Aquaculture Research

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

Journal:	*Aquaculture Research*
Manuscript ID:	ARE-OA-08-Oct-583.R1
Manuscript Type:	Original Article
Date Submitted by the Author:	
Complete List of Authors:	Murchie, Karen; Carleton University, Biology Danylchuk, Sascha; Cape Eleuthera Institute Pullen, Chris; Carleton University, Biology Brooks, Edd; Cape Eleuthera Institute Shultz, Aaron; Cape Eleuthera Institute Suski, Cory; University of Illinois, Department of Natural Resources and Environmental Sciences Danylchuk, Andy; Cape Eleuthera Institute Cooke, Steven; Carleton University, Biology
Keywords:	bonefish, holding, physiology, stress, transport

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

Running head: Capture, transport, and long-term holding of bonefish

Karen J. Murchie[1,2*], Sascha E. Danylchuk[2], Christopher E. Pullen[1], Edd Brooks[2], Aaron D. Shultz[2], Cory D. Suski[2,3], Andy J. Danylchuk[1,2], and Steven J. Cooke[1,2,4]

Deleted: ,3

Deleted: 4

Deleted: 5

[1]Fish Ecology and Conservation Physiology Laboratory, Department of Biology, Carleton University, Ottawa, ON, Canada

[2]Flats Ecology and Conservation Program, Cape Eleuthera Institute, Eleuthera, The Bahamas

[3]Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana, IL, USA

Deleted: [3]Bonefish and Tarpon Unlimited, Islamorada, FL, USA¶ [4]

[4]Institute of Environmental Science, Carleton University, Ottawa, ON, Canada

Deleted: [5]Institute

K.J.M., Fish Ecology and Conservation Physiology Laboratory, Department of Biology, Carleton University, 1125 Colonel By Dr., Ottawa, Ontario, Canada K1S 5B6. Telephone: 613-520-2600 ext. 4377. Email: <kmurchie@connect.carleton.ca>

S.E.D., Cape Eleuthera Institute, 498 SW 34th St, Ft. Lauderdale, Florida, USA, 33315. Telephone: 242-359-7625. Email: <saschaclark@ceibahamas.org>

C.E.P., Fish Ecology and Conservation Physiology Laboratory, Department of Biology, Carleton University, 1125 Colonel By Dr., Ottawa, Ontario, Canada K1S 5B6. Telephone: 613-520-2600 ext. 4377. Email: <cpullen@golder.com>

E.B., Cape Eleuthera Institute, 498 SW 34th St, Ft. Lauderdale, Florida, USA, 33315. Telephone: 242-359-7625. Email: <eddbrooks@ceibahamas.org>

A.D.S., Cape Eleuthera Institute, 498 SW 34th St, Ft. Lauderdale, Florida, USA, 33315. Telephone: 242-359-7625. Email: <aaronshultz@ceibahamas.org>

C.D.S., Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, 1102 S. Goodwin Ave., Urbana, Illinois, USA, 61801. Telephone: 217-244-2237. Email: <suski@uiuc.edu>

A.J.D., Cape Eleuthera Institute, 498 SW 34th St, Ft. Lauderdale, Florida, USA, 33315. Telephone: 242-359-7625. Email: <andydanylchuk@ceibahamas.org>

S.J.C., Fish Ecology and Conservation Physiology Laboratory, Department of Biology and Institute of Environmental Science Carleton University, 1125 Colonel By Dr., Ottawa, Ontario, Canada K1S 5B6. Telephone: 613-520-2600 ext. 4377. Email: <steven_cooke@carleton.ca>

Deleted: ,

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.

Deleted: Indeed, there is little known on this topic for any wild tropical or subtropical marine fish species.

Keywords: bonefish; holding; physiology; stress; transport

Introduction

In recent years, the apparent world-wide decline in marine fish populations (e.g., Pauly, Alder, Bennett, Christensen, Tyedmers & Watson 2003; Pauly, Watson & Alder 2005; Worm, Barbier, Beaumont, Duffy, Folke, Halpern, Jackson, Lotze, Micheli, Palumbi, Sala, Selkoe, Stachowicz & Watson 2006) has renewed interest in the development of techniques for holding fish in captivity to enable culture for wild stock enhancement (e.g., Blankenship & Leber 1995; Leber 2004; Bell, Bartley, Lorenzen & Loneragan 2006; True, Loera & Castro 1997), captive food production (i.e., mariculture; De Silva 1998; Naylor, Goldburg, Primavera, Kautsky, Beveridge, Clay, Folke, Lubchenco, Mooney, & Troell. 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, Thomas, Arnold & Trant 1987; Garcia, Hilomen-Garcia & Emata 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, Woodley & Cech 2006; Hur, Park & Chang 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, Forsyth, Mazur & Iwama 2000; Barton 2000), and a range of temperate, non-salmonid freshwater species (e.g., Pankhurst, Wells & Carragher 1992; Waring, Stagg & Poxton 1996), with proportionately fewer data on tropical and sub-tropical fish (De Silva 1998; Grutter & Pankhurst 2000; Biswas, Seoka, Takii, Maita & Kumai 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, Bénech & Marmulla 2002). Furthermore, marine stock enhancement and mariculture are considered challenging and knowledge is not as advanced as for freshwater taxa (De Silva 1998; Leber, Kitada, Svåsand & Blankenship 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 & Alevizon 1983; Pfeiler, Pardon & Crabtree 2000). Estimates suggest that recreational angling for bonefish is a billion dollar per year industry in the Florida Keys alone (Humston 2001). Bonefishing can easily support the economy of coastal communities in small island nations such

Deleted: ; Humston 2001

Deleted: Estimates in Florida suggest that each bonefish has a lifetime value of $75 000 (Ault, Moret, Luo, Larkin, Zurcher & Smith 2006), although this fails to consider the ecological services that they may provide (Costanza, d’Arge, de Groot, Farber, Grasso, Hannon, Limburg, Naeem, O’Neill, Paruelo, Raskin, Sutton & van den Belt 1997; Holmlund & Hammer 1999). Recreational angling for bonefish as The Bahamas, where tourism is responsible for 60% of the gross domestic product (Buchan 2000; Danylchuk, Danylchuk, Cooke, Goldberg, Koppelman & Philipp 2008). Despite their recognized economic value, very little is known about the ecology, physiology, or population dynamics of bonefish (Ault, Humston, Larkin, Perusquia, Farmer, Luo, Zurcher, Smith, Barbieri & Posada 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 & 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 & 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 & Pinshow 2002; Costa & 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, Snodgrass & Harnden (1998) 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. 1). Preliminary genetic analyses on bonefish from this area indicated that all bonefish specimens were *Albula vulpes* (Danylchuk, Danylchuk, Cooke, Goldberg, Koppelman & Philipp 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 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 & 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

Deleted: (S. Cooke, Carleton University, unpubl. data).

have been previously validated as a reliable tool for fish field physiology (Venn Beecham, Small & Minchew 2006; Mandelman & Farrington, 2007) and specifically for bonefish (Cooke, Suski, Danylchuk, Danylchuk, Donaldson, Pullen, Bulté, O'Toole, Murchie, Koppelman, Shultz, Brooks & Goldberg 2008).

Deleted: In press

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 & 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, Cooke, Suski & Philipp 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. Shade^{TM}, 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

Deleted: netted from the holding tank 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.

Deleted: where they

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 & 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 & 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). 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), 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 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 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 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). 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 5), however Chi-square analysis revealed no significant differences (P's>0.05) between the treatment groups.

Deleted: observations of behavioural and physical abnormalities occurred. N

Deleted:

Deleted: occurred during the study

Deleted:

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.

Deleted: Guided tours through the CEI holding facility or occupation by CEI staff only allowed for minimal disturbance to the tanks.

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.

Deleted: for long periods of time

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, Afonso & Vijayan 2006). The level of hyperglycemia detected in moribund bonefish is below measured values for exercised bonefish (Suski, Cooke, Danylchuk, O'Connor, Gravel, Redpath, Hanson, Gingerich, Murchie, Danylchuk & Goldberg 2007). Control values for glucose in this study (4.2 mmol l^{-1}) were similar to those reported for bonefish by Friedlander, Caselle, Beets, Lowe, Bowen, Ogawa, Kelley, Clitri, Lange & Anderson (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, Cooke, McKinley & Moccia. 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, Wendelaar Bonga & Balm, 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, Van Puymbroeck, Van Den Sande & Vanderborght 1990; Pearson & Stevens 1991). Frisch & 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.

Fish exposed to stress commonly exhibit changes in physical appearance (e.g., True *et al.* 1997) and behaviour (Huntingford, Adams, Braithwaite, Kadri, Pottinger, Sandøe & Trunbull 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 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, Brobbel, Davidson, Forsyth, & Tufts 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.

Acknowledgements

We gratefully acknowledge C. Maxey and the staff, students, and volunteers of the Cape Eleuthera Institute and The Island School for logistical support and assistance with field work and tank maintenance. In particular, S. Langosch, G. Walton, A. Oronti, T. Voorhees, K. Svelling, C. Dziuba, T. Ambrecht, M. Ayres, P. Bassett, S. Calvin, J. Lord, and S. Thompson. We also thank other research staff including D. Philipp, J. Koppelman, J. Claussen, M. Philipp, M. Philipp, G. Bulté, M. Donaldson, A. Gingerich, M. Gravel, K. Hanson, C. O'Connor, A. O'Toole, and T. Redpath. Thanks to W. Nalley for designing and manufacturing the fish cradle. J. Linder (Aventix Canada) provided training on the i-STAT and J. Mandelman shared his experience using field diagnostic tools. This project was supported by a grant from Bonefish and Tarpon Unlimited and the Charles A. and Anne Morrow Lindbergh Foundation. Additional financial support was provided by the Canadian Foundation for Innovation, the Ontario Research Fund, Carleton University, the Cape Eleuthera Foundation, and the University of Illinois. K. Murchie was supported by a Natural Sciences and Engineering Research Council CGSD fellowship. We also thank B. Richards (Scientific Anglers), J. Shulin (Temple Fork Outfitters), and The Bahamas Department of Marine Resources for their support.

References

Ackerman, P. A., Forsyth, R. B., Mazur, C. F. & Iwama, G. K. (2000). Stress hormones and the cellular stress response in salmonids. *Fish Physiology and Biochemistry* **23**, 327-336.

Ault, J. S. (2008). The world biology of tarpon and bonefish. CRC Press, Boca Raton.

Ault, J. S., Humston, R., Larkin, M. F., Perusquia, E., Farmer, N. A., Luo, J., Zurcher, N., Smith, S. G., Barbieri, L. R. & Posada, J. M. (2008). Population dynamics and resource ecology of Atlantic tarpon and bonefish. In: *The world biology of tarpon and bonefish*. (ed. by J. S. Ault), pp. 217-258. CRC Press, Boca Raton.

Baras, E., Bénech, V. & Marmulla, G. (2002). Outcomes of a pilot fish telemetry workshop for developing countries. *Hydrobiologia* **483**, 9-11.

Deleted: Ault, J. S., Moret, S., Luo, J., Larkin, M. F., Zurcher, N. & Smith, S. G. (2006). Florida Keys bonefish population census. Rosentiel School of Marine and Atmospheric Science, Miami, Florida.¶
¶

Barthel, B. L., Cooke, S. J., Suski, C. D. & Philipp, D. P. (2003). Effects of landing net mesh type on injury and mortality in a freshwater recreational fishery. *Fisheries Research* **63**, 275-282.

Barton, B. A. (2000). Salmonid fishes differ in their cortisol and glucose responses to handling and transport stress. *North American Journal of Aquaculture* **62**, 12-18.

Bell, J. D., Bartley, D. M., Lorenzen, K. & Loneragan, N. R. (2006). Restocking and stock enhancement of coastal fisheries: potential, problems and progress. *Fisheries Research* **80**, 1-8.

Biswas, A. K., Seoka, M., Takii, K., Maita, M. & Kumai, H. (2006). Stress response of red sea bream *Pagrus major* to acute handling and chronic photoperiod manipulation. *Aquaculture* **252**, 566-572.

Blankenship, H. L. & Leber, K. M. (1995). A responsible approach to marine stock enhancement. *American Fisheries Society Symposium* **15**, 167-175.

Brett, J. R. (1995). Energetics In: *Physiological ecology of Pacific salmon.* (ed. by C. Groot, L. Margolis, & W. C. Clarke), pp. 1-68. UBC Press, Vancouver.

Bruger, G. E. & Haddad, K. D. (1986). Management of tarpon, bonefish, and snook in Florida. In: *Multi-jurisdictional management of marine fisheries*. (ed. by R. H. Stroud), pp. 53-57. National Coalition for Marine Conservation Inc)

Buchan, K. C. (2000). The Bahamas. *Marine Pollution Bulletin* **41**, 94-111.

Casselman, S. J. (2005). Catch-and-release angling: a review with guidelines for proper fish handling practices. Fish and Wildlife Branch, Ontario Ministry of Natural Resources. Peterborough, Ontario.

Chandroo, K. P., Cooke, S. J., McKinley, R. S. & Moccia, R. D. (2005). Use of electromyogram telemetry to assess the behavioural and energetic responses of rainbow trout, *Oncohynchus mykiss* (Walbaum) to transportation stress. *Aquaculture Research* **36**, 1226-1238.

Colton, D. E. & Alevizon, W. S. (1983). Feeding ecology of bonefish in Bahamian waters. *Transactions of the American Fisheries Society* **112**, 178-184.

Cooke, S. J., Suski, C. D., Danylchuk, S. E., Danylchuk, A. J., Donaldson, M. R., Pullen, C., Bulté, G., O'Toole, A., Murchie, K. J., Koppelman, J. B., Shultz, A. D., Brooks, E. & Goldberg, T. L. (2008). Effects of different capture techniques on the physiological condition of bonefish *Albula vulpes* evaluated using field diagnostic tools. *Journal of Fish Biology* **73**, 1351-1375.

Deleted:

Deleted: In press

Deleted: (

Deleted:)

Deleted: physiology

Deleted: 00

Deleted: 000-000

Deleted: 1

Cooke, S. J. & Philipp, D. P. (2004). Behavior and mortality of caught-and-released bonefish (*Albula* spp.) in Bahamian waters with implications for a sustainable recreational fishery. *Biological Conservation* **118**, 599-607.

Costa, D. P. & Sinervo, B. (2004). Field physiology: physiological insights from animals in nature. *Annual Review of Physiology* **66**, 209-238.

Crabtree, R. E., Snodgrass, D. & Harnden, C. (1998). Survival rates of bonefish, *Albula vulpes*, caught on hook-and-line gear and released based on capture and release of captive fish in a pond in the Florida Keys. In: *Investigation into nearshore and estuarine gamefish abundance, ecology and life history in Florida, Five year Technical Report to the US Fish and Wildlife Service, Sport Fish Restoration Project F-59*. pp. 252-254. Florida Marine Research Institute, St. Petersburg.

Deleted: Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R. V., Paruelo, J., Raskin, R. G., Sutton, P. & van den Belt, M. (1997). The value of the world's ecosystem services and natural capital. *Nature* **387**, 253-260.¶
¶

Danylchuk, A. J., Danylchuk, S. E., Cooke, S. J., Goldberg, T. L., Koppelman, J. & Philipp, D. P. (2008). Ecology and management of bonefish (*Albula spp*) in the Bahamian Archipelago. In: *The world biology of tarpon and bonefish*. (ed. by J. S. Ault), pp. 73-92. CRC Press, Boca Raton.

Danylchuk, A. J., Danylchuk, S. E., Cooke, S. J., Goldberg, T. L., Koppelman, J. & Philipp, D. P. (2007a). Post-release mortality of bonefish (*Albula vulpes*) exposed to different handling practices during catch-and-release angling in South Eleuthera, Bahamas. *Fisheries Management and Ecology* **14**, 149-154.

Danylchuk, S. E., Danylchuk, A. J., Cooke, S. J., Goldberg, T. L., Koppelman, J. & Philipp, D. P. (2007b). Effects of recreational angling on the post-release behavior and predation of bonefish

(*Albula vulpes*): the role of equilibrium status at the time of release. *Journal of Experimental Marine Biology and Ecology* **346**, 127-133.

Day, R. W. & Quinn, G. P. (1989). Comparisons of treatments after an analysis of variance in ecology. *Ecological Monographs* **59**, 433-463.

De Silva, S.S. (1998). Tropical mariculture: current status and prospects. In: *Tropical mariculture*. (ed. by S. S. De Silva), pp. 1-16. Academic Press, London.

Friedlander, A. M., Caselle, J. E., Beets, J., Lowe, C. G., Bowen, B. W., Ogawa, T. K., Kelley, K. M., Clitri, T., Lange, M. & Anderson, B. S. (2008). Biology and ecology of the recreational bonefish fishery at Palmyra Atoll National Wildlife Refuge with comparisons to other Pacific islands. In: *The world biology of tarpon and bonefish*. (ed. by J. S. Ault), pp. 27-56. CRC Press, Boca Raton.

Frisch, A. J. & Anderson, T. A. (2000). The response of coral trout (*Plectropomus leopardus*) to capture, handling and transport and shallow water stress. *Fish Physiology and Biochemistry* **23,** 23-34.

Garcia, L. M. B., Hilomen-Garcia, G. V. & Emata, A. C. (2000). Survival of captive milkfish *Chanos chanos* Forsskal broodstock subjected to handling and transport. *Aquaculture Research* **31**, 575-583.

Goldstein, D. L. & Pinshow, B. (2002). Taking physiology to the field: using physiological approaches to answer questions about animals in their environments. *Physiological Biochemistry and Zoology* **79**, 237-241.

Grutter, A. S. & Pankhurst, N. W. (2000). The effects of capture, handling, confinement and ectoparasite load on plasma levels of cortisol, glucose and lactate in the coral reef fish *Hemigymnus melapterus. Journal of Fish Biology* **57**, 391-401.

Humston, R. (2001). Development of movement models to assess the spatial dynamics of fish populations. PhD Thesis, Rosentiel School of Marine and Atmospheric Science, University of Miami.

Huntingford, F. A., Adams, C., Braithwaite, V. A., Kadri, S., Pottinger, T. G., Sandøe, P. & Trunbull, J. F. (2006). Current issues in fish welfare. *Journal of Fish Biology* **68**, 332-372.

Hur, J. W., Park, I.-S. & Chang, Y. J. (2007). Physiological responses of the olive flounder, *Paralichthys olivaceus*, to a series stress during the transportation process. *Ichthyological Research* **54,** 32-37.

Iwama, G. K., Afonso, L. O. B. & Vijayan, M. M. (2006). Stress in fishes. In: *The physiology of fishes. 3rd edn*. (ed. by D. H. Evans & J. B. Caiborne), pp. 319-242. CRC Press, Boca Raton.

Larson, L. L. (1995). A portable restraint cradle for handling large salmonids. *North American Journal of Fisheries Management* **15**, 654-656.

Leber, K. M. (2004). Marine stock enhancement in the USA: status, trends and needs. In: *Stock enhancement and sea ranching: developments, pitfalls and opportunities. 2nd edn.* (ed. by K. M. Leber, S. Kitada, T. Svåsand, & H. L. Blankenship), pp. 11-24. Blackwell Scientific Publications, Oxford.

Leber, K. M., Kitada, S., Svåsand, T. & Blankenship, H. L. (2004). *Enhancement and sea ranching: developments, pitfalls and opportunities. 2nd edn.* Blackwell Scientific Publications, Oxford.

Mandelman, J. W. & Farrington, M. A. (2007). The estimated short-term discard mortality of trawled elasmobranch, the spiny dogfish (*Squalus acanthias*). *Fisheries Research* **83,** 238-245.

Maule, A. G., Schreck, C. B., Bradford, C. S. & Barton., B. A. (1988). Physiological effects of collecting and transporting emigrating juvenile chinook salmon past dams on the Columbia River. *Transactions of the American Fisheries Society* **117**, 245-261.

Deleted: ~~Holmlund, C. M. & Hammer, M. (1999). Ecosystem services generated by fish populations. *Ecological Economics* **29**, 253-268.¶~~
¶

Naylor, R. L., Goldburg, R. J., Primavera, J. H., Kautsky, N., Beveridge, M. C. M., Clay, J., Folke, C., Lubchenco, J., Mooney, H. & Troell, M. (2000). Effect of aquaculture on world fish supplies. *Nature* **405**, 1017-1024.

Pankhurst, N. W., Wells, R. M. G. & Carragher, J. F. (1992). Effects of stress on plasma cortisol levels and blood viscosity in blue mao mao, *Scorpis violaceus* (Hutton), a marine teleost. *Comparative Biochemistry and Physiology A* **101**, 335-339.

Pauly, D., Alder, J., Bennett, E., Christensen, V., Tyedmers, P. & Watson, R. (2003). The future for fisheries. *Science* **302**, 1359-1361.

Pauly, D., Watson, R. & Alder, J. (2005). Global trends in world fisheries: impacts on marine ecosystems and food security. *Philosophical Transactions of the Royal Society of London B* **360**, 5-12.

Pearson, M. P. & Stevens, E. D. (1991). Size and hematological impact of the splenic erythrocyte reservoir in rainbow trout, *Onchorynchus mykiss. Fish Physiology and Biochemisty* **9**, 39-50.

Pfeiler, E., Pardon, D. & Crabtree, R. E. (2000). Growth rate, age and size of bonefish from the Gulf of California. *Journal of Fish Biology* **56**, 448-453.

Portz, D. E., Woodley, C. M. & Cech, J. J. (2006). Stress-associated impacts of short-term holding on fishes. *Reviews in Fish Biology and Fisheries* **16**, 125-170.

Robertson, L., Thomas, P., Arnold, C. R. & Trant, J. M. (1987). Plasma cortisol and secondary stress responses of red drum to handling transport, rearing density, and a disease outbreak. *Progressive Fish Culturist* **49**, 1-12.

Ruane, N. M., Wendelaar Bonga, S. E. & Balm, P. H. M. (1999). Differences between rainbow trout and brown trout in the regulation of the pituitary-interrenal axis and physiological performance during confinement. *General and Comparative Endocrinology* **115**, 210-219.

Sokal, R. R. & Rohlf, F. J. (1995). *Biometry. 3rd edn*. W. H. Freeman & Company, New York.

Suski, C. D., Cooke, S. J., Danylchuk, A. J., O'Connor, C., Gravel, M., Redpath, T., Hanson, K. C., Gingerich, A., Murchie, K. J., Danylchuk, S. E. & Goldberg, T. L. (2007). Physiological disturbance and recovery dynamics of bonefish (*Albula vulpes*), a tropical marine fish, in response to variable exercise and air exposure. *Comparative Biochemistry and Physiology A.* **148,** 664-673.

True, C. D., Loera, A. S. & Castro, N. C. (1997). Acquisition of broodstock of *Totoaba macdonaldi*: field handling, decompression, and prophylaxis of an endangered species. *Progressive Fish Culturist* **59**, 246-248.

Venn Beecham, R. B., Small, C. & Minchew, C. D. (2006). Using portable lactate and glucose meters for catfish research: acceptable alternatives to established laboratory methods? *North American Journal of Aquaculture* **68**, 291–295.

Waring, C. P., Stagg, R. M. & Poxton, M. G. (1996). Physiological responses to handling in the turbot. *Journal of Fish Biology* **48**, 161-173.

Wedemeyer, G. A. (1996). *Physiology of fish in intensive culture systems.* Chapman &Hall, New York.

Wendelaar Bonga, S. E. (1997). The stress response of fish. *Physiological Reviews* **77,** 591-625.

Wilkie, M. P., Brobbel, M. A., Davidson, K., Forsyth, L. & Tufts, B. L. (1997). Influences of temperature upon the postexercise physiology of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 503-511.

Witters, H. E., Van Puymbroeck, S., Van Den Sande, I. & Vanderborght, O. L. J. (1990). Haematological disturbances and osmotic shifts in rainbow trout, *Oncorynchus mykiss* (Walbaum) under acid and aluminum exposure. *Journal of Comparative Physiology B* **160**, 563-571.

Wood, C.M. (1991). Acid-base and ion balance, metabolism, and their interactions, after exhaustive exercise in fish. *Journal of Experimental Biology* **160**, 285-308.

Worm, B., Barbier, E. B., Beaumont, N., Duffy, J. E., Folke, C., Halpern, B. S., Jackson, J. B. C., Lotze, H. K., Micheli, F., Palumbi, S. R., Sala, E., Selkoe, K., Stachowicz, J. J. & Watson, R. (2006). Impacts of biodiversity loss on ocean ecosystem services. *Science* **314**, 787-790.

Box 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, Schreck, Bradford & Barton 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 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. 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)
February 17	Plum Creek	24	3.2 cm mesh	5	0	coolers	60
February 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]
February 19	Starved Creek	23	3.2 cm and 0.6 cm	8	0	flow-through cage	60
February 20	Kemps Creek	21	3.2 cm and 0.6 cm	70	0	flow-through cage	45
February 23	Broad Creek	21	3.2 cm and 0.6 cm	3	0	flow-through cage	45
February 23	Half Sound	22.5	3.2 cm and 0.6 cm	47	0	flow-through cage	100[3]
March 16	Broad Creek	22.5	3.2 cm and 0.6 cm	21	0	flow-through cage	120[4]

Deleted: *

Formatted: Superscript

Deleted: **

Formatted: Superscript

Deleted: ***

Formatted: Superscript

Deleted: ****

Formatted: Superscript

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

Formatted: Superscript

Deleted: *

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

Formatted: Superscript

Deleted: **

[3] approximately 650 m from seining location to truck

Formatted: Superscript

Deleted: ***

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

Deleted: ****

Formatted: Superscript

Table 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
February 17	Plum Creek	Truck	3	25	0	Half of the trip on un-paved roads, half of the trip on poorly maintained paved roads
February 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.
February 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.
February 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
February 23	Broad Creek	Boat	20	20	0	Frequent water changes in the coolers on the way
February 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.
March 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).

Deleted: *

Formatted: Superscript

Deleted: *

Formatted: Superscript

Table 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
February 17	Plum Creek	5	0	Fish used for other physiological experiments and euthanized within 5 d of capture
February 18	Starved Creek	41	33	Remaining fish held
February 19	Starved Creek	8	0	Three fish used for other physiological experiments and euthanized within 5 d of capture. Remaining fish held
February 20	Kemps Creek	70	0	Fish used for used for other physiological experiments and euthanized within 5 d of capture. Remaining fish held
February 23	Broad Creek	3	0	Remaining fish held
February 23	Half Sound	47	6	Fish used for other physiological experiments and euthanized within 5 d of capture. Remaining fish held
March 16	Broad Creek	21	0	Fish used for handling experiment included in this study

Table 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 %

Figure Captions

Fig. 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 for details).

Fig. 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 were 7 in each treatment except 12 for the moribund fish and 8 for the holding tank.

Fig. 1.

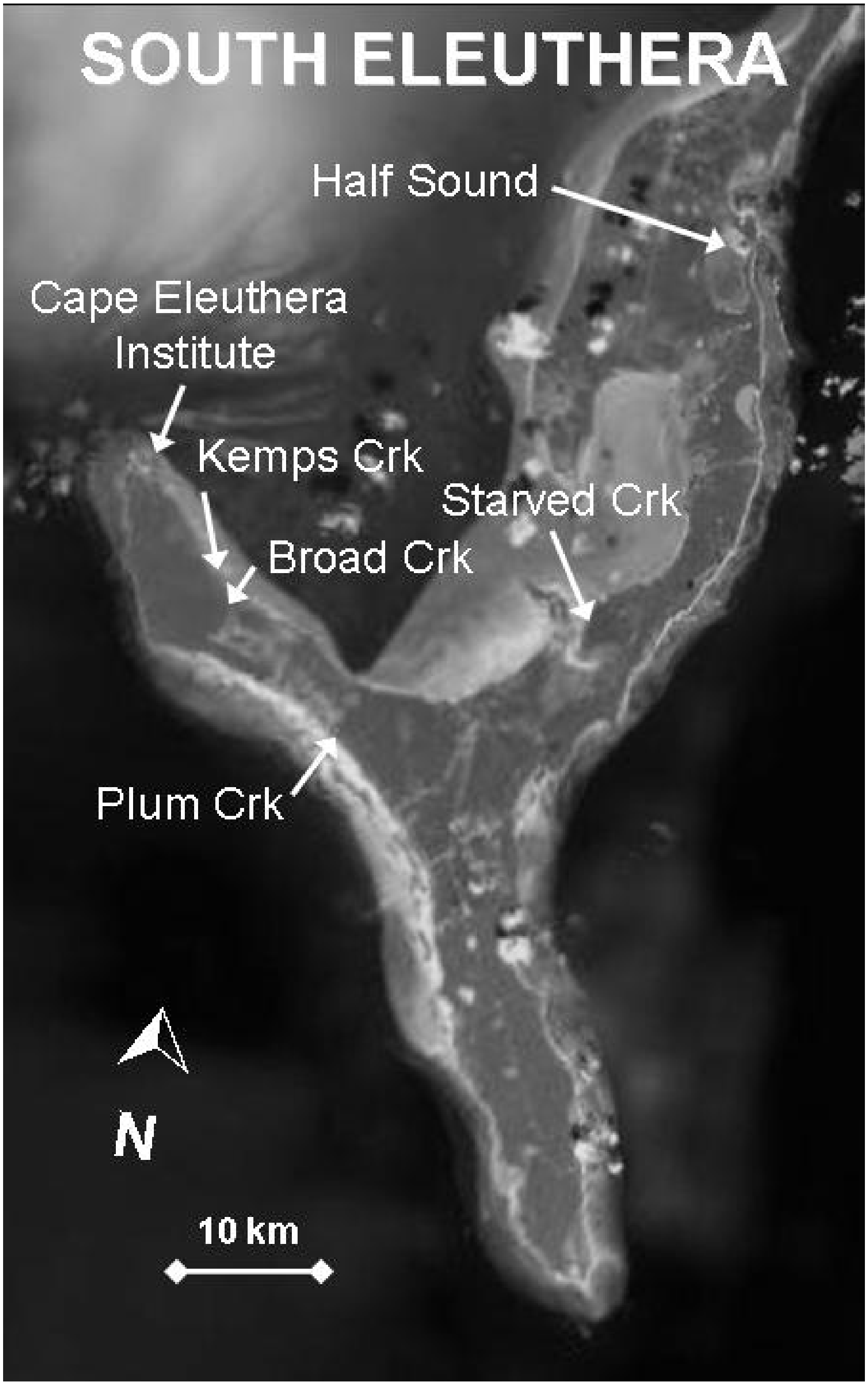
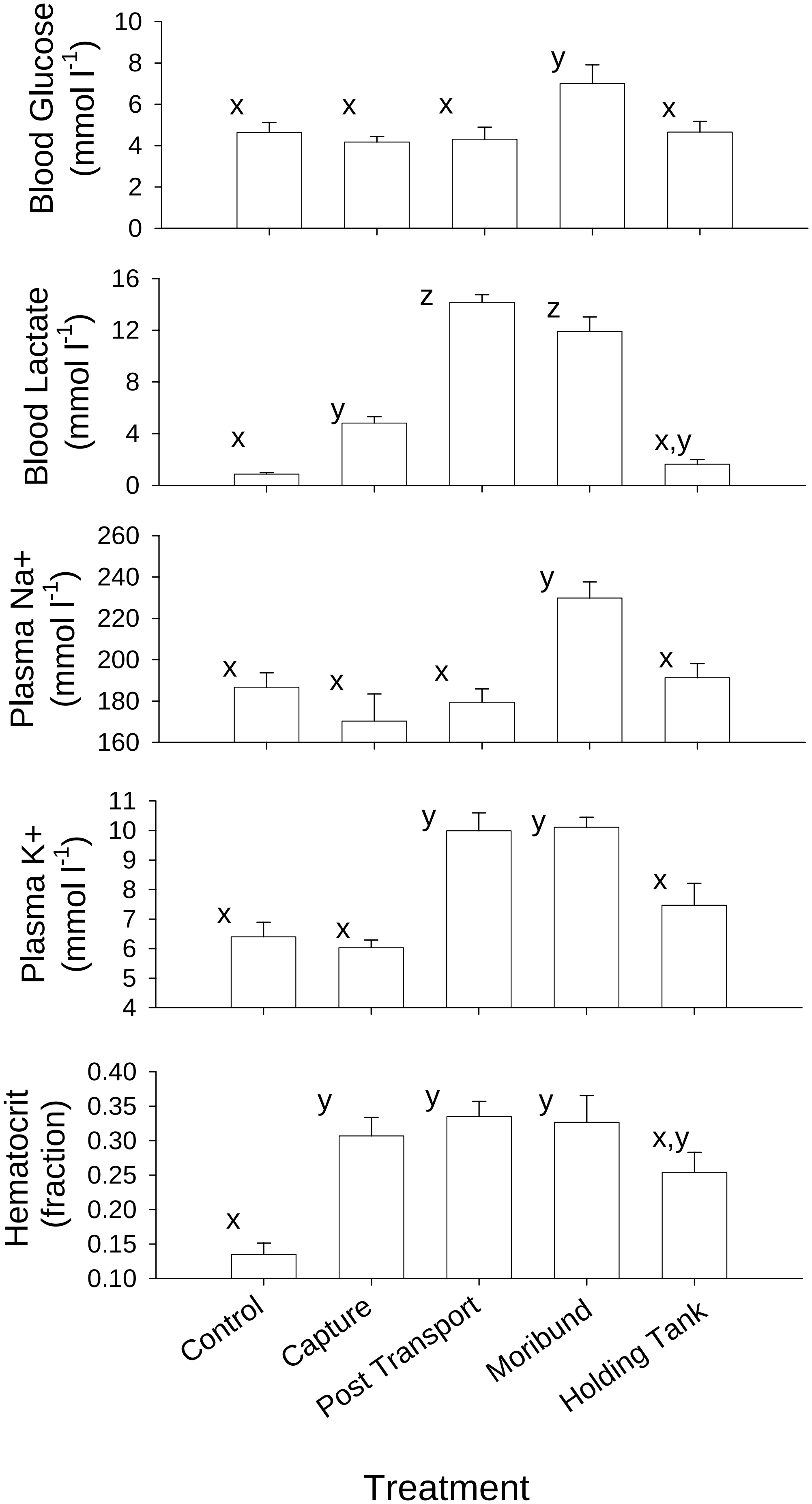

SOUTH ELEUTHERA
Half Sound
Cape Eleuthera Institute
Kemps Crk
Starved Crk
Broad Crk
Plum Crk
N
10 km

Fig. 2.


Blood Glucose (mmol l^{-1})
0
2
4
6
8
10
x
x
x
y
x
Blood Lactate (mmol l^{-1})
0
4
8
12
16
x
y
z
z
x,y
Plasma Na+ (mmol l^{-1})
160
180
200
220
240
260
x
x
x
y
x
Plasma K+ (mmol l^{-1})
4
5
6
7
8
9
10
11
x
x
y
y
x
Hematocrit (fraction)
0.10
0.15
0.20
0.25
0.30
0.35
0.40
x
y
y
y
x,y
Control
Capture
Post Transport
Moribund
Holding Tank
Treatment