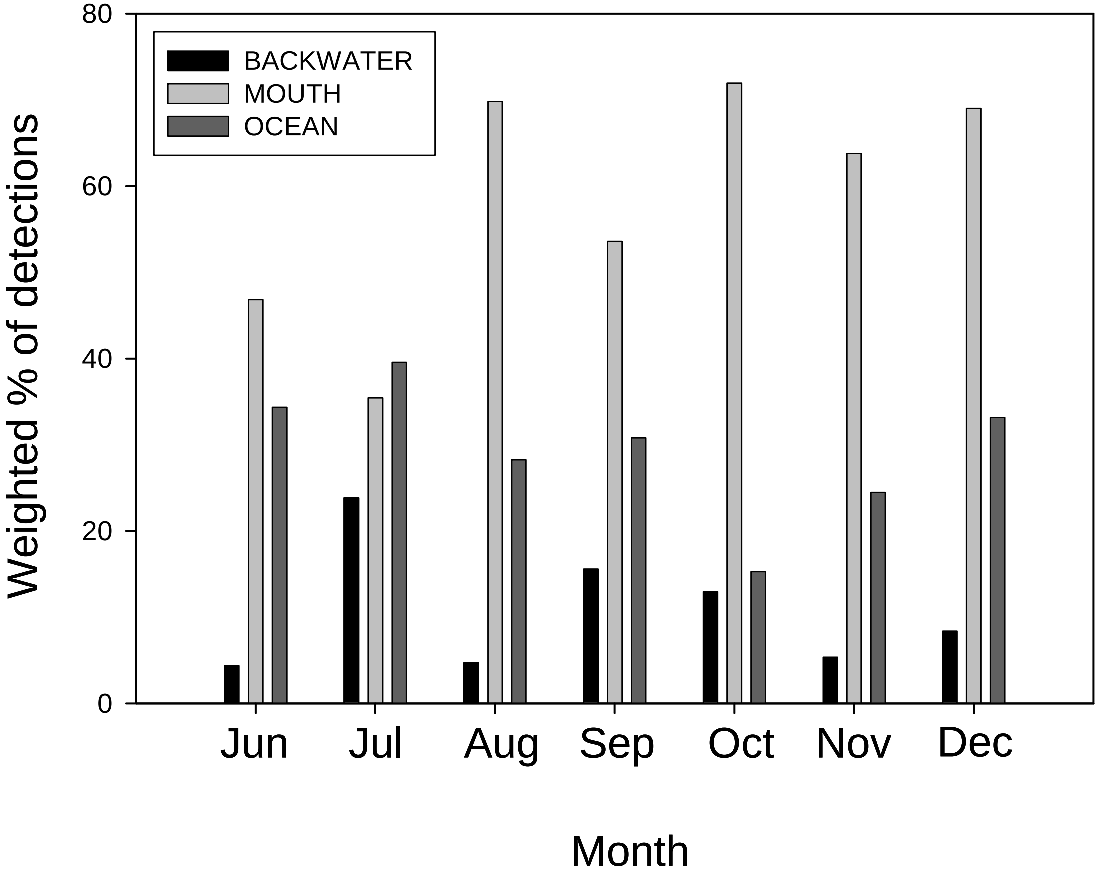


Figure 3.4: Scatterplot of arcsine transformed proportions of bonefish detections in tidal creek backwaters between June and December 2007 versus maximum daily water temperatures. The resultant model was arcsine root proportion of detections = -0.054 + 0.006 maximum water temperature ($r^2 = 0.090$, F = 33.19, $p < 0.0001$)

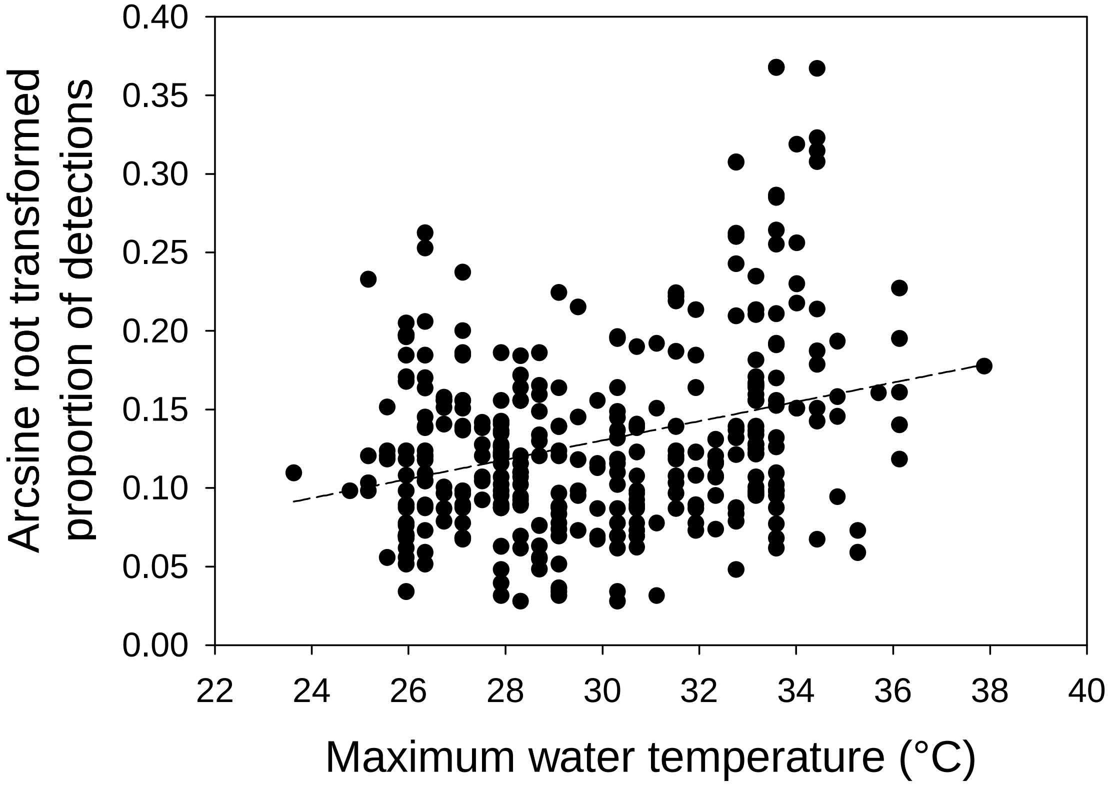
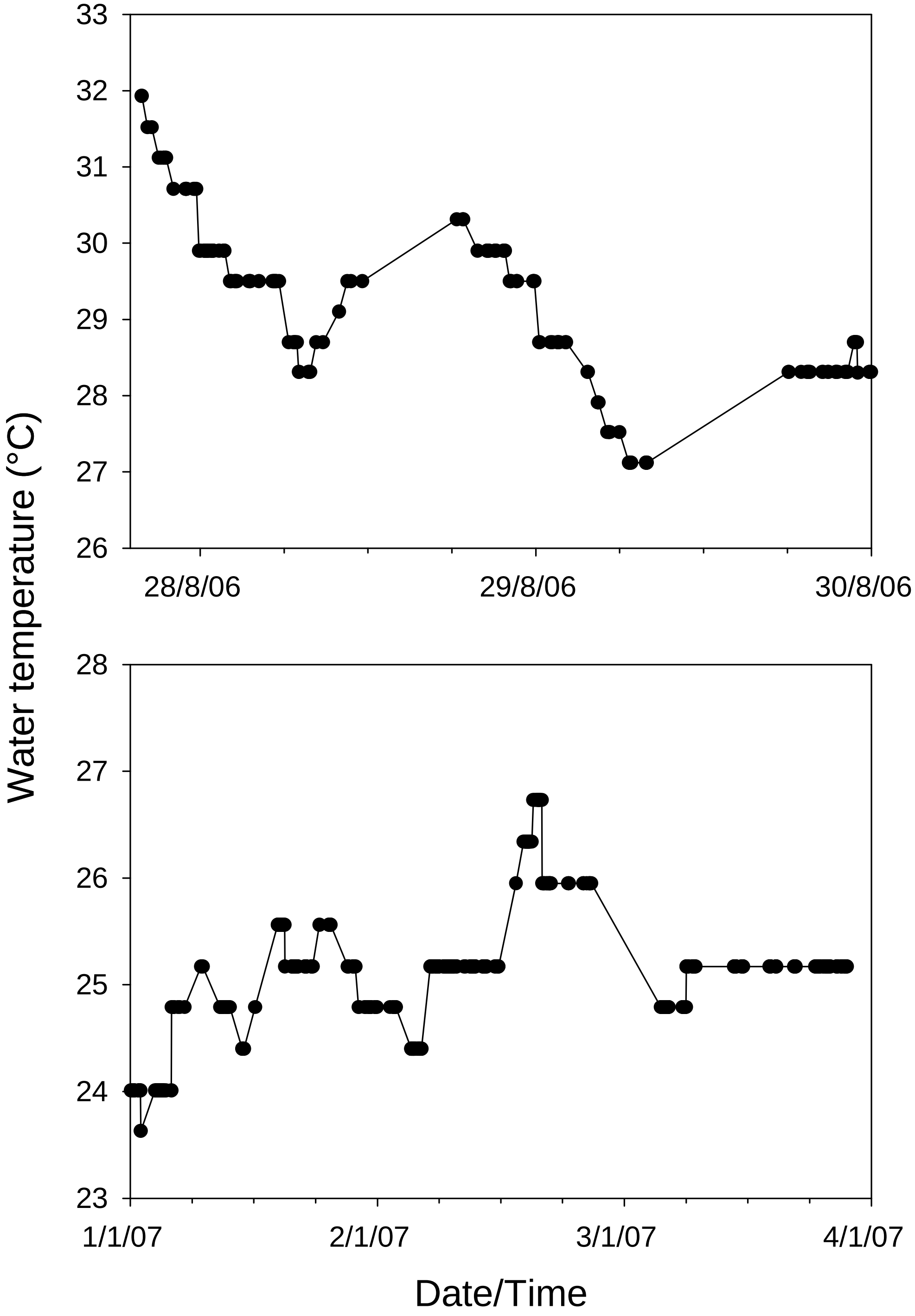


Figure 3.5: Corresponding water temperatures recorded over a 72 hour period for each detection of an individual bonefish in August 2006 (panel a) and January 2007 (panel b). Dates are given as dd/mm/yy



Chapter 4: Seasonal energetics and condition of bonefish from different subtropical tidal creeks in Eleuthera, The Bahamas

Abstract

Body composition and fish health indices of bonefish (*Albula vulpes*) were investigated to elucidate energy dynamics in poorly studied tropical tidal flats environments. In general, bonefish were composed of 72% water, 21% protein, 4% ash, and 3% lipid, which is consistent with the wet weight values of the majority of freshwater and marine fish studied to date. Significant inverse relationships between water and lipid content of whole body, gonad and liver tissues revealed that the percentage of water is a good indicator of the relative amounts of stored energy in bonefish, and may be used as a surrogate for lipid content in future studies. The liver was the main storage site for lipids, containing more than two times the fat in the whole body. While both abiotic (e.g., season, location) and biotic (e.g., sex, size) factors contributed to the predictive power of general linear models generated for all constituent analyses, there were no significant differences in whole body or liver lipid content between seasons which may be explained by a consistent food supply year round. There was however a significant relationship between lipid content and body size linked to season. Lipid content decreased in the winter and increased in the summer with increasing body size, a trend which can be explained by the timing of the reproductive season in bonefish. Seasonal changes in condition factor and gonadosomatic indices were also linked to the winter spawning season, with decreasing body condition and increasing gonad development in the winter. Observed site specific differences in lipid content and liver somatic indices of bonefish

may be accounted for by local trophodynamics as well as subtle differences in life history strategies. Energy reserve and fish health indices data collected in this study may provide a useful baseline for future comparative work and help to elucidate fish energetics in dynamic tropical tidal flats systems.

Introduction

In any aquatic environment the physico-chemical properties and dynamic nature of water (e.g., high thermal conductance and viscosity, low oxygen solubility) can place constraints on how organisms function (Domenici et al., 2007). Given the tight connection between fish and their surroundings (e.g., water temperature; Fry, 1967), the fluctuating nature of environmental variables can influence how individuals partition energy between growth, reproduction, and survival, ultimately affecting fitness (Claireaux and Lefrançois, 2007). The resulting strategies adopted by fish living in fluctuating environments can be a combination of physiological and behavioral traits, and specific insights into how these strategies function is fundamental to the conservation and management of fish populations.

How fish cope with fluctuating environments while still meeting the energetic requirements for life functions can be gained by estimating proximate body composition (e.g. water, lipids, proteins, carbohydrates, ash, energy density), and by using indicators of fish health (Love, 1970; Busacker et al., 1990). Estimates of proximate body composition can be valuable for modeling the changes in seasonal energy dynamics and are necessary for bioenergetics-based modeling to track energy flow into and out of fish (Adams and Breck 1990; Madenjian et al. 2006). The different variables examined in proximate body composition can lend clues to a variety of aspects of a fish's current life history. For example, changes in protein content are considered a measure of sustainable growth, whereas the presence or absence of lipids indicates the storage of potential chemical energy (Busacker et al., 1990). Other indicators of nutritional or energy status can be used to complement proximate body composition analysis, such as Fulton's condition factor and various organosomatic indices. Unfortunately, the energetic consequences of environmental stressors has primarily been investigated in freshwater fishes or anadromous salmonids (e.g. Cunjak and Power, 1986; Fechhelm et al., 1995; Simpkins et al., 2003; Madenjian et al., 2006). Studies involving the use of sub-tropical or tropical fish have typically been focused on aquaculture applications (e.g., Ellis et al. 1996) rather than natural environments (e.g., Montgomery and Galzin, 1993; Wuenschel et al., 2006). Tropical tidal flats in particular are regions of the marine environment where water temperature, salinity, and dissolved oxygen are in constant flux (Nybakken and Bertness, 2005) thus creating physiological challenges for its resident fish (Reise, 1985). As such, indicators of energy and/or nutritional status would provide novel insight into the consequences of living in these dynamic aquatic systems.

Bonefish (*Albula* spp.) serve as an interesting model for the study of energy dynamics in tidal marine flats systems. With a circumtropical distribution and a popular recreational fishery throughout, bonefish play an essential role in many local economies (Pfeiler et al., 2000; Ault, 2008; Danylchuk et al., 2008). In addition to economic importance, it is hypothesized that bonefish provide a number of fundamental services to the tidal flats ecosystems in which they inhabit. Bonefish move into shallow flats to feed on invertebrates and small fish during high tide, and then retreat to deeper water at low tide (Colton and Alevizon, 1983a,b; Humston et al., 2005). Through their movements and feeding habits, bonefish effectively connect the mosaic of tidal flats habitats and have been found to limit the depth distribution of some of their prey items (see Engstrom, 1984). Despite their known economic value and hypothesized key ecological importance, there are critical gaps in the scientific knowledge surrounding the biology of bonefish. This dearth of information hampers not only effective conservation and management plans for the species, but also an understanding of community trophodynamics and productivity of tropical tidal flats.

The purpose of this study was to examine the body composition and condition of bonefish (*A. vulpes*). Specific objectives were to: (1) determine energy partitioning between tissues in relation to abiotic (e.g., season, location) and biotic (e.g., sex, size) factors and (2) quantify baseline levels of bonefish health and condition. Collectively, we will elucidate the energy dynamics of fish in tropical tidal flats systems and also provide detailed information on the biology of bonefish.

Materials and methods

This study took place in south Eleuthera, The Bahamas (N 24° 50' 05" and W 76° 20' 32") in a number of tidal creek systems (Kemps, Broad, Starved) and tidal embayments (Half Sound), as well as the Cape Eleuthera Institute (CEI) research facility (Figure 4.1). Preliminary genetic analyses on bonefish from this area indicated that all specimens were *Albula vulpes* (Danylchuk et al., 2007). Telemetry data for bonefish in the study area show that bonefish from Broad and Kemps creek mix frequently, thus allowing samples from these two locations to be combined (Murchie et al., In Review).

All research was conducted in accordance with the policies of the Canadian Council on Animal Care under an approval granted by the Carleton University Animal Care Committee (Protocol B07-03, B07-05, and B07-06).

Sample collection and initial processing

Bonefish were collected by seine net for proximate body composition analysis between August 27, 2006 and August 25, 2007. Sampling occurred at two distinct periods; in August to represent the ‘summer’ season (May 1 - October 31) where ambient water temperatures are on average 29°C, and in February and March to represent ‘winter’ (November 1 - April 30) when the mean water temperature is 23°C (Murchie et al., In Review). Fish were transported to the laboratory at CEI for processing. All fish were measured to the nearest millimeter total length and wet weight was measured to the nearest gram (g). The viscera, gonads, and liver were removed, weighed to the nearest 0.1 g, and placed into individual labeled storage bags. The carcass of the fish was weighed to the nearest gram and then ground to a homogenous mixture using a hand-operated meat grinder. The ground sample was thoroughly mixed and run through the grinder again. After a second mixing, a sub-sample of the homogenous mixture was extracted into a labeled storage bag and sealed. The organs and ground samples were placed into a larger labeled bag, sealed, and frozen. Samples were shipped back to the Fish Ecology and Conservation Physiology Laboratory at Carleton University, Canada where they remained frozen until further processing for water, lipid and ash (trace minerals).

Proximate body composition analysis and energy density

Water content was determined for whole body, liver, and gonads by placing 2 ± 0.02 g of the sample in a crucible and drying overnight (18 hours) to a constant mass at 80°C. Samples were cooled in a desiccator and re-weighed to the nearest 0.0001 g to determine water content (Crossin and Hinch, 2005). Dried samples were crushed into a fine powder using a glass pestle, and a portion (0.2 g) was used in the lipid extraction procedure. The lipid content of the whole body, liver, and gonads of all fish was determined using the Smedes and Askland (1999) modification of the chloroform-methanol extraction technique developed by Bligh and Dyer (1959). Samples were combined thoroughly with chloroform, methanol, and distilled water in a 1:2:0.8 mL ratio and placed in an ultrasonic bath for 15 minutes. An additional 1 mL of chloroform and distilled water were added and the samples centrifuged at 1500 rpm for 10 minutes. The solvent layer, containing the lipids, was extracted using a pipette and filtered through sodium sulfate and quartz wool into pre-weighed aluminum boats. The extraction procedure was repeated on the supernatant, and extracted lipids were left overnight in the fume hood to allow the chloroform to evaporate. The aluminum weigh boats were dried for one hour at 60°C, and re-weighed to the nearest 0.0001 g to calculate the percent of lipids by dry mass. These values were then converted to an expression of percent lipid by wet mass. All samples were analyzed in duplicate and differences between replicates never exceeded 2%. Ash and protein were determined on whole body samples only due to the mass of tissue required. Methods outlined by Crossin and Hinch (2005) were used to determine ash and protein content. After replicate samples (described above) were analyzed for water content, the crucibles containing dried sample were combusted for two hours in a muffle furnace between 500-600°C. After the samples cooled in a dessicator, the remaining ash was weighed to determine the percent of trace minerals by wet mass. As above, differences between duplicate samples never exceeded 2%. The percent of whole body protein was determined by the relationship

$$C_P = 100 - (C_W + C_L + C_A),$$

where C_W, C_L, and C_A are percent water, lipid and ash, respectively (Berg et al. 1998; Hendry et al., 2000).

Using the determined fraction of whole body lipid and protein of each individual fish, whole body energy density (d; in MJ kg^{-1}) could be calculated using the following equation:

$$d = fD_f + pD_p,$$

where f and p are the fraction of lipids and proteins from the samples expressed in g kg^{-1}, respectively, and Df and Dp are the energy density of lipids and protein in fish expressed in MJ g^{-1} (Breck, 2008). Values for the energy density of lipids and protein in fish were taken from Brett and Groves (1979) and were 0.0362 MJ g^{-1} and 0.0201 MJ g^{-1}, respectively.

To calculate fish health indices such as Fulton's condition factor (K) (Anderson and Neumann, 1996), gonadosomatic index (GSI), and liver somatic index (LSI) (Barton et al., 2002), the following equations were used:

K = whole body weight (g) / total length^3 (mm) x 10^5

GSI = gonad weight (g) / whole body weight (g) x 100

LSI = liver weight (g) / whole body weight (g) x 100

Data analysis

All statistical analyses on collected and derived data were completed using JMP 7.0.2 (SAS Institute, Cary, NC). Maximal type-1 error rates were set at α=0.05. Normality and homogeneity of variance assumptions were evaluated using Shapiro-Wilk or Kolmogorov Smirnov goodness-of-fit tests and Levene's test, respectively (Sokal and Rohlf, 1995). Regression analyses were used to determine any relationships between the percentages of water and lipid in various tissues and the percentage of water and protein in whole body (see Salam and Davies, 1994). A general linear model (GLM) was used to assess whether proximate composition (e.g., % water, % lipid, % ash, % protein) and energy density in whole body, gonad, and liver tissue varies for bonefish between season, location, sex, and/or size. Tukey-Kramer HSD tests were performed to determine where significant differences lie between means (Day & Quinn, 1989). Relationships between indices of fish condition and proximate composition were evaluated using regression analyses (e.g., Kaufman et al., 2007). The effects of season, location, and sex on K were evaluated using a three-way ANOVA, followed by Tukey-Kramer HSD tests. To evaluate the effects of season, location, and sex on GSI and LSI, the proportional data (gonad or liver weight divided by total fish weight) was arcsine root transformed and evaluated using a three-way ANOVA, followed by Tukey-Kramer HSD tests. Total length was not included in these analyses since all health indices incorporate some measure of fish size into the metric.

Results

Proximate body condition and energy density

A total of 174 bonefish (442 ± 47 mm total length; mean ± SD) were captured and processed for proximate body composition. It should be noted that an additional 21 bonefish were captured in the summer season but were not included in the data set as sex could not be determined. The wet weight of bonefish generally consists of 72% water, 21% protein, 4% ash, and 3% lipid (71.8 ± 3.4, 21.1 ± 2.2, 4.0 ± 1.3, 3.1 ± 1.5 %; mean ± SD, respectively). The mean energy density of bonefish whole body is 5.4 ± 0.8 MJ kg^{-1}. On average, bonefish livers have higher lipid content (7.4 ± 2.2 %) than whole body, but the percentage of fat is highest in gonads (13.4 ± 15.0 %). Significant negative relationships exist between the percentages of lipid versus water for whole body, gonad, and liver tissues (Figure 4.2). The equations of the line are as follows for whole body, gonad, and liver, respectively: % lipids = 25.2 – 0.308 * % water ($r^2 = 0.5$, F = 169.12, $p<0.0001$), % lipids = 78.7 – 1.027 * % water ($r^2 = 0.9$, F = 1591.47, $p<0.0001$), and % lipids = 51.898 – 0.633 * % water ($r^2 = 0.427$, F = 128.23, $p<0.0001$). As with lipid content, protein stores in whole body also exhibited the same strong negative relationship with whole body water content (% protein = 58.9 – 0.527 * % water, $r^2 = 0.6$, F = 298.27, $p<0.0001$).

Patterns in proximate composition of various tissue samples were not similar across all sample locations (Table 4.1). To best understand the influence of season, location, sex, and size of the bonefish on the various tissue constituents, the results of the GLM must be examined. The GLM for whole body water content was significant (F = 9.341, p<0.0001), with 58.9% of the variability in the model explained. An interaction between the season and location terms occurred in the model (F = 8.825, p = 0.0002) (Table 4.1). Bonefish from Broad/Kemps Creek and Half Sound had no seasonal differences in water content, whereas fish from Starved Creek had higher values in the summer versus the winter (Figure 4.3a). A GLM was produced for whole body lipid content with an r^2 value of 0.5 (F = 7.917, p<0.0001). Location and an interaction between season and total length (mm) were significant terms in the model (F = 4.505, p = 0.013, and F = 14.745, p = 0.0002, respectively) (Table 4.2). Lipid content was higher in bonefish sampled from Starved Creek versus those from Broad/Kemps Creek (Figure 4.3b). A relationship exists between the lipid content and total length of bonefish in both seasons, but in opposite directions (Figure 4.4a, b). Fat content increased with fish size in the summer (WB % lipids = -0.581 + 0.007 * TL, r^2 = 0.104, F = 4.185, p = 0.048) and decreased in the winter (WB % lipids = 11.9 - 0.020 * TL, r^2 = 0.266, F = 48.533, p<0.0001). Variation in whole body ash content was explained by the GLM (r^2 = 0.2, F = 1.855, p = 0.015). An interaction between season and location was found (F = 3.955, p = 0.021) (Table 4.2). There was no difference in % ash between seasons for Broad/Kemps and Half Sound, whereas bonefish from Starved creek had highest ash content in the winter relative to the summer (Figure 4.3c). The GLM for whole body protein content was significant (F =3.884, p<0.0001), with 37.3% of the variability in the model explained. An interaction between the season and location terms occurred in the model (F = 8.346, p = 0.0004) (Table 4.2). Protein content only varied significantly by season at Starved Creek, with the highest values recorded in the winter season (Figure 4.3d). Energy density of bonefish was predicted by the GLM ($r^2 = 0.528$, F = 7.290, $p<0.0001$). An interaction between season and total length was found in the model (F = 6.501, p = 0.012) (Table 4.2). There was no relationship between energy density and bonefish size for the summer season ($r^2 = 0.082$, F = 3.217, p = 0.081) (Figure 4.4c). However, there appears to be a slight tendency for energy density to decrease with increasing total length during the winter (WB energy density = 9.01 - 0.008 * TL, $r^2 = 0.2$, F = 24.907, $p<0.0001$) (Figure 4.4d).

Regarding the water and lipid content in bonefish gonads, the GLMs produced explained a significant portion of the variability ($r^2 = 0.4$, F = 4.852, $p<0.0001$; $r^2 = 0.4$, F = 3.770, $p<0.0001$. respectively). An interaction was found between season and total length for both gonad constituents (Table 4.3). No relationship was found between water content and total length (Figure 4.5a, b) or lipid content and total length (Figure 4.5c, d) for either season ($p<0.05$). A GLM was produced for both water ($r^2 = 0.2$, F = 1.981, p = 0.008), and lipid ($r^2 = 0.3$, F = 2.176, p = 0.003) content for bonefish livers. For both liver constituents, there were no significant terms in the model (Table 4.4).

Fish health indices

Condition factor ranged from 0.928 to 1.571 and was significantly higher in the summer than the winter for both males and females ($p<0.0001$) (Table 4.5, Table 4.6). Location was also a significant factor in the three-way ANOVA for K (p = 0.028) (Table

4.6), with bonefish being in higher condition at Half Sound compared to Starved Creek (Figure 4.6a). Winter GSI levels were greater than values reported for the summer in both sexes ($p<0.0001$) (Table 4.5, Table 4.6). Location and an interaction between season and location were also significant variables for GSI (Table 4.6). Gonadosomatic index was different between bonefish collected from Starved Creek (mean = 3.064) and Half Sound (mean = 0.791) (Figure 4.6b) (all $p <0.05$). When further examined by season and location, summertime values for GSI did not vary across locations, but in the winter, GSI values were higher in Starved Creek, followed by Broad/Kemps Creek, and Half Sound (Figure 4.6c). Liver somatic indices ranged from 0.178 to 1.005, and differed between the sexes ($p = 0.010$) (Table 4.5, Table 4.6). LSI values were higher in bonefish from Starved Creek compared to Half Sound ($p<0.05$) (Figure 4.6d). Furthermore, Starved Creek bonefish had significantly higher LSI values in the summer (mean = 0.642) and significantly lower LSI values in the winter (mean = 0.499) than the other two locations (Figure 4.6e). A positive relationship exists between K and whole body fat content for bonefish ($K = 1.28 + 0.016 * \text{WB \% lipids}$, $r^2 = 0.06$, $F = 11.150$, $p = 0.001$) (Figure 4.7).

Discussion

This study represents the first assessment of the nutritional status of wild bonefish, and more generally, one of the few energetic studies focusing on any wild tropical fish inhabiting tidal marine flats. In general, proximate body composition values determined for bonefish (72% water, 21% protein, 4% ash, and 3% lipid) are consistent with wet weight values of the majority of freshwater and marine fish studied to date (70-

80% water, 20-30% protein, 2-12% lipid; Love, 1970). A well documented trend is the inverse relationship between water and fat (see Craig, 1977; Hartman and Brandt, 1995; Kaufman et al., 2007). For bonefish, the percentage of water is a good indicator of the relative amounts of energy stored in the whole body with a lower percentage of water equating to a greater lipid and protein content. Significant inverse relationships between water and fat in bonefish were also documented for gonad and liver tissues. Depending on the energetic status of the fish (Idler and Bitners, 1959; Love, 1970), tissue water content has often been recommended or used as a surrogate for lipid or energy content in a number of fish species (e.g., Salam and Davies, 1994; Plante et al., 2005; Trudel et al., 2005) since water replaces catabolized lipids. Future studies of bonefish may benefit from the use of the predictive equations derived for these relationships as determining water content of various tissues is much less labor intensive and costly than determining lipid content.

Lipid deposition is the most efficient mode of energy storage as it can easily be metabolized when needed (Jobling, 1994) and can be depleted without reducing the physical performance of the fish (Hendry et al., 2000). Contrasting the percentage of lipid content in the various tissues is useful for determining the main compartment of lipid storage. The location of lipid storage varies between fish species but includes the liver, between muscle myomeres, in the mesentery, along the lateral line, or at the base of fins (Arrington et al., 2006). Bonefish livers appear to be the main site for lipid storage as the mean density value (7.4%) was more than double the content of whole body (3.1%). Even when examined by season the liver lipid density values were 2-fold greater than that of the carcass. In addition to serving as a storage depot for lipids, the liver is the major site of lipid biosynthesis (Henderson and Tocher, 1987) which may contribute to the higher values. Regardless of the main location of lipid storage, evidence of its presence indicates surplus energy is available for future maintenance, growth, and reproduction (Kaufman et al., 2007). Because it is often assumed that body condition indices are good indicators of the lipid status of fish (Kaufman et al. 2007), the relationship between K and the percentage of whole body lipid was examined for bonefish. Indeed a positive relationship (F = 11.150, p = 0.001) was detected, but only 6% of the variability in K was explained by whole body lipid content.

The highest amount of stored energy in bonefish was in muscle protein, at 21%. This is similar to results documented by Hendry et al. (2000) for sockeye salmon (*Oncorhynchus nerka*). Muscle protein is important for structural and performance-related functions (e.g., swimming) (Hendry et al., 2000), and deposition of protein is the most effective way to grow since each gram of protein also binds 3-4 g of water (Jobling, 1994). From a life history perspective, allocating more energy to protein storage and thus growth may be more important for bonefish since a greater body size may decrease the susceptibility of predation by sharks and barracuda that also reside in tropical marine flats (Cooke and Philipp, 2004). Additionally, since bonefish appear to spend a great deal of time foraging while moving in and out of the tidal flats (Colton and Alevizon, 1983b), the turnover rates for consumed energy are likely high. Future studies that investigate the daily field activity budget of bonefish would be beneficial in quantifying the amount of time fish are foraging versus avoiding predation, as activity is variable and could significantly contribute to the total energy budget (Boisclair and Leggett, 1989). A laboratory study which investigates the tissue turnover time and metabolic rate of bonefish would also provide clues to bonefish energetics.

Both abiotic (e.g., season, location) and biotic (e.g., sex, size) factors influenced proximate body composition in a variety of ways. While not all factors were significant predictors of the various constituents, all contributed to the predictive power of the general linear models. As a main effect, season was not a significant variable. In particular, there was no significant difference in whole body or liver lipid content between seasons. While such changes are expected and observed in northern temperate regions where fish require the use of stored energy to fuel metabolic activity during harsh winter conditions (e.g., brook trout, *Salvelinus fontinalis;* Cunjak and Power, 1986; Atlantic salmon, *Salmo salar*; Shackley et al., 1994; broad whitefish, *Coregonus nasus*; Fechhelm et al., 1995), species inhabiting subtropical or tropical waters in which food abundance is more stable seasonally may focus energy allocation into growth rather than storage (Wuenschel et al., 2006). Consistent with this, we found no seasonal differences in whole body lipid, water, ash, protein, or GSI. However, interactions between season and location were found. For example, somatic protein and ash were lower and somatic water higher, in bonefish sampled from Starved Creek in the summer. Starved Creek fish also had higher somatic lipid levels than fish from the other locations. This may be due to site-specific differences in diet, prey availability, and/or subtle differences in life history strategies (Love, 1970; Cunjak and Power, 1986; Hoey et al., 2007). Starved Creek lies 1.2 km from the settlement of Rock Sound, so potential anthropogenic inputs of nutrients could possibly affect trophodynamics at this locale. Future investigations of trophodynamics and nutrient sources using stable isotope analysis would provide further insight.

An interaction between season and size was a significant predictor in the GLM of bonefish whole body lipid and energy density as well as gonad lipid and water content. The percentage of whole body lipid as well as energy density increased significantly with bonefish size in the summer and decreased in the winter. These decreases in whole body lipid content and energy density in the winter can be explained by the timing of the reproductive season (Crabtree et al., 1997; Danylchuk et al., CEI, unpublished data). Fish health indices such as K and GSI also reflected the winter reproductive period with lower fish condition in the winter versus the summer, and higher GSI in the winter versus the summer. Liver lipid and water concentrations were not significant variables in the GLM for LSI, but location, sex, and an interaction between season and location were significant predictors. Differences between bonefish at Half Sound and Starved Creek were determined for LSI and those differences could be due to the site specific differences in prey content. Sex differences in LSI are unlikely to be caused by differences in energy storage strategies since no sex differences were detected for liver protein or water content, but it may be possible that female bonefish acquire different types of lipids than males. Analysis of the fatty acid composition of bonefish livers in future studies could elucidate this difference. In general, it is suggested that a future study examining the proximate body composition of bonefish on a monthly basis be conducted to further parse out the potential combined effects of reproductive timing and water temperature (Montgomery and Galzin, 1993; Kurita, 2003).

In conclusion, an assessment of the proximate body composition and condition of bonefish improved our understanding of strategies that fish use for making a living in tropical tidal flats environments. Ultimately, data from this study will serve as the basis for the development and parameterization of a bioenergetics model for bonefish, providing fisheries managers with a useful tool for understanding bonefish production (Hansen et al., 1993). In addition, a bonefish bioenergetics model could be used in combination with bioenergetics models for other tropical flats organisms (e.g., lemon sharks (*Negaprion brevirostris*); Sundström and Gruber, 1998) to study predator-prey relations and truly understand ecosystem-level trophodynamics (Adams and Breck, 1990; Hansen et al. 1993). Ideally proximate body composition data such as we have reported in this study would be combined with information on trophic relationships (from stable isotope analyses), feeding ecology (from field studies), field activity levels, and growth to generate a comprehensive understanding of bonefish bioenergetics and trophic relations. Our observations also provide a baseline for future biomonitoring programs. Since bonefish are benthivores occupying shallow coastal habitats, they may be appropriate candidates to use as bioindicators (Leamon et al., 2000; Plante et al., 2005). Energy reserve and fish health indices data from Eleuthera may provide a useful baseline for bonefish populations in areas such as Florida where human development along the coastline is much more substantial. However caution should be exercised since there are numerous physiological and environmental factors that can influence the data as observed in this study.

Tables

Table 4.1: Proximate composition (% water, % lipid, % ash, % protein; by wet mass) and energy density (MJ kg^{-1}) in whole body, gonad, and liver tissue of female and male bonefish sampled in summer and winter in various locations in Eleuthera, The Bahamas. Values are given as the mean (± SD).

				Whole body					Gonad		Liver	
Season	Location	Sex	N	Water	Lipid	Ash	Protein	Energy density	Water	Lipid	Water	Lipid
Summer	Broad/Kemps Creek	F	4	72.6 ± 2.2	1.7 ± 0.6	4.4 ± 0.8	21.4 ± 1.3	4.9 ± 0.3	67.1 ± 5.1	8.6 ± 3.4	70.2 ± 0.9	5.7 ± 1.2
		M	8	72.6 ± 3.2	2.2 ± 1.0	4.3 ± 1.1	20.9 ± 2.4	5.0 ± 0.8	53.7 ± 18.6	23.7 ± 25.3	70.5 ± 0.9	5.7 ± 0.8
	Half Sound	F	3	73.2 ± 0.6	3.3 ± 0.5	3.7 ± 0.7	19.8 ± 0.8	5.2 ± 0.2	70.6 ± 2.0	7.6 ± 2.1	70.3 ± 1.0	6.6 ± 0.5
		M	7	74.4 ± 2.6	3.2 ± 1.2	3.6 ± 0.8	19.0 ± 1.2	5.0 ± 0.7	55.4 ± 18.5	20.8 ± 19.0	70.8 ± 2.4	6.1 ± 1.8
	Starved Creek	F	6	75.5 ± 2.0	2.8 ± 1.1	3.0 ± 0.3	18.7 ± 1.3	4.8 ± 0.5	61.5 ± 12.8	17.8 ± 13.6	72.4 ± 1.6	6.8 ± 0.7
		M	10	74.5 ± 3.0	3.5 ± 1.4	3.3 ± 0.8	18.7 ± 1.7	5.0 ± 0.7	41.6 ± 18.7	39.9 ± 20.0	71.1 ± 2.1	7.5 ± 1.9
Winter	Broad/Kemps Creek	F	21	73.1 ± 2.8	2.6 ± 1.0	3.8 ± 1.2	20.6 ± 2.0	5.1 ± 0.6	60.6 ± 6.6	12.0 ± 3.5	71.2 ± 1.8	7.9 ± 1.9
		M	43	71.5 ± 2.3	3.3 ± 1.4	4.0 ± 1.4	21.2 ± 2.1	5.5 ± 0.7	66.9 ± 12.0	11.3 ± 14.0	70.0 ± 2.0	7.9 ± 2.1
	Half Sound	F	15	73.8 ± 2.1	1.9 ± 0.8	3.4 ± 0.5	20.8 ± 1.7	4.9 ± 0.5	65.1 ± 7.2	10.5 ± 5.6	70.1 ± 1.9	8.0 ± 1.9
		M	14	74.7 ±	1.8 ±	3.4 ±	20.2 ±	4.7 ± 0.4	64.1 ±	14.1 ±	70.8 ±	7.4 ±

			1.9	0.9	0.5	1.6		10.8	13.3	2.2	2.1
Starved Creek	F	13	69.1 ± 2.5	3.6 ± 1.6	4.7 ± 1.5	22.7 ± 1.8	5.9 ± 0.7	58.4 ± 13.0	13.6 ± 12.0	71.7 ± 2.4	5.3 ± 1.5
	M	30	67.8 ± 2.4	4.5 ± 1.3	4.7 ± 1.4	23.0 ± 2.0	6.3 ± 0.7	73.6 ± 9.9	5.2 ± 11.1	69.1 ± 2.8	7.8 ± 2.7

Table 4.2: Evaluation of significant terms in the general linear models examining if proximate composition (% water, % lipid, % ash, % protein; by wet mass) and energy density (MJ kg^{-1}) for bonefish whole body is dependent on season, location, sex, and/or size. Note that total length (mm) = TL. All significant values are designated with an asterisk.

	Water		Lipid		Ash		Protein		Energy density	
Term	F	p	F	p	F	p	F	p	F	p
Season	0.885	0.349	1.206	0.274	0.177	0.685	0.636	0.426	1.535	0.217
Location	0.193	0.825	4.505	0.013*	0.879	0.417	0.742	0.478	0.912	0.404
Sex	0.154	0.695	0.510	0.476	0.340	0.561	0.268	0.605	0.017	0.895
TL	0.0002	0.988	0.191	0.663	0.473	0.493	0.041	0.840	0.175	0.677
Season*Location	8.825	0.0002*	0.699	0.499	3.955	0.021*	8.346	0.0004*	1.769	0.174
Season*TL	2.278	0.133	14.745	0.0002*	0.320	0.572	0.002	0.962	6.501	0.012*
Season*Sex	0.335	0.564	0.276	0.600	0.319	0.573	0.424	0.516	0.006	0.937
Location*TL	0.284	0.753	1.990	0.140	0.205	0.815	0.018	0.982	1.023	0.362
Location*Sex	0.603	0.549	0.018	0.982	0.212	0.809	0.433	0.649	0.236	0.790
TL*Sex	0.587	0.445	0.021	0.884	0.045	0.833	0.787	0.376	0.232	0.631
Season*Location*TL	1.427	0.243	0.075	0.928	1.378	0.255	0.375	0.688	0.321	0.726
Season*Location*Sex	1.310	0.273	0.010	0.990	0.786	0.456	0.797	0.453	0.297	0.744
Season*TL*Sex	1.856	0.175	0.334	0.564	0.121	0.728	1.269	0.262	1.235	0.268
Location*TL*Sex	0.451	0.638	0.334	0.715	0.092	0.913	0.880	0.417	0.076	0.927
Season*Location*TL*Sex	0.915	0.403	0.117	0.890	0.058	0.943	1.300	0.276	0.813	0.445

Table 4.3: Evaluation of significant terms in the general linear models examining if proximate composition (% water, % lipid; by wet mass) for bonefish gonads is dependent on season, location, sex, and/or size. Note that total length (mm) = TL. All significant values are designated with an asterisk.

	Water		Lipid	
Term	F	p	F	p
Season	0.046	0.830	0.000	0.995
Location	1.165	0.315	1.581	0.209
Sex	0.021	0.884	0.090	0.765
TL	2.107	0.149	2.001	0.159
Season*Location	2.958	0.055	2.812	0.063
Season*TL	6.807	0.010*	4.703	0.032*
Season*Sex	0.651	0.421	0.164	0.686
Location*TL	1.888	0.155	0.986	0.376
Location*Sex	0.104	0.901	0.159	0.853
TL*Sex	1.142	0.287	1.332	0.250
Season*Location*TL	0.465	0.629	1.101	0.335
Season*Location*Sex	0.532	0.589	0.501	0.607
Season*TL*Sex	0.958	0.329	1.659	0.200
Location*TL*Sex	0.159	0.853	0.038	0.963
Season*Location*TL*Sex	2.770	0.066	2.106	0.125

Table 4.4: Evaluation of significant terms in the general linear models examining if proximate composition (% water, % lipid; by wet mass) for bonefish livers is dependent on season, location, sex, and/or size. Note that total length (mm) = TL.

	Water		Lipid	
Term	F	p	F	p
Season	0.688	0.408	1.354	0.247
Location	0.872	0.420	0.254	0.776
Sex	0.000	0.998	0.080	0.778
TL	0.033	0.856	0.017	0.895
Season*Location	0.635	0.531	2.797	0.065
Season*TL	1.310	0.254	0.492	0.484
Season*Sex	0.155	0.695	0.153	0.697
Location*TL	1.799	0.169	0.885	0.415
Location*Sex	0.834	0.437	0.719	0.489
TL*Sex	0.164	0.686	0.052	0.820
Season*Location*TL	0.766	0.467	1.803	0.168
Season*Location*Sex	0.018	0.982	0.016	0.984
Season*TL*Sex	1.004	0.318	2.365	0.126
Location*TL*Sex	0.490	0.614	1.195	0.306
Season*Location*TL*Sex	0.555	0.576	0.275	0.760

Table 4.5: Health indices (Fulton's condition factor (K), gonadosomatic index (GSI), and liver somatic index (LSI) for female and male bonefish collected in the summer and winter in Eleuthera, The Bahamas.

Health index	Females		Males	
	Summer (n = 13)	Winter (n = 49)	Summer (n = 25)	Winter (n = 87)
K	1.38 ± 0.08	1.30 ± 0.09	1.40 ± 0.10	1.31 ± 0.09
GSI	0.39 ± 0.20	2.65 ± 2.43	0.25 ± 0.13	2.41 ± 2.22
LSI	0.59 ± 0.20	0.56 ± 0.18	0.45 ± 0.16	0.51 ± 0.17

Table 4.6: Three-way ANOVA results evaluating the influence of season, location, and sex on various fish health indices (e.g., K, GSI, LSI) of bonefish. All significant values are designated with an asterisk.

	K		GSI		LSI	
Term	F	p	F	p	F	p
Season	25.118	<0.0001*	35.569	<0.0001*	0.925	0.338
Location	3.667	0.028*	6.074	0.003*	6.605	0.002*
Sex	0.439	0.508	0.881	0.349	6.718	0.010*
Season*Location	2.681	0.072	6.554	0.002*	9.650	0.0001*
Season*Sex	0.006	0.938	0.271	0.603	1.146	0.286
Location*Sex	0.824	0.441	0.983	0.377	0.113	0.893
Season*Location*Sex	0.753	0.473	1.212	0.300	0.599	0.551

Figures

Figure 4.1: Study area in south Eleuthera, The Bahamas showing the locations of the tidal creeks (Kemps, Broad, Starved) and tidal embayments (Half Sound), as well as the Cape Eleuthera Institute (CEI). The inset map displays the entire island of Eleuthera with the study area highlighted.

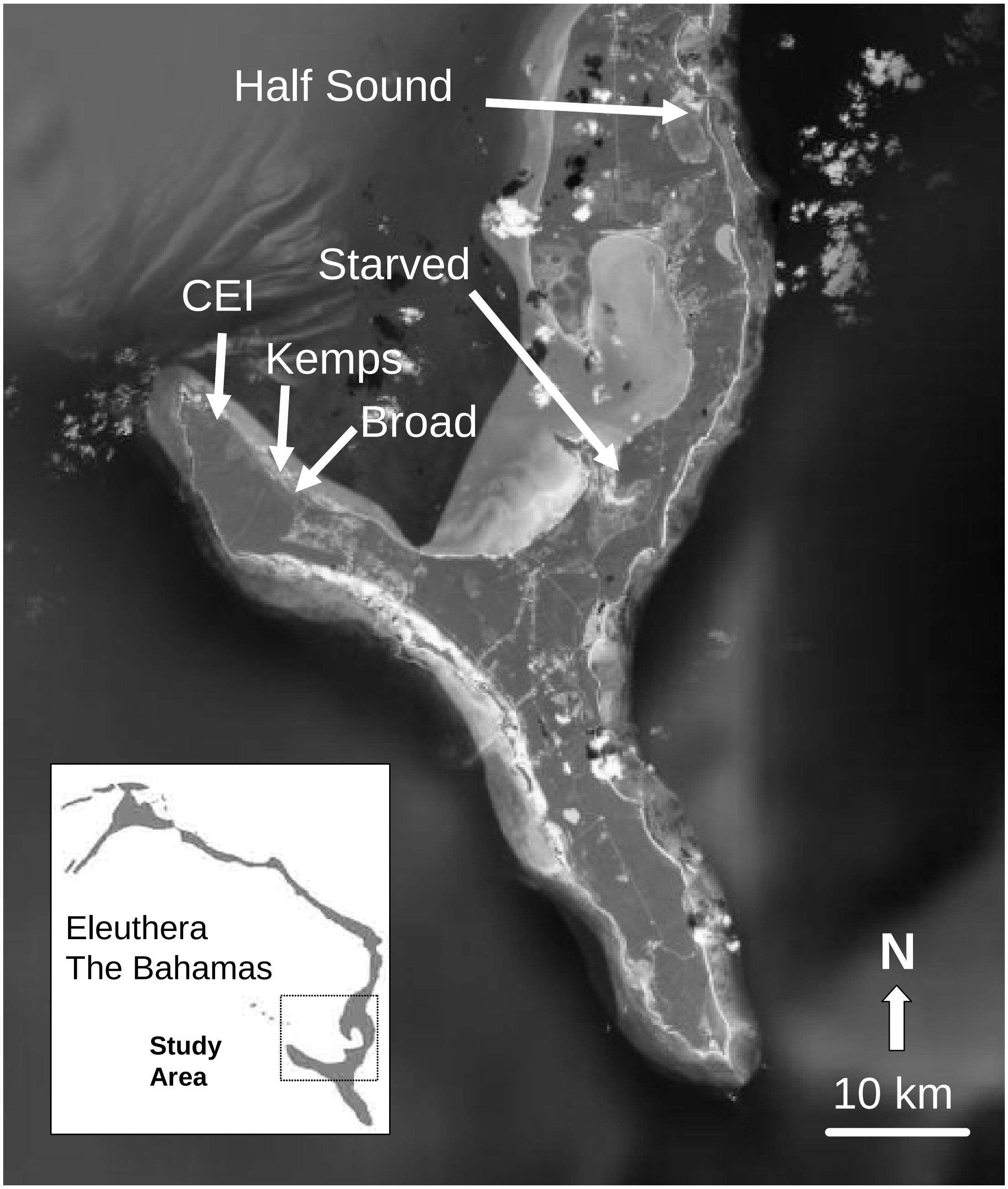


Figure 4.2: Relationship between % water and % lipid by wet mass for (a) whole body, (b) gonad, and (c) liver samples for bonefish collected from Eleuthera, The Bahamas. The equation of the line for whole body is % lipids = 25.2 – 0.308 * % water (r^2 = 0.5, F = 169.12, $p<0.0001$). The equation of the line for gonad is % lipids = 78.7 – 1.03 * % water (r^2 = 0.9, F = 1591.47, $p<0.0001$). The equation of the line for liver is % lipids = 51.9 – 0.633 * % water (r^2 = 0.4, F = 128.23, <0.0001).

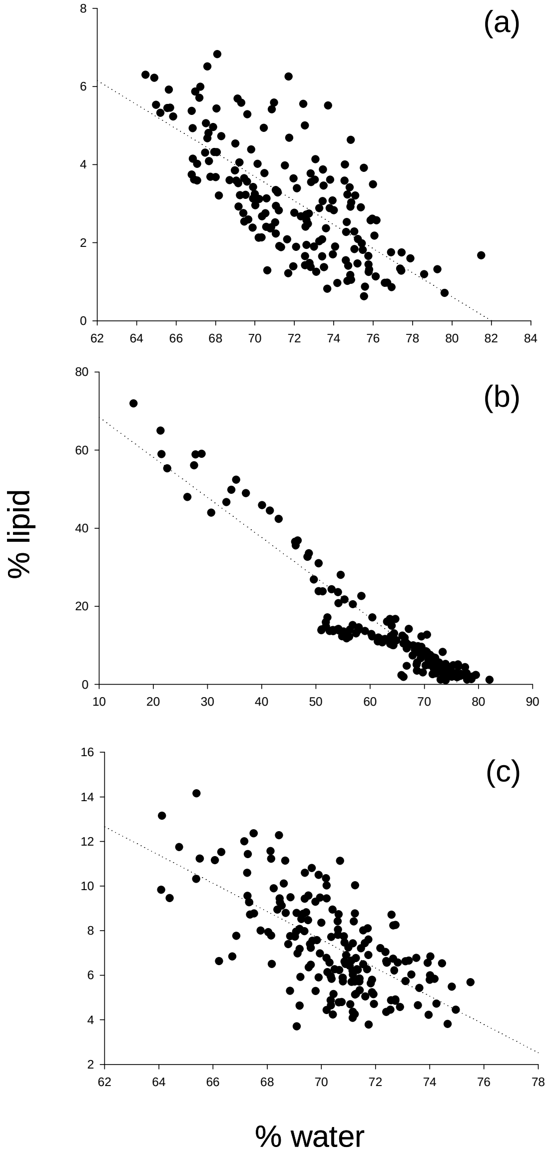


Figure 4.3: Mean body constituent analyses ((a) % water, (b) % lipid, (c) % ash, (d) % protein) of whole body samples of bonefish collected from Broad/Kemps Creek, Half Sound, and Starved Creek in the summer (closed circle) and winter (open circle) seasons. Error bars represent standard error. Levels not connected by the same letter are significantly different (p<0.05). Note letters in bold are for the summer season samples.

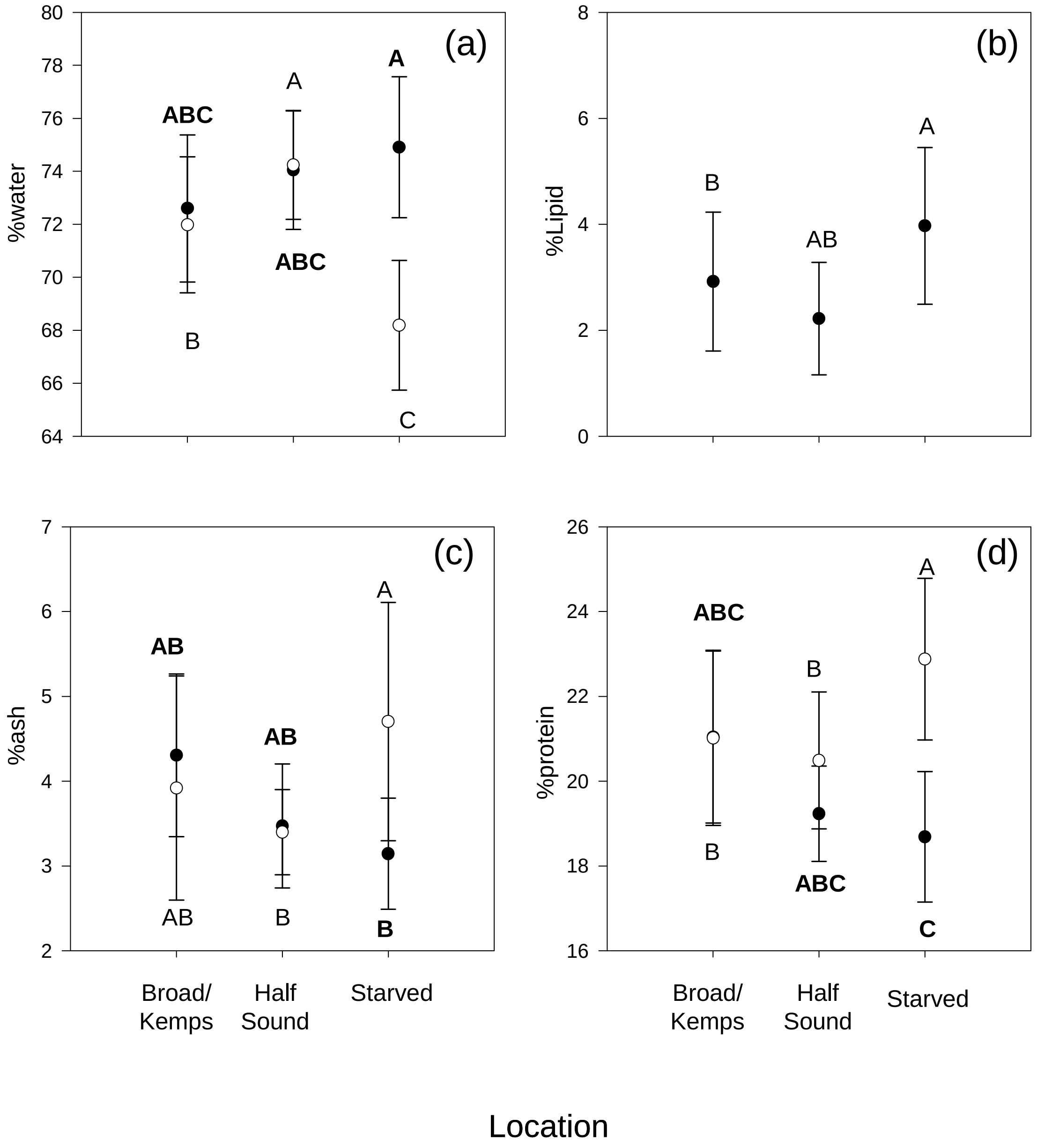


Figure 4.4: Relationship between whole body % lipid or energy density (MJ kg^{-1}) and total length (mm) for bonefish sampled in the summer (panels a and c, respectively) and the winter (panels b and d, respectively) in Eleuthera, The Bahamas. The equation of the line for the summer is WB % lipids = -0.581 + 0.007 * TL ($r^2 = 0.1$, F = 4.185, p = 0.048). The equation of the line for the winter is WB % lipids = 11.9 - 0.020 * TL (r^2 = 0.3, F = 48.533, p<0.0001). The equation of the line for the winter is WB energy density = 9.01 - 0.008 * TL (r^2 = 0.2, F = 24.907, p<0.0001).

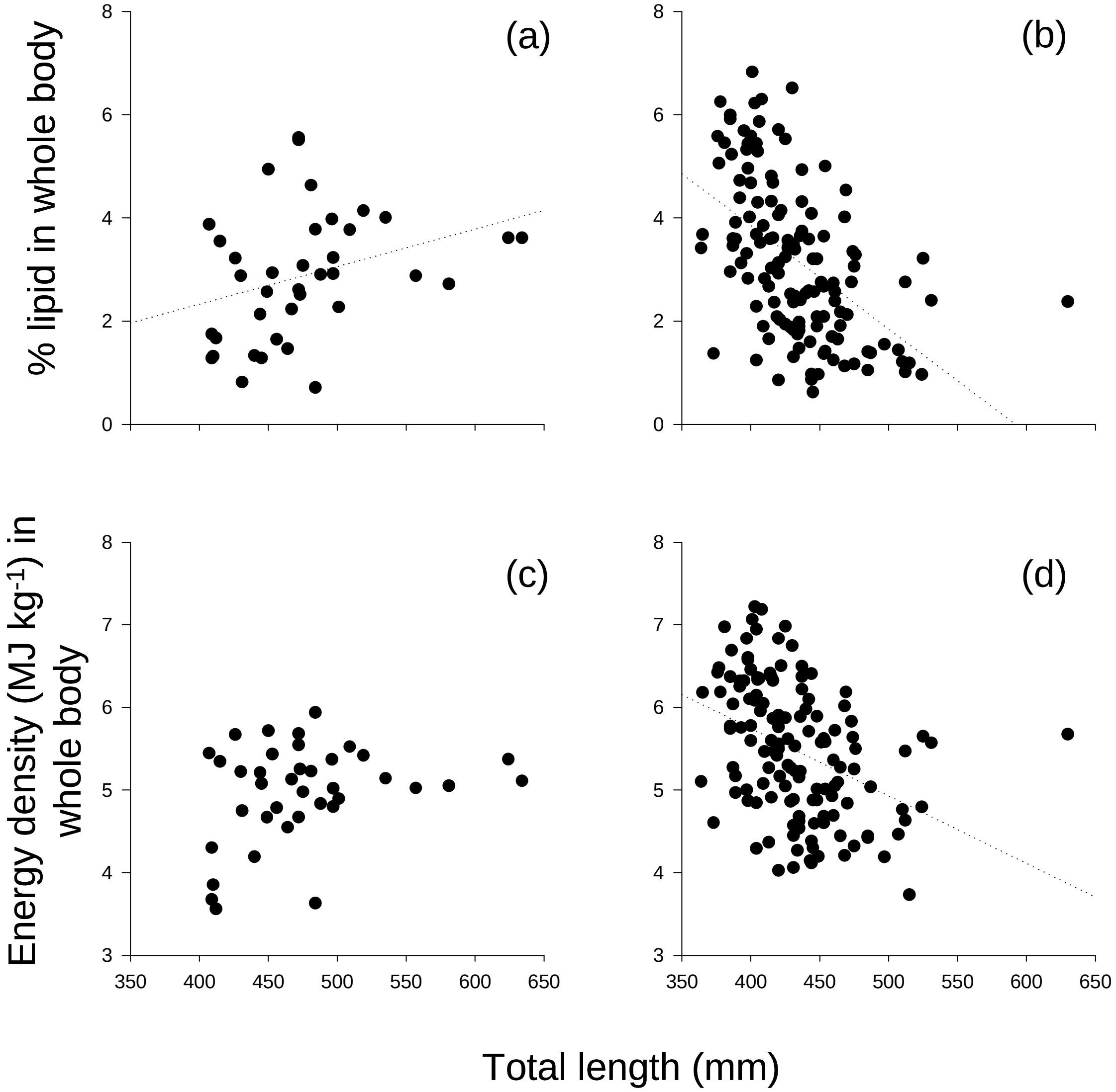


Figure 4.5: Relationship between gonad % water or % lipid by wet mass and total length (mm) for bonefish sampled in the summer (panels a and c, respectively) and the winter (panels b and d, respectively), in Eleuthera, The Bahamas.

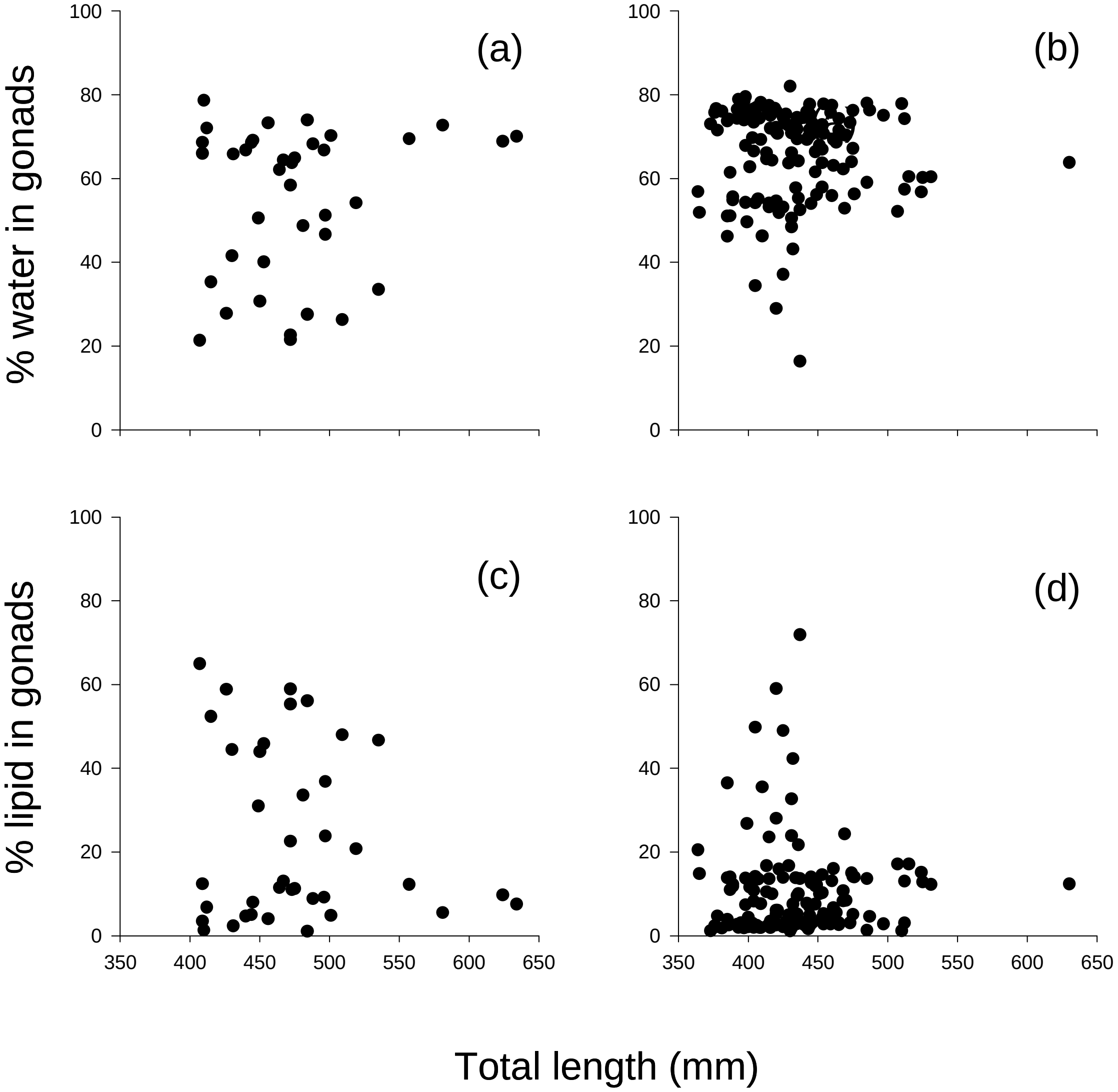


Figure 4.6: Mean fish health indices of bonefish sampled from Broad/Kemps Creek, Half Sound, and Starved Creek. Error bars represent standard error. Levels not connected by the same letter are significantly different ($p<0.05$). Panel (a) is condition factor (K), (b) is gonadosomatic index (GSI), (c) is GSI divided by season, (d) is liver somatic index (LSI), and (e) is LSI divided by season Note that the summer season is represented by closed circles, whereas winter is represented by an open circle, and that letters in bold are for the summer season samples.

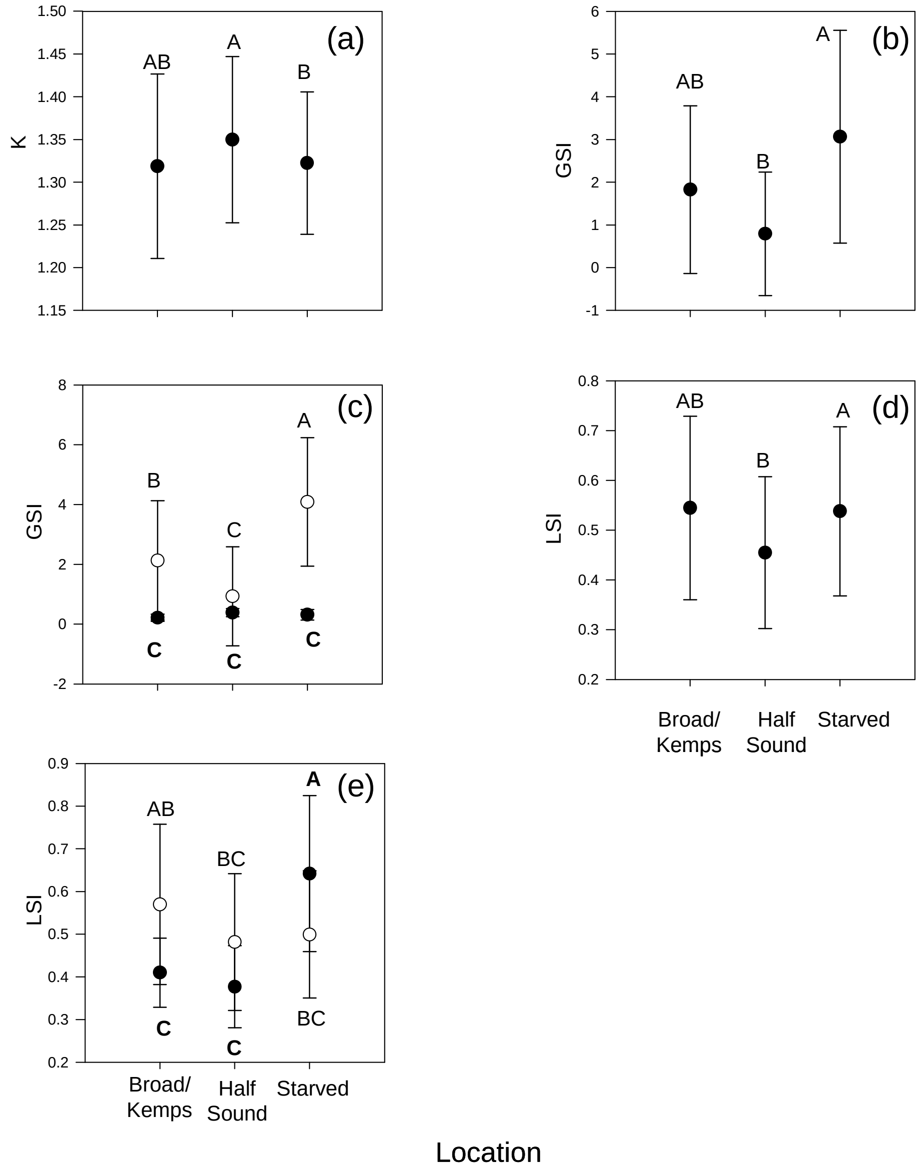
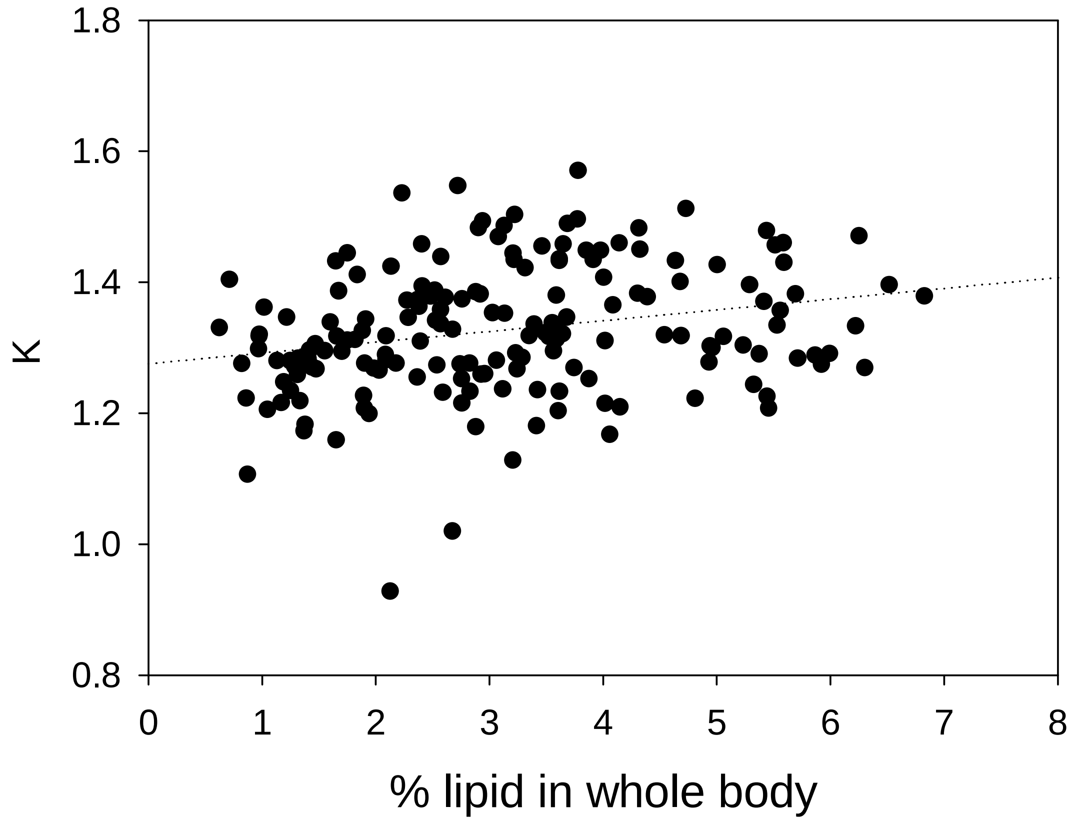


Figure 4.7: Relationship between condition factor and % lipid of whole body bonefish, by wet mass. The equation of the line is K = 1.28 + 0.016 * WB % lipids ($r^2 = 0.06$, F = 11.150, p = 0.001).



Chapter 5: Bonefish (*Albula vulpes*) movement patterns in tidal creeks and coastal waters of Eleuthera, The Bahamas

Abstract

Bonefish (*Albula* spp.) are a group of fishes that inhabit tropical and subtropical marine waters worldwide. Large gaps in our understanding of the biology of these fishes exist despite their economic importance as a sport fish and their potential role in the ecological functioning of coastal systems. Using a passive acoustic telemetry array, we monitored the movement patterns of *A. vulpes* along the north coast of Cape Eleuthera, The Bahamas. Because we were successful in detecting a number of individuals for periods exceeding six months, we observed several previously unknown behaviours of bonefish. Data indicate that bonefish undergo periods of site fidelity where they repeatedly visit the same tidal creeks, interspersed with transient periods in which they utilize large areas of shallow coastal habitats. Relative activity space, as measured by minimum linear dispersal, median distance travelled, and mean number of receivers visited daily, did not vary with fish size or sex. Although school-fidelity exists in bonefish, it may be influenced by school size, the duration in which an individual has been in the school, and the frequency with which schools mix. Although seasonal movements toward the seaward portion of the array likely correspond with spawning, confirmation will require further investigation. In general, the movement patterns of bonefish we observed demonstrate the importance of this species in the connectivity between nearshore and offshore habitats.

Introduction

With over 60% of the world's population living in a coastal band 50 km wide, and half of these residents in developing countries (Barnabé and Barnabé-Quet, 2000), degradation of coastal environments is widespread. Mangrove forests, which once covered more than 200 000 km^2 of coastline in tropical and subtropical latitudes (Duke et al., 2007), have been heavily exploited by humans for aquaculture, timber, industry, tourism and other coastal developments (Alongi, 2002; Blaber, 2007). Destruction of mangroves results not only in the loss of ecosystem services provided by such habitats, but also indirect losses of services provided by the habitats connected to mangroves (Boaden and Seed, 1985; Kathiresan and Bingham, 2001). Over the decades, public and political recognition of this major transformation in the coastal zone has been scant (Valiela et al., 2001). It was not until after the 2004 Indian Ocean tsunami that the importance of mangroves for coastal protection received attention, as the greatest loss of human life occurred in areas where there was no vegetation to absorb wave energy (Williams, 2005). With the increased perception of the value of these natural resources (Williams, 2005) and the current momentum for ecosystem-based management plans (Hofmann and Gaines, 2008), there is a critical need for life history data on the organisms occupying nearshore coastal areas.

Flats are shallow (< 4m) tropical and subtropical coastal marine environments that represent a transition zone between the land and sea. This ecotone consists of a number of habitat types including mangrove creeks, seagrass beds, algal and sand plains, as well as patch reefs. Traditionally, habitats comprising the flats environment have been studied as independent entities within the context of a larger coastal ecosystem. For example, mangrove forests and seagrass meadows have received much individual attention from scientists because of their high productivity, ability to stabilize sediment, and provision of key habitats for fish and invertebrate taxa (e.g. Heck et al., 2003; Bujang et al., 2006; Blaber, 2007). However, because of the regular submersion and exposure of mangrove habitats associated with semidiurnal tides in most tropical and subtropical areas, few fish can use these areas exclusively. Instead, fish must move in and out of these areas, using alternative habitats such as seagrass beds when mangroves are unavailable at low tides (Sheaves, 2005). As such, the habitat mosaic comprising flats systems are inherently interconnected not only through physicochemical processes associated with tidal cycles, but also through the biota that move between them (Moberg and Folke, 1999; Semeniuk, 2005; Mumby, 2006).

Because the movement of organisms promotes energy flow across habitat boundaries (Depczynski et al., 2007; Gaines et al., 2007), knowledge of the spatial distribution of animals is fundamental to the basic understanding of ecological functioning within an ecosystem. Although locating sufficient food to meet the energy requirements for daily life plays a critical role in the distributional limits of a species (Diana, 2004), other biotic (e.g., predators) and abiotic factors (e.g., temperature) are also important (Reise, 1985). To understand and/or predict the distribution of organisms among various available habitats, it is necessary to track individuals moving about freely in their natural environment (Claireaux et al., 1995). Empirical studies of the unrestrained movement of animals are facilitated through the use of biotelemetry, where physiological function under natural conditions can be investigated (Goldstein and Pinshow, 2002; Cooke et al., 2004).

Bonefish (*Albula* spp.) are a group of fishes that not only demonstrate the interconnectedness of the habitats found in tropical tidal flats and tidal creeks, but also the connectivity of the flats with other marine ecosystems. Throughout much of their distribution in subtropical and tropical area, it is thought that they move into shallow flats to feed on invertebrates and small fish during high tide, and then move into deeper water presumably to digest and defecate at low tide (Colton and Alevizon, 1983a,b; Humston et al., 2005). Bonefish also carry the distinction of being a popular sport fish and thus play an important role in many local economies (Pfeiler et al., 2000; Ault, 2008; Danylchuk et al., 2008). To date, there have been three published studies using biotelemetry to study the movement patterns of *A. vulpes*; two studies in Florida (Humston et al., 2005; Larkin et al., 2008), and one study in The Bahamas (Colton and Alevizon, 1983b). There is also an additional study on a congeneric species (*A. glossodonta*) in an area of the central Pacific approximately 1600 km south of Hawaii (Friedlander et al., 2008). Unfortunately, because most of these studies were hampered by low samples sizes of fish studied ($n \leq 3$) or by a limited time (mean = 5.3 days) that fish were at large, they were unable to conduct rigorous analyses of the data that would allow them to evaluate temporal trends.

The objective of this study was to document the spatial ecology of bonefish (*A. vulpes*) in tidal flats and tidal creek areas near Cape Eleuthera, Eleuthera, The Bahamas. Using acoustic telemetry we investigate patterns of habitat use, relative activity space of individuals, schooling behaviour, and the influence of tidal cycles. Furthermore, we evaluate the diel and seasonal movement patterns of bonefish to reveal any temporal trends in their use of habitat. Collectively this work will serve to fill a critical knowledge gap in bonefish ecology, provide a basis for understanding how energy moves through flats ecosystems, and will be useful for developing ecosystem-based management plans.

Materials and methods

Study site

This study was conducted along a 23 km section of the north coast of Cape Eleuthera, Eleuthera, The Bahamas (N 24° 50' 05" and W 76° 20' 32") (Fig. 5.1). A number of tidal flats and four distinct tidal creeks (Page Creek, Kemps Creek, Broad Creek, and Starved Creek) are located along this stretch of coastline. The creeks contain a mosaic of habitats including mangroves, seagrass, sand, algal plains, and patch reefs, with sharp calcium carbonate outcroppings throughout (Danylchuk et al., 2007a). Semi-diurnal tides occurred with a maximum daily range of 0.8 m.

Fish capture, transmitter implantation, and passive monitoring

Preliminary genetic analyses on bonefish from the study area indicated that all specimens were *A. vulpes* (Danylchuk et al., 2007a). Between November 1, 2005 and March 16, 2007, 47 bonefish (495 ± 45 mm total length; mean ± SD) were implanted with acoustic transmitters (model V13 coded tags, 13 mm diameter, 36 mm long, 6 g, 700 day battery life (Vemco Inc., Shad Bay, NS.). To capture bonefish, various sized seine nets (0.6 cm mesh, 46 m long; 1.3 cm mesh, 46 m long; 3.2 cm mesh, 76 m long; 7.0 cm mesh, 61 m long) were deployed at tidal creek mouths to intercept bonefish on an outgoing tide. When a school of bonefish approached, the net was moved quickly to encircle the fish. Captured bonefish were dip-netted or passed by hand into flow-through holding pens (1.3 m x 0.8 m x 1.25 m, 3.1 cm extruded plastic mesh) submerged in a minimum of 0.6 m of water where they were held until surgery. Bonefish were anesthetized with MS-222 prior to surgery (approx. 100 ppm) and then placed on a surgery table where the gills were supplied with a maintenance dose of MS-222 (approx. 50 ppm) in recirculating seawater. To implant the transmitter, a small (2-3 cm) incision was made to one side of the ventral midline, posterior to the pectoral fins. After being disinfected with an iodine solution, the transmitter was inserted and gently guided into the coelomic cavity, toward the pectoral fins. The incision was closed with 3-4 simple interrupted sutures using monofilament absorbable suture material (Ethicon 3-0 PDS II, Johnson and Johnson, New Jersey). The length of the fish (mm) was measured, and when possible, the sex was determined via internal examination. The entire procedure generally took less than five minutes. Bonefish were held for up to one hour in the flow-through net pens to recover following anesthetization. Transmitter-implanted fish were released simultaneously with a group of untagged bonefish from the same school from which they were captured.

To track the movements of transmitter-implanted bonefish, a series of 27 hydrophone receivers (VR2 and VR2W models, Vemco Inc., Shad Bay, NS) were placed strategically throughout the study area (Fig. 5.1). Individual receivers were anchored to a short piece of rebar cemented into a concrete block. Receivers in water greater than 1 m deep at low tide and in open water were positioned vertically in the water column. In water that was shallower than 1 m deep at low tide and at narrow choke points, receivers were deployed horizontally or 5-10 degrees above horizontal, with the hydrophone orientated to maximize coverage. Range tests were performed upon the initial deployment of the receivers, revealing that receivers deployed in water greater than 1 m deep had a coverage radius of 250 m, whereas receivers in shallow water or positioned horizontally had a coverage radius as small as 30 m due to shoreline confinement. Wind and wave conditions as well as tidal cycles influence the detection range of individual receivers (Heupel et al. 2006). Although the range of coverage for receivers in shallow water or positioned horizontally was considerably less, they did provide the necessary coverage to monitor choke points (i.e., creek mouths) and as such we did not correct for receiver range in our data analysis. Even when the tides were at slack low, all of the receivers were covered by at least 20 cm of water and could still receive signals from tagged fish. Receivers were deployed between November 2005 and May 2008 (Table 5.1) and were visited regularly to download data and to clean the hydrophone of accumulated plant material. Data used in this study were collected from November 1, 2005 to February 18, 2008 (i.e., the date of the last download). All procedures used in this study were in accordance with the policies of the Canadian Council on Animal Care as administered by the Carleton University Animal Care Committee (Protocol B07-03, B07-05, and B07-06).

Defining ‘site’ and other spatial extents

For the purpose of this study, a ‘site’ is defined as the individual hydrophone receiver location. To elucidate trends in movement patterns at larger spatial scales, strategic groupings of receiver locations were selected within the study site. Receiver locations were grouped into seven ‘areas’ along the shoreline that were associated with one of the tidal creeks, points, or large open flats (Powell Point, Page Creek, Kemps Creek, Broad Creek, Red Point, Poison Flats and Starved Creek) (Table 5.1). Receiver locations were also grouped into three habitat zones (i.e., within-creek, nearshore, and offshore). ‘Within-creek’ receivers were located in creek mouths or the backwaters of creeks; ‘near-shore’ receivers were located 200 m or less from shore but were outside of the creeks; and, ‘offshore’ receivers were located more than 200 m from shore (Table 5.1).

Data analysis

For each receiver, the total number of detection records was tallied from the date of deployment to the last date downloaded (February 18, 2008). To account for the fact that not all receivers were deployed for an equal length of time, the total number of detections was divided by the number of days the receiver was deployed. The total number of detections per days deployed was compared for receivers in each of the three habitat zones (i.e., within-creek, nearshore, and offshore) using a repeated measures analysis of variance (ANOVA). Detection records were further sorted by transmitter identity, date, and time so that individual habitat preferences and movement patterns could be elucidated. The relative activity space of bonefish was examined by comparing the sites most frequented to the location of tagging, as well as by calculating the median distance traveled and minimum linear dispersal by each individual. The median distance traveled was determined by calculating the distance between the receiver with the highest percentage of detections and all other receivers visited, and then taking the median of those distances. The minimum linear dispersal is defined as the straight line distance between the two most distant receivers which detected the individual (see Chapman et al., 2005). Distance measurements were made using Mapsource version 6.13.7 (Garmin). In addition, the minimum, maximum, and mean number of receivers that individual bonefish visited daily was calculated for the study period. The relationship between bonefish size and median distance traveled, minimum linear dispersal, and the mean number of receivers visited daily was examined using linear regression analysis. Plots of individual fish movement over time were examined to determine if there were repeatable patterns in movements on a daily to seasonal basis. Comparisons of plots over time allowed an assessment of the propensity for fish to be detected with each other as well as the duration of their synchronous movements, which is relevant to schooling behavior. Swimming speed was estimated by dividing the distance between two receivers detecting a bonefish by the elapsed time between detections. All statistical analyses on collected and derived data were completed using JMP 7.0.2 (SAS Institute, Cary, NC). Maximal type-1 error rates were set at $\alpha=0.05$.

Results

Tracking characteristics

Forty-seven bonefish (7 males, 8 females, 32 of unknown sex) were captured from either Broad Creek or Kemps Creek and were surgically implanted with acoustic transmitters (Table 5.2). Two of the bonefish (#101 and #4073) were not ever detected after release, and 12 other bonefish were not detected beyond one week after release (Table 5.2). In contrast, 15 bonefish were at large for periods of six months or more

(Table 5.2). The maximum number of days over which an individual was detected within the hydrophone array was 611 days for bonefish #4079, resulting in a total of 63 533 detections (Table 5.2). In general, 27 bonefish were detected more than 75% of the days they were at large (Table 5.2).

Habitat use and site fidelity

The receiver with the highest number of detections per days deployed was R04 which is located nearshore, east of Powell Point in a channel near the back of the Cape Eleuthera Marina (Fig. 5.2). Receivers R10 and R11 had the second and third highest number of detections per days deployed, and they were located outside of the mouth and in the mouth of Kemps Creek, respectively (Fig. 5.2). The entire 23 km study area was utilized by the tagged bonefish as all receivers detected the presence of one or more tagged individuals during the course of the study (Fig. 5.2). There was no significant difference ($F=2.85$, $p>0.05$; repeated measures ANOVA) in the number of detections per days receivers were deployed for bonefish within the three habitat zones (i.e., within-creek (0.31 ± 0.15), nearshore (0.66 ± 0.14), and offshore (0.19 ± 1.15); mean ± SE).

Although no bonefish utilized the entire study area, five bonefish (#108, #928, #2377, #2382, and #2383) were detected at 20 or more receivers (Table 5.3). The mean number of receivers used by bonefish during the study period was 11. The total number of tidal creeks that bonefish utilized varied from zero to three. A consistent trend noted was that when bonefish were detected in more than one creek, they always used creeks adjacent to each other (Table 5.4). Broad Creek was used by 34 bonefish, Kemps Creek by 23 bonefish, and Page Creek and Starved Creek by seven and three individuals, respectively (Table 5.4). A relatively high degree of site fidelity (with most of the detections located at a receiver within 1500 m of the tagging location) was displayed by 62% of detected bonefish in this study (Table 5.4). Trends in site fidelity were further examined by calculating the median distance traveled and minimum linear dispersal for each individual. Fish that were only detected at one receiver were not included in the analysis. The range in median distance traveled was 118 to 10 691 m (Table 5.4). There was no significant relationship between the median distance traveled and the total length of the individual ($r^2=0.005$, $p>0.05$). Minimum linear dispersal ranged from 235 to 15 498 m (Table 5.4), and was not significantly related to bonefish body size ($r^2=0.001$, $p>0.05$). Measures of daily activity level and site fidelity were also examined by calculating the minimum, maximum, and mean number of receivers that individual bonefish were detected at on a daily basis. The minimum number of receivers ranged from one to five; the maximum ranged from one to fifteen; and the overall mean number of receivers visited daily by bonefish was three (± 1.5; SD) (Table 5.4). There was no significant relationship between the mean number of receivers visited daily and bonefish body size ($r^2=0.067$, $p>0.05$).

School fidelity

Four mass tagging efforts were conducted during the study period; two in August 2006 and two in February/March 2007. Because fish were captured in a school leaving either Broad Creek or Kemps Creek on an outgoing tide, and then released as a group (including untagged conspecifics from the same school), it was possible to investigate the cohesion of the school through time. Within hours of being released from Kemps Creek on February 20, 2007, tagged individuals broke into two sub-schools with half of the school heading east towards Poison Flats (R24, R25, and R26) or Starved Creek (R27), or west to R04 near Powell Point (Fig. 5.3). Over a few days most of the individuals were detected mainly within those areas, but then two bonefish, one from each sub-school headed back toward the release site and each other. Bonefish #936 was detected later joining individuals that were located at Poison Flats, and bonefish #2376 was last detected in transit as it headed west towards Powell Point. Although the duration of detection post-tagging ranged for individuals tagged on February 20, 2007, one individual from each sub-school (i.e., bonefish #2381 from the 'west' sub-school and #2383 from the 'east' sub-school) was at large for over 280 days while being detected for greater than 90% of those days (Table 5.2). Bonefish #2381 and #2383 continued to remain in separate sub-schools utilizing different habitats within the study area for a majority of the time they were detected, but occasionally their paths crossed at R04 or at a receiver in the Kemps Creek and Broad Creek areas (Fig. 5.4).

When bonefish remain with other individuals from the school in which they were captured, movements tend to be relatively synchronized over time (Fig. 5.5). Bonefish #108 and #109 display repeatable and synchronous patterns of movement throughout the hydrophone array during the two months they were detected together. Between August 28, 2006 and October 29, 2006 bonefish #108 and #109 moved together from a receiver offshore of Page Creek (R07) to ones inside and outside of Kemps Creek and Broad Creek, and then to receivers as far east as Red Point (Fig. 5.5).

Inter- and intra-individual variability in movement patterns and seasonal trends

Repeatable patterns of movement were apparent in all tagged individuals, but the duration of these repeatable patterns varied, as did the amount of relative activity space that individuals occupied. Although inter- and intra-individual variability was demonstrated for fish #2381 and #2383 above (Fig. 5.4), plots of four bonefish tagged on March 16, 2007 can also be examined to assess their movement patterns over time (Fig. 5.6). Bonefish #934 exhibited periods of time when the majority of detections were focused around Poison Flats (i.e., April-May 2007), whereas at other times movements were more widely distributed throughout the array (i.e., Sept 2007; February 2008) (Fig. 5.6a). While bonefish #934 was detected at Poison Flats, bonefish #937 and bonefish #2377 also had overlapping detections in the same location (R26) (Fig. 5.6a, b, d). Although R10, which is outside of the mouth of Kemps Creek, received the largest number of detections for both #937 and #2377, both individuals exhibited varying patterns of movements through the rest of the study area (Fig. 5.6b, d). Bonefish #2375 exhibited a relatively consistent activity pattern between the time of tagging and mid-August 2007, at which point movements involved more detections outside of Kemps Creek followed by a visit off of Broad Creek, and a disappearance from the array from September 11 to November 28, 2007 (Fig. 5.6c). Further detections of bonefish #2375 were at receivers between the Powell Point to Broad Creek areas.

Because bonefish #4079 was detected for all of 2007, plots of its movement patterns were also examined (Fig. 5.7). Similar observations of repeatable movement patterns for distinct time periods are observed, as well as changes in the amount of relative activity space that the fish is using (Fig. 5.7). Bonefish #4079 uses the Broad Creek area most heavily between mid-June to the end of August, whereas the rest of the year detections are rare or non-existent. Although this is typically when water temperatures peak compared to the rest of the year, this trend is not apparent in other tagged individuals. However, when examining the data for any other 'seasonal' type trends between the other tagged individuals for which there is a large data set for (i.e., #934, #937, #2375, #2377, #2381, and #2383), the only consistent observation is that bonefish are only detected at R01, the most westerly receiver in the array, between early November and late January (see Figs. 5.4, 5.6, 5.7).

Tidal influence and swimming activity

Bonefish only occupied the backwater portions of tidal creeks when the tide was high and generally retreated to deeper coastal waters on a falling tide (Fig. 5.8). This trend is apparent across daily and seasonal time scales. Bonefish, however, were also found in other nearshore areas during high tides, such as the channel behind the Cape Eleuthera Marina (R04) (see bonefish #2381, Fig. 5.8).

Swimming speeds were estimated for bonefish #2381 during its detection in the tidal creeks (between R19 & R13, and R12 & R11) versus when it was travelling from offshore of Page or Kemps Creek towards R04. On average, bonefish #2381 swam at approximately 0.08 m s^{-1} while in the backwaters of the creeks compared to 0.37 m s^{-1} when making westerly movements to R04. In contrast speeds of up to 2.33 m s^{-1} were estimated when this individual travelled between offshore of Kemps (R09) towards Broad Creek (R14) on February 9, 2008.

Discussion

This study provides the longest continuous monitoring of bonefish movement patterns through the use of a passive acoustic telemetry array, with a total of 327 719 detections to base inferences on. Previous research by Larkin et al. (2008) successfully monitored three bonefish between 90 to 96 days, whereas our study detected one bonefish for a total of 611 days, and eight other individuals for a period between 237 to 353 days. The benefit to the extended monitoring period is more seasonal trends and individual variations in movement patterns can be evaluated. Evidence of site fidelity and transient behaviour were observed in our transmitter-implanted bonefish both for individual fish as well as groups of fish. The range in minimum linear dispersal recorded for bonefish in this study was from 235 m (bonefish #4075) to 15.5 km (bonefish #928) (Table 5.4). For all individual bonefish which were tracked over six months, repeatable diel movements were observed (i.e., site fidelity), as well as forays to more distant receivers and disappearance from the array (i.e., transient behaviour) (see Figs. 5.4, 5.6, 5.7). Our results are contradictory to all three other studies on *A. vulpes* movements which suggest bonefish are either transient or site attached. The lack of observed mixed behaviours of bonefish in these studies, however, is likely an artifact due to the duration of individual monitoring, and/or the tracking methodology. For example, Colton and Alevizon (1983b) concluded that bonefish were highly transient in nature after having limited success with manually tracking 13 transmitter-implanted bonefish by boat or with recapturing any conventionally tagged bonefish in a mark-recapture program. Out of 13 fish, only three were located after 24 hours, with two being monitored for a total of 16 and 30 hours, respectively, and one fish being tracked for a total of 32 hours over a period of 100 days. Humston et al. (2005) tagged 11 bonefish and monitored their movements via an automated hydrophone array. Unfortunately only eight fish were detected post release. Of the eight fish, two of the fish were suspected to be dead or to have shed their transmitters based on highly localized or no movements. Another four individuals left the study area after only one to four days, suggesting transient behaviour. The remaining two fish were located within the array for a total of 40 and 61 days, respectively, suggesting site fidelity (Humston et al., 2005). Larkin et al. (2008), who also incorporated analyzed data from a nine year mark recapture program, found substantial variability between individual movement patterns with some fish moving >100 km (i.e., transient), versus others that moved ≤ 2 km from where they were tagged (i.e., strong site attachment). Unfortunately the authors provide limited information on individual movement patterns of acoustically tagged fish as they were picked up in the array, but the majority of fish (78%) were detected for less than 30 days, limiting the opportunity to examine movement patterns over a greater temporal scale.

Intra-specific variation in movement patterns has been documented for many marine animals (see Quinn and Brodeur, 1991), and shifts between site fidelity and transient behaviour may occur for reasons related to the establishment of home ranges of sufficient size to meet life requirements (see Morrissey and Gruber, 1993a; Carfagno and Weatherhead, 2008). The relatively short duration of effective monitoring in previous studies may also reflect high levels of post-release predation. In this study we also noted that a proportion of fish were only tracked for a very short duration (hours to a week). Although it is not possible to know with certainty the fate of those animals, research has revealed that exercise and handling stress associated with catch-and-release fishing can induce post-release mortality in bonefish by lemon sharks (*Negaprion brevirostris*) and barracuda (*Sphyraena barracuda*) (Cooke and Philipp, 2004; Danylchuk et al., 2007a; 2007b). Despite our attempts to provide extended recovery times, to optimize surgical procedures, and to minimize stress, it was still likely that some post-release predation occurred. We also noted thermal-specific trends in the post-release fate of individuals, with a lower percentage of the fish tagged at warmer temperatures being tracked for longer periods compared to fish tagged at cooler temperatures (Murchie, unpublished data). This observation lends further support to the conclusion that post-release mortality, more than migration from the study site, accounts for the loss of animals.

In many animals, including fish, body size (Kramer and Chapman, 1999) and/or sex (Paukert et al., 2004) can influence home range. In this study, there was no relationship between the size of bonefish and the size of its relative activity space as determined by the median distance travelled, the minimum linear dispersal, or the mean number of receivers visited daily. We did, however, only use a relatively small size range of bonefish for tagging. Although the sizes of fish tagged are representative of those typically captured in south Eleuthera (see Murchie et al., 2009), we cannot exclude the possibility that smaller or larger fish may display different behaviours. Regardless of body size, however, it is likely that movement patterns would still be dependent on feeding habits. Diet studies on *A. vulpes* show similar dietary composition for bonefish across a range of sizes, but more large prey items are consumed more frequently as gape size increases (Crabtree et al., 1998a). Typically stomach contents are more a reflection of the type of habitat in which the fish had been feeding rather than the size of the fish itself (Colton and Alevizon, 1983a). Similarly, no obvious trends in sex-related movement patterns emerged from our data, despite the fact that most of the long-term monitoring data came from individuals for which sex was known. There is no evidence to date to suggest that movement patterns should be different between male and female bonefish. Although a study of *A. vulpes* in Florida found significantly different growth models between males and females, the differences were small and individual growth parameters were not significantly different (Crabtree et al., 1998a).

Schooling behaviour is common among fishes (Klimley and Holloway, 1999) and the benefits of such behaviour (e.g., predator avoidance, foraging) have been well documented (see Partridge, 1982; Krause, 1993). Anecdotally (based on angling lore and our observations), bonefish are typically found in schools, but to date no one has investigated school fidelity in this group of fishes. Results from our study suggest that the structure of schools of bonefish is dynamic. The duration in which individuals remain with one school before joining another varies, and observations are limited by the duration with which the tagged fish remain at large within the hydrophone array. In our study, individuals captured in a school, tagged, and released simultaneously either stayed together with school-mates for periods of hours to days to months, or had movement patterns that were typically distinct from other fish with temporary interludes of overlap. One possible explanation for the varying degrees of school fidelity observed may be that it is too challenging for fish in large schools to stay together because they cannot possibly recognize 200 or more individuals (Griffiths and Magurran, 1997). Thus, if schools overlap frequently there are many opportunities for individuals to switch groups (Ward et al., 2002). The amount of time that individuals are together in a school, and thus have time to become familiar with each other, will also influence schooling preferences

(Griffiths and Magurran, 1997). Sharp (1978) found that tuna (*Thunnus* spp.) school-mates are often related and are of similar size, implying a common birth date and location. Similar tests could be conducted for bonefish via genetic analysis of fin clips taken during implantation of acoustic transmitters in future studies.

The association of movement patterns of bonefish and tidal cycle were noted by both Colton and Alevizon (1983b) and Humston et al. (2005). Colton and Alevizon (1983b) observed bonefish accessing shallow waters of tidal flats during high tide, and moving to deeper water on an ebbing tide. Humston et al. (2005) found that bonefish stage in an area prior to high tide, and then disappear from detection following peak high tides, likely into shallow interior areas of tidal flats where no receivers were located. Our data indicate similar movement patterns with the tides; particularly that bonefish only enter tidal creek areas during periods of rising/high tide and leave as tides fall. This observation is not surprising because during low tide, water depths within the backwaters of tidal creeks are too shallow for bonefish to occupy. During periods of high tides, however, bonefish are not found exclusively in tidal creeks or flats but can still be found in waters that are accessible through the entire tidal cycle such as R04 (3 m deep).

The use and/or avoidance of deep water channels by bonefish has been debated, with some evidence that these habitats are used as routes between foraging areas and/or areas of thermal refuge (see Humston et al., 2005). Receiver R04 received the overall greatest number of detections per days deployed during this study, suggesting that these deeper water channels areas are of importance to bonefish. While the risk of exposure to predators at R04 compared to within-creek habitats has not been quantified, bonefish could encounter predators in both areas. Juvenile lemon sharks have been shown to exhibit a high degree of site fidelity within tidal creek locations along the north coast of Cape Eleuthera (Murchie, unpublished data), and anecdotal observations of both sharks and dolphins have been made at R04. Visual surveys via snorkeling have documented bonefish feeding in this area (Murchie, unpublished data). Use of the area for both feeding and migration are plausible and a more detailed examination of the habitat characteristics in the future is worthwhile.

The only discernable pattern in season trends that emerged from the data was the large-scale movement of bonefish to the most westerly-located receiver at the mouth of the Cape Eleuthera Marina between November and late January. The timing of these movements corresponds to the proposed spawning period for bonefish in The Bahamas (Danylchuk et al., 2008). Unfortunately this receiver was not deployed until mid May 2007, limiting the opportunity for a full year's worth of data to be examined within this data set, but still no detections were made on this receiver during months outside the proposed reproductive period. An extension of the receiver array to include areas west (seaward) of this location and to more offshore sites is warranted to explore possible spawning migrations. Ault et al. (2002) suggested that in Florida bonefish are not found on shallow flats during the warmest or coldest periods of the year. Our results in The Bahamas indicate otherwise, as our study detected many bonefish well up on the tidal flats and in the tidal creeks throughout the year.

Relative measures of bonefish swimming speed were calculated for fish travelling from the backwater portion of tidal creeks to the creek mouth as well as fish travelling from offshore receivers towards R04, to compare to a single relative swimming speed reported by Humston et al. (2005). In our study, bonefish appear to swim more slowly (e.g., 0.08 m s^{-1}) while in the backwaters of the creeks compared to when making westerly movements to R04 (e.g., 0.37 m s^{-1}). Humston et al. (2005) calculated a quick movement between two receivers 400 m apart to be at a relative swimming speed of 1.66 m s^{-1}. In comparison, bonefish 2381 was estimated to have a relative swimming speed of 2.33 m s^{-1} when travelling between receivers 600 m apart. Overall these estimates are crude, but do suggest that there are times when fish move more slowly, such as in tidal creeks when the focus of the movements is on feeding, versus other times when fish move more quickly when the purpose of the movements may be to travel between foraging sites. Further examination of bonefish activity levels and relative swimming speeds in bonefish in the wild could be accomplished with acoustic transmitters with accelerometers (see Tsuda et al., 2006; Whitney et al., 2007).

Bonefish are presumed to play an important role in the connectivity of habitats in coastal systems and such information is critical for effective ecosystem management on a seascape level (Verweij and Nagelkerken, 2007). This study documented a number of bonefish movements between tidal creeks, tidal flats, and along the open coastline of South Eleuthera. The extensive use of nearshore habitats by bonefish is the basis for concern because these areas are typically most vulnerable to habitat alteration and degradation (Blaber, 2007). While a marine protected area (MPA) in this study area may provide some protection to bonefish populations in South Eleuthera, there are locations outside of the current array that bonefish are occupying to fulfill some life requirements. Continued research into the basic ecology of bonefish will not only aid in the management of this fishery, but provide more information into coastal ecosystem dynamics.

Tables

Table 5.1: Summary of the hydrophone receiver array on the north coast of Cape Eleuthera, The Bahamas. Receivers were roughly numbered sequentially from west to east.

Area	Receiver #	Habitat zone	Easting	Northing	Description of receiver location	Date deployed
Powell Point	R01	Nearshore	18363943	2747548	50 m south of the mouth of the Cape Eleuthera marina	19-May-07
	R02	Nearshore	18364563	2747868	200 m offshore of Powell Point	9-Oct-06
	R03	Offshore	18365028	2748038	400 m offshore receiver #R04	9-Oct-06
	R04	Nearshore	18364898	2747390	Near a small channel that cuts into the back portion of the Cape Eleuthera Marina	9-Oct-06
Page Creek	R05	Within-creek	18367203	2745362	Inside the mouth of Page Creek	16-Feb-06
	R06	Within-creek	18367360	2745256	Inside the backwaters of Page Creek	9-Oct-06
	R07	Nearshore	18367592	2745659	Nearshore, east of Page Creek	16-Feb-06
	R08	Offshore	18367891	2746016	Offshore, northeast of Page Creek	9-Oct-06
Kemps Creek	R09	Offshore	18368465	2745239	400 m offshore of the mouth of Kemps Creek	9-Oct-06
	R10	Nearshore	18368248	2744887	Outside the mouth of Kemps Creek	2-Nov-05
	R11	Within-creek	18368118	2744691	Inside the mouth of Kemps Creek	16-Feb-06
	R12	Within-creek	18368163	2744388	Inside the backwaters of Kemps Creek	9-Oct-06
Broad Creek	R13	Nearshore	18369031	2743970	Outside the west mouth of Broad Creek	2-Nov-05
	R14	Nearshore	18369392	2744189	200 m offshore between the east and west mouth of Broad Creek	16-Feb-06
	R15	Offshore	18369678	2744547	In-between the east and west mouth of Broad Creek, 400 m offshore	9-Oct-06
	R16	Within-	18369297	2743602	Inside the west mouth of Broad Creek	2-Nov-05

		creek				
	R17	Nearshore	18369630	2743734	Outside the east mouth of Broad Creek	2-Nov-05
	R18	Within-creek	18369525	2743509	Inside the east mouth of Broad Creek	2-Nov-05
	R19	Within-creek	18369719	2743300	Inside the backwaters of Broad Creek	9-Oct-06
	R20	Nearshore	18370258	2743423	East of the east mouth of Broad Creek	16-Feb-06
	R21	Offshore	18370431	2743793	600 m offshore of the east mouth of Broad Creek	8-Oct-06
Red Point	R22	Nearshore	18371406	2743039	200 m offshore of Red Point	16-Feb-06
	R23	Offshore	18371599	2743434	600 m offshore of Red Point	8-Oct-06
Poison Flats	R24	Offshore	18376416	2742584	1000 m offshore, southwest of Starved Creek	8-Oct-06
	R25	Offshore	18376734	2742269	600 m offshore, southwest of Starved Creek	8-Oct-06
	R26	Nearshore	18376913	2742032	200 m offshore, southwest of Starved Creek	8-Oct-06
Starved Creek	R27	Within-creek	18380193	2744888	Inside the mouth of Starved Creek	8-Oct-06

Table 5.2: Summary of the tagging, biological, and monitoring data for the 47 bonefish used in this study, collected from the north coast of Cape Eleuthera, The Bahamas

Date tagged	Location tagged	Transmitter ID	Total length (mm)	Sex	Date last detected	Total # of days at large	Total # of days detected	% of days detected while at large	Total # of detections
01-Nov-05	Broad Creek	4073	590	Uknown	n/a	n/a	n/a	n/a	n/a
18-Feb-06	Kemps Creek	4075	550	Uknown	18-Feb-08	731	260	36	20489
19-Feb-06	Broad Creek	4076	400	Uknown	19-Feb-07	366	1	<1	1
		4077	400	Uknown	04-Sep-06	198	9	5	1245
	Kemps Creek	4078	540	Uknown	24-Feb-06	6	6	100	601
		4079	490	Uknown	30-Jan-08	711	611	86	63533
27-Aug-06	Kemps Creek	101	500	Uknown	n/a	n/a	n/a	n/a	n/a
		104	500	Uknown	27-Aug-06	1	1	100	40
		108	440	Uknown	10-Jan-07	137	132	96	8910
		109	440	Uknown	30-Oct-06	65	60	92	1522
		4080	495	Uknown	30-Jan-08	522	30	6	1700
		4081	480	Uknown	27-Aug-06	1	1	100	44
		4082	530	Uknown	06-Jan-08	498	82	17	2804
		4086	450	Uknown	27-Oct-06	62	10	16	407
		4087	420	Uknown	20-Feb-07	178	5	3	62
		4088	450	Uknown	02-Sep-06	7	7	100	233
28-Aug-06	Broad Creek	102	550	Uknown	22-Feb-07	179	107	60	1008
		103	530	Uknown	01-Sep-06	5	5	100	112
		105	460	Uknown	01-Sep-06	5	5	100	187
		106	500	Uknown	19-Sep-06	23	8	35	222
		107	450	Uknown	04-Feb-07	161	43	27	1099

		110	490	Uknown	02-Sep-06	6	6	100	549
		111	500	Uknown	28-Aug-06	1	1	100	2
		112	460	Uknown	02-Sep-06	6	6	100	472
		113	500	Uknown	30-Aug-06	3	3	100	97
		4089	550	Uknown	01-Sep-06	5	5	100	190
		4090	450	Uknown	02-Sep-06	6	6	100	881
20-Feb-07	Kemps Creek	928	515	Female	10-Apr-07	50	50	100	5596
		933	515	Male	13-May-07	83	64	77	3499
		936	480	Male	05-Apr-07	45	44	98	4837
		2376	465	Male	25-Mar-07	34	6	18	1077
		2378	570	Male	01-Mar-07	10	7	70	1702
		2379	530	Female	30-Dec-07	314	142	45	5200
		2381	520	Female	13-Feb-08	359	353	98	52480
		2382	475	Female	16-Feb-08	362	272	75	16035
		2383	515	Male	30-Nov-07	284	259	91	41683
		2385	520	Female	01-May-07	71	68	96	7354
16-Mar-07	Broad Creek	926	475	Unknown	06-May-07	52	38	73	1074
		927	555	Unknown	29-Mar-07	14	13	93	268
		929	560	Female	09-May-07	55	3	6	150
		931	515	Unknown	05-Apr-07	21	5	24	149
		932	540	Female	29-Sep-07	198	189	96	11069
		934	460	Male	18-Feb-08	340	278	82	14748
		937	460	Unknown	16-Feb-08	338	237	70	10010

2375	560	Female	18-Feb-08	340	252	74	22947
2377	455	Uknown	14-Feb-08	336	255	76	21362
2384	480	Male	17-Mar-07	2	2	100	69

Table 5.3: Proportions of use of each tidal creek and flats area along the north coast of Cape Eleuthera, The Bahamas by the individual bonefish used in this study. Note that all receiver locations are listed from west to east and that empty fields indicate that the individual bonefish was not detected at that receiver. Bonefish 101 and 4073 are not included as they were never detected post-release.

Area	Receiver #	4075	4076	4077	4078	4079	104	108	109	4080	4081	4082	4086	4087
Powell Point	R01					<1								
	R02					<1		5						
	R03					<1		1						
	R04					24		12	1					
Page Creek	R05			<1		<1		<1	<1	1				
	R06					<1		<1	1					
	R07		100	5	100	47		12	10	1		1	4	9
	R08					2		2				97		
Kemps Creek	R09					<1		6	<1					
	R10	98		7		3	100	6	16	9	100	2	13	38
	R11	2		14		19		16	15	3			6	
	R12					2		<1						
Broad Creek	R13			32		<1		8	24	11		<1	34	17
	R14			1		<1		1	1	1		<1		
	R15							<1						
	R16			4		<1		1	1	1			6	
	R17					<1								2
	R18			13		<1		2	3	7			10	
	R19					1		<1						
	R20			14				9	12	9			12	12
	R21							1		45				
Red Point	R22			10				14	15	11			14	23
	R23							4						

Poison Flats	R24
	R25
	R26
Starved Creek	R27

4088	102	103	105	106	107	110	111	112	113	4089	4090	928	933	936
	<1													
														<1
												2		39
				8										
		1	1	2				<1		1	<1	1		3
												<1		2
												4	<1	2
12	<1	2	15	3		6		8		15	8	4	2	2
				3								4	1	<1
10	2	8	10	44	<1	25		8		10	27	2	3	
5	<1	30	39	1		33		7		28	5	1	2	<1
												<1	1	
	<1			3		2						4	<1	
	4											5	7	<1
	4	2	1	4	<1	13	47	6	5	15	3	3	1	
	35											4	1	
54	21	35	24	30	4	18	53	52	90	22	35	1	2	<1
												<1	<1	<1
19	<1	22	9		96	2		18	4	9	21	1	1	<1
												<1	<1	<1
													<1	<1
	2											2	30	30
	32											6	35	21
												58	14	

2376	2378	2379	2381	2382	2383	2385	926	927	929	931	932	934	937	2375
			<1											<1
			<1				1				<1			
			<1				14							<1
	86		49	<1	2	89	<1				6	<1		1
			<1											
														<1
<1	8	3	2	1	<1	2	<1		7		<1	1		<1
<1	3	1	<1	<1	<1	1			1			<1		<1
7	1	3	18	<1	7	4	<1	1				1	1	7
4	1	2	12	11	3	1	<1	2	20		<1	13	45	16
<1	2	55	10	57	<1	<1					<1	17	21	4
			2	3	<1							3	6	4
<1		1	2	2	3	<1	1	2	14	31	60	11	3	33
1		1	1	<1	1	<1	<1	1	3		5	1		6
1		<1	<1	<1	<1			2			<1		<1	<1
		2	1	1	2	<1		10		2	7	3	1	8
2		1	<1	1	5	3	5	31	29	47	4	<1	<1	4
		3	1	1	3	1	<1	2		1	4	2	1	6
		4	2	1	7				2		12	4	<1	10
1		1	<1	<1	15		3	2	24	18	<1	1	<1	1
3		<1		<1	2		1							
2		<1		<1	1		<1	<1				1	<1	
3		<1		<1	<1			3				<1		
8				<1	1							<1		
30		4		7	18		12	2				9	4	
37		5		15	29		61	42				34	18	
		13												

2377	2384
<1	
<1	
<1	
13	
2	
1	4
13	13
25	
21	
4	
6	9
1	1
<1	2
2	6
1	34
2	
3	
<1	17
<1	2
<1	10
<1	
<1	
1	
5	

Table 5.4: Indicators of the relative activity of bonefish tagged along the north coast of Cape Eleuthera, The Bahamas.

Bonefish 101 and 4073 are not included as they were never detected post-release. Note that n/a means not applicable.

Date tagged	Location tagged	Transmitter ID	Receiver with highest proportion of detections	Total # of creeks used	Creeks used	Median distance travelled (m)	Minimum linear dispersal (m)	Minimum # of receivers visited daily	Maximum # of receivers visited daily	Mean (±SD) # of receivers visited daily
18-Feb-06	Kemps Creek	4075	R10	1	Kemps	118	235	1	2	1.1 ± 0.3
19-Feb-06	Broad Creek	4076	R07	0	n/a	n/a	n/a	1	1	1.0*
		4077	R13	3	Page, Kemps, Broad	1185	4802	4	9	6.3 ± 1.5
	Kemps Creek	4078	R07	0	n/a	n/a	n/a	1	1	1.0 ± 0
		4079	R07	3	Page, Kemps, Broad	2271	7170	1	7	2.4 ± 1.4
27-Aug-06	Kemps Creek	104	R10	0	n/a	n/a	n/a	1	1	1.0*
		108	R11	3	Page, Kemps, Broad	1605	8691	2	14	6.2 ± 2.6
		109	R13	3	Page, Kemps, Broad	1343	7828	1	8	4.8 ± 1.7
		4080	R21	3	Page, Kemps, Broad	1233	4802	1	10	2.2 ± 2.8
		4081	R10	0	n/a	n/a	n/a	1	1	1.0*
		4082	R08	0	n/a	1184	2324	1	4	1.1 ± 0.4
		4086	R13	2	Kemps,	1185	4627	1	8	4.9 ± 2.6

					Broad					
		4087	R10	0	n/a	1503	4627	1	3	2.0 ± 1.0
		4088	R20	0	n/a	1211	3659	2	5	3.3 ± 1.1
28-Aug-06	Broad Creek	102	R19	1	Broad	954	13659	1	7	1.7 ± 0.9
		103	R20	1	Broad	1211	4627	3	6	4.0 ± 1.4
		105	R14	1	Broad	1156	4627	2	6	4.8 ± 1.6
		106	R13	3	Page, Kemps, Broad	1163	3618	1	8	4.8 ± 2.6
		107	R22	1	Broad	1575	2551	1	3	1.1 ± 0.3
		110	R14	1	Broad	693	3659	4	6	4.7 ± 1.0
		111	R20	1	Broad	369	738	2	2	2.0*
		112	R20	1	Broad	1211	4627	2	7	4.0 ± 1.9
		113	R20	1	Broad	738	1939	2	3	2.7 ± 0.6
		4089	R14	1	Broad	1156	4627	3	6	4.6 ± 1.1
		4090	R20	1	Broad	1211	4627	3	7	4.8 ± 1.5
20-Feb-07	Kemps Creek	928	R27	3	Kemps, Broad, Starved	10691	15498	1	14	3.8 ± 3.6
		933	R26	3	Kemps, Broad, Starved	7392	11733	1	9	2.0 ± 1.7
		936	R04	1	Kemps	5749	13316	1	9	2.4 ± 1.7
		2376	R26	1	Kemps	7569	10002	2	13	5.2 ± 4.0
		2378	R04	1	Kemps	3729	4202	1	5	2.3 ± 1.4
		2379	R11	3	Kemps, Broad, Starved	1789	12625	1	11	1.5 ± 1.5
		2381	R04	3	Page, Kemps, Broad	4202	7543	1	10	3.8 ± 1.7
		2382	R11	2	Kemps, Broad	1789	13156	1	8	2.5 ± 1.3

		2383	R26	2	Kemps, Broad	7660	13156	1	12	4.2 ± 2.4
		2385	R04	2	Kemps, Broad	4202	5980	1	7	2.0 ± 1.2
16-Mar-07	Broad Creek	926	R26	1	Broad	7824	13659	1	5	2.2 ± 1.2
		927	R26	1	Broad	7534	9036	1	6	2.7 ± 1.9
		929	R17	1	Broad	672	3480	1	8	4.3 ± 3.5
		931	R17	1	Broad	358	1343	1	4	2.0 ± 1.4
		932	R13	2	Kemps, Broad	960	7224	1	7	2.7 ± 1.3
		934	R26	2	Kemps, Broad	7776	13156	1	8	2.7 ± 1.4
		937	R10	2	Kemps, Broad	1729	9036	1	7	2.2 ± 1.0
		2375	R13	3	Page, Kemps, Broad	1185	7543	1	9	3.9 ± 1.6
		2377	R10	2	Kemps, Broad	2021	14094	1	9	3.7 ± 1.7
		2384	R17	1	Broad	752	4606	5	6	5.5 ± 0.7

Figures

Figure 5.1: Study area along the north coast of Cape Eleuthera, Eleuthera, The Bahamas, showing the locations of the 27 hydrophone receivers (black squares), and the various tidal creeks. Receivers were roughly numbered sequentially from west to east. The inset map displays the entire island of Eleuthera with the study area highlighted.

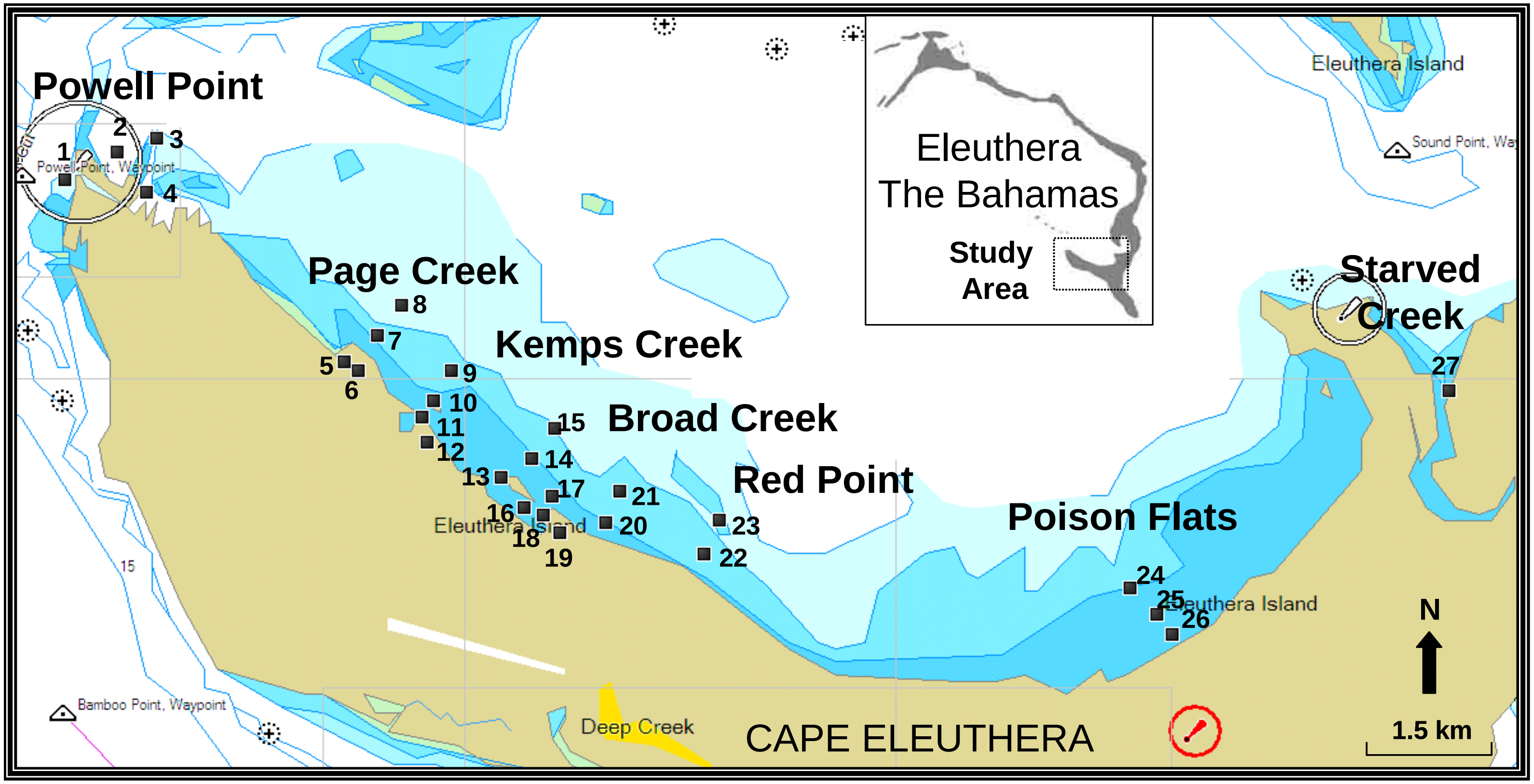


Figure 5.2: The number of detections of bonefish per days deployed of each receiver, located roughly from west to east along the north coast of Cape Eleuthera, The Bahamas.

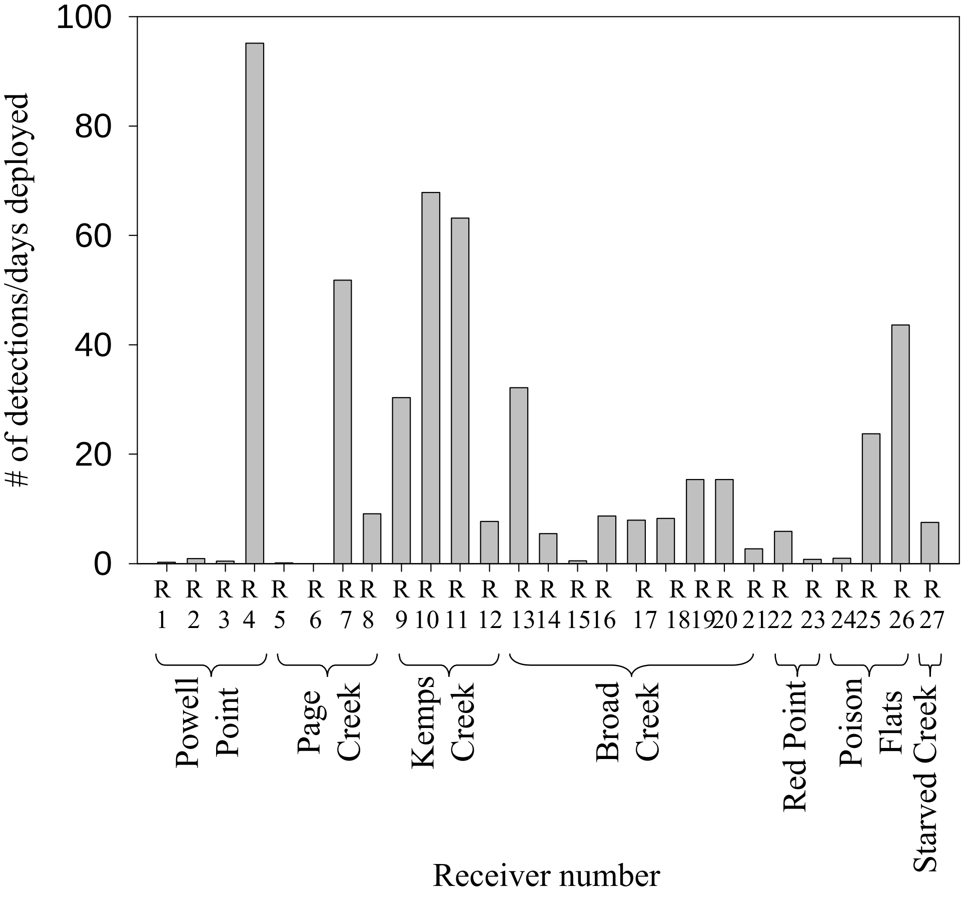
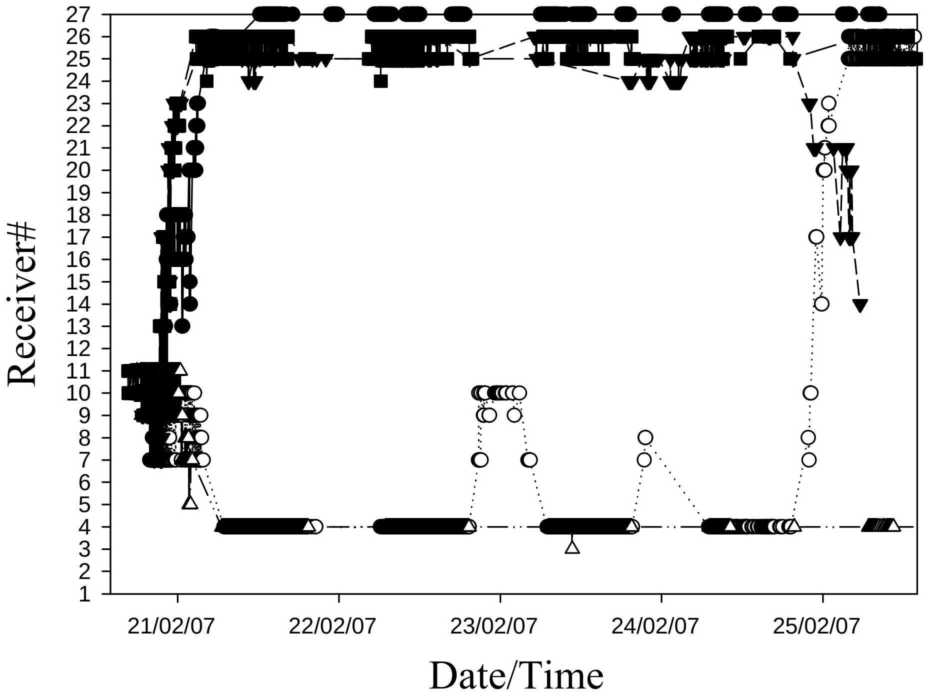


Figure 5.3: Movement patterns of bonefish throughout the hydrophone array for five days post release from Kemps Creek on February 20, 2007. Bonefish 928 (solid circle), 936 (open circle), 2376 (solid triangle), 2381 (solid square), and 2383 (open triangle) are included in the plot to illustrate the variation in movement. The other five tagged individuals released with the school have overlapping movement patterns with one of the above (i.e., bonefish 933 & 2379 have similar patterns to 2383, and bonefish 2378, 2382 & 2385 have similar patterns to 2381 for this time period). Dates are given as dd/mm/yy.



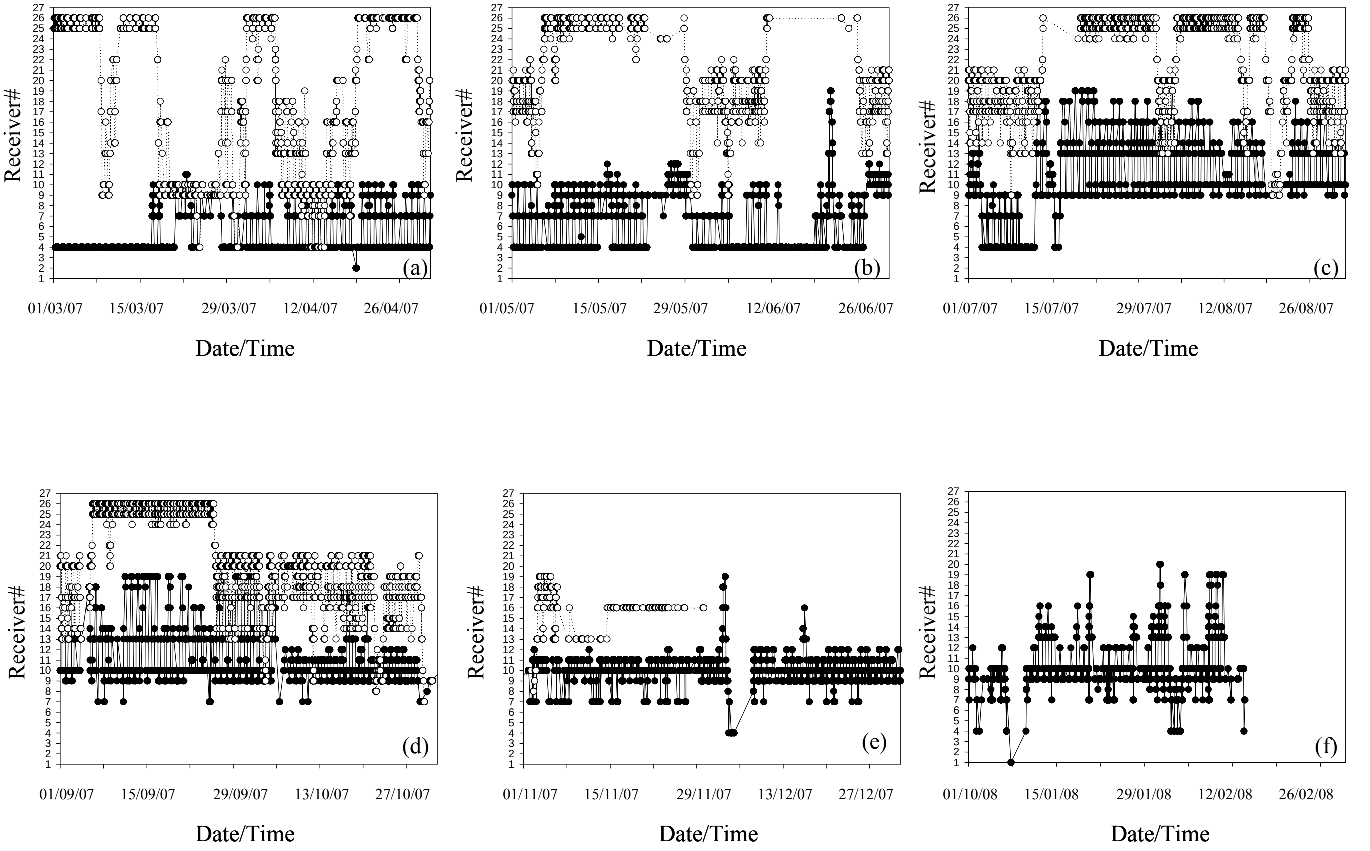


Figure 5.4: Movement patterns of bonefish 2381 (solid circle) and 2383 (open circle) over their time at large within the study area. Panels a-f cover the months of March-April, May-June, July-August, September-October, November-December 2007, and January-February 2008, respectively. Dates are given as dd/mm/yy.

Figure 5.5: Movement patterns of bonefish 108 (solid circle) and 109 (open circle). Each plot represents a two-week time series of detections. Dates are given as dd/mm/yy.

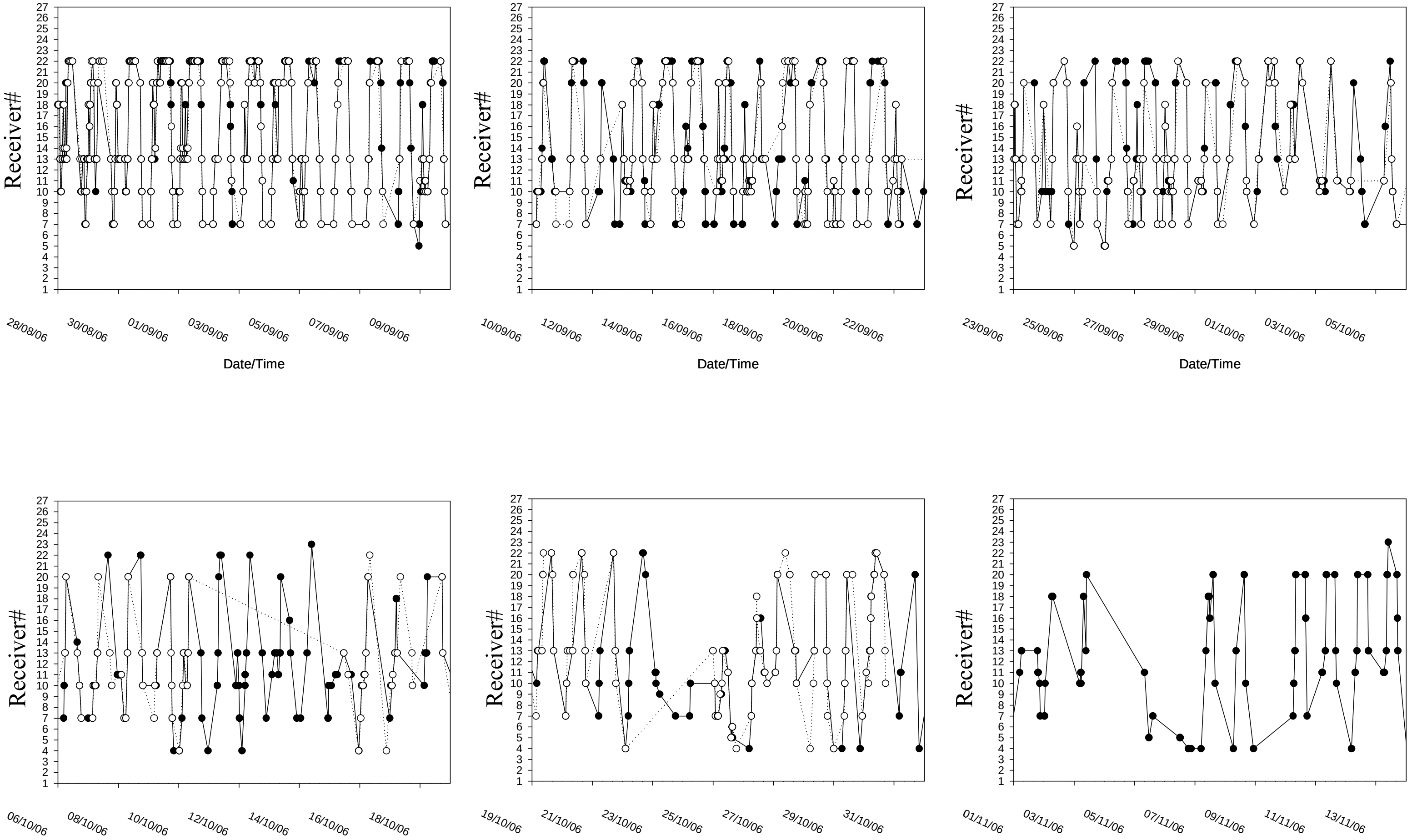


Figure 5.6: Movement patterns of four bonefish tagged and released from Broad Creek on March 16, 2007. Plots represent all detections of bonefish 934 (panel a), 937 (panel b), 2375 (panel c), and 2377 (panel d) from the tagging date till the last download period in February 2008. Dates are given as dd/mm/yy.

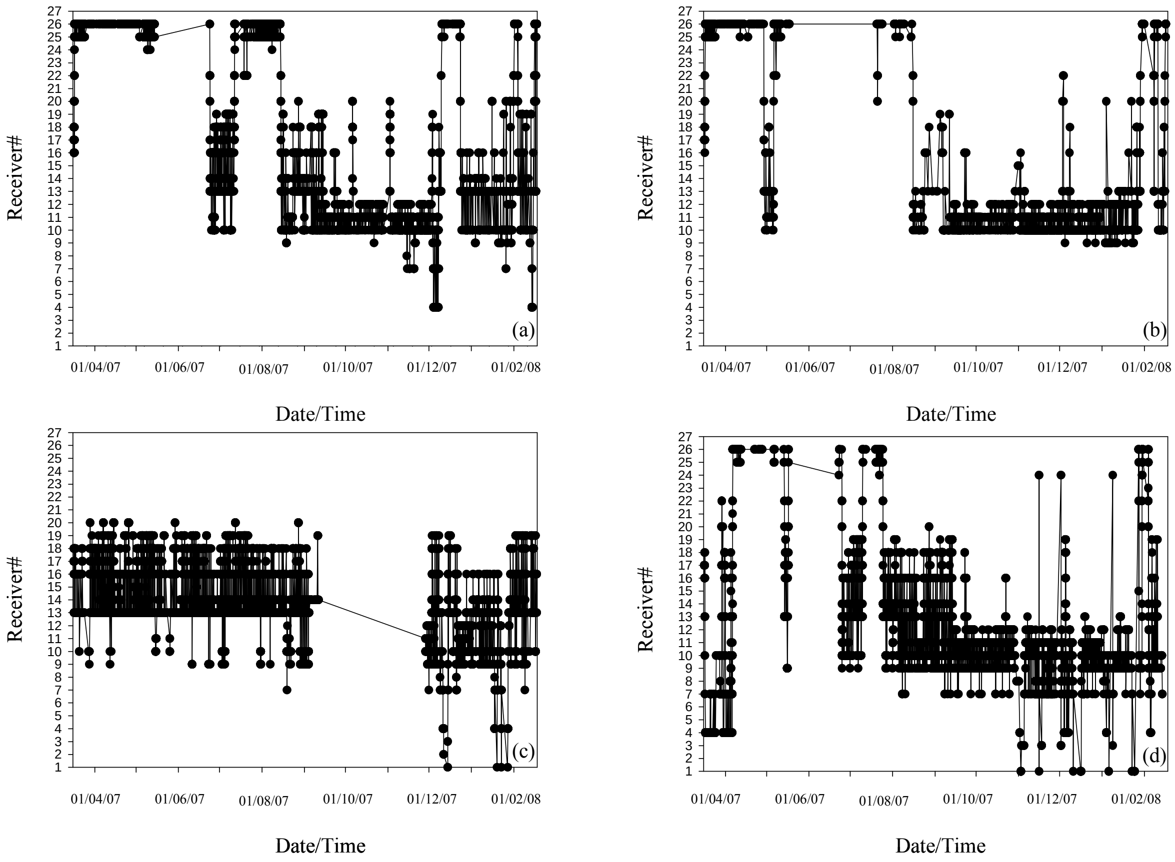


Figure 5.7: Movement patterns of bonefish 4079 as it was detected through the hydrophone array during the entire year of 2007. Panels a-f cover the months of January-February, March-April, May-June, July-August, September-October, and November-December 2007, respectively. Dates are given as dd/mm/yy.

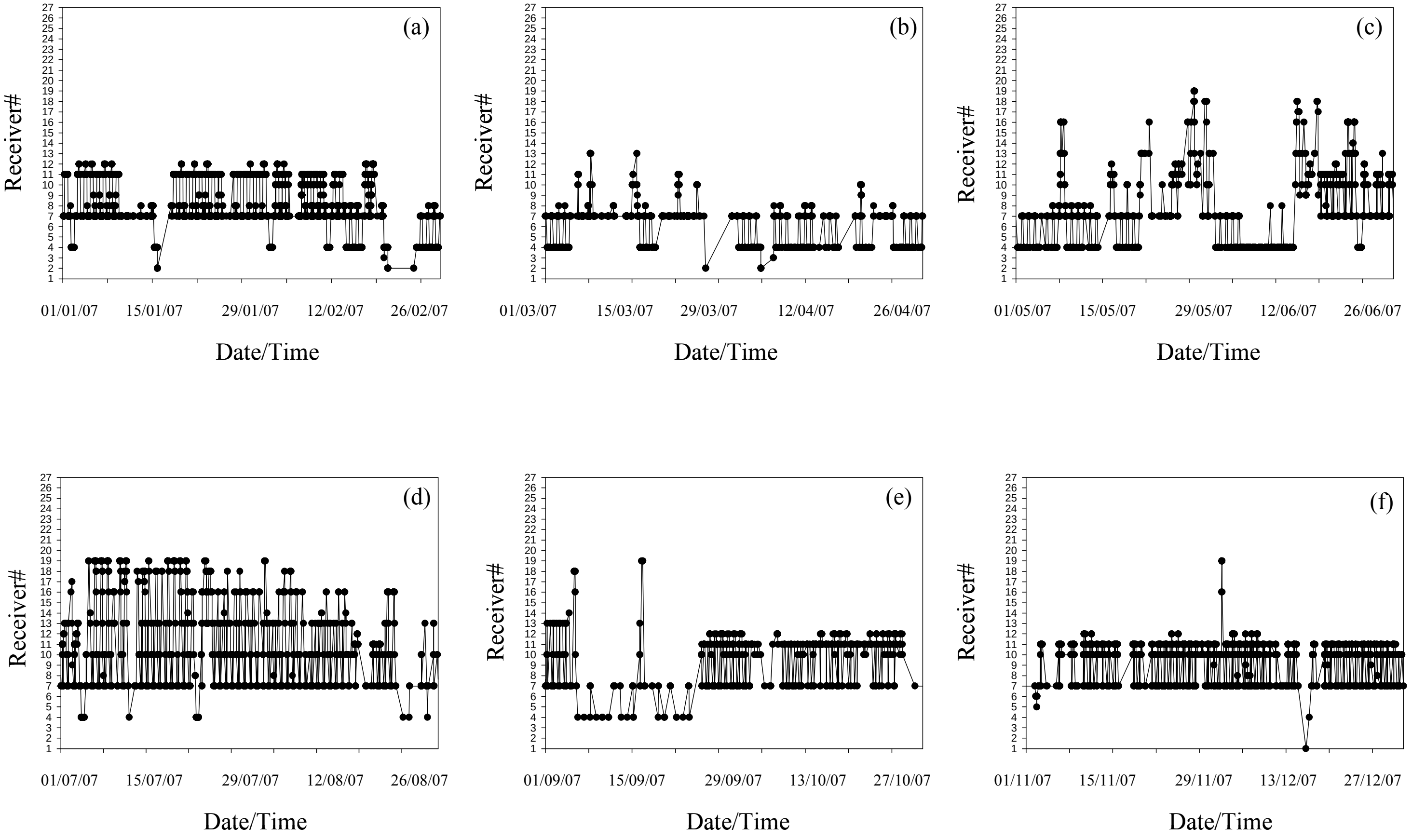
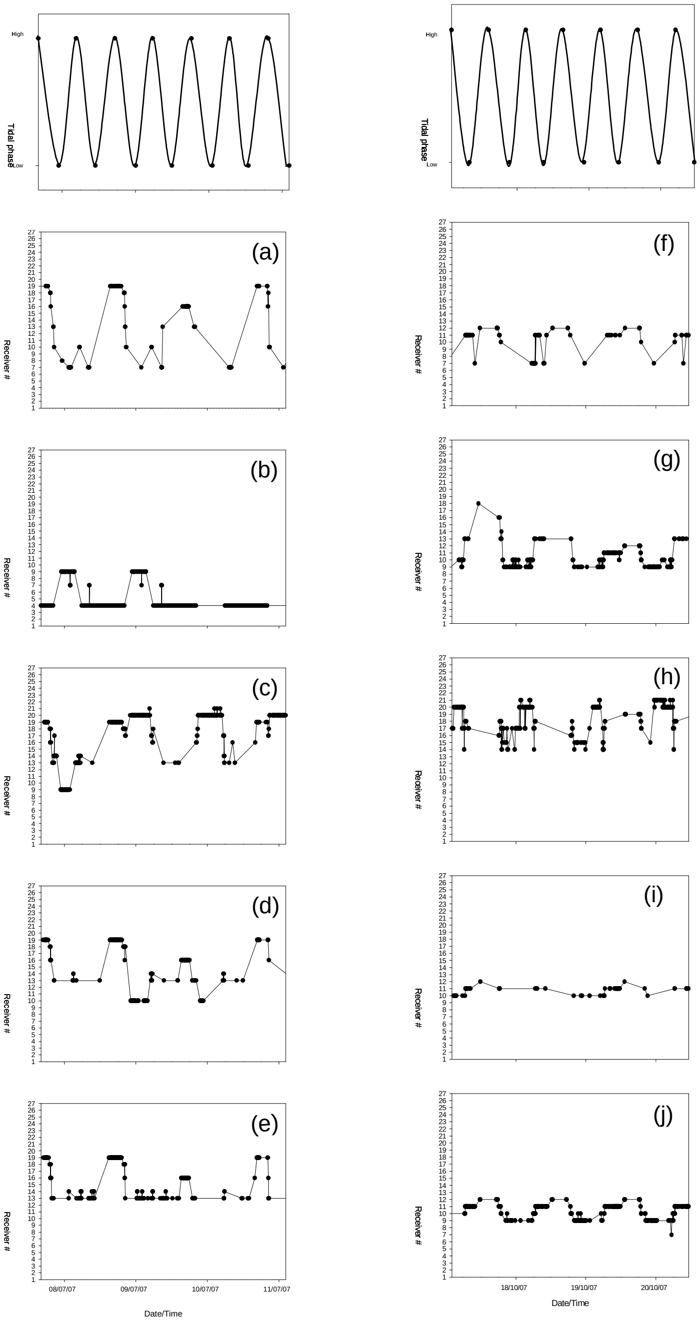


Figure 5.8: Movement patterns of bonefish associated with tidal cycles. Panels a-e represent a time series of detections of bonefish 4079, 2381, 2838, 934, and 2375 between July 7-11, 2007, whereas panels f-j represent a time series of detections of bonefish 4079, 2381, 2838, 937, and 2377 between October 17-20, 2007. Dates are given as dd/mm/yy.



Chapter 6: Estimates of field activity and metabolic rates of bonefish (*Albula vulpes*) in coastal marine habitats using tri-axial accelerometer transmitters and intermittent flow respirometry

Abstract

We tested the utility of acoustic tri-axial acceleration transmitters in combination with ethogram and respirometry studies to quantify the activity patterns and field metabolic rates of free-swimming bonefish (*Albula vulpes*) in the coastal waters of Eleuthera, The Bahamas. Bonefish were found to exhibit relatively low activity levels in the field with no evidence of diel patterns or inter-sexual variation. Low activity levels reported by the accelerometers reflected low swimming speeds and intermittent swimming behaviours (i.e., swim-then-drift) that maximized their energy efficiency. Such behaviours were also observed when conducting ethograms on bonefish held in a large field mesocosm. Laboratory derived standard metabolic rates and maximum metabolic rates during recovery from exercise were combined with acceleration transmitter calibrations to determine that individual bonefish typically operate at between 40 to 60% of their estimated metabolic scope in the field. However, occasionally acceleration values in the field were indicative of high intensity bursting activity that exhausted the majority of their estimated metabolic scope (0.7% of all field observations exceed 90% of metabolic scope). Data gathered in this study provide a critical starting point for the development of a bioenergetics model for bonefish which will ultimately lend clues into how fish make a living in tropical tidal flats environments. Given that this paper is one of the first to use transmitters rather than archival loggers to collect data on

tri-axial acceleration on fish, we also discuss the opportunities and limitations of using this new technology for marine fisheries research.

Introduction

Energy is an important commodity for all organisms, and is the currency most often employed in analyses of animal behaviour (Townsend and Calow, 1981). How animals partition energy into different life functions, and thus how they make a living, can be represented by balanced energy equations (Soofiani and Hawkins, 1985). Animals making energetic choices that increase survival will be favored, and as such, the balanced energy equation is strongly influenced by natural selection (Diana, 2004; Wilson et al., 2006). Estimating the complete energy budget of a free-living organism, however, has presented challenges to scientists, particularly when assessing the energetic costs of activity in fish (see Briggs and Post, 1997a). As the cost of activity may represent a large and variable component of the fish's energy budget (Boisclair and Sirois, 1993), the methods used to measure locomotion need to be effective in free-swimming fish in the wild (Briggs and Post, 1997b; Cooke et al. 2004).

Previous studies have explored the utility of a variety of biotelemetry sensors including heart-rate (e.g., Lucas et al., 1991), tail-beat frequency (e.g., Ross et al., 1981), and axial muscle electromyograms (EMGs) (e.g., Briggs and Post, 1997a, b; Cooke et al., 2004). However, both heart-rate (electrocardiogram (ECG)) and EMG transmitters require precise surgical implantation of electrodes and significant handling of the animal (Whitney et al., 2007). More recently, the use of animal-borne acceleration data loggers for studying free-swimming fish and other animals is gaining popularity (Wilson et al., 2007; Shepard et al. 2008). Because locomotion occurs when animals expend energy to contract muscles which leads to body acceleration, the accurate measurement of acceleration should be a good proxy for energy expenditure during activity (Halsey et al., 2009). Indeed, acceleration data loggers have been successful in elucidating homing migrations and spawning behaviour in salmon (Tanaka et al., 2001; Tsuda et al., 2006; respectively), diel activity patterns in whitetip reef sharks (*Triaenodon obesus*) (Whitney et al., 2007), and general activity patterns in rainbow trout (*Onchorhynchus mykiss*) (Kawabe et al., 2003a) and Japanese flounder (*Paralicthys olivaceus*) (Kawabe et al., 2003b). Acceleration data loggers have their limits as well, requiring retrieval of the logger to access the data (Ropert-Coudert and Wilson, 2005). Only recently has the technology of onboard processing improved sufficiently to encode and transmit tri-axial accelerometer data efficiently. With acceleration transmitters the data are sent to acoustic hydrophone receivers, extending the use of these devices to species and/or environments where recaptures are difficult.

Knowledge of the activity patterns and energetic requirements of marine species is becoming increasingly important for modeling ecosystems and managing populations (Lowe, 2002; Fitzgibbon et. al., 2007). This is particularly true for species occupying coastal habitats, since over half of the world's population lives in these areas (Barnabé and Barnabé-Quet, 2000). Habitat degradation is widespread where humans exploit resources such as mangrove forests (Alongi, 2002; Blaber, 2007). Studying the behaviour and activity patterns of a species that not only occupies coastal marine environments, but is also the object of an economically important recreational fishery may provide insight into individual and population level processes, which may ultimately influence the effectiveness of conservation and management strategies.

Bonefish (*Albula* spp.) are a group of benthivorous fishes found in tropical tidal flats and tidal creeks (Colton and Alevizon 1983a,b; Humston et al., 2005). Throughout much of their circumtropical distribution bonefish also carry the distinction of being a popular sport fish and thus play an important role in many local economies (Pfeiler et al., 2000; Ault, 2008; Danylchuk et al., 2008). To date, there has been no known study which has examined bonefish activity patterns and behaviour beyond traditional positional biotelemetry studies (see Colton and Alevizon, 1983b; Humston et al., 2005; Friendlander et al., 2008; Larkin et al., 2008), all of which have had limited spatial (often on the order of 500m accuracy of positioning) and temporal resolution (fish tracked at infrequent intervals and often for short duration), thus making it impossible to evaluate fine scale activity patterns or estimate energy expenditure.

The objective of this study was to quantify the field activity and metabolic rates of bonefish (*A. vulpes*) in tidal flats and tidal creek areas near Cape Eleuthera, Eleuthera, The Bahamas. Using acoustic tri-axial acceleration transmitters, we investigated the influence of sex and photoperiod on the activity patterns of wild bonefish and compared results to laboratory and field calibrations. Furthermore, we catalogued the discrete behaviours of bonefish held in a natural wetland mesocosm to produce an activity time budget. Static respirometry was used to determine standard metabolic rate and maximum metabolic rate during recovery after exercise. When combined with data from accelerometers, we estimated the field energetics of bonefish.

Materials and methods

Study site

This study was conducted along a 15 km section of the north coast of Cape Eleuthera, Eleuthera, The Bahamas (N 24° 50' 05" and W 76° 20' 32"), as well as the Cape Eleuthera Institute (CEI) research facility (Fig. 6.1). The coastline in this area is composed of tidal creeks, sandy bays, mangroves, and jagged calcium carbonate outcroppings. Preliminary genetic analyses on bonefish from this area indicated that all specimens were *A. vulpes* (Danylchuk et al., 2007a). All procedures used in this study were in accordance with the policies of the Canadian Council on Animal Care as administered by the Carleton University Animal Care Committee (Protocol B07-03, B07-05, and B07-06).

Acceleration transmitter experiments

On December 8, 2008, 10 bonefish (527 ± 36 mm total length; mean ± SD) were implanted with acoustic tri-axial acceleration transmitters. Water temperatures were 22.5°C at time of capture. The transmitters (model V9AP-2L coded tags, 46 mm long, 3.3 g in air, 160 day battery life; Vemco Inc., Shad Bay, NS.) measure acceleration in the X, Y, and Z axes at a rate of five samples per second with a 25 second sampling period. This model of tag calculates a value (vector, measured in g-force) that represents the root mean square of acceleration from each of the three axes over time (i.e., $g - force = \sqrt{x^2 + y^2 + z^2}$). The value in g-force is converted to m/s^2 by multiplying by 9.8. The transmitter was programmed to transmit randomly at an interval ranging from 45 to 135 seconds, with an average of 90 seconds. The transmitters alternated in the transmission of acceleration and depth information, however the depth data were not used in the current study as most fish resided exclusively in shallow waters (i.e., < 2m).

To capture bonefish for implantation with the acceleration transmitters, various sized seine nets were deployed at the mouth of a tidal creek to intercept fish on an outgoing tide (as per Murchie et al., 2009). When a school of bonefish approached, the net was moved quickly to encircle the fish. Captured bonefish were dip-netted or passed by hand into flow-through holding pens (1.3 m × 0.8 m × 1.25 m, 3.1 cm extruded plastic mesh) submerged in a minimum of 0.6 m of water where they were held until surgery. Bonefish were anesthetized with MS-222 prior to surgery (approx. 100 ppm) and then placed on a surgery table where the gills were supplied with a maintenance dose of MS-222 (approx. 50 ppm) in recirculating seawater. To implant the transmitter, a small (2-3 cm) incision was made to one side of the ventral midline, posterior to the pectoral fins. After being disinfected with an iodine solution, the transmitter was inserted and gently guided into the coelomic cavity, toward the pectoral fins. The transmitter was always oriented the same way for each fish (i.e., accelerometer sensors facing anteriorly). The incision was closed with 3-4 simple interrupted sutures using monofilament absorbable suture material (Ethicon 3-0 PDS II, Johnson and Johnson, New Jersey). The length of the fish (mm) was measured, and the sex was determined via internal examination. The entire procedure generally took less than five minutes. Bonefish were held for up to one hour in the flow-through net pens to recover following anesthetization. Transmitter-implanted fish were released simultaneously with a group of untagged bonefish (at least n=10) from the same school from which they were captured. All surgeries were completed by the same surgeon who had previously implanted more than 2000 telemetry devices in fish.

A series of 13 hydrophone receivers (VR2 and VR2W models, Vemco Inc., Shad Bay, NS) deployed prior to December 8, 2008 were used to record acceleration values as transmitter-implanted fish swam through the study area (Fig. 6.1). Receiver locations covered tidal flats, the mouths of tidal creeks, and other nearshore areas bonefish have been found to frequent (Murchie, unpublished data). Individual receivers were anchored to a short piece of rebar cemented into a concrete block. Receivers in water greater than 1 m deep at low tide and in open water were positioned vertically in the water column. In water that was < 1 m deep at low tide and at narrow choke points, receivers were deployed horizontally or 5-10 degrees above horizontal, with the hydrophone orientated to maximize coverage. Range tests were performed upon the initial deployment of the receivers, revealing that receivers deployed in water greater than 1 m deep had a coverage radius of 250 m, whereas receivers in shallow water or positioned horizontally had a coverage radius as small as 30 m due to shoreline confinement. Wind and wave conditions, as well as tidal cycles, influence the detection range of individual receivers (Heupel et al., 2006). Although the range of coverage for receivers in shallow water or positioned horizontally was considerably less, they did provide the necessary coverage to monitor choke points (i.e., creek mouths) and as such we did not correct for receiver range in our data analysis. Receivers were visited regularly to download data and to clean the hydrophone of biofouling material.

To determine the range of acceleration values that could be generated for implanted bonefish, a laboratory calibration was conducted. Additional bonefish captured by seine net on December 8, 2008 were transported to the CEI research facility following protocols outlined in Murchie et al. (2009). Fish were held in large (3.7 m diameter × 1.25 m height; 13 180 L) circular holding tanks that were aerated and continuously supplied with fresh sea water (1800 L/hr) at ambient temperatures. Tanks were housed in a covered open-sided outdoor facility with natural photoperiod, but the tanks themselves were uncovered. Bonefish were maintained on a diet of sinking pellets (13 mm Zeigler, USA) (Murchie et al., 2009). Seven laboratory-acclimated bonefish (419 ± 17 mm total length; mean ± SD) were implanted with acceleration transmitters (as described above) between January 14 and January 17, 2009, and were left to recover from surgery in a smaller holding tank (1.6 m diameter × 0.85 m height; 1400 L) for a minimum of 12 hours before experimentation. Acceleration values for swimming, bursting, and stationary behaviours were generated. Swimming values were determined by using a VR100 portable receiver (Vemco Inc., Shad Bay, NS) and VR110 hydrophone to log transmitted acceleration data while bonefish swam leisurely in the holding tank for a minimum of 30 minutes. Bonefish were then captured and placed individually in an inflatable children's wading pool (1.5 m wide, 19 cm deep) with the portable hydrophone. Once a depth transmission occurred, which is visible on the VR100, bonefish were chased by tail grabbing (Suski et al., 2007) to provoke them to burst. Bonefish were chased in this manner until the acceleration transmission occurred (approximately two minutes on average). After being chased, bonefish rested in the pool and stationary values were recorded. Two burst and two resting values were recorded for each fish. Because there were only three accelerometer transmitters to be used between the seven fish for calibration, bonefish were then euthanized with a lethal dose of MS-

222. An additional stationary value for dead bonefish was recorded and the transmitter removed.

The last three bonefish implanted with acceleration transmitters were marked with T-bar anchor tags in unique colour combinations and then released into a natural wetland mesocosm after their calibration values were recorded. The wetland is immediately adjacent to CEI and receives water continuously (approx. 1900 L/hr) discharged from a flow-through wetlab facility. The total area of the wetland is approximately 250 m^2 and is vegetated primarily with red mangrove (*Rhizophora mangle*) and black mangrove (*Avicennia germinans*). The wetland is also intermittently connected to the ocean during spring tides and fish enter the system at that time. Typical inhabitants include schoolmaster snapper (*Lutjanus apodus*), yellowfin mojarra (*Gerres cinereus*), juvenile great barracuda (*Sphyraena barracuda*), as well as a variety of small-bodied fish species, and one young-of-the-year lemon shark (*Negaprion brevirostris*). Six bonefish were introduced in the wetland in September 2007 to determine whether the wetland could be used as a potential mesocosm. The fish thrived and were all alive in January 2009 suggesting the wetland was a suitable habitat. The three acceleration transmitter-implanted bonefish were introduced to their conspecifics in the wetland on January 17, 2009. The fish immediately joined the schooling resident bonefish. The bonefish typically remained in the deepest section of the wetland (approximately 0.5 m) which was close to 25 m^2. On January 29, 2009 a VR2W receiver was placed in the wetland to record acceleration values from the three tagged fish. The receiver was positioned vertically by burying it in the substrate so only the top 20% of the receiver was visible.

Ethogram study

Although it is not typically possible to observe detailed bonefish behaviour for long periods of time in the wild, the wetland provided an ideal environment to develop an ethogram for bonefish. A wooden foot-bridge stretched across the width of the wetland and was directly adjacent to the deepest area where the bonefish most often resided. From the bridge, an observer could easily watch the behaviour of the school or isolate the behaviour of an individual. Unfortunately the coloured sheath of the T-bar anchor tags on two of the acceleration transmitter-implanted bonefish fell off within a few days of release, but the anchor tags on the third fish remained. Between January 19, 2009 and January 24, 2009, bonefish were observed for a total of three hours to determine their distinct behaviours. Focal sampling (see Martin and Bateson, 1993) of the remaining marked bonefish (i.e., the one with the remaining T-bar anchor tags) occurred for 10 minute intervals during 20 morning (7:10-9:20), 20 noon (11:33-13:13), and 20 pre-dusk (17:41-18:52) monitoring periods between March 25, 2009 and May 15, 2009. During the observation period, the order and duration of specific behaviours was recorded.

Respirometry study

Because the basis for metabolism is the conversion of glucose and oxygen into carbon dioxide, water and energy (Diana, 2004), determining the standard metabolic rate (SMR) and maximum metabolic rate during recovery after exercise (MMR_R) would serve as a set of metabolic bounds in which bonefish operate. Using the remaining 10 bonefish (407 ± 42 mm total length; mean ± SD) held in captivity, SMR and MMR_R were determined using computerized, intermittent-flow respirometry (LoligSystems, Hobro, Denmark) (Steffensen, 1989). The system consisted of four glass chambers (746 mm length x 140 mm wide) outfitted with fiber optic oxygen probes immersed in a tank (3.09 m length × 0.65 m width × 0.17 m height) of aerated sea water at ambient temperatures. Each glass chamber was connected to two aquarium pumps; one for recirculating water through the chamber, and one for flushing ambient, oxygenated water into the chamber. The total volume per set up, including the glass chamber, two pumps, and all associated tubing was 11.48 L. Experiments were designed such that oxygen consumption in each individual chamber was quantified within 26 min cycles that consisted of a 10 min measurement phase, a 15 min 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 recirculated over the fiber optic oxygen probes to ensure adequate mixing. The change in oxygen concentration (α) for each chamber was calculated as slope ($\Delta O_{2\text{saturation}}/\Delta t$), and oxygen consumption rate (MO_2, mg O_2 kg^{-1} h^{-1}) for each fish was calculated by:

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

where V_{resp} is the volume of each glass chamber minus the volume of the fish (L), β is oxygen solubility (adjusted daily for both temperature and barometric pressure), and M_b is the fish mass (kg) measured before placing in the respirometer chamber. During each trial, the coefficient of determination (r^2) for all slope measurements was >0.95. All calculated dissolved oxygen values were corrected for background oxygen consumptions generated for each specific fish and chamber prior to commencing experiments. Calibration of the fiber optic oxygen probes occurred with oxygen-free water and fully saturated water regularly through the experiments. Data were recorded with AutoResp software (Version 1.4, Steffensen, 1989; Schurmann and Steffensen, 1997). Fish were not fed 24 hours prior to experimentation. SMR values were calculated as the average of six lowest values recorded between 20:00 and 06:00 as very minimal human disturbance occurred in the wetlab during these hours (Schurmann and Steffensen, 1997; Gingerich et al., 2010). After 06:00, individual fish were removed from their chamber, exercised continuously for 4 minutes by tail grabbing (Suski et al., 2007), and then put back in their chamber to measure MMR_R. MMR_R was determined as the highest value recorded over a six hour recovery period. A six hour recovery period was selected as bonefish were found to require four hours to return to baseline blood chemistry values following exhaustive exercise (Suski et al., 2007). While the true metabolic scope (i.e., MMR-SMR) could not be calculated since a true MMR was not determined, an estimate of metabolic scope (i.e., MMR_R-SMR) was calculated and field data expressed as a percent of estimated scope as per Priede (1977). Respirometry trials were conducted between February 7, 2009 and February 12, 2009.

Data analysis

All statistical analyses on collected and derived data were completed using JMP 8.0.2 (SAS Institute, Cary, NC). Maximal type-1 error rates were set at $\alpha=0.05$. Normality and homogeneity of variance assumptions were evaluated using Shapiro-Wilk or Kolmogorov Smirnov goodness-of-fit tests and Levene's test, respectively (Sokal and Rohlf 1995). Acceleration data derived from the wild and wetland environment were evaluated for differences in photoperiod and sex (wild only) using repeated measures analysis of variance (ANOVA). Photoperiod was divided into day (06:00-17:59) and night (18:00-5:59) and divisions were based on sunrise and sunset timing data from a weather station located on Cape Eleuthera. Linear regression was used to generate an equation of the line relating oxygen consumption to acceleration following determination of SMR and MMR_R. Mean acceleration from free-swimming bonefish in the wild was incorporated into the equation to derive an average field metabolic rate.

Results

Acceleration experiments

Laboratory calibration of acceleration transmitters provided baseline values to which the wild and wetland acceleration data could be compared (Table 6.1). A dead bonefish in a cooler of water gave an acceleration of 0.06 ± 0.01 m/s^2, whereas a stationary alive bonefish had an acceleration value of 0.37 ± 0.14 m/s^2. Acceleration values for swimming (routine) and bursting bonefish were 0.60 ± 0.18 m/s^2 and 3.47 ± 0.00 m/s^2, respectively. Bursting activity exceeded the measurement capacity of the transmitter providing an effective endpoint for activity. A ‘low activity’ range was defined as the mean value for stationary alive bonefish minus the standard deviation up to the swimming bonefish plus the standard deviation (i.e., 0.23-0.78 m/s^2).

In the wild, five female and five male bonefish were implanted with acceleration transmitters (Table 2). Although the transmitters had an estimated battery life of 160 days, a number of fish were detected until the end of May/beginning of June 2009 (Table 6.2). Although bonefish #136 was detected until May 31, 2009, moving between 11 of the 13 receivers in the array, the acceleration data was consistently low (0.02 ± 0.02 m/s^2; mean ± SD) and less than that of a dead bonefish. As such, data for bonefish #136 were excluded from further analyses based on the assumption that the acceleration sensor had failed. Because there were four bonefish detected into May/June, plots of average monthly accelerations and associated standard deviation error bars were plotted to determine if there were any ‘seasonal’ trends, but none were detected. Temperature data collected from a data logger (Hobo-H8 temperature logger, Onset Computer Corporation, ± 0.7°C accuracy, range of -20°C to 70°C) encased in a waterproof housing just offshore of CEI revealed the mean water temperature in the field between December 8, 2008 and June 1, 2009 was 23.7 ± 2.5°C. A histogram was constructed for all acceleration data from the wild (Fig. 6.2). The mean acceleration value from free-swimming fish in the wild was 0.65 ± 0.43 m/s^2. The majority (76%) of acceleration values fell in the ‘low activity’ range (i.e., 0.23-0.78 m/s^2). Bursting activity was detected in the wild and made up 0.4% of all readings. Acceleration values did not differ between the sexes (F=3.453, p=0.105). There was no difference in daytime (0.69 ± 0.09 m/s^2; mean ± SE) or nighttime (0.61 ± 0.09 m/s^2; mean ± SE) acceleration values for wild bonefish (F=0.425, p=0.524).

A similarly shaped histogram was generated from bonefish acceleration data from the wetland (Fig. 6.3). The mean acceleration value for bonefish in the wetland was 0.78 ± 0.37 m/s^2. The majority (60%) of acceleration values fell in the ‘low activity’ range (i.e., 0.23-0.78 m/s^2), and bursting composed 0.5% of all readings. Nighttime acceleration values (0.87 ± 0.01 m/s^2; mean ± SE) were higher than daytime acceleration values (0.69 ± 0.01 m/s^2; mean ± SE) (F=70.428, $p<0.05$).

Ethogram study

Seven distinct behaviours were catalogued for bonefish in the wetland mesocosm (Table 6.3). The behaviours consisted of the following categories; stationary, swimming, drifting, bursting, flashing, nose dip, and face wedge (Table 6.3) (Fig. 6.4). Swimming accounted for the highest percentage (51.74%) of observed behaviour, followed by drifting (24.17%), and nose dip (16.96%) behaviours (Table 6.3). The observed bonefish tended to spend more time being stationary at mid-day, and more time bursting during pre-dusk hours (Table 6.3). More time was spent exhibiting nose dip and face wedge behaviours in the morning versus the other two time periods (Table 6.3). The average duration of most types of behaviour was less than 12 s, with only swimming behaviour lasting on average 16 s (Table 6.4). Because individual behaviours were short-lived combined with the nature of how acceleration values are recorded and transmitted, we were unable to assign distinct acceleration signatures to the observed behaviours.

Respirometry study

Respirometry trials occurred at a mean water temperature of 20.6 ± 0.7°C. SMR was determined for 10 bonefish, while MMR_R was measured for seven. The three bonefish that were not measured for MMR_R would not adequately respond to exercise challenges and therefore were not subjected to further experimentation to avoid misrepresentation of data. The lowest recorded SMR was 59.6 mg O_2 kg^{-1} h^{-1}, and the highest SMR was 209.1 mg O_2 kg^{-1} h^{-1}. MMR_R ranged from 234.3 to 362.5 mg O_2 kg^{-1} h^{-1} (Table 6.5). By pairing laboratory-derived resting and bursting bonefish values with SMR and MMR_R, an equation of the line relating the two variables together was generated. The equation of the line is: oxygen consumption = 117.382 + 49.779 * acceleration ($r^2 = 0.745$, $F = 35.019$, $p < 0.0001$). With a mean acceleration of 0.65 m/s^2 for free-swimming bonefish in the wild, mean oxygen consumption is estimated as 149.7 mg O_2 kg^{-1} h^{-1} at 20.6°C. As such, bonefish, on average, were only using 51.6% of their available estimated metabolic scope (i.e., 149.7 mg O_2 kg^{-1} h^{-1}/290.1 mg O_2 kg^{-1} h^{-1} *100). The percentage of available estimated metabolic scope varied somewhat between individuals, but most fish used between 40 and 60% of their estimated scope the majority of time (Fig. 6.5). However, 0.7% of all observations exceeded 90% of estimated metabolic scope.

Discussion

This study was the first attempt to quantify the field activity patterns of free-swimming bonefish in the wild using acoustic tri-axial acceleration transmitters. Because this is one of the few studies that have used acceleration transmitters rather than archival data logger for examining behaviour of wild fishes, it is worth contrasting the two types of electronic tags (Table 6.6). There are some clear disadvantages to using acceleration transmitters rather than data loggers. In particular, with the transmitters used in this study, data are averaged across multiple vectors (3-axes) and over a 25 second sampling time. Given that most bursting activity lasts on the order of seconds, it is not surprising that most of the acceleration data collected consisted of low values. Nonetheless, many fish did ‘max out’ the device in the wild on numerous occasions. The fact that the transmitter reached measurement capacity at bursting is also a limitation in this study, as it is unknown if acceleration values at maximum swimming speeds are actually greater than 3.47 m/s^2 for bonefish. However, this can be remedied in the future by providing the manufacturer with user-defined acceleration ranges. Another constraint with the acceleration transmitter is the proportion of time the transmitter is actually sampling. With the transmitters used in this study, data was recorded for 25 seconds out of an average period of 180 seconds, thus only measuring acceleration approximately 15% of the time. Acceleration data loggers on the other hand allow researchers to collect data on multiple axes at once and detect swimming activity from body undulations; however, loggers must be retrieved to access data and intense sampling frequency can lead to rapid battery depletion (e.g., days as opposed to months with the transmitter). Clearly there is a tradeoff here of which researchers must be aware. Transmitters last longer and data can be received remotely, but the data are not of high resolution. However, in the absence of any fine-scale data on the activity patterns of bonefish, data collected through the use of acceleration transmitters did provide considerable insights. The smallest available archival logger with tri-axial accelerometry sensors are still too big for even the largest bonefish used in our study. Moreover, annually we tend to only recover a small number of animals that we have previously tagged making the use of archival loggers for bonefish an expensive proposition that may yield very little data.

As mentioned above, the majority of acceleration values for bonefish were low (i.e., between 0.23-0.78 m/s^2). While these results may have been influenced by the functional capacity of the transmitters (i.e., averaging over 25 sec period), the field observations are supported by ethogram data from the wetland mesocosm which found bursting activity to represent only 1.22% of total observed behaviour. Using acoustic telemetry, Block et al. (1992) found that blue marlin (*Makaira nigicans*), while capable of high swimming speeds, typically spent most of their time swimming slowly. Weihs

(1984) suggested that the most energy-efficient swimming speed for a fish will be near the low end of their range. Even choosing an intermittent swimming style such as bursting-and-drifting can be energetically advantageous over constant swimming provided that the fish does not ram ventilate (Videler and Weihs, 1982). Indeed, bonefish in the wetland commonly followed up bursting or swimming behaviours with drifting. It is likely this activity pattern also contributed to lower acceleration values observed for bonefish in the wild.

Energetic efficiency plays a role in natural selection, and it has been suggested that a relationship between activity level and mortality may be the basis of that mechanism (Preide, 1977). Because animals need to operate within their scope for activity (Fry, 1947), any time they function at the limits of these bounds, their probability of death increases (Priede, 1977). As such, fish working at moderate power outputs are assumed to have a low probability of mortality due to natural causes (Priede, 1977). Transmitter-implanted bonefish in the wild tended to spend the majority of their time operating at between 40 and 60% of their estimated metabolic scope. Although there clearly are some limitations with how we related acceleration values to oxygen consumption (i.e., assumed a linear relationship between the two variables), we feel that this is a reasonable approach given the linear relationship between metabolism and acceleration documented in cormorants (Wilson et al., 2006), and the linear relationship between oxygen consumption and heart rate documented in fish studies (Preide, 1983; Lucas et al., 1991). We also acknowledge that we have not determined a true maximum metabolic rate since the transmitter not only reached their limit when we observed maximum metabolic rate (i.e., during chasing), but also because bursting is not aerobic, our estimates likely incorporate some element of anaerobic costs not measured here as well as the excess post-exercise oxygen consumption (see Lee et al., 2003). A future study should simultaneously measure acceleration and oxygen consumption of bonefish under controlled conditions in a swim flume equipped with respirometry apparatus to more accurately define the relationship between acceleration and metabolic rate, and the true metabolic scope for activity could be determined.

The lack of difference in activity level between day and night from both our ethology and field acceleration data was not surprising as bonefish movement patterns are more often be associated with tidal cycles (Colton and Alevizon 1983b; Humston et al., 2005; Murchie, unpublished data), and semi-diurnal tides occurred in the study area. No sex-related differences in activity patterns were observed through the use of acceleration transmitters. No differences in movement patterns between the sexes were observed using long-term positional telemetry on bonefish in the study area (Murchie, unpublished data). However, future studies should investigate potential difference in activity patterns of the sexes in offshore locations where bonefish are hypothesized to spawn during winter months (see Danylchuk et al., 2008).

To our knowledge this was the first attempt to catalogue the discrete behaviours typically employed by bonefish. While observations on individuals occurred in a mesocosm environment, we believe these observations likely encompassed many of the behaviours exhibited by bonefish in the wild. When conducting an ethogram it is important not to infer the function of the behaviour as it could be misleading or potentially bias the observer (Martin and Bateson, 1993). After completing the observations however, it can be suggested that swimming, drifting, and bursting are clearly linked to locomotion, while ‘nose dip’ and ‘face wedge’ behaviours are associated specifically with food acquisition. The purpose of flashing behaviour is likely a comfort movement related to body care (Colgan, 1993). Ethograms provide a tool for future comparative studies (e.g., Müller et al., 1998), and are particularly worthwhile when considering costs and benefits of specific behaviours (Grantner and Taborsky, 1998). Future work with either refined acceleration transmitters, or even the use of acceleration archival data loggers, may reveal more details about fish behaviour in the wild (see Sakamoto et al., [2009] for ethograms generated from acceleration data from free-ranging birds).

In general, the information gathered on the behaviour and activity patterns of bonefish provides an effective starting point for the generation of a bioenergetics model for *A. vulpes*, particularly since no efforts have been made to date to generate metabolic data for this species. Production of a bioenergetics model for bonefish would not only provide fisheries managers with a useful tool for understanding bonefish production (Hansen et al., 1993) but would further assist scientists in understanding sub-tropical coastal ecosystem dynamics. Acceleration transmitters have a role in the ecologist’s toolbox for elucidating activity patterns for fish that are not easily monitored, but researchers must understand their limitations prior to conducting studies.

Tables

Table 6.1: Laboratory-derived acceleration values for stationary, swimming, and bursting behaviours in bonefish (419 ± 17 mm total length; mean ± SD) (n = 7). Values are given as the mean (± SD).

Behaviour	Acceleration (m/s^2)
Stationary dead bonefish*	0.06 ± 0.01
Stationary alive bonefish	0.37 ± 0.14
Swimming	0.60 ± 0.18
Bursting	3.47 ± 0.00

*n = 4

Table 6.2: Summary of the tagging, biological, and monitoring data for the 10 wild bonefish implanted with acceleration transmitters and released along the north coast of Cape Eleuthera, The Bahamas, December 8, 2008.

Transmitter ID	Total length (mm)	Sex	Date last detected	# of detections
128	555	Female	15-Dec-08	495
130	578	Female	17-Jan-09	2035
132	475	Male	01-June-09	3453
134	510	Male	11-Dec-08	52
136	520	Male	31-May-09*	2897*
138	590	Female	18-Mar-09	2865
140	520	Female	10-Dec-08	152
142	515	Female	31-May-09	6891
144	495	Male	27-May-09	2766
146	515	Male	31-May-09	4434

*The accelerometer sensor failed in transmitter 136 and was therefore not included in the analyses.

Table 6.3: Summary of the behaviours exhibited by bonefish in the wetland mesocosm, and the total duration, in seconds, that each behaviour was observed during morning, noon, and pre-dusk monitoring periods. Note each observation period was 10 min, and there were 20 observation periods at each time of day.

Behaviour	Description	Morning (7:10-9:20)	Mid-day (11:33-13:13)	Pre-dusk (17:11-18:52)	Total duration (s) and percentage (%) of total observed behaviour
Stationary	not moving horizontally	556	807	446	1809 (5.03%)
Swimming	steady horizontal movement while moving the caudal fin	5990	6193	6444	18 627 (51.74%)
Drifting	gliding through the water without movement of the caudal fin	2914	2979	2807	8700 (24.17%)
Bursting	fast horizontal swim, short duration	83	95	261	439 (1.22%)
Flashing	rolls onto side, get a flash of silver as the sun reflects off of the scales, returns to horizontal position	39	68	95	202 (0.56%)
Nose dip	nose of the fish dips toward the substrate	2326	1849	1930	6105 (16.96%)
Face wedge	face of the bonefish is wedged into the substrate	92	9	17	118 (0.33%)
				Total	360 000 (100%)

Table 6.4: Average duration (± SD) of individual behaviours exhibited by bonefish in the wetland mesocosm, at the Cape Eleuthera Institute in The Bahamas.

Behaviour	Average duration ± SD (s)
Stationary	11.7 ± 8.7
Swimming	16.4 ± 12.3
Drifting	11.01 ± 6.2
Bursting	8.0 ± 3.9
Flashing	7.5 ± 4.3
Nose dip	11.1 ± 7.5
Face wedge	9.1 ± 4.1

Table 6.5: SMR, MMR_R, and estimated metabolic scope for laboratory-acclimated bonefish at 20.6 ± 1.4°C. SMR was calculated as the average of six lowest values recorded between 20:00 and 06:00. MMR_R was determined as the highest value recorded over a six hour recovery period after 4 minutes of exhaustive exercise. The estimated metabolic scope was calculated as the difference between the SMR and MMR_R. Three fish did not exercise after SMR trials so MMR_R, and thus scope could not be determined.

Total length (mm)	Weight (g)	SMR (mg O_2 kg^{-1} h^{-1})	MMR_R (mg O_2 kg^{-1} h^{-1})	Estimated metabolic scope (mg O_2 kg^{-1} h^{-1})
458	810	59.6	234.3	174.8
416	932	117.2	247.2	130.1
430	802	118.0	277.5	159.5
404	508	100.6	280.7	180.1
417	887	186.6	295.3	108.7
330	420	209.1	333.3	124.2
340	348	159.5	362.5	203.0
393	590	135.3		
432	897	161.5		
445	679	74.4		

Table 6.6: A comparison of the utility of acceleration data loggers versus acceleration transmitters. Note that for both loggers and transmitters it is possible to adjust device settings. We have attempted to generalize with approximate comparisons between the daily diary device (Wilson et al., 2007) and the Vemco V9AP-2L used in the current study.

Comparison of	Logger	Transmitter
Sampling frequency for acceleration	Often between 5 and 32 Hz (with variable rates specified for different channels).	Currently samples at 5 Hz but averages over user-determined period (25 seconds in this study).
Data retrieval	Data can only be retrieved when devices are recovered which may not be possible for free-swimming fish, particularly in open systems. Pop-off technology may be employed with the loggers to aid in retrieval.	Data can be transmitted remotely so animals do not need to be recaptured. However, data is only collected when fish is in the range of a hydrophone.
Number of channels	Loggers can typically record data from multiple sensors on different channels simultaneously and store it until analysis. That means that for acceleration data one can record all axes at once as well as data on water temperature, depth, orientation, etc.	Although data can be recorded on multiple channels, some on board processing is required as not all data can be transmitted simultaneously. In the case of the device used in this study, all three axes of acceleration were integrated into a single metric, thus reducing resolution. Additional sensors (e.g., depth or temperature) can be added but that reduces the time that will be devoted to recording and transmitting data on acceleration.
Potential to correlate with metabolic rate	Given that it is possible to detect tail beats from tri-axial accelerometry data, it is possible to establish relationships between tail beats and oxygen consumption.	Potential to correlate with metabolic rate more limited due to the averaging of the transmitter and inability to specifically quantify tailbeats. Note that it is possible to only record one or two axes.
Commercial availability	Available for some.	Yes.
Longevity and	The majority of acceleration	Because of the reduced resolution

size of device	loggers that would work on a large fish (approx 5kg or larger) without burdening the animal would only last for 1 day assuming that resolution is set to record multiple channels at 8 Hz.	in sampling time and the number of channels recorded, the devices can last >150 days.

Figures

Figure 6.1: Study area along the north coast of Cape Eleuthera, Eleuthera, The Bahamas, showing the locations of the 27 hydrophone receivers (black squares), and the location of the Cape Eleuthera Institute research facility (black star). The inset map displays the entire island of Eleuthera with the study area highlighted.

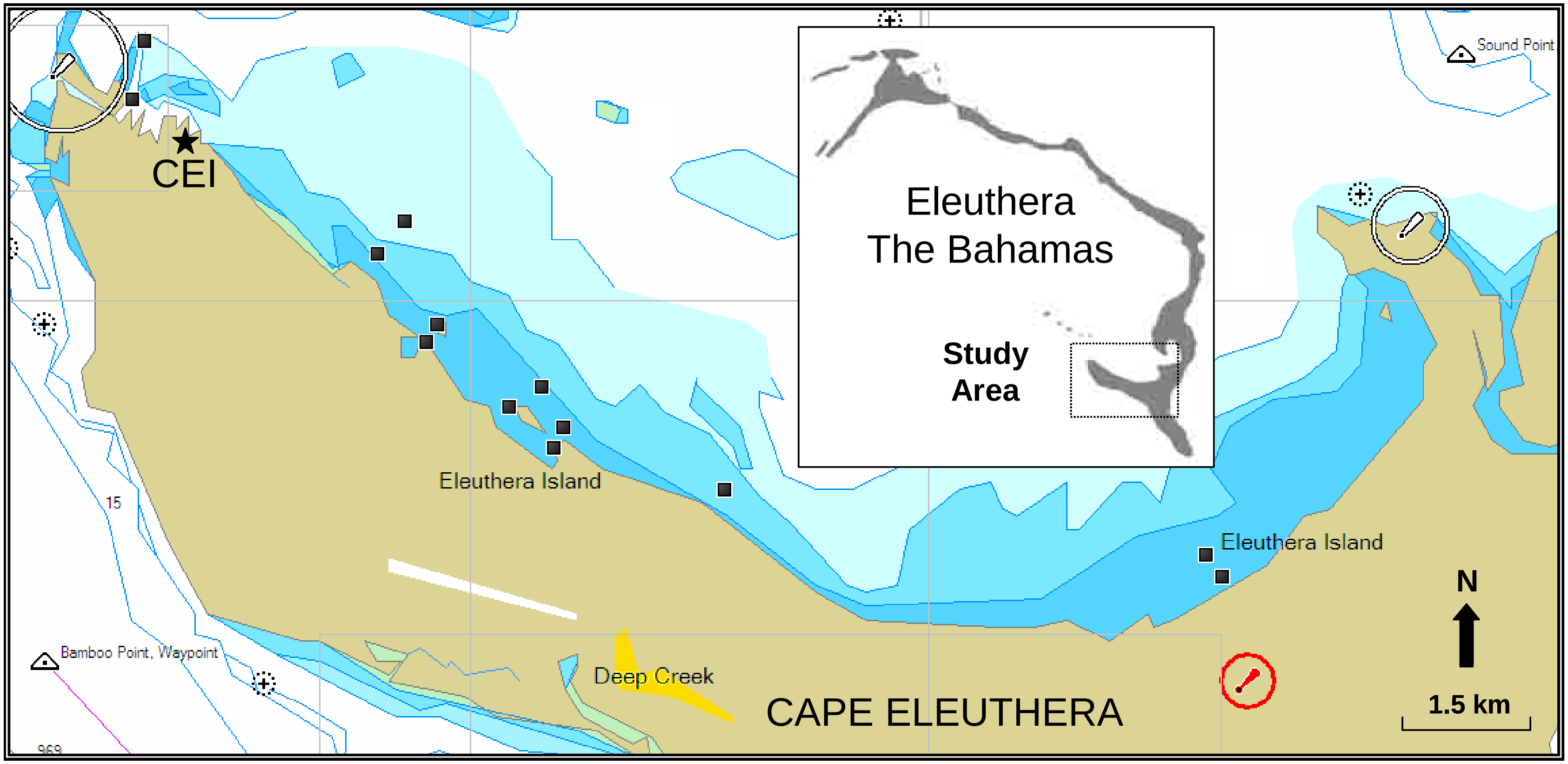


Figure 6.2: Histogram of acceleration activity from free-swimming bonefish in the wild. Values for stationary, low activity, and bursting from laboratory calibrations were overlaid on the histogram for reference.

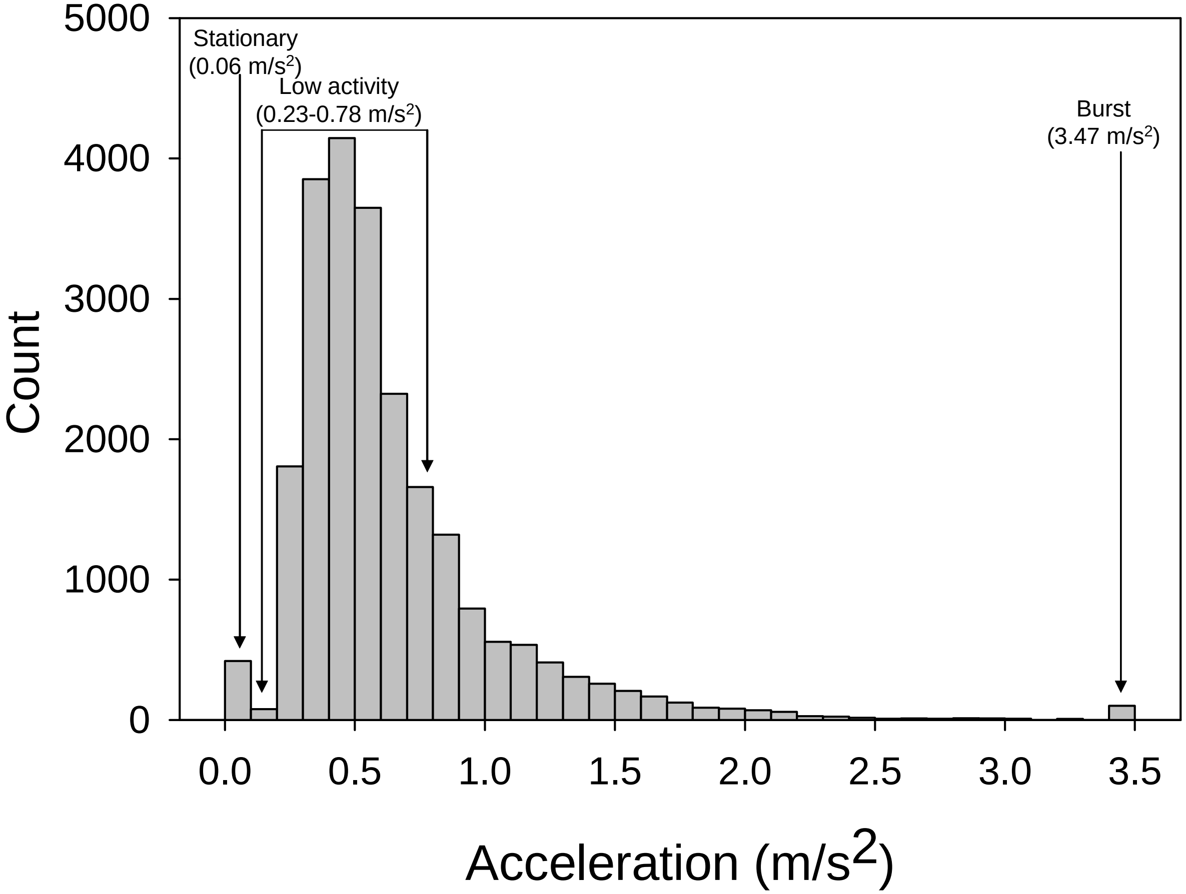


Figure 6.3: Histogram of acceleration activity from free-swimming bonefish in the wetland mesocosm. Values for stationary, low activity, and bursting from laboratory calibrations were overlaid on the histogram for reference.

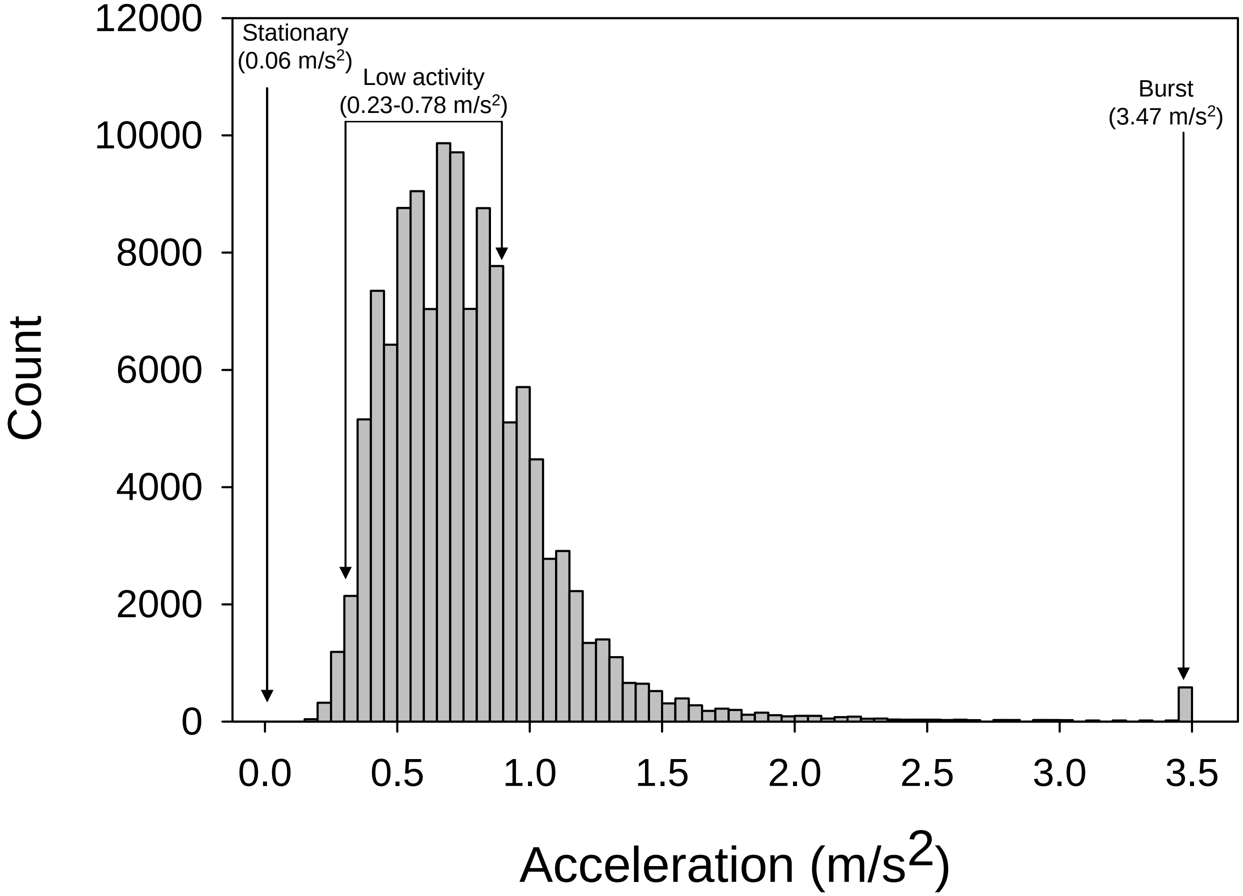


Figure 6.4: Photographs demonstrating a) swimming behaviour (and schooling); b) nose-dip behaviour; and, c) face wedge behaviour of bonefish held in a large wetland mesocosm. See Table 6.3 for a description of each behaviour.

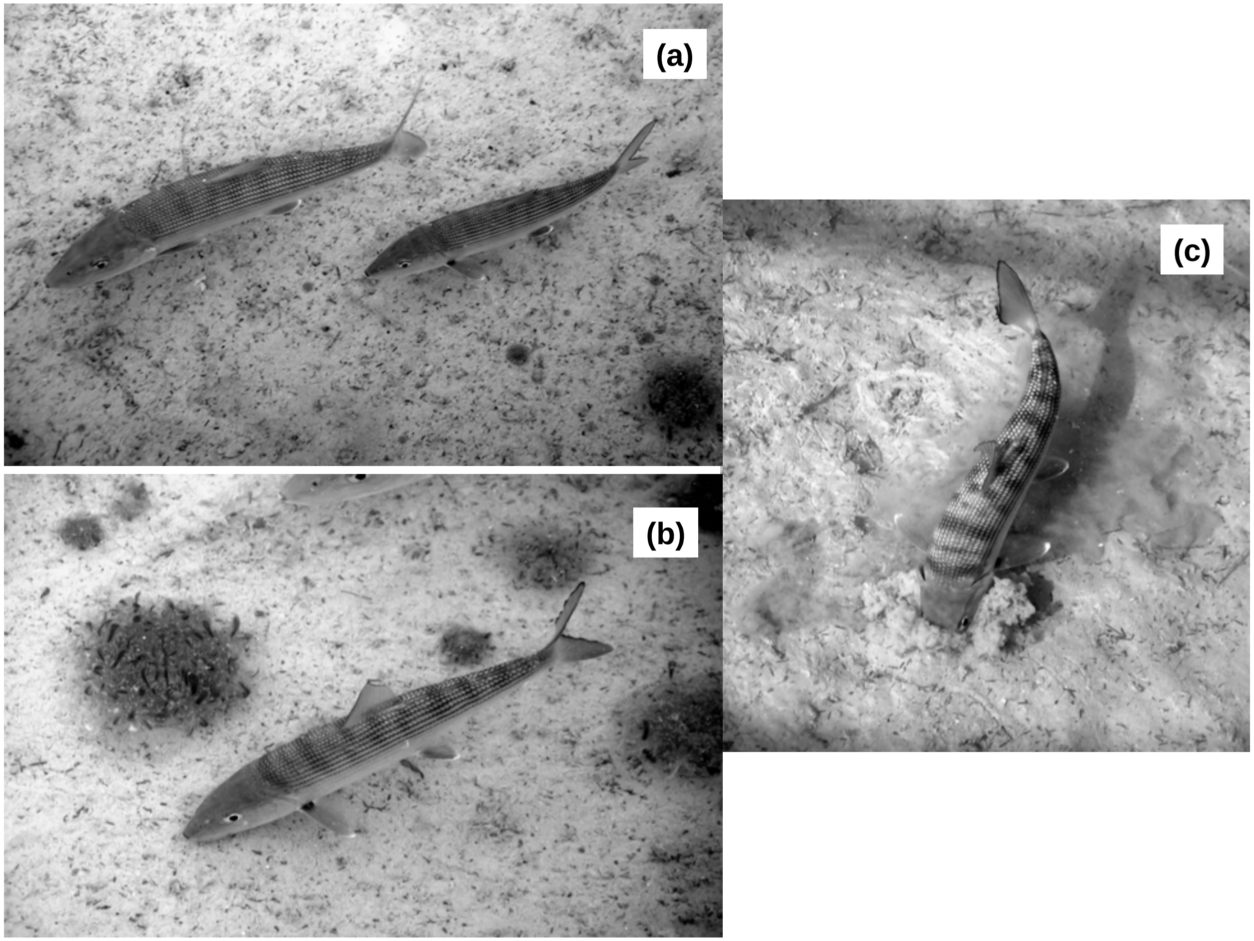
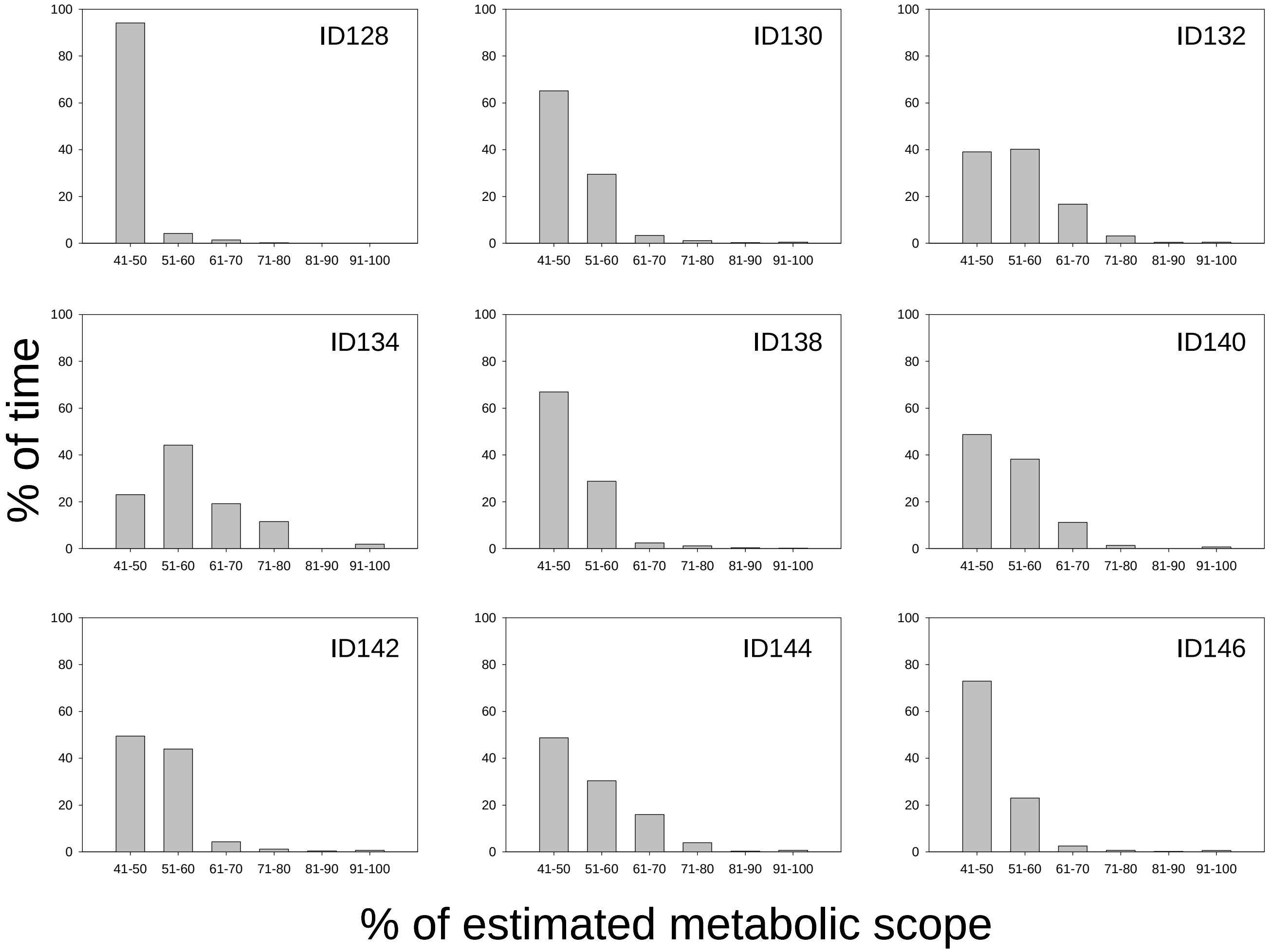


Figure 6.5: Histograms of the percentage of time individual acceleration transmitter-implanted bonefish were at varying percentages of their estimated metabolic scope in the wild.



Chapter 7: General discussion and future research directions

The dearth of knowledge surrounding the physiological ecology and behaviour of fishes in subtropical and tropical tidal flats is an impediment to the management of these coastal marine systems, and limits assessments of their resiliency to future disturbances (see Hughes et al., 2005; Hofmann and Gaines, 2008). With this in mind, the goal of this thesis was to use bonefish (*Albula vulpes*) as a model to determine and describe the environmental relations of fish in tropical tidal flats, and examine how energy is transported through these ecosystems. By using a number of research tools (i.e., blood chemistry analysis, proximate body composition analysis, fish health indices, biotelemetry, ethograms, and respirometry), insight into organismal adaptations to living in stochastic environments has been gained, as well as a mechanistic explanation for the movement of energy between a mosaic of habitats.

Telemetry data from this thesis supports what anglers have long known about bonefish movement patterns; bonefish enter tidal flats and tidal creeks when water levels rise, and leave these areas on the ebb (Chapter 5). Indeed, we used this knowledge to our advantage when we wanted to capture large numbers of individuals for sampling by setting a seine net across the mouth of a creek to intercept fish on an incoming our outgoing tide. There is no question tidal flats ecosystems are important to bonefish. In general, animals should select environments that supply required resources (e.g., food, shelter, mates, etc.) (Matthews, 1990), as selection of optimal habitats should lead to increased reproductive success and survival (Itzkowitz, 1991). Energy intake, refuge from predation, and competitor avoidance are balanced against costs such as energy expenditure, predation risk, and agonistic interactions (Power, 1984; Petty and Grossman, 1996). As indicated by the lack of seasonal differences in whole body or liver lipid content, bonefish appear able to acquire sufficient food from tidal flats and/or creeks year round (Chapter 4). The occasional transient behaviour of bonefish, observed in Chapter 5, may enable fish to assess habitat suitability in other places, or perhaps allow resource recovery in preferred areas (Morrissey and Gruber, 1993b). While bonefish certainly do encounter predators such as lemon sharks and barracuda in these habitats, their risks are likely balanced against the abundance and diversity of predators in offshore areas (i.e., other shark species, sharks of larger size, dolphins).

Bonefish were found to occupy flats habitats that approached their laboratory determined temperature thresholds without apparent significant physiological consequences or mortality, except when exposed to additional stressors (Chapter 3). It is recognized that a fish would not be in a habitat that they were not adapted to (Helfman et al., 2009), and clearly bonefish can withstand the associated temperature fluctuations in the shallow water habitat. Physiological tolerance and behavioral adaptations in combination may be responsible for their ability to exploit backwater portions of tidal creeks when temperatures peak. Indeed, such a combination has been suggested as the mechanism for fishes inhabiting hyperthermal rockpools that have water temperatures meeting or exceeding their thermal tolerances (see Fangue et al., 2001). The synthesis of telemetry and water temperature data from within the backwater portions of tidal creeks revealed that the presence of bonefish in these habitats was positively associated with increasing temperatures. This occurrence is consistent with the need for more food energy to fuel increased metabolic demands associated with warmer temperatures (Hochachka and Somero, 1973).

Maximizing energy efficiency appears to be a key strategy for bonefish. Repeatable movement patterns and periods of site fidelity exhibited by bonefish certainly limit the amount of energy expended for foraging (Chapter 5), and the use of intermittent swimming styles, such as swimming-then-drifting, observed in bonefish is energetically advantageous (Videler and Weihs, 1982) (Chapter 6). Frequent utilization of swimming speeds near the low end of their range in concurrence with swimming-then-drifting behaviours translated into the use of only 40-60% of their estimated metabolic scope (Chapter 6). Given that bonefish are constantly on the move, and are susceptible to predation, their strategy for preferentially storing energy as muscle protein makes sense (Chapter 4). Increased deposition of muscle protein is not only important for structural and performance-related functions such as swimming (Hendry et al., 2000), but is also the most effective method of growth since each gram of protein also binds 3-4 g of water (Jobling, 1994).

In terms of the movement of energy through tidal flats systems, the repeatable movement patterns and occasional forays of bonefish to offshore locales demonstrates the connectivity of these habitats, and provides a mechanism for energy flow. Even the ‘face-wedging’ feeding behaviour observed in the ethogram study of Chapter 6 demonstrates bioturbation which could be important in regulating sediment processes of tidal flats ecosystems. When taken into account that these activities may be performed by hundreds of bonefish in a school, the scale of connectivity and the likelihood of these fish acting as ecosystem service providers (see Holmlund and Hammer, 1999; Kremen, 2005) is high.

While future research objectives were indentified for each individual chapter of the thesis, the overall future research directions to further elucidate the strategies of fish living in tropical tidal flats environments, and the movement of energy through these systems is outlined below. Although useful data has been collected to feed into a bioenergetics model, a complete model can not be assembled at this point. A paired laboratory and field study examining consumption rates of bonefish, along with a field and laboratory study of bonefish growth rates would provide enough data so that the energy lost due to egestion and excretion could be calculated (see Adams and Breck, 1990). A bioenergetics model for bonefish would not only provide more insight into the basis of adaption and behaviour of these flats inhabitants, but could also be used to evaluate the effects of heterogeneous environments resulting from either natural or anthropogenic disturbances to fish growth (Wuenshel et al., 2005). Bioenergetics models are additionally useful for determining fundamental ecological services that organisms provide (e.g., Roth et al., 2004), and are important tools for many facets of fisheries management (e.g., estimation of fisheries production) (Adams and Breck, 1990; Diana, 2004). Manipulative field experiments could include translocation studies of bonefish where fish are captured in one creek and placed in another creek on the same side of the island, or they could be relocated from the Caribbean side of the island to the Atlantic (and vice versa). Translocation studies would be useful for assessing homing behaviour in bonefish, possible shifts in feeding strategies between sites, and general behavioural changes between sites. Experiments where replicated areas within tidal flats are excluded from bonefish access could be used to assess the influence that bonefish have on structuring prey density and abundance. Stable isotope analyses could additionally provide information on the flow of energy through tidal flats ecosystems by assessing aquatic foodweb structures and the feeding ecology of constituent fish populations (Gu et al., 1996; Kwak and Zedler, 1997, Layman et al., 2007). Stable isotope analysis would additionally aid in the demonstration of the bonefish's role in the connectivity of tidal flats with other ecosystems and illustrate specifically where bonefish are feeding while in certain areas. Also, an assessment of the quality and quantity of prey items along a continuum from mangroves, seagrass, sand and algal plains and into the open ocean could help determine whether there is any energetic benefit to feeding within the tidal flats and/or tidal creeks versus offshore. While predation risks to bonefish are perceived to be less within the shallow confines of tidal flats and tidal creek areas, comparisons of predator diversity and abundance could be made between tidal creeks and offshore areas to confirm suspicions. Combined laboratory and field assessments of oxygen tolerances would also be beneficial to understanding the role of other abiotic factors in structuring the distribution of bonefish within the flats. Future research should continue to strive to use field physiology to answer questions about free-swimming fish in the wild (see Goldstein and Pinshow, 2006), particularly through the use of technological advances in telemetry equipment. For example, the use of an overlapping acoustic telemetry array in a number of creek and flats areas would allow for the exact position of a bonefish to be detected. By accompanying each of the receivers with a water depth logger, temperature logger, and dissolved oxygen logger, movements in relation to these abiotic factors could be examined. The use of transmitters equipped with temperature sensors would also provide more specific information about the thermal habitats bonefish are exposed to. Other tidal creek inhabitants such as juvenile lemon sharks, that have been found to be very site fidelic (Morrissey and Gruber, 1993a,b; Murchie et al., unpublished data), could be outfitted with 'business card tags' which employ mobile peer-to-peer technology (see Holland et al., 2009) that allow the tagged sharks to exchange codes among each other and detect other tagged fish with one-way coded acoustic transmitters (i.e., bonefish). The use of these tags could provide insights into the timing, frequency, and duration of interactions between individuals, allowing assessments of both intra- and interspecific behaviours (Holland et al., 2009).

Although the physiological ecology and behaviour of bonefish were the focus of this thesis, it is likely that many similar strategies are employed by other fishes in the tidal flats, given that few fish species can use these habitats exclusively due to tidal processes (see Sheaves, 2005; Mumby, 2006). This is evidenced by the by-catch in the seine net when sampling for bonefish. Mullet, schoolmaster snapper, juvenile lemon sharks, barracuda, needlefish, mojarra, and checkered puffer are all common catch in tidal creeks on an outgoing tide (Murchie, personal observation). All of these species would be exposed to similar abiotic conditions as bonefish while occupying these areas. While feeding strategies vary, the basic need to balance energetic costs versus gains while in the flats is the same. With that said, I would encourage more research into other species of fish occupying these ecosystems as there will likely be some subtle and some more drastic differences in strategies. Certainly the thermal tolerance experiments and proximate body composition analyses could easily be repeated on some of the abundant smaller-bodied fish such as the checkered puffer and mojarra to contrast results to bonefish. Also, smaller-bodied fish may be better suited to laboratory experimentation over bonefish which required a labour-intensive capture and laboratory acclimation process. Adding to the knowledge base for fish species in subtropical and tropical tidal flats in general will also enhance the comparisons of collected data to relevant species data, rather than having to draw from freshwater or temperate fish studies which commonly occurred in this dissertation.

In closing, the knowledge gained from the individual studies not only expanded our knowledge on the energetic strategies of tropical and subtropical flats inhabitants, but specifically feeds into conservation and management strategies of bonefish. Clearly tropical tidal flats ecosystems are important habitats for bonefish and require protection to maintain the populations of these economically important sportfish. Such protection could come in the form of Marine Protected Areas, which would at least benefit sub-adult and adult stages of bonefish. Given the decline of fish stocks, particularly noted in Florida (Ault et al., 2008), the information gained of general aquaculture practices of bonefish (Chapter 1) could be useful for potential stock enhancement. Also, the combined effects of handling and temperature stress on bonefish has direct implications for catch-and-release angling during the warmer months, and a potential closed season for bonefishing should be considered to help protect the fishery.

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