Aquaculture Research

Aquaculture Research, 2009, **40**, 1538–1550 doi: 10.1111/j.1365-2109.2009.02255.x

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

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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 haematological levels as indicated by changes in blood glucose, lactate, haematocrit 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 sub-tropical and tropical marine 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; True, Loera & Castro 1997; Leber 2004; Bell, Bartley, Lorenzen & Loneragan 2006), 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 sub-tropical 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 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 and Harnden (1998) involved holding 11 adult bonefish in an outdoor pond and repeatedly angling them over a 1 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 (17 February to 17 April 2007). The second phase was an assessment of handling and long-term holding of bonefish (20 April to 14 September 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).

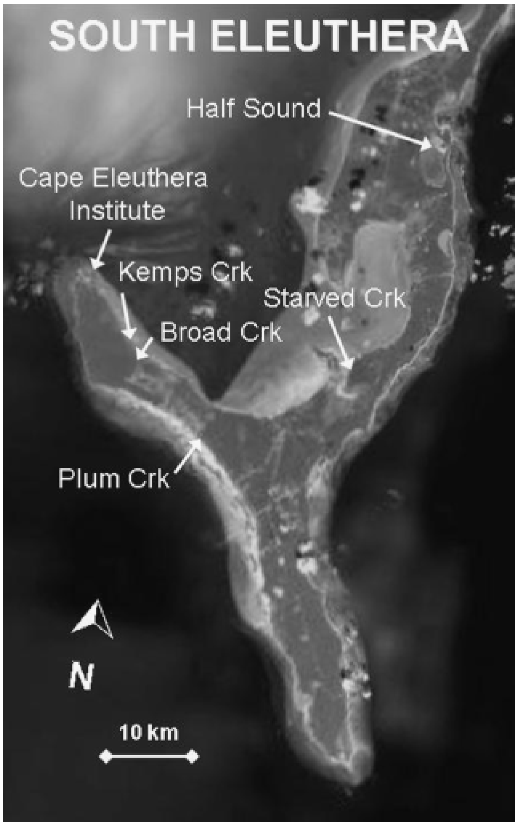

Figure 1 Map (developed using Google Earth) of study sites on Eleuthera, The Bahamas. Laboratory holding facilities were located at the Cape Eleuthera Institute. The various creeks represent locations where fish were sampled from (see Table 2 for details).

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 × 0.8 m × 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 W) and a 1 hp aeration pump (Sweetwater model S41; 15 V; 3450 rpm; Aquatic ecosystems, Apopka, FL, USA). 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 min).

Holding tanks at the CEI

Upon arrival to CEI, bonefish were transferred to small (1.6 m diameter × 0.85 m height; 1400 L) or 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 h^{-1}) 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 before 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 h 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 (colouration), 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

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 h to obtain control values. Fish were not introduced into the chambers until 48–72 h post transport. All fish were from Kemps Creek ($n = 7$)
Capture	Fish were sampled within 5 min 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 (18 February 2007) ($n = 5$)
Post-transport	Fish were sampled immediately following a 50 min transport (approximately 150 min 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 (18 February 2007) ($n = 12$)
Holding tank	Fish sampled from holding tanks via dip net between 48 and 72 h post-transport. Sample fish were from mixed populations ($n = 8$)

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 h to generate control (resting) physiological values for comparison. Secondary stress response parameters (glucose, lactate, sodium and potassium concentrations, and haematocrit) were examined for each blood sample. To live sample bonefish for blood, individuals were restrained by hand in supine position (without the use of anaesthetic) in a foam-lined trough filled with seawater at a depth to completely submerge their gills. Using a 21 G 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, USA). After the blood was drawn (typically < 45 s), it was held in an ice-water slurry until analysis. Total length (to the closest millimetres) 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, Indianapolis, IN, USA) and lactate (Lactate Pro LT-1710 portable lactate analyzer, Arkray, Kyoto, Japan) meters. Sodium, potassium and haematocrit concentrations were measured using the i-STAT point of care devise (Heska, 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, 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).

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 discolouration (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 20 April 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 s 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, NV, USA) 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 coloured 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 days and fed a diet of queen conch offal.

Following return to the holding tank, fish were first observed for 1 min 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, Danylchuk, Cooke, Goldberg, Koppelman & Philipp 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 discolouration, 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 (20 April to 11 May 2007), with daily observations during week 1, and every other day for weeks 2 and 3.

Data analysis

Differences in fish length were compared between the treatment groups using a one-way ANOVA followed by a Tukey–Kramer HSD test (Day & Quinn 1989). All analyses were performed using JMP 6.0.2 (SAS Institute) and the level of significance (α) for all tests was 0.05. Occurrences of physical abnormalities of fish from each of the three treatment groups were pooled over the 21 day observation period and divided by the number of possible observations to give a frequency of occurrence and were compared for differences via χ^2 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 (11 May 2007) fish were weaned off a diet of queen conch offal and switched to a commercially available sinking pellet (6 mm, Skretting, Bayside, New Brunswick, Canada) until 24 June 2007, then switched to a larger sinking pellet (13 mm Zeigler, Gardners, PA, 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 to 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 18 February 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

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 before transport (minutes)
February 17	Plum Creek	24	3.2 cm mesh	5	0	Coolers	60
February 18	Starved Creek	22	0.6, 1.3, 3.2 and 7 cm mesh used but all fish captured in 7 cm mesh	41	1*	Flow-through cage	150†
February 19	Starved Creek	23	3.2 and 0.6 cm	8	0	Flow-through cage	60
February 20	Kemps Creek	21	3.2 and 0.6 cm	70	0	Flow-through cage	45
February 23	Broad Creek	21	3.2 and 0.6 cm	3	0	Flow-through cage	45
February 23	Half Sound	22.5	3.2 and 0.6 cm	47	0	Flow-through cage	100‡
March 16	Broad Creek	22.5	3.2 and 0.6 cm	21	0	Flow-through cage	120§

*Only 1 fish died directly from gilling, but 39 of the 41 fish captured were gilled or entangled in the net.
†Due to strong tidal flow and storm surge fish were exercised in the flow for the duration of holding.
‡Approximately 650 m from seining location to truck.
§Longer duration due to inserting transmitters in 10 bonefish.

shore to ensure a minimum depth of 0.6 m on an outgoing tide. The duration of holding before transport ranged from 45 to 170 min, 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 18 February 2007 at Starved Creek resulted in the fish being subjected to strong storm surges for the last 30 min 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 to 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 to 95 min 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 with 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 (18 February 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 h 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 colouration changed dramatically from a normal silver-white colour to dark olive. Within 12 h post-capture fish demonstrated fin erosion and haemorrhaging 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

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* (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 min of the trip down very rough, bush trail and 25 min on poorly maintained paved roads. After 20 min into the trip, approximately 100 L of water was exchanged in the tank
February 19	Starved Creek	Truck	5	65	0	40 min of the trip down very rough, bush trail and 25 min on poorly maintained paved roads. After 20 min into the trip, approximately 150 L of water was exchanged in the tank
February 20	Kemps Creek	Truck	40	15	0	15 min 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 min on unpaved roads, 25 min on paved roads, 10 min 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 1/4 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

*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 4 Summary of 24 h mortality of wild bonefish held in captivity at the Cape Eleuthera Institute in The Bahamas

Date (2007)	Location	Number of bonefish captured	Number of mortalities after 24 h holding in tanks	Comments
February 17	Plum Creek	5	0	Fish used for other physiological experiments and euthanized within 5 days 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 days of capture. Remaining fish held
February 20	Kemps Creek	70	0	Fish used for used for other physiological experiments and euthanized within 5 days 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 days of capture. Remaining fish held
March 16	Broad Creek	21	0	Fish used for handling experiment included in this study

Note that all fish that succumbed to death were fully analysed for genetic sampling, length, weight, ageing (otoliths and scales removed), health indices, gut content analysis, stable isotope analysis and proximate body composition.

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^{-1}), and were minimally disturbed by human observation quickly resumed schooling behaviour and silver-white colouration.

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). Haematocrit values varied among treatments (ANOVA, $F = 6.24$, $df = 4{,}35$, $P < 0.05$). In general, haematocrit levels were elevated in the capture, post-transport and moribund fish relative to the fish held in tanks or in sensory deprivation chambers.

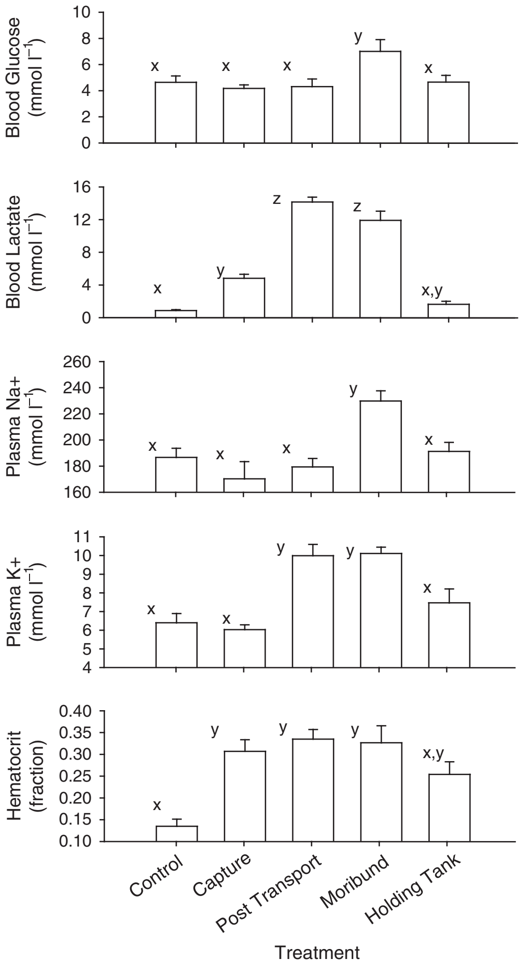

Figure 2 Physiological responses of bonefish to various handling, transport, and holding conditions. Dissimilar letters indicated significant differences (Tukey's Post Hoc Test, $P < 0.05$). Sample sizes for each treatment group are indicated in Table 1.

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 s 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 s 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 3-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 discolouration) than the bare hand and gloved hand treatments (Table 5), however χ^2 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 and 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. Approxi-

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 day observation period at the Cape Eleuthera Institute in The Bahamas

	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 discolouration	34.74	34.38	29.47

Note that χ^2 analysis found no significant differences in the frequency of physical disturbances between the three handling methods (P's > 0.05).

mately 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 before (moribund) or post-death compared with 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 and 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, haematocrit levels are often elevated (Ruane, Wendelaar Bonga & Balm 1999). In this study, haematocrit values were significantly higher in the capture, post transport and moribund tank, relative to control fish levels. Elevations in haematocrit 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 and Anderson (2000) found similar increases in haematocrit 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 discolouration 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 haematological changes. The duration of the effects appears to be < 72 h 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 1). 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., 21–24 °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.

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 min of travel, $\leq$30 kg m^{-3}; $>$15 min of travel, $\leq$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 h 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.

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 sub-tropical 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.

Acknowledgments

We gratefully acknowledge C. Maxey and the staff, students, and volunteers of the CEI 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.

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