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Effects of different capture techniques on the physiological condition of bonefish *Albula vulpes* evaluated using field diagnostic tools

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A study was conducted on bonefish *Albula vulpes* in The Bahamas to assess the utility of portable physiological diagnostic tools (i-STAT analyser with E3+ ion and haematology cartridge, ACCU-CHEK glucose meter and Lactate Pro lactate meter) for field physiology applications in remote locations. Physiological values derived from portable diagnostic tools were significantly related to values obtained from standard laboratory techniques [glucose ($r^2 = 0{\cdot}96$), packed cell volume (PCV; $r^2 = 0{\cdot}33$), Na^+ ($r^2 = 0{\cdot}28$), K^+ ($r^2 = 0{\cdot}71$) and Cl^- ($r^2 = 0{\cdot}15$)]. Actual values (*i.e.* intercepts), however, tended to deviate slightly between the two techniques. Nonetheless, these tools showed promise for documenting relative differences among fishes experimentally exposed to treatments inducing different levels of 'stress'. These tools were then used to characterize the effects of different capture techniques on the stress response of *A. vulpes*. *Albula vulpes* captured in seines and then temporarily held in pens were physiologically sampled between 1 and 45 min postcapture to evaluate postcapture stress dynamics. Blood glucose and lactate as well as PCV and haemoglobin (Hb) increased rapidly after capture but stabilized at maximal values by *c.* 20 min postcapture. When angled, larger *A. vulpes* took longer to exhaust and land than did smaller individuals. In addition, there was a positive relationship between the magnitude of increase in lactate and the duration of the angling event, implying that anglers can reduce stress by minimizing the duration of the fight. Fish sampled before and after a simulated angling treatment displayed clear increases in blood lactate, K^+, PCV and Hb, providing some of the first data on how individual *A. vulpes* respond to angling stress. In summary, this study revealed that techniques are now available for conducting field physiological studies on *A. vulpes* and possibly other species in remote locales, and that haematological and biochemical indicators of physiological disturbance vary with the intensity of the angling event.

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INTRODUCTION

Bonefish *Albula* spp. are one of the most sought after, but elusive, game fishes in the world. Recreational fisheries that target bonefish are characterized by highly specialized and skilled anglers who often fish with guides or outfitters (Policansky, 2002). *Albula* spp. fisheries are also distinguished by the fact that almost all *Albula* spp. captured by anglers are released upon capture (Humston, 2001; Policansky, 2002). The popularity of *Albula* spp. recreational fisheries and the wide circum-tropical distribution of this group of fishes make them important elements of many local economies (McIntosh, 1983; Humston, 2001; Ault *et al.*, 2002; Danylchuk *et al.*, 2007*a*). Bonefishing lodges and guide service industries have been developed in remote regions of the South Pacific (*e.g.* Christmas Island), the Indo-Pacific seas (*e.g.* the Seychelles), the Caribbean (*e.g.* Mexico, Belize, Cuba and The Bahamas) and the U.S.A. (*e.g.* Florida Keys and Hawaii). From an ecological perspective, *Albula* spp. are benthivorous and are thought to play an important role in the movement of energy in coastal regions (Ault *et al.*, 2002). As such, there is great interest in sustaining or enhancing *Albula* spp. populations for their ecological and economic values (Cooke *et al.*, 2006*a*).

Despite the importance of *Albula* spp. to the recreational angling industry, little is known about the effects of different angling practices on these fishes (Ault *et al.*, 2002; Cooke & Philipp, 2007). At present, only a few studies have explicitly examined issues associated with the effectiveness of catch-and-release strategies for bonefish (Crabtree *et al.*, 1998; Cooke & Philipp, 2004; Danylchuk *et al.*, 2007*b*, *c*). Collectively, these studies provide information on post-release behaviour and survival, but little information on the sublethal physiological effects of catch-and-release angling. Danylchuk *et al.* (2007*c*) revealed that *Albula* spp. that lost equilibrium were six times more likely to be preyed upon after release than conspecifics that did not lose equilibrium, suggesting that a physiological disturbance may be linked with equilibrium loss. Sublethal effects associated with catch-and-release angling may reduce the biological fitness of angled individuals (Cooke *et al.*, 2002; Cooke & Suski, 2005; Arlinghaus *et al.*, 2007), which may, in turn, reduce recruitment. Understanding the effects of catch-and-release angling is essential for basic fisheries management (Wydoski, 1977), especially in light of recent evidence suggesting that some local *Albula* spp. populations are experiencing declines in abundance and shifts in size structure (Bruger & Haddad, 1986; Ault *et al.*, 2002).

Physiological tools are being used with increasing success to support studies on fish conservation (Wikelski & Cooke, 2006; Young *et al.*, 2006; Cooke & Schramm, 2007). Many fish species, including *Albula* spp., however, reside in remote regions where conventional laboratories for analysing physiological samples are unavailable (Wydoski & Wedemeyer, 1976; Iwama *et al.*, 1995; Morgan & Iwama, 1997). New methods in field physiology are therefore in

demand (Costa & Sinervo, 2004). In human and veterinary medicine, many new innovations in 'point-of-care' diagnostic tools have recently been developed that can be applied in a physician's or veterinarian's office or in the home of a patient, providing nearly immediate results and obviating the need to send samples to an external laboratory for analysis (St-Louis, 2000). These tools have been extensively tested for humans (Erickson & Wilding, 1993; Jacobs *et al.*, 1993; Pyne *et al.*, 2000) and domestic animals (cats and dogs; Grosenbaugh *et al.*, 1998; Looney *et al.*, 1998; Cohn & McCaw, 2000; Wess & Rausch, 2000) but have only recently been tested for their applicability to fisheries research (Wells *et al.*, 2003; Kojima *et al.*, 2004; Mandelman & Farrington, 2007), aquaculture (Wells & Pankhurst, 1999; Venn Beecham *et al.*, 2006) and aquatic animal medicine (Harrenstien *et al.*, 2005). This type of approach would allow fisheries scientists to collect physiological data at field sites (*e.g.* stream bank, boat and shore), no matter how remote, and to acquire previously unattainable data.

A study was executed to assess the utility of portable diagnostic tools for field physiology applications in remote locations using the bonefish *Albula vulpes* (L.) as a model. Beyond assessing the efficacy of the field diagnostic tools, additional research focused on the effects of different capture techniques on *A. vulpes* to yield baseline information on their physiological stress response. Specifically, fish were captured by seine and then sampled between 1 and 45 min postcapture to assess short-term physiological dynamics. In addition, some fish were angled to assess the physiological consequences of angling on *A. vulpes*. Some fish were sampled before and after angling to document individual-level responses to angling stress. Collectively, this work will serve as a basis for future research activity on *A. vulpes* and other recreational game fish species in remote locations around the globe.

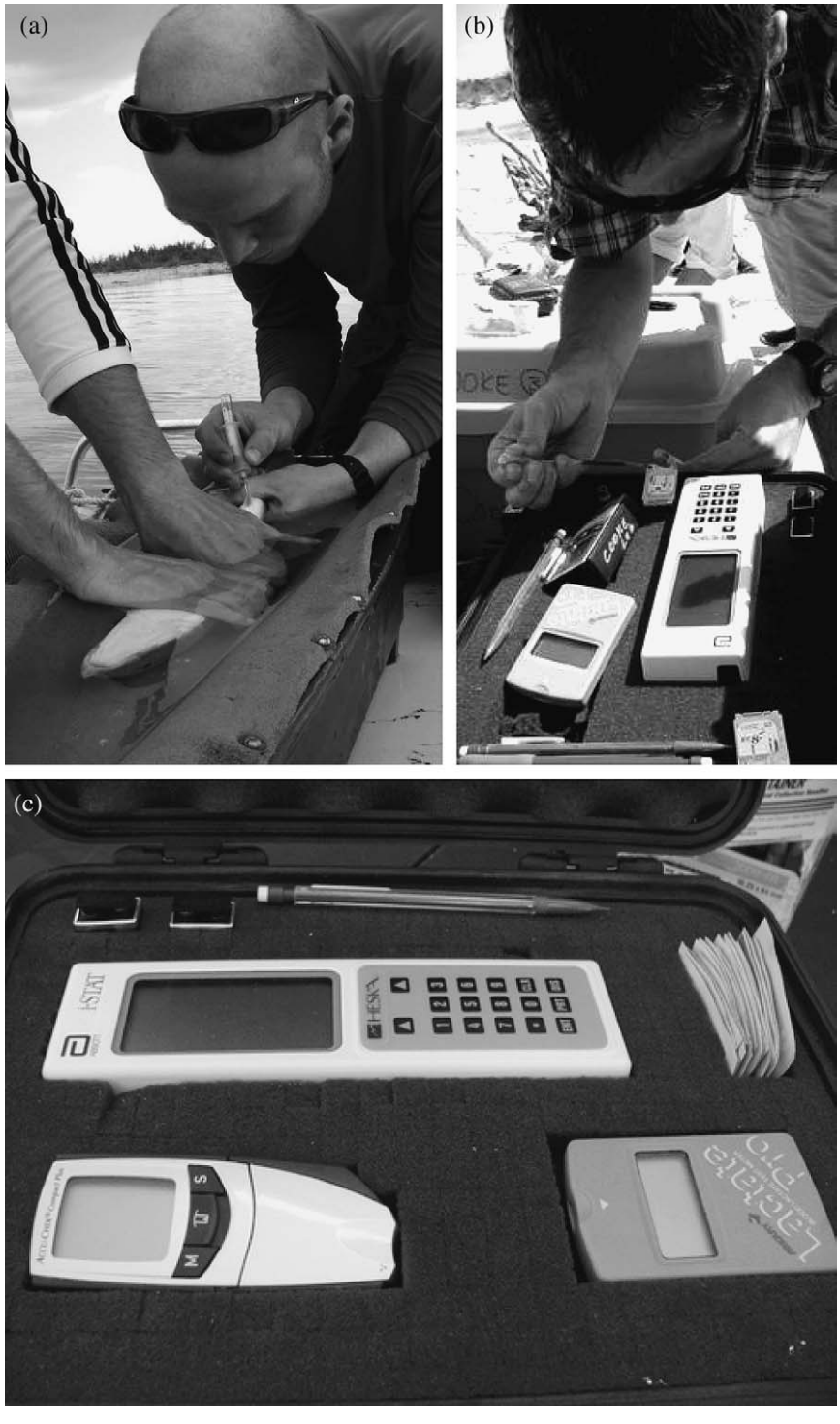
MATERIALS AND METHODS

STUDY SYSTEM

This study was conducted in south Eleuthera, The Bahamas (24°50′ N; 76°20′ W) in a number of tidal creek systems (Plum, Kemps, Broad and Starved), tidal embayments (Half Sound), as well as at the Cape Eleuthera Institute (CEI) seawater research facility. The shoreline of Eleuthera is composed of small, sandy bays and sharp calcium carbonate outcroppings. The tidal creeks are characterized by mosaics of sandy beach and turtle grass beds *Thalassia testudinum* (König) surrounded by tracts of red mangrove *Rhizophora mangle* (L.). All research activity was conducted in accordance with the Canadian Council on Animal Care under an approval granted by Carleton University (protocol B07-05 and B07-06).

VALIDATION OF FIELD DIAGNOSTIC TOOLS

Captive adult *A. vulpes* held in the Cape Eleuthera Institute seawater facility were exposed to a range of conditions designed to induce a gradient of physiological disturbance. Fish used for this experiment had not previously been sampled for blood and were not used in subsequent experiments (*e.g.* the angling simulations) described in this paper. Fish were either netted directly from tanks or were transferred by hand and placed supine in a padded, V-shaped, water-filled trough that was frequently refreshed



with buckets of fresh sea water. One team member held the fish mid-body while another team member obtained a small non-lethal blood sample (1·5 ml) *via* caudal venipuncture [B-D vacutainer, 3 ml vial, lithium heparin, 21 gauge needle (BD, Franklin Lakes, NJ, U.S.A.); Fig. 1(a)]. Blood samples were immediately placed in a water-ice slurry and analysed prior to processing and storage. Fish total lengths (L_T; to the nearest mm) were recorded and fish were then released into a common tank for subsequent experiments.

SEINE CAPTURE STRESS

Multiple fish from schools were captured by deploying seines (13 mm mesh, 46 m long; 32 mm mesh, 76 m long; 70 mm mesh, 61 m long) at creek mouths to intercept fish as they either entered or left the stream systems with incoming and outgoing tides, respectively. Seines were left in place until a school of *A. vulpes* approached at which time the seine was closed around the school. Fish were then individually netted (dip-net) or passed by hand into 1·3 m^2 flow-through holding pens (1·3 × 0·8 × 1·25 m tall, 31 mm extruded plastic mesh). Non-lethal blood samples were collected between 1 and 45 min postcapture, as described above. Fish were then either released or retained in the CEI seawater system for future studies. Water temperature was measured during the period that fish were retained in the holding pen.

ANGLING SIMULATIONS

Angling simulations were conducted to assess the immediate and prolonged effects of angling on haematological–physiological variables. Although simulated angling differs from natural angling events in that there was not an element of surprise, it enabled the angling event to be controlled. Specifically, a single angler 'played fish', which ensured that the duration of the angling event reflected the physical capacity of the fish and not the varying abilities of different anglers. Furthermore, using a team of seven researchers, it was possible to ensure rapid handling of fish and expedient blood collection (<1 min). A previous study focused on Atlantic salmon *Salmo salar* L. similarly used simulated angling (Anderson *et al.*, 1998) to control for inter-angler variation.

Fish were captured as above (but not sampled) and transported from the field to the seawater facility at CEI and provided 48 h to recover from the stress of initial capture and transport. At the facility, fish were held in large circular tanks (3·7 m diameter × 1·25 m height, 13 400 l) that were continuously supplied with fresh sea water (1800 l h^{-1}). Fish were individually netted from the tanks, placed in 60 l coolers, and transported 100 m by electric cart to the shoreline. Coolers were carried into the water where an in-water sampling station had been established. Fish were individually hooked in the left or right upper lip (using a # 6 circle hook with no offset) while in water and then released in *c.* 0·75 m of water. The circle hook was attached to a 4·54 kg leader and floating fly line (number 9 weight) with backing on a saltwater fly reel equipped with a drag and mounted on a 9 weight, 2·74 m fly rod.

During the first few seconds of release, fish were coaxed away to initiate the fight. Fish were angled in a region dominated by sandy substratum and where *A. vulpes* have been often observed. As fish were angled in the ocean, predation during the angling event was possible. The angler was instructed to 'fight the fish' until it was in a condition that it could be easily landed by hand. Once landed [hand placed anterior to the

FIG. 1. Photographs of field physiology techniques and tools. (a) Fish held in water-filled trough while phlebotomy is performed with a vacutainer. Fish were not anaesthetized prior to sampling and none of the sampling was lethal. (b) Pipettor used to load blood into i-STAT cartridge on a beach beside where fish were captured in a seine. (c) All field physiology tools [*i.e.* i-STAT (top of photograph), glucose meter (bottom left of photograph) and lactate meter (bottom right of photograph)] were kept in a waterproof hard plastic case with a waterproof seal to prevent damage from salt water and temperature.

dorsal fin on the ventral surface and the fish briefly (5 s) lifted out of the water to simulate landing and hook removal] the fish was passed to an assistant holding a small knotless nylon net (in the water). Within 10 s, the fish was transferred to a sampling trough filled with sea water and immediately sampled for blood (as above), measured (total length, L_T, to nearest mm) and unhooked. Fish were then returned to large circular holding tanks and observed for 24 h post-angling. A sub-set of fish ($n = 8$) was sampled for blood (as above both immediately prior to and immediately after angling).

FIELD DIAGNOSTICS AND LABORATORY ANALYSES

After blood sample collection, lactate and glucose levels were measured on whole blood by adding 10 μl of blood to hand-held glucose (ACCU-CHEK glucose meter; Roche Diagnostics, Basel, Switzerland) and lactate (Lactate Pro LT-1710 portable lactate analyser; Arkray Inc., Kyoto, Japan) meters [Fig. 1(b), (c)]. Appropriate standards and calibrations were used with meters prior to analysis as per manufacturer guidelines. The Lactate Pro has previously been validated as a reliable tool for field physiology (Pyne *et al.*, 2000; Mizock, 2002) including for fishes (Venn Beecham *et al.*, 2006). Hence, the Lactate Pro was not evaluated in this study. Sodium, potassium, chloride, packed cell volume (PCV) and haemoglobin (Hb) concentrations were measured using the i-STAT point of care device (Heska Corporation, Fort Collins, CO, U.S.A.). Whole blood was diluted by 25% using distilled water and was immediately aliquotted (60 μl) to an i-STAT E3+ cartridge for analysis. The remaining whole blood was centrifuged (Clay Adams Compact II centrifuge; Corning, NY, U.S.A.) at 10 000 *g* for 5 min. PCV was measured following centrifugation as the proportion of packed red blood cells to the total volume of sample. Plasma samples were separated by pipette, transferred to 1·5 ml standard micro test tubes and immediately stored in a liquid nitrogen dry shipper (at a minimum of −80° C) until laboratory analyses were conducted. The Roche Hitachi 917 analyser, with appropriate Roche reagents, was used for laboratory assays of plasma glucose, Na^+, K^+ and Cl^-. Glucose analysis methodologies were based on Trinder's glucose oxidase method and ion analysis methodologies relied on the ion selective electrode principle (Wagner & Congleton, 2004). All laboratory assays were conducted in an animal science diagnostics laboratory (Vita-Tech, Markham, Ontario, Canada that is accredited by the Veterinary Laboratory Association Quality Assurance Program and the Canadian Food Inspection Agency, and is compliant with the ISO 9001:2000 Quality Management System). All field assays were conducted using whole blood, while all laboratory assays used plasma only. Cl^- analysis was only conducted for the purpose of contrasting field and laboratory measurement tools. It is important to recognize that at times data derived from plasma and whole blood are compared, which requires caution with interpretation.

STATISTICAL ANALYSIS

To assess the performance of the field diagnostic tool, linear regression analysis (model 1) was conducted. For this analysis, the laboratory values were assumed to be the 'gold standard' enabling an evaluation of whether the slopes differed statistically from one (which would be the expectation if both tests gave uniformly scaled results across the measurement ranges) and whether the intercepts differed significantly from zero (which would be the expectation if the two measurement techniques were calibrated to the same absolute physiological values). Regression analysis was also used to evaluate the relationship between postcapture stress responses and the time since capture. For these analyses, it was assumed that relationships between variables may not be linear, and a combination of second order polynomial and second parameter exponential rise was applied to maximum fit lines. In all cases, the line that is the best fit to the data (defined by the highest r^2 value) is reported. To assess the relationship between L_T and angling duration, linear regression was used. Additional assessments were also conducted to evaluate the relationship between angling duration and various

physiological variables again using second order polynomial and second parameter exponential rise to maximum fit lines. To assess changes in individual physiological variables measured before and after angling, paired *t*-tests were used. All analyses were conduced using JMP 4.0 (SAS Institute, Cary, NC, U.S.A.) and were assessed for significance at $\alpha = 0{\cdot}05$. Because of multiple comparisons in some analyses (*i.e.* the pre- and post-angling paired *t*-tests), however, sequential Bonferroni corrections were conducted where appropriate, although uncorrected *P*-values are presented as well (Cabin & Mitchell, 2000).

RESULTS

ASSESSMENT OF FIELD DIAGNOSTIC TOOLS

Blood samples were collected from 56 fish (mean ± S.E. 449 ± 47 mm L_T) exposed to a range of conditions (*e.g.* various intensities of exercise and various durations of air exposure, resting and recovery) and water temperatures (ranging from 21 to 25° C).

For glucose, there was a significant positive relationship between the laboratory and field-derived values (mmol l^{-1}) [$n = 55$, $r^2 = 0{\cdot}96$, $P < 0{\cdot}001$; Fig. 2(a)]. In addition, the intercept did not differ significantly from zero ($P > 0{\cdot}05$). Pairwise analysis revealed that field diagnostic values were on average 0·47 mmol l^{-1} higher than values derived from laboratory techniques (Table I).

Positive relationships were observed between i-STAT and laboratory measurements (mmol l^{-1}) for K^+, Na^+ and Cl^- [K^+, $n = 55$, $r^2 = 0{\cdot}71$, $P < 0{\cdot}001$, Fig. 2(c); Na^+, $n = 56$, $r^2 = 0{\cdot}28$, $P < 0{\cdot}001$, Fig. 2(d); Cl^-, $n = 39$, $r^2 = 0{\cdot}15$, $P < 0{\cdot}05$, Fig. 2(e)]. For all ions, however, the intercepts were significantly different from zero (Table I). For Na^+, the i-STAT values were consistently higher than the laboratory (mean difference of 11·03 mmol l^{-1}; Table I) values, whereas i-STAT values for K^+ and Cl^- were generally lower than those obtained in the laboratory (mean difference of 0·64 mmol l^{-1} for K^+ and mean difference of 1·19 mmol l^{-1} for Cl^-).

The PCV measurements obtained with the i-STAT were correlated with those values obtained from centrifuging the blood and measuring PCV manually [$n = 28$, $r^2 = 0{\cdot}33$, $P < 0{\cdot}01$; Fig. 2(b)]. The intercept, however, differed from zero ($P < 0{\cdot}001$; Table I).

POSTCAPTURE STRESS DYNAMICS IN SEINE-CAUGHT *ALBULA VULPES*

To characterize the postcapture stress response of *A. vulpes* caught in a seine, fish were sampled from various locations (Table II) over a range of sampling times (between 1 and 45 min postcapture) while being held *in situ* using a mesh pen. Water temperatures at time of seine capture were on average 22·5 ± 0·3° C (range of 21·2–25·0° C) and the mean ± S.E. L_T of fish from which blood samples were collected was 463 ± 12 mm ($n = 27$). Generally, all physiological indicators sampled changed across the 45 min postcapture period, reflecting a stress response induced by the capture event. For example, blood lactate and glucose exhibited significant relationships (best characterized by a two parameter exponential rise to maximum fit line) with time postcapture [lactate,

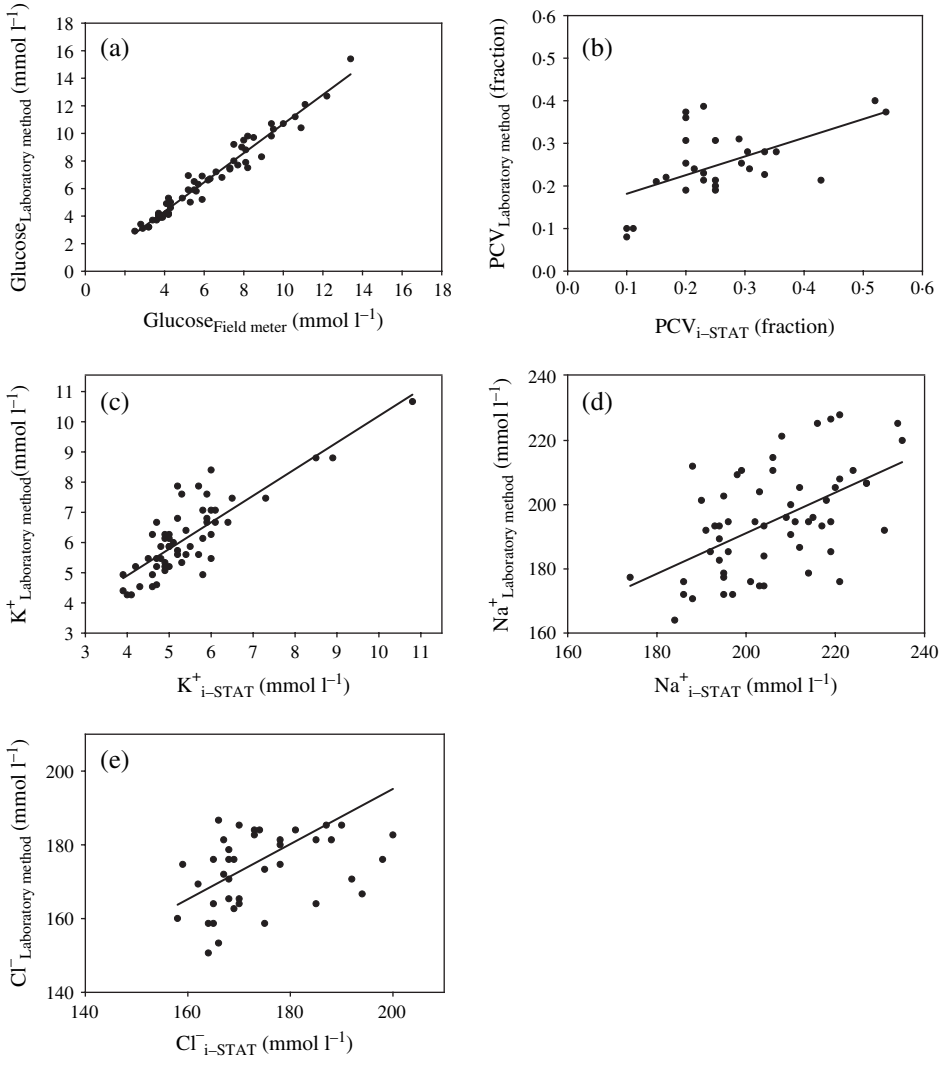


FIG. 2. Assessment of the relationship between values derived from field diagnostic tools and laboratory techniques for *Albula vulpes*. (a) Glucose field values were determined using an ACCU-CHEK glucose meter, while all other field values (b) packed cell volume (PCV), (c) potassium, (d) sodium and (e) chloride were determined using the i-STAT diagnostic device. Comparative laboratory values were determined using the Roche Hitachi 917 clinical laboratory analyser for all but PCV (determined using a centrifuge to reveal PCV). Note that all field assessments were completed using whole blood, whereas laboratory techniques were based on plasma. The curves were fitted by: (a) $y = 0{\cdot}092 + 1{\cdot}060x$, (b) $y = 0{\cdot}138 + 0{\cdot}439x$, (c) $y = 1{\cdot}420 + 0{\cdot}868x$, (d) $y = 63{\cdot}834 + 0{\cdot}641x$ and (e) $y = 111{\cdot}952 + 0{\cdot}350x$.

$n = 25$, $r^2 = 0{\cdot}69$, $P < 0{\cdot}001$; glucose, $n = 26$, $r^2 = 0{\cdot}49$, $P < 0{\cdot}001$; Fig. 3(a), (b)]. There was a plateau by *c.* 15 min postcapture for glucose and 20 min postcapture for lactate, with no evidence that these concentrations of metabolites were decreasing by 45 min postcapture. Ionic response was less consistent. There was no significant relationship between time postcapture and blood

TABLE I. Statistical output for linear regression analysis (model I) of physiological values derived from field diagnostic tools (independent variable) and laboratory assays or techniques (dependent variable)

				CI of intercept				CI of slope		
Variable	n	r^2	Intercept	Lower 95%	Upper 95%	P (intercept)	Slope	Lower 95%	Upper 95%	P (slope)
Glucose (mmol l^{-1})	55	0·96	0·092	−0·328	0·512	0·663	1·059	0·999	1·121	**<0·001**
Na^+ (mmol l^{-1})	56	0·28	62·834	4·420	121·248	**0·036**	0·641	0·359	0·923	**<0·001**
K^+ (mmol l^{-1})	56	0·71	1·420	0·562	2·279	**0·002**	0·868	0·714	1·023	**<0·001**
Cl^- (mmol l^{-1})	38	0·15	111·952	62·437	161·468	**<0·001**	0·350	0·067	0·634	**0·017**
PCV (fraction)	29	0·33	0·138	0·066	0·209	**<0·001**	0·439	0·184	0·694	**0·002**

PCV, packed cell volume. Significant values are in bold.

TABLE II. Summary of seining activity in Eleuthera, The Bahamas after which fish were held in a pen and randomly sampled between 1 and 45 min postcapture

Date	Location	Water temperature (° C)	Number sampled
17 February 2007	Plum Creek	24–25	5
18 February 2007	Starved Creek	22–23	7
19 February 2007	Starved Creek	21–22	5
20 February 2007	Kemps Creek	21–22	2
23 February 2007	Broad Creek	22–24	1
23 February 2007	Half Sound	22–24	7

Na^+ concentrations [second order polynomial, $n = 24$, $r^2 = 0{\cdot}20$, $P > 0{\cdot}05$; Fig. 3(c)]. There was a significant relationship between time postcapture and blood K^+ concentrations [second order polynomial, $n = 25$, $r^2 = 0{\cdot}23$, $P < 0{\cdot}05$; Fig. 3(d)]. The PVC and Hb shared a similar relationship to the metabolites in that they too were best characterized by a two-parameter exponential rise to maximum-fit line with both parameters increasing rapidly in the first minutes after capture and then stabilizing by *c.* 10 min postcapture [PCV, $n = 23$, $r^2 = 0{\cdot}22$, $P < 0{\cdot}05$; Hb, $n = 23$, $r^2 = 0{\cdot}22$, $P < 0{\cdot}05$; Fig. 3(e), (f)].

POST-ANGLING STRESS RESPONSE OF *ALBULA VULPES*

In total, 20 *A. vulpes* (mean ± S.E. = 471 ± 11 mm L_T) were angled for mean ± of 183 ± 16 s at a water temperature of 23° C. Water temperatures in the holding tanks where fish were held before and after angling were also 23° C as the water intakes for the seawater facility were only 200 m away from the angling site. The duration of the angling event ranged from 75 to 339 s and was positively related to the size of the fish with larger individuals requiring more time to land ($n = 20$, $r^2 = 0{\cdot}38$, $P < 0{\cdot}01$; Fig. 4). Most angling events were characterized by several long swimming bursts (up to 20 s) with the frequency and length of the burst typically higher for larger fish. Despite being angled until the fish were able to be landed by hand by the angler (*i.e.* exhausted), none of the fish lost equilibrium. The duration of the angling event influenced the extent of physiological disturbance. There was a positive linear relationship between the duration of the angling event and blood glucose concentration [$n = 19$, $r^2 = 0{\cdot}33$, $P = 0{\cdot}01$; Fig. 5(a)] and a similar relationship for blood lactate, although the relationship for glucose was better described using a two-parameter exponential rise to maximum-fit line [$n = 20$, $r^2 = 0{\cdot}31$, $P < 0{\cdot}05$; Fig. 5(b)]. There were no trends in ionic concentrations relative to fight duration [linear regressions, Na^+, $n = 20$, $r^2 = 0{\cdot}01$, $P > 0{\cdot}05$; K^+, $r^2 = 0{\cdot}006$, $P > 0{\cdot}05$; Fig. 5(c), (d)]. The PCV and Hb concentrations did not increase significantly with the duration of angling [PCV, linear regression, $n = 18$, $r^2 = 0{\cdot}07$, $P > 0{\cdot}05$, Fig. 5(e); linear regression, $n = 17$, $r^2 = 0{\cdot}21$, $P > 0{\cdot}05$, Fig. 5(f)]. None of the 20 angled *A. vulpes* died immediately following the angling event (*i.e.* no immediate mortality) or during the first 24 h while being held in an experimental tank (*i.e.* no short-term mortality).

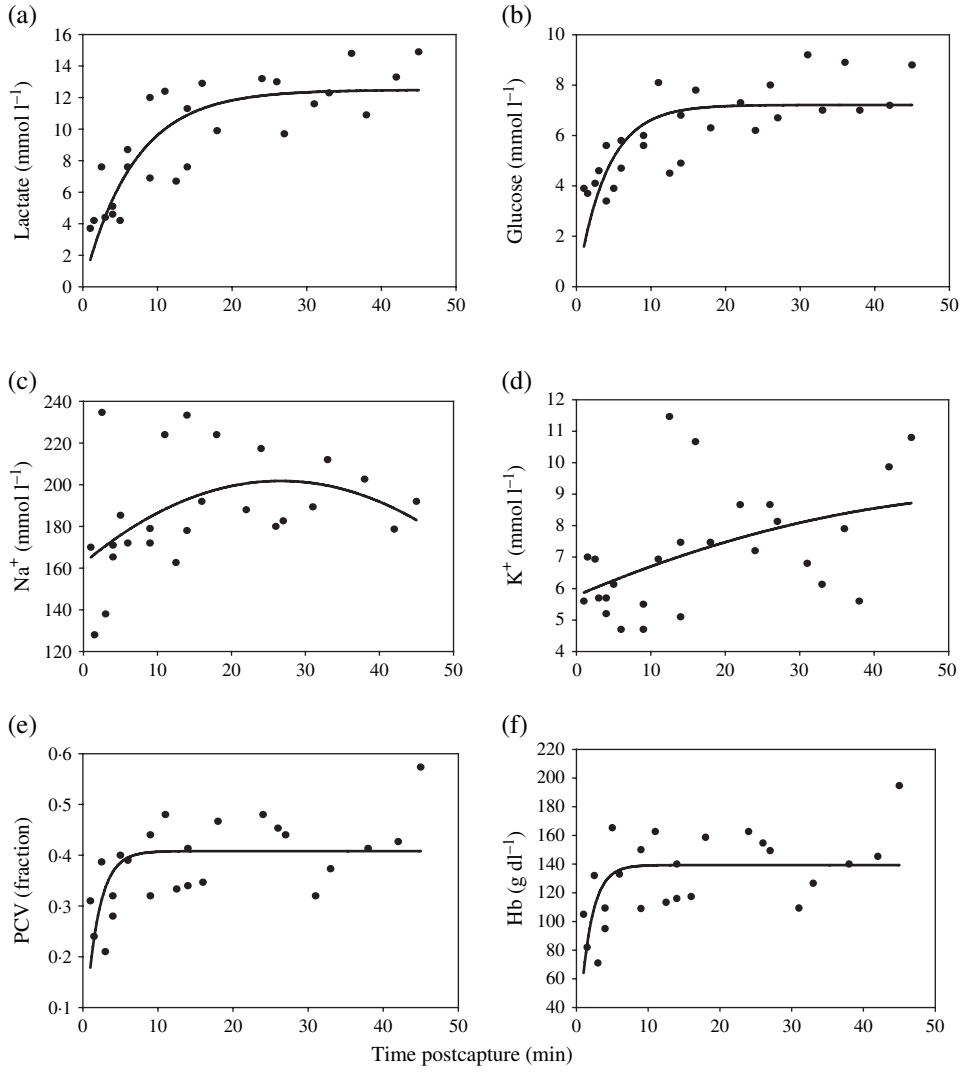


FIG. 3. Short-term (*i.e.* <45 min) postcapture stress dynamics in field-captured *Albula vulpes*. Visualized data represent the relationship between the time postcapture (by seine) and sampling for various physiological variables including (a) lactate, (b) glucose, (c) sodium, (d) potassium, (e) packed cell volume (PCV) and (f) haemoglobin (Hb). Data were determined from whole blood using field diagnostic tools. Lines represent two-parameter exponential rise to maximum fit (a), (b), (e), (f) and (c), (d) second order polynomials. The curves were fitted by: (a) $y = 12{\cdot}489\ (1 - e^{-0{\cdot}147x})$, (b) $y = 7{\cdot}213\ (1 - e^{-0{\cdot}249x})$, (c) $y = 162{\cdot}336 + 2{\cdot}966x - 0{\cdot}056x^2$, (d) $y = 5{\cdot}772 + 0{\cdot}101x + 0{\cdot}001x^2$, (e) $y = 0{\cdot}408\ (1 - e^{-0{\cdot}575x})$ and (f) $y = 139{\cdot}310\ (1 - e^{-0{\cdot}617x})$.

A sub-set of fish ($n = 8$, mean ± S.E. L_T 443 ± 11 mm) were sampled for blood immediately before and immediately after angling (mean ± S.E. duration of angling event = 166 ± 22 s) to evaluate the immediate physiological consequences of angling. Concentrations of blood lactate rose significantly during

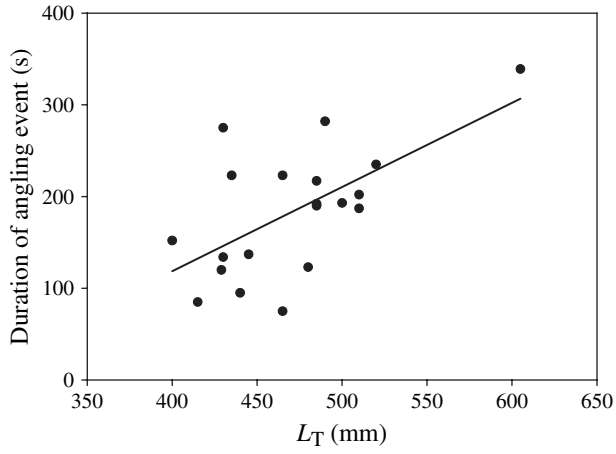


FIG. 4. Relationship between total length (L_T) of *Albula vulpes* and the duration of the angling event. The curve was fitted by: $y = -248{\cdot}300 + 0{\cdot}917x$.

angling [Table III and Fig. 6(a)], whereas glucose did not [Fig. 6(b)]. Na^+ failed to exhibit a consistent response to angling [Fig. 6(c)]. There was a significant increase, however, in K^+ between pre- and post-angling periods [Table III and Fig. 6(d)]. The PCV and Hb concentrations both increased significantly in response to angling [Table III and Fig. 6(e), (f)]. Interestingly, there was significant individual variation in response to the angling-related stressors for all variables (see data in Fig. 6).

DISCUSSION

There is a need to understand the response of fishes to stress associated with recreational and commercial fishing. There are few tools, however, that enable the collection and analysis of physiological data in field settings to address these pressing conservation issues (Wikelski & Cooke, 2006). Here, the performance of field diagnostic tools was evaluated for assessing the stress dynamics and short-term consequences of angling stressors on *A. vulpes*. Collectively, the data reveal that novel field diagnostic tools are reliable for the assessment of some physiological variables and thus provide an opportunity to conduct physiological studies at remote field settings and, in turn, to develop strategies for reducing the negative physiological consequences of different capture and handling techniques.

ASSESSMENT OF FIELD DIAGNOSTIC TOOLS

In general, physiological values obtained using portable diagnostic tools in the field had strong relationships with values obtained from using standard laboratory-derived techniques. Portable tools for the measurement of fish lactate and glucose have previously been used in the field. Wells & Pankhurst (1999) and Venn Beecham *et al.* (2006) studied lactate and glucose in rainbow trout *Oncorhynchus mykiss* (Walbaum) and channel catfish *Ictalurus punctatus*

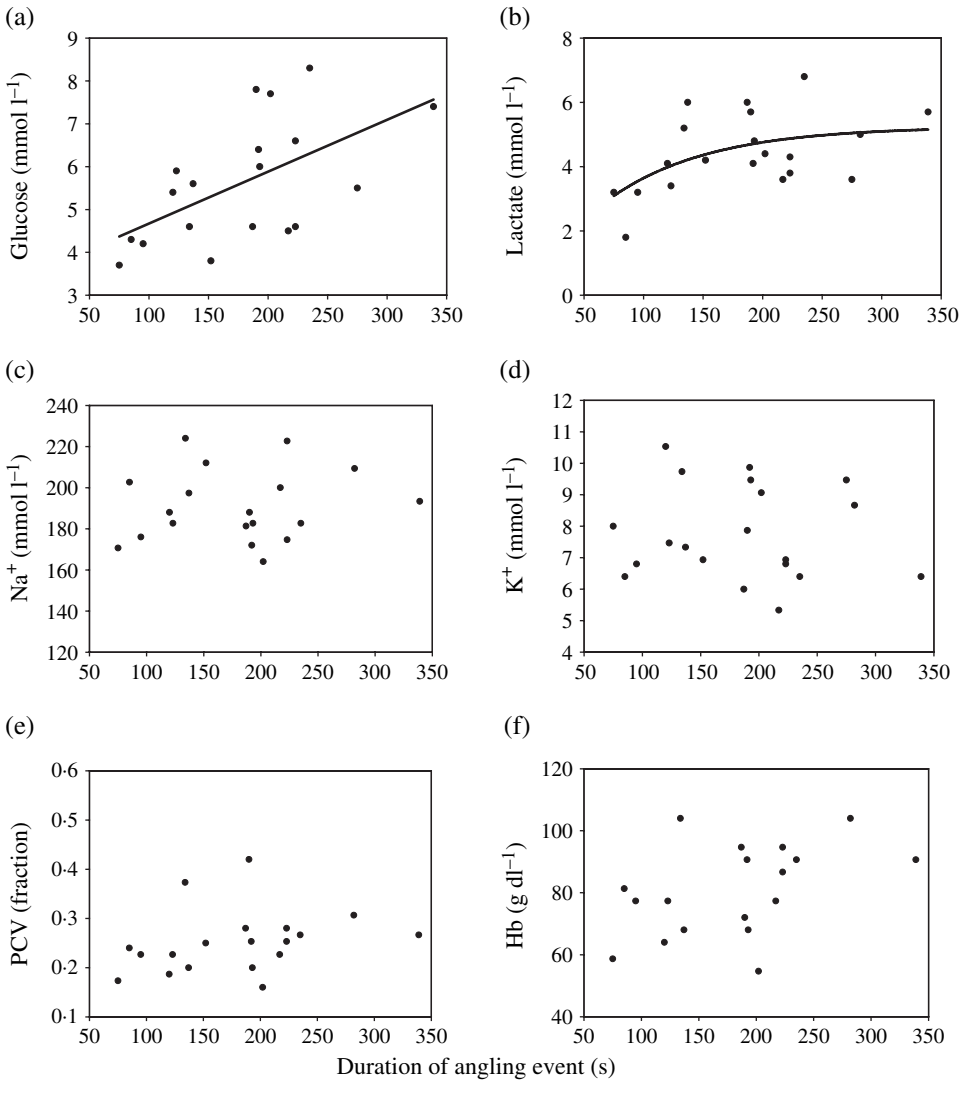


FIG. 5. Post-angling stress response of *Albula vulpes* relative to the duration of the angling event. Physiological variables including (a) glucose, (b) lactate, (c) sodium, (d) potassium, (e) packed cell volume (PCV) and (f) haemoglobin (Hb). Data were determined from whole blood using field diagnostic tools. Curves represent (a) linear regressions or (b) second order polynomials and were fitted by: (a) $y = 3{\cdot}460 + 0{\cdot}012x$ and (b) $y = 5{\cdot}248\ (1 - e^{-0{\cdot}0118x})$.

(Rafinesque), respectively, and found that these portable devices yielded repeatable and reliable results. Each of these studies observed that the field-derived values for lactate and glucose were consistently lower than established laboratory-derived values but both methods yielded similarly correlated values. Field-derived values for glucose in the current study closely approximated, but were higher on average, than laboratory-derived methods. Field-derived values were for whole blood glucose concentrations, while laboratory-derived values

TABLE III. Comparison of the statistical output for paired *t*-tests evaluating the physiological change of *Albula vulpes* between pre- and post-angling sampling periods. Significance was assessed at the $P < 0{\cdot}05$ level (significant values italicized) and after Bonferroni correction (*i.e.* $<0{\cdot}008$)

Factor	Mean difference	*t* statistic	d.f.	*P*
Glucose (mmol l^{-1})	0·30	−2·29	7	0·055
Lactate (mmol l^{-1})	3·02	−8·16	7	**<0·001**
Na^+ (mmol l^{-1})	2·66	−0·37	6	0·719
K^+ (mmol l^{-1})	1·39	−4·81	6	**0·003**
PCV (fraction)	0·06	−3·63	6	0·011
Hb (g dl^{-1})	14·02	−3·02	6	0·023

Hb, Haemoglobin; PCV, packed cell volume; significant values in bold.

were based on plasma glucose concentrations, which may account for the difference in mean glucose concentrations. These results contribute to a growing body of evidence (Iwama *et al.*, 1995; Wells & Pankhurst, 1999; Venn Beecham *et al.*, 2006) that portable tools for measuring lactate and glucose are reliable and offer a rapid means of accurately assessing relative, but not necessarily absolute, lactate and glucose values. The field-based approach for measuring these variables is now validated enabling future studies to adopt this approach as a rapid, precise means of assessing relative lactate and glucose concentrations outside of the laboratory.

The i-STAT point-of-care device has been used to assess PCV (Harrenstien *et al.*, 2005), Na^+ (Harrenstien *et al.*, 2005), Cl^- (Harrenstien *et al.*, 2005) and K^+ (Kojima *et al.*, 2004; Harrenstien *et al.*, 2005) for fish species in other studies. In this study, values for K^+ showed strong relationships between field-based and laboratory-based approaches, whereas moderate to weak relationships existed for PCV, Na^+ and Cl^-. The actual values, however, tended to deviate between the two measurement techniques. The deviations in actual values for PCV, Na^+, K^+ and Cl^- are probably explained by the fact that the i-STAT point-of-care device is calibrated for blood from mammals and not for the blood of ectotherms (Kojima *et al.*, 2004; Harrenstien *et al.*, 2005; Mandelman & Farrington, 2007). Furthermore, PCV and ions were measured from whole blood using the i-STAT device but were measured from plasma samples for laboratory assays, which may account for the discrepancies observed between actual values obtained from the two methods. Similar to Harrenstien *et al.* (2005), the current study found that Na^+ concentrations were higher for the field-based method than the laboratory-based method, while the opposite was true for K^+ and Cl^-. Kojima *et al.* (2004) obtained expected K^+ concentrations for *O. mykiss* across a range of treatments using the i-STAT, although these values were not compared to laboratory-derived methods. Even following the 25% dilution of whole blood, the field values of Cl^- in the current study approached the maximum reportable value of the i-STAT (140 mmol l^{-1}). Harrenstien *et al.* (2005), who used undiluted whole blood, found that all measured values of Cl^- in black rockfish *Sebastes melanops* Girard and blue rockfish *Sebastes mystinus* (Jordan & Gilbert) exceeded the reportable value of the i-STAT device. These findings show that the relative values of

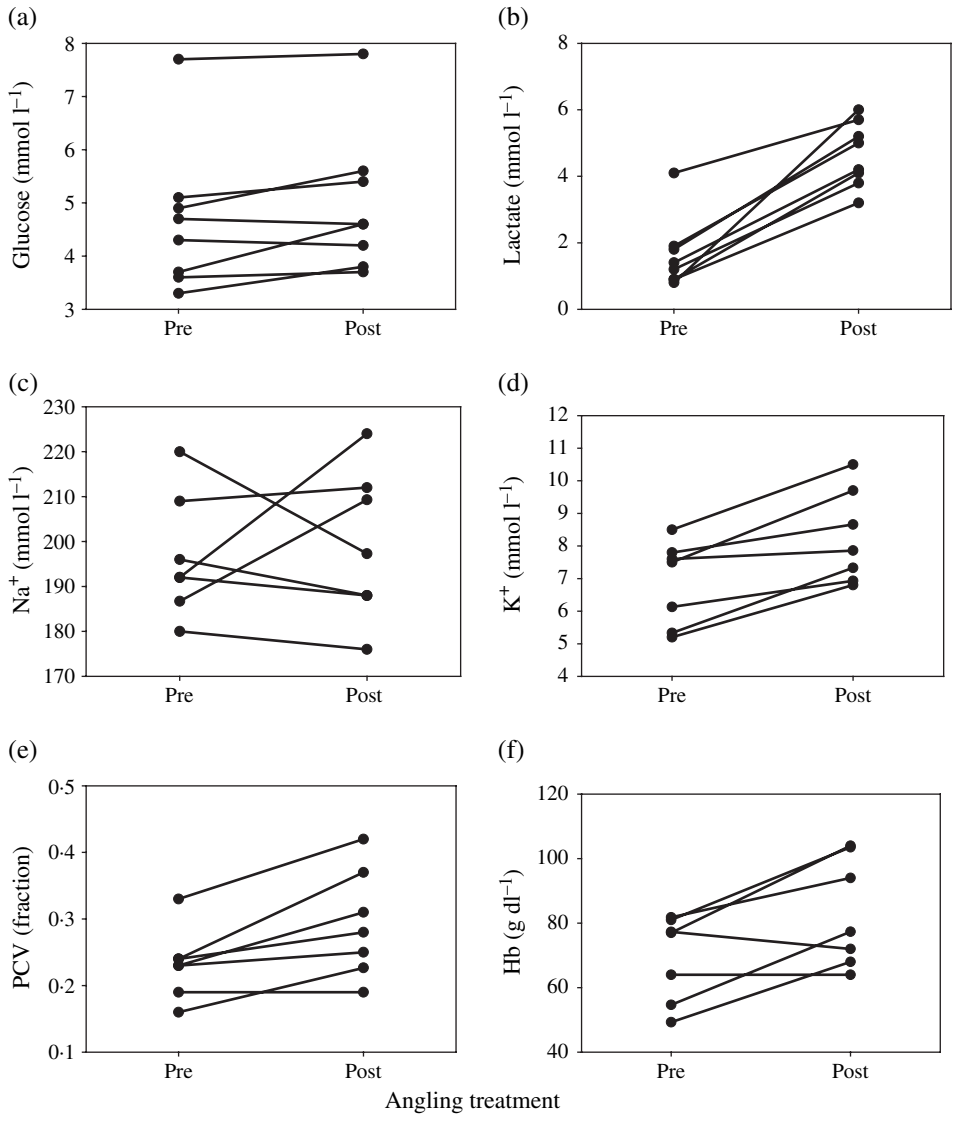


FIG. 6. Individual physiological status of pre- and post-angling of *Albula vulpes*. Lines connect individuals and represent the paired *t*-test analysis described in the text and in Table III. Physiological variables include (a) lactate, (b) glucose, (c) sodium, (d) potassium, (e) packed cell volume (PCV) and (f) haemoglobin (Hb). Data were determined from whole blood using field diagnostic tools.

PCV and ions (Na^+, K^+ and, in some applications, Cl^-) can be assessed rapidly using field-based diagnostic techniques.

CONSEQUENCES OF STRESS ON SHORT-TERM PHYSIOLOGICAL STATUS OF *ALBULA VULPES*

There has been a growing interest in documenting the postcapture physiology of fishes in both the commercial and recreational fisheries sectors (Chopin

& Arimoto, 1995; Cooke & Cowx, 2006). Such research has helped to yield information to reduce by-catch mortality and to enhance the welfare status of fishes that are captured and released (Bartholomew & Bohnsack, 2005; Arlinghaus *et al.*, 2007; Cooke & Sneddon, 2007). Although seines are rarely used in shallow waters in a commercial context where *A. vulpes* would be captured and subsequently released (other than for research purposes), netting provides the opportunity to document the short-term (<1 h) physiological consequences of a stressful event on *A. vulpes* in a field setting. It is important to note, however, that because fish from different creek systems and across multiple days were compared, the data may be inherently more variable than if all fish were sampled from the same system on the same day. Seining for *A. vulpes* induces burst swimming as fish attempt to escape from the net, similar to behaviours observed during angling. When exposed to stress and anaerobic exercise, a cascade of neuroendocrine changes (primary stress response) result in a number of changes in secondary physiology that were quantified in this study (Mazeaud *et al.*, 1977).

In the current study, clear elevations in blood glucose and lactate, PCV and several ions (Na^+ and K^+) were documented in *A. vulpes* following capture and during short-term captivity in the field. Lactate production occurs in response to anaerobic metabolism that can be induced by hypoxia (including air exposure) as well as strenuous exercise (Driedzic & Kiceniuk, 1976; Kieffer, 2000). *Albula vulpes* were minimally handled after capture and were only briefly exposed to air (seconds) during movement from the seine to holding pen. Furthermore, water quality was maintained by using flow-through holding pens. While held captive in the pen, fish probably experienced confinement stress, but this would be unlikely to result in elevated blood lactate concentrations given that fish did not tend to struggle and had ample oxygenated water. Hence, the lactate dynamics recorded in this study were probably reflective of the post-exercise (capture) conditions of the fish. Rising blood lactate concentrations were documented 20–30 min post-disturbance followed by a plateau for the remainder of the 45 min monitoring period similar to what has been reported for the subtropical coral trout *Plectropomus leopardus* (Lacepède) (Frisch & Anderson, 2000) and the temperate marine lingcod *Ophiodon elongatus* Girard (Milston *et al.*, 2006). The magnitude of the lactate concentrations (*i.e.* maximal) was *c.* 12 mmol l^{-1} which compares to values obtained in other fish species following exposure to capture stress from various gear types [*e.g.* coho salmon *Oncorhynchus kisutch* (Walbaum) captured in a gillnet, Farrell *et al.*, 2001; sockeye salmon *Oncorhynchus nerka* (Walbaum) captured in a purse seine, Cooke *et al.*, 2006*b*; spiny dogfish *Squalus acanthias* L. captured in otter trawls, Mandelman & Farrington, 2007; various marine pelagics captured by rod and reel, Wells & Davie, 1985; Wells *et al.*, 1986; Skomal, 2007].

Hyperglycaemia is induced by catecholamines and corticosteroids (Barton, 2000) and probably arose in *A. vulpes* in the current study from a combination of the actual capture event as well as confinement stress. Typically, an initial increase in plasma glucose concentrations results from catecholamine stimulation (Vijayan & Moon, 1992), whereas more sustained levels of elevated glucose tend to be mediated by cortisol (Vijayan & Moon, 1992). Hyperglycaemia is a common response to stress in most fish species (Barton, 2000) and varies

in response to the severity and duration of the stressor (as well as the species, size and nutritional status; Barton, 2000), but in general it stabilizes within several hours post-stress. In this study, blood glucose values for *A. vulpes* stabilized within 20 min postcapture similar to what has been reported for a variety of marine fishes in response to handling related stress including *P. Leopardus* (Frisch & Anderson, 2000), damselfish *Acanthochromis polyacanthus* (Bleeker) (Begg & Pankhurst, 2004) and *O. elongatus* (Milston *et al.*, 2006). For *A. vulpes*, it is unclear whether glucose concentrations would have increased further if the holding period had been extended. The extent of the hyperglycaemia observed in this study is consistent with values recorded for a number of marine and freshwater species following capture (*e.g. O. mykiss* captured by anglers, Meka & McCormick, 2005; *O. nerka* captured by purse seine, Cooke *et al.*, 2006*b*). Because glucose levels in *A. vulpes* plateaued within 20 min of capture, this may suggest that cortisol values may have also stabilized. There is only one study on cortisol dynamics in *A. vulpes* (Friedlander *et al.*, 2007), however, that study involved confining fish in pens and reported relatively high levels of cortisol throughout a 48 h holding period and thus provides little information on the cortisol dynamics at fine temporal scales (as monitored in the present study) and independent of a chronic stressor (*i.e.* confinement).

Blood ion concentrations in *A. vulpes* generally increased in response to the different stressors, however, the response was less consistent among individuals than was observed for metabolites. This probably reflects a combination of inherent individual biological variation (Cooke *et al.*, 2006*b*) as well as the performance of the field physiology tools for quantifying ionic status. Ionic responses to capture stress were also different from metabolites in that the relationships did not plateau. Postcapture alterations of Na^+ and K^+ were best described by polynomial relationships, although the relationship was only significant for K^+. Unfortunately, there are no relevant comparative studies on other marine species to assess comparable time courses at this temporal scale. For *A. vulpes*, absolute levels (ranging from immediately postcapture to 45 min postcapture) of Na^+ (160–210 mmol l^{-1}) were generally consistent with values observed for most marine fishes (*e.g. O. kisutch*, Farrell *et al.*, 2001; *O. nerka*, Cooke *et al.*, 2006*b*; *O. elongatus*, Milston *et al.*, 2006; *S. acanthias*, Mandelman & Farrington, 2007). The K^+ ranges for *A. vulpes* (*c.* 5–10 mmol l^{-1}), however, tended to be higher than in other species (usually maximal values of *c.* 5 mmol l^{-1}; *e.g. O. nerka* Cooke *et al.*, 2006*b*, *O. kisutch*, Farrell *et al.*, 2001, *O. elongatus*, Milston *et al.*, 2006). Unfortunately, there are few studies of the ionic status of tropical marine fishes associated with capture and handling stress to determine if the high K^+ values for *A. vulpes* are typical of other fishes from this region.

In this study, PCV and Hb values increased to maximal values by 10 min postcapture and then remained stable (but elevated) during the 45 min monitoring period. Similar elevations in these haematological variables have been observed for two species of coral trout *P. leopardus* and *Plectropomus maculatus* (Bloch) after capture stress (Frisch & Anderson, 2000, 2005). For these coral trout, however, the values tended to increase rather linearly after capture, reaching maximal values at *c.* 60 min postcapture. Not all marine species

respond to capture stress with changes in PCV and Hb as has been noted by Pankhurst *et al.* (1992). The increase in PCV observed in the current study may reflect a loss of water (Avella *et al.*, 1991) but may also be a result of red blood cells released from splenic contractions (Yamamoto *et al.*, 1980) or from erythrocytic swelling (Nikinmaa, 1983). Because PCV and Hb appeared to reach maximal values by 10 min post-stress, it is not possible to exclude any of these mechanisms as they all respond rapidly to stress. For *A. vulpes*, absolute levels (ranging from immediately postcapture to 45 min postcapture) of PCV (*c.* 0·20–0·45 fraction PCV) and Hb (60–140 g dl^{-1}) were consistent with values observed for most marine fishes [*e.g. P. leopardus* and *P. maculatus*, Frisch & Anderson, 2005; common dentex *Dentex dentex* (L.), Morales *et al.*, 2005]. Control values collected from *A. vulpes* held in sensory deprivation chambers for 24 h, however, yielded mean PCVs of *c.* 0·10 (Suski *et al.*, 2007). All the PCV values recorded in the present study, even if immediately after angling, yielded values at least twice this control value from Suski *et al.* (2007) suggesting that there were nearly instantaneous splenic contractions during stress.

CONSEQUENCES OF ANGLING ON PHYSIOLOGICAL STATUS OF *ALBULA VULPES*

By quantifying the response of fishes to different angling durations, it is possible to derive quantitative information about the magnitude of the stress (Wells *et al.*, 1984) and to use such information to develop strategies to reduce sublethal effects and to enhance survival (Cooke *et al.*, 2002). Several previous studies have documented that the duration a fish is angled depends on the size of the fish (*e.g. S. salar*, Thorstad *et al.*, 2003; *O. mykiss*, Meka & McCormick, 2005; *Albula* spp., Cooke & Philipp, 2004). No studies, however, have explicitly controlled for the angler (which is known to influence injury; Dunmall *et al.*, 2001) and gear to provide a robust assessment of the relationship between fish size, angling duration and the subsequent physiological disturbance. In this study, there was a strong relationship between the size of the fish and the duration of the angling event when the angler (*i.e.* the same angler fought all the fish) and the gear (*i.e.* the same gear was used for all angling events) were controlled. Beyond the 20 fish used for simulated angling, an attempt was made to angle two more fish but they escaped. Interestingly, those fish were both >600 mm L_T and would have represented the second and third largest fish that were angled. It is not uncommon to lose larger fish, which fight harder than their smaller conspecifics, and this evidence provides further support that the simulated angling events were realistic.

Relationships between the duration of the angling event and the degree of physiological disturbance were also observed. The rapid bursting behaviour of *Albula* spp. after hook-up is fuelled primarily through anaerobic metabolism (Kieffer, 2000), which leads to the accumulation of metabolites, acid–base disturbance, depletion of tissue energy stores and alterations to ionic and osmotic status (Wells *et al.*, 1984). The extent of physiological disturbance tends to reflect the magnitude of an angling event in a number of freshwater (Gustaveson *et al.*, 1991; Kieffer *et al.*, 1995; Thompson *et al.*, 2002; Thorstad *et al.*, 2003)

and marine (Skomal, 2007) fishes. For *A. vulpes*, blood lactate increased significantly with increased angling duration in a curvilinear manner. Skomal (2007) also developed regression models that directly related physiological changes to the duration of the capture event for blue shark *Prionace glauca* (L.) and he showed that blood pH decreased in a curvilinear manner with increased angling duration (for periods of up to 1 h), indicating an acidosis. Similar relationships between lactate accumulation and angling duration have been documented for a variety of freshwater and marine fish species (Gustaveson *et al.*, 1991; Kieffer *et al.*, 1995; Gallman *et al.*, 1999; Thompson *et al.*, 2002; Meka & McCormick, 2005) but were also absent for some species [*e.g.* no relationship between plasma lactate levels and angling duration for kahawai *Arripis trutta* (Forster); Davidson *et al.*, 1997].

In marine fishes, anaerobic exercise typically results in an influx of ions (such as Na^+ and K^+) and loss of water (Avella *et al.*, 1991). Several studies have documented ionic and osmotic disturbances associated with angling activity or other forms of anaerobic exercise (Gonzalez & McDonald, 1992; Gallman *et al.*, 1999; Thompson *et al.*, 2002). This study, however, did not observe such relationships for Na^+ or consistently for K^+ (it did increase post-capture with the seine and pre- and post-simulated angling). Interestingly, there was a trend towards a slight increase in PCV and in Hb concentrations with longer angling durations. The rapid onset of the increase in PCV may indicate contributions from both splenic contractions and erythrocytic swelling.

This is the first study to sample fish exposed to angling outside of a laboratory repeatedly (*i.e.* repeated sampling of cannulated fish exposed to chasing to simulate angling and then recovered in a 'black box'). The repeated sampling of individuals provides a powerful technique for assessing individual responses to angling induced stress and could serve as a tool for assessing different techniques for minimizing the impact of angling stressors. Interestingly, when repeatedly sampling individuals, different individual responses to angling stress were noted perhaps reflecting genetic or biotic differences (*e.g.* population, sex and feeding history). Although few studies have used angling simulations in the field (Anderson *et al.*, 1998), this approach may prove to be a valuable means of collecting relevant data on catch and release from diffuse fishes such as *Albula* spp. with low encounter and capture rates. Although this study has focused on techniques that collect physiological data in field situations, there are still logistic issues associated with the immediate sampling of angled fishes in the field. Nonetheless, sampling fishes in the field and linking post-release behaviour and fate with physiological status would also be extremely insightful, acknowledging that field sampling would introduce more variability associated with different anglers, different gear types and probably less expedient phlebotomy.

There is probably opportunity to reduce the magnitude of physiological disturbance by reducing the duration of the angling event through changing angler behaviour or gear (heavier tackle). At elevated water temperatures (fish were angled at a moderate 24° C) or when coupled with other stressors (*e.g.* air exposure), the duration of the angling event may become more important. It is also important to note that it was not possible to separate the effects of angling duration and fish size in this study, a similar problem faced by Thorstad *et al.* (2003). Previous research (Kieffer *et al.*, 1996) on largemouth bass *Micropterus*

salmoides (Lacepède) and brook charr *Salvelinus fontinalis* (Mitchill) has revealed that fishes experience size-specific responses to anaerobic exercise (*e.g.* magnitude of disturbance and recovery time). Only through controlled experimentation will it be possible to separate the effect of size and physiological disturbance. For example, if all larger fishes experience greater physiological disturbance than small fishes independent of the degree of exercise, then attempts to reduce angling duration will be less prudent.

Compared to freshwater and temperate marine fishes, little is known about the stress response and comparative physiology of tropical fishes (Frisch & Anderson, 2000). With the increased realization that physiology can inform fisheries management (Young *et al.*, 2006) and conservation (Wikelski & Cooke, 2006) on issues such as commercial and recreational fishing discards, there is a need for tools that enable field physiology on fishes in regions where tropical marine fishes reside (Costa & Sinervo, 2004). This study revealed that a number of commercially available field diagnostic tools provide new opportunities for studying fish physiology in remote locales. At a minimum, these tools provide researchers the opportunity to compare relative differences among treatments (Morgan & Iwama, 1997; Wells & Pankhurst, 1999) and when calibrated, they may provide detailed information on absolute differences (Wells *et al.*, 2003; Venn Beecham *et al.*, 2006). In this study, data derived from these tools provided the first detailed assessment of stress responses in *A. vulpes* in the context of the short-term consequences of capture-related stressors (seining and angling). This work will serve as the basis for controlled laboratory and field experimentation to evaluate the physiological correlates of post-release mortality (*i.e.* predation) for *A. vulpes* and to develop strategies to reduce the sublethal consequences of angling. A similar approach could be applied to other important game or commercial fishes in tropical or other remote regions around the world in an effort to enhance the sustainability of all fisheries sectors (Cooke & Cowx, 2006).

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