Chemical excretions of angled bonefish *Albula vulpes* and their potential use as predation cues by juvenile lemon sharks *Negaprion brevirostris*

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Bonefish *Albula vulpes* ($n = 7$) exercised to exhaustion and air exposed for 1 min as part of a catch-and-release angling event were found to excrete both ammonia and urea, but cortisol and lactate were below detectable levels. Urea made up a greater proportion of total nitrogen excretion from these fish at all time points following an angling event. When captive juvenile lemon sharks *Negaprion brevirostris* ($n = 12$) were exposed to a 30 s pulse of these chemicals [ammonia (500 mM), cortisol (20 µg l^{-1}), lactate (6 mM) or urea (3 mM)], they showed a significant reduction in the frequency of resting behaviours when exposed to ammonia and urea than when exposed to control water. It appears that products excreted by *A. vulpes*, particularly ammonia and urea, may provide an olfactory cue for the post-release predation of *A. vulpes* by *N. brevirostris* during catch-and-release angling events.   © 2010 The Authors

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INTRODUCTION

Angling is a popular pastime worldwide, and recently, there has been an increase in awareness of the role recreational fisheries play in the decline of fish populations (Cooke & Cowx, 2006). Many anglers practise catch-and-release whereby the fish is returned to the water without harvest (Cooke *et al*., 2002; Bartholomew & Bohnsack, 2005; Pelletier *et al*., 2007). Some regulatory bodies advocate catch-and-release angling as a method for protecting stocks, while many anglers also practice catch-and-release voluntarily (Policansky, 2002; Pelletier *et al*., 2007). Both of these attitudes

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are based on the assumption that released fishes survive the angling event and that returning fishes to the water has a reduced effect on a population compared with harvesting (Arlinghaus *et al.*, 2007). Studies, however, indicate that catch-and-release angling may result in mortality (Broadhurst *et al.*, 2005; Stunz & McKee, 2006), while sublethal effects can include changes in physiology (Suski *et al.*, 2003*a*), reproductive behaviour (Suski *et al.*, 2003*b*) and swimming performance (Schreer *et al.*, 2005). Increased knowledge of the sublethal effects caused by angling would be of use in animal welfare science and for fisheries management (Arlinghaus *et al.*, 2007).

In addition to physical injury from hooking, there are several factors that can induce a stress response in angled fishes. These include the exercise associated with capture (Lowe & Wells, 1996) and air exposure during photography or weigh-in (Ferguson & Tufts, 1992). Metabolic disturbances following simulated angling (exercise and air exposure) of bonefish *Albula vulpes* (L.) include elevated blood lactate (Suski *et al.*, 2007*a*; Cooke *et al.*, 2008). During burst swimming fishes may exhibit highly elevated muscle lactate concentrations (Wood, 1991; Kieffer *et al.*, 2001) with a subsequent rise in plasma lactate concentrations as a result of diffusion of lactate from the tissues. Numerous authors report elevated plasma cortisol levels after angling (Pankhurst & Dedual, 1994; Bartholomew & Bohnsack, 2005; Meka & McCormick, 2005; Cooke *et al.*, 2006) or simulated angling (Suski *et al.*, 2006). Concentrations of circulating nitrogenous waste products such as ammonia or urea may also be elevated following exercise or angling stressors (Todgham *et al.*, 2001; Suski *et al.*, 2007*b*). Furthermore, elevated cortisol levels influence the excretion of both ammonia and urea (Chan & Woo, 1978; Wood, 1991; McDonald & Wood, 2004), suggesting a possible link between these three chemicals.

Changes in chemicals associated with angling stress have been primarily documented as internal changes within the blood or blood plasma of fishes. Less is known about the potential excretion of these chemicals into the water during and after angling events or about the potential ecological effects of these excretions. Of particular interest is the effect these chemicals might have on potential predators of angled fishes. After exercise, lactate is usually cleared from white muscle (Wood, 1991) with little or no release to the external environment (Holeton & Heisler, 1983; Holeton *et al.*, 1983; Wood, 1988) although recently lactate excretion in the urine of fathead minnows *Pimephales promelas* Rafinesque has been documented (Ekman *et al.*, 2007). Cortisol excretion to the external environment occurs in rainbow trout *Oncorhynchus mykiss* (Walbaum) following stress (Ellis *et al.*, 2004) and an increase in excretion of nitrogenous waste is seen in response to chasing and stressful situations (Walsh *et al.*, 1994; Wakefield *et al.*, 2004).

Bonefishes *Albula* spp. are a group of commercially important sportfishes, with a circumtropical distribution. The strong swimming ability of *Albula* spp. makes them popular with recreational anglers (Cooke & Philipp, 2004). In Florida alone, bonefishing is worth several billion dollars annually (Humston, 2001) and they can be the economic mainstay for many tourism-based communities in developing countries such as The Bahamas (Danylchuk *et al.*, 2007*a*). The recreational angling industry presents an interesting model since most participants voluntarily practice catch-and-release (Cooke & Philipp, 2004; Danylchuk *et al.*, 2007*b*). *Albula* spp. are found inhabiting nearshore flats and shallow, tidal creek systems (Alexander, 1961). Lemon sharks *Negaprion brevirostris* (Poey) and barracuda *Sphyraena barracuda* (Edwards) also occupy these areas of shoreline, and once >50 cm total length (L_T)

are considered possible predators of *Albula* spp. (Cooke & Philipp, 2004; Danylchuk *et al*., 2007*a*).

Although low mortality (4%) for caught-and-released *Albula* spp. has been reported for fishes held in an enclosed body of water (Crabtree *et al*., 1998), this mortality rate is unlikely to be representative of the natural environment where predators are present. In fact, more recent studies have demonstrated post-release mortality rates of 8–39% in natural tidal creeks and flats (Cooke & Philipp, 2004; Danylchuk *et al*., 2007*a*, *c*), with higher predation rates occurring where the abundance of *N. brevirostris* was high (Cooke & Philipp, 2004). It is well known that sharks have highly developed olfactory systems (Moss, 1977) and is, therefore, possible that *N. brevirostris* use stress chemical cues to track caught-and-released *A. vulpes* even when no physical damage to the fish has occurred (Danylchuk *et al*., 2007*c*). The excretion of chemicals during stress in *A. vulpes* is yet to be investigated. Based on results from other fish species, however, it is possible that cortisol, ammonia, urea or lactate may be excreted by *A. vulpes* in response to angling stress. *Negaprion brevirostris* hunting *A. vulpes* released after angling events have been observed tracing the path of their prey, rather than taking a direct approach (Danylchuk *et al*., 2007*c*), suggesting olfactory tracking. As such, the aim of this study was to examine this possibility by addressing two questions: 1) what, if any, chemicals do *A. vulpes* release into the water following the stress of catch-and-release angling? 2) Do *N. brevirostris* behaviourally respond to the presence of stress chemicals released by *A. vulpes*? It was hypothesized that angling would produce a stress response in *A. vulpes* including excretion of stress chemicals known from other fish species. It was also predicted that *N. brevirostris* would show a positive attraction and distinct hunting response when exposed to typical stress chemicals.

MATERIALS AND METHODS

STUDY AREA

Field and laboratory work was conducted at the Cape Eleuthera Institute (CEI) from February to June 2008. All *A. vulpes* and *N. brevirostris* used in this study were captured off the northern coast of Cape Eleuthera, The Bahamas (24° 50′ N; 76° 20′ W). This coastline consists of sandy bays, shallow, tidal mangrove creeks and rocky limestone outcrops. Initially thought to be one species, genetic analysis has revealed several different, morphologically similar species of *Albula* (Colborn *et al*., 2001; Bowen *et al*., 2007). Previous work (A. J. Danylchuk, S. J. Cooke, T. L. Goldberg, C. D. Suski, J. B. Koppelman & D. P. Phillipp, unpubl. data) indicates that all *Albula* spp. found in this area are *A. vulpes*.

EXCRETIONS OF ANGLED *A. VULPES*

Albula vulpes were angled to exhaustion, either from a skiff or by wading, using standard fly fishing equipment (8 and 9 weight fly rod and reel combinations, with # 4 and # 6 sized flies that imitated small shrimps or crab). Duration of the fight time was recorded to the nearest 10 s. Fish were measured [L_T and fork length (L_F) to the nearest 5 mm]. Mass (M, g) was estimated from L_T (mm) according to the following equation: $M = 5.86\ L_T - 1841.20$, based on previous *A. vulpes* data (K. Murchie, unpubl. data). The mean ± s.e. L_T of fish caught was $433.0 ± 11.8$ mm ($n = 10$).

Albula vulpes were exposed to air for a period of 1 min after capture to simulate the actions of a typical recreational angler and then placed into a cooler (*c*. 30 l) containing *c*. 20 l of

freshly collected sea water. Aeration was provided by a recirculating bilge pump. Water samples (1·5 ml) were taken immediately after the fish was placed into the cooler (0 min) and then at 1, 5, 10 and 30 min and stored at $-20°$ C until later analysis. In addition, at 30 min, larger (500 ml) samples were taken for use as test substances in shark behavioural experiments. *Albula vulpes* manipulation occurred *in situ*, at the angling location. For comparative purposes, samples of sea water from outside the container were also taken at each angling location.

Water samples were transported frozen on dry ice to the University of Plymouth, U.K. Urea and ammonia concentrations were determined by the diacetyl monoxime (Rahmatullah & Boyde, 1980) and salicylate–hypochlorite (Verdouw *et al*., 1978) methods, respectively. Ammonia and urea concentrations were calculated as mmol-N and total nitrogen (N_T) was obtained by adding total ammonia-nitrogen (N_{TA}) to total urea-nitrogen (N_{TU}) for each time point. Lactate concentrations were determined using an enzymatic method (Gutmann & Wahlefeld, 1974). Due to the small sample volume, samples for cortisol analysis were pooled across all replicate individuals for each time period. Cortisol was extracted from the water samples according to the methods of Ellis *et al*. (2004, 2007) and concentrations determined by a commercial ELISA (DRG diagnostics; http://www.drg-diagnostics.de/) that has previously been used for the determination of cortisol in fish plasma (King *et al*., 2005; Sloman *et al*., 2008). The proportions of N_{TA} and N_{TU} were calculated as percentages of N_T.

NEGAPRION BREVIROSTRIS BEHAVIOUR

Juvenile *N. brevirostris* ($n = 12$; mean $\pm$ S.E. $L_T = 679·0 \pm 33·0$ mm) were captured using standard spinning rods, wire leaders and size 12–13, barbless, circle hooks. Each *N. brevirostris* was caught and transported to the laboratory individually in a large cooler (140 l) containing sea water, with *c*. 30% water exchange every 10 min. Transit times were between 11 and 92 min.

The mixing of salt water and chemicals during test conditions was assessed using an inert dye (one tablet in 1 l tank water; Bright dyes, Fluorescent Green–Yellow, Kingscote Chemicals; http://kingscotechemicals.com/). Dye tests indicated that in the presence of *N. brevirostris*, dispersal within the tank (*i.e.* <5% of dye remaining visible in the input quadrant) occurred within 5 min.

Following transport, *N. brevirostris* experiments took place in the aquatic holding facility at CEI. They were housed individually in 3·5 m diameter tanks, supplied with a continuous flow-through (*c*. 10 l min^{-1}) of filtered, aerated sea water. Tank volume was *c*. 9000 l of water (mean $\pm$ S.E. dissolved oxygen: $5·38 \pm 0·07$ mg l^{-1}, $24·80 \pm 0·15°$ C and salinity: $37·90 \pm 0·06$). The outdoor laboratory allowed for a natural photoperiod ($24°$ 50′ N). *Negaprion brevirostris* were allowed a minimum of 5 days recovery before any experimental manipulation. Feeding occurred on days 3 and 5 of this acclimation period, and 6 days after, ensuring animals were hungry during test periods. Tanks were siphoned every other day, but >24 h before the next test.

The tanks were divided visually into quarters to allow quantification of activity by methods similar to those used in other fish behaviour studies (Sloman *et al*., 2006; Baron *et al*., 2008). Each of the resulting quarters contained an input tube and was assigned a number for ease of identification. Input tubes were 150 cm lengths of plastic piping (13 mm internal diameter), secured to the tank wall at the mid-point of the quarter.

It was important to use biologically relevant concentrations of the test substances for *N. brevirostris* treatments. There is a limited amount of data, however, on the metabolic products of *A. vulpes*. Consequently, ammonia, cortisol and urea concentrations were calculated based on concentrations released during stress or exercise in other fish species. Final test substances were cortisol (20 μg l^{-1}), sodium lactate (6 mM), ammonium sulphate (500 mM) and urea (3 mM, as nitrogen; Sigma-Aldrich; www.sigmaaldrich.com) (Kong *et al*., 1998; Meka & McCormick, 2005; Suski *et al*., 2007*a*, *b*). Water was also collected from coolers containing angled *A. vulpes* for 30 min (*A. vulpes* water). Water entering the tank at the inflow was collected and used as a control. All substances were used in 100 ml volumes and were stored refrigerated ($4°$ C) but allowed to warm to ambient temperature before use.

Aeration and water flow were turned off for the duration of all behavioural tests. To ensure behavioural tests were double-blind, assistants were used to simultaneously pour liquid

through the four input tubes. One tube had one of the test substances and the remaining three had tank water (dummy substances). In the control tests, all four input tubes received tank water. As a result, both the identity and location of the chemical input were unknown to all observers and recorders. *Negaprion brevirostris* are well known for their ability to learn in captivity (Clark, 1961; Sabalones *et al*., 2004) so the location of the test substance-containing tube was randomized. Tests of all substances on an individual *N. brevirostris* took place over 6 successive days, and the order of substances was also randomized. Substances were delivered manually over the initial 30 s period of testing, and in such a way as to not be visible to the fish.

During the test period, the fish's location in the tank was recorded by an observer concealed from the fish's view by a screen. The time the fish spent in each quarter was determined by recording the times at which the fish moved to a new quarter of the tank. Movement to a new quarter was defined at the point when the anterior of the animal crossed the quarter boundary. This information was relayed from the observer to a recorder, who was unable to see into the tank. The frequency of distinct behaviours was also noted (Table I). The test period lasted 60 min from the first addition of substances to the tubes. One substance was tested per day, with a minimum of 24 h between tests. After each test period, tubes were flushed with freshly collected tank water.

DATA ANALYSIS

All data were tested for normality using Kolmogorov–Smirnov tests, and for homogeneity of variance with Levene's test, appropriate parametric or non-parametric tests were subsequently used. Statistical comparisons of seawater samples with 0 min cooler samples were made using independent *t*-tests. Differences in the change in chemical concentration between all sampling times were tested for significance using Friedman tests. Pair-wise comparisons between sampling times were performed using Wilcoxon signed-rank tests. Friedman tests were also used for statistical analysis of the *N. brevirostris* data to analyse differences in time spent swimming, time spent in the treatment quadrant and the incidence of behaviours across all treatments. Statistics were calculated using SPSS (version 16.0; www.spss.com). All values are means ± S.E. and significance for all tests was set at $P = 0.05$.

RESULTS

EXCRETIONS OF ANGLED *ALBULA VULPES*

Samples for three *A. vulpes* were ruptured during trans-Atlantic transport. The remaining samples ($n = 7$) were from fish with a L_T of 425.0 ± 13.8 mm and a mass of 0.65 ± 0.08 kg. None of these fish were bleeding at the time of placement

TABLE I. Behaviours exhibited by captive, juvenile *Negaprion brevirostris*

Behaviour	Definition
Resting	Complete cessation of forward movement, resting on the tank floor
Rolling	Slow lateral turn onto the side, while swimming in mid or surface water
Turning	Fast turn taking place within one body length[a]
Circling	Swimming in a tight circling motion, within one or two body lengths
Biting	Complete opening and closing of the mouth
Head shake	Vigorous lateral movement of the head, greater than that normally associated with swimming motion
Head raise	Elevation of the head, breaking the water surface

[a]Not to be confused with a general change in direction.

into the cooler, but two had lost equilibrium. For those fish that lost equilibrium, equilibrium was regained in <30 s, and all *A. vulpes* used in this study survived to release. Fight times were 254.29 ± 14.82 s. Of the three fish that were used to produce *A. vulpes* water for *N. brevirostris* experiments (L_T 443.33 ± 28.91 mm; *M* 0.71 ± 0.17 kg) only one lost equilibrium.

Both cortisol and lactate concentrations were below levels detectable in water. There was no significant difference in ammonia (independent *t*-test, d.f. = 10, $P >$ 0.05) between free sea water and 0 min samples taken as the fish was added to the cooler. In contrast, the concentration of urea was significantly higher in 0 min samples than in free sea water (independent *t*-test, d.f. = 10, $P < 0.05$). Water ammonia concentrations varied significantly across time points (Friedman test, d.f. = 5, $P < 0.01$). Specifically, ammonia concentrations were significantly lower at 0 min than at all other time points. Additionally, at 0, 1, 5 and 10 min the ammonia levels were significantly lower than at 30 min (Wilcoxon signed-rank test, d.f. = 5, $P < 0.05$). There was also a significant temporal trend in water ammonia concentrations, with an average increase of 0.52 µmol l^{-1} min^{-1} [$P < 0.05$; Fig. 1(a)].

Urea concentrations in the water surrounding the *A. vulpes* did not vary significantly across any time points (Friedman test, d.f. = 5, $P > 0.05$). There was a significant trend, however, towards increasing urea concentrations at later time points, with an average increase of 0.77 µmol l^{-1} min^{-1} [$P < 0.001$; Fig. 1(b)]. At all time points N_{TU} exceeded N_{TA} (Fig. 2), and at 0 min this was significantly so (Wilcoxon signed-rank test, d.f. = 4, $P < 0.05$).

NEGAPRION BREVIROSTRIS BEHAVIOUR

Negaprion brevirostris did not spend significantly more time in quadrants where chemicals were added in any time period (Friedman test, d.f. = 5, $P > 0.05$). When the 1 h long time period was analysed as a whole, different treatments resulted in *N. brevirostris* resting at significantly different frequencies (Friedman test, d.f. = 5, $P < 0.01$). A significantly greater incidence of resting occurred during the control in comparison to both ammonia and urea treatments (Wilcoxon signed-rank test, d.f. = 5, $P < 0.01$), indicating a greater frequency of active movements in response to both ammonia and urea. Significantly less resting also occurred in cortisol and lactate treatments in comparison to *A. vulpes* water [Fig. 3(e)]. No overall significant difference in the incidence of resting behaviour was found for the initial 5 min period (Friedman test, d.f. = 5, $P > 0.05$). No significant difference (Friedman test, d.f. = 5, $P > 0.05$) was found in the incidence of behaviours other than resting (Fig. 3). Rolling behaviour was only exhibited by one animal and consequently the data set was so small that it was not considered statistically viable and is not presented here.

DISCUSSION

EXCRETIONS OF ANGLED *A. VULPES*

This study indicates that *A. vulpes* do excrete ammonia and urea after angling. Although the *N. brevirostris* behavioural experiments revealed a large amount of individual variation (among fish), the data do suggest that *N. brevirostris* respond
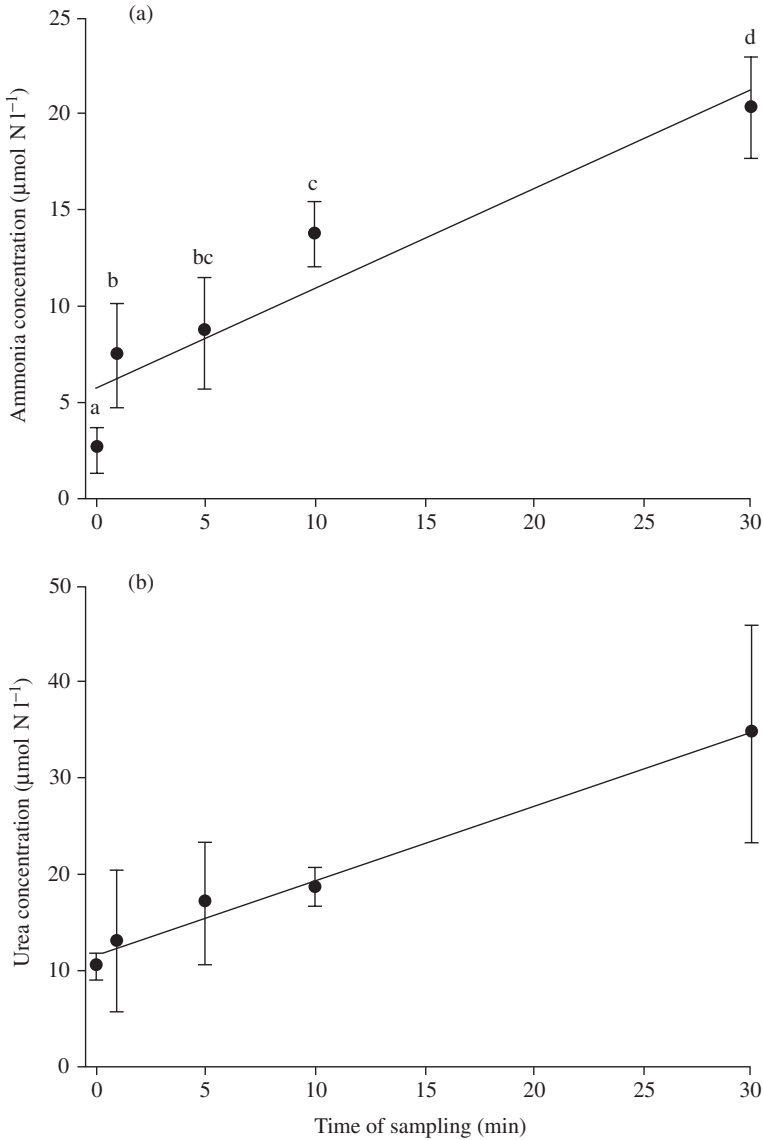
FIG. 1. Concentrations of (a) ammonia and (b) urea in water surrounding *Albula vulpes* at various times after angling and 1 min air exposure. Values are means $\pm$ S.E. ($n = 7$). Where significant differences occur ($P < 0.05$, as determined by pair-wise Wilcoxon signed-rank tests) they are indicated by mismatching lowercase letters. The curves were fitted by (a) $y = 5.70 + 0.52x$ ($r^2 = 0.89$, $P < 0.05$) and (b) $y = 11.68 + 0.76x$ ($r^2 = 0.99$, $P < 0.001$).

to known fish stress chemicals. Specifically, it appears that *N. brevirostris* rest less frequently and, therefore, increase frequency of active movements when exposed to ammonia and urea as opposed to control sea water. This result indicates that *N. brevirostris* may detect these chemicals in sea water and use them as cues to locate *A. vulpes*.
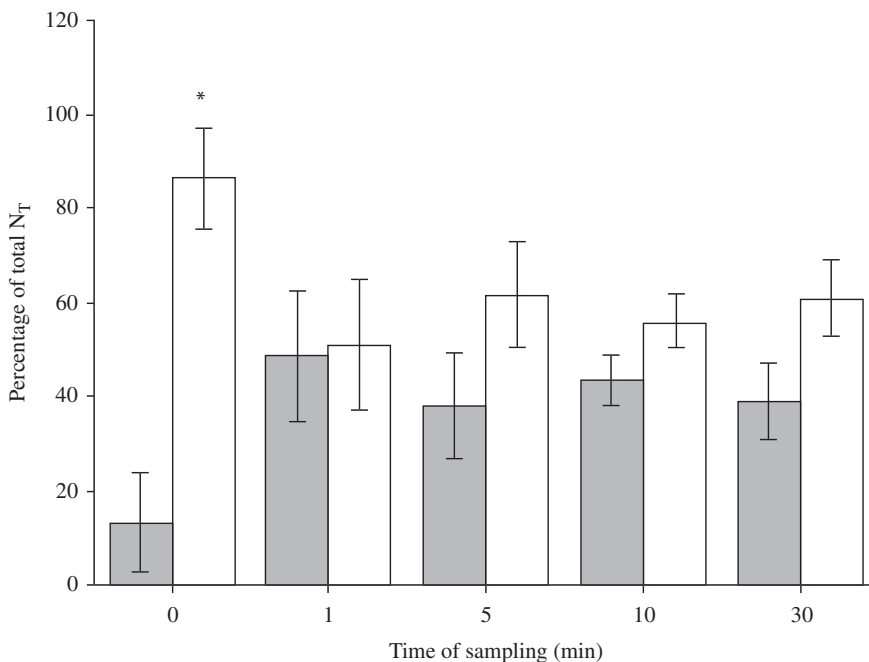
FIG. 2. Mean ± s.e. ammonia (▨) and urea (□) as a percentage of total nitrogen (N_T) at each sampling time.
*, significant difference between ammonia and urea concentrations at time of sampling.

Ammonia is readily excreted by fishes across the gill membrane, particularly in response to physical exertion (Wakefield *et al.*, 2004) and it is, therefore, unsurprising to find that ammonia was excreted by angled *A. vulpes*. At all five sampling times, however, N_{TU} exceeded N_{TA} suggesting that *A. vulpes* can be ureotelic, certainly under the stressful conditions examined here. Production of urea occurs *via* three principal pathways: 1) the breakdown of arginine or uric acid [argininolysis or uricolysis, respectively, as seen in most ammoniotelic teleosts (Wright, 1995)], 2) *de novo* synthesis of urea [*via* the complete ornithine–urea cycle, as in most ureotelic fishes (Mommsen & Walsh, 1989; Saha & Ratha, 2007)] and 3) a decrease in ammoniotely resulting in an increased proportion of nitrogenous waste as urea [thought to occur *via* the trapping of ammonia by glutamine synthetase (GNS) (Walsh & Milligan, 1995)]. Enzymatic analysis of *A. vulpes* tissues for GNS, other enzymes of the ornithine–urea cycle and allantoicase (the primary enzyme for uricolysis) might reveal the dominant mechanism.

A number of hypotheses have been presented for ureotely in marine teleosts including crypsis, social signalling, avoidance of hyperammonia toxicity and a generalized stress response involving elevated cortisol levels (Walsh & Milligan, 1995; Wood *et al.*, 1995; Iwata *et al.*, 2000; Sloman *et al.*, 2005). Of these hypotheses, it is most likely that ureotely in *A. vulpes* is the result of a generalized stress response following angling as angling induces elevated cortisol in many fish species (Bartholomew & Bohnsack, 2005; Meka & McCormick, 2005; Cooke *et al.*, 2006). Production of urea is energetically expensive (Walsh *et al.*, 2004), which may add to the burden
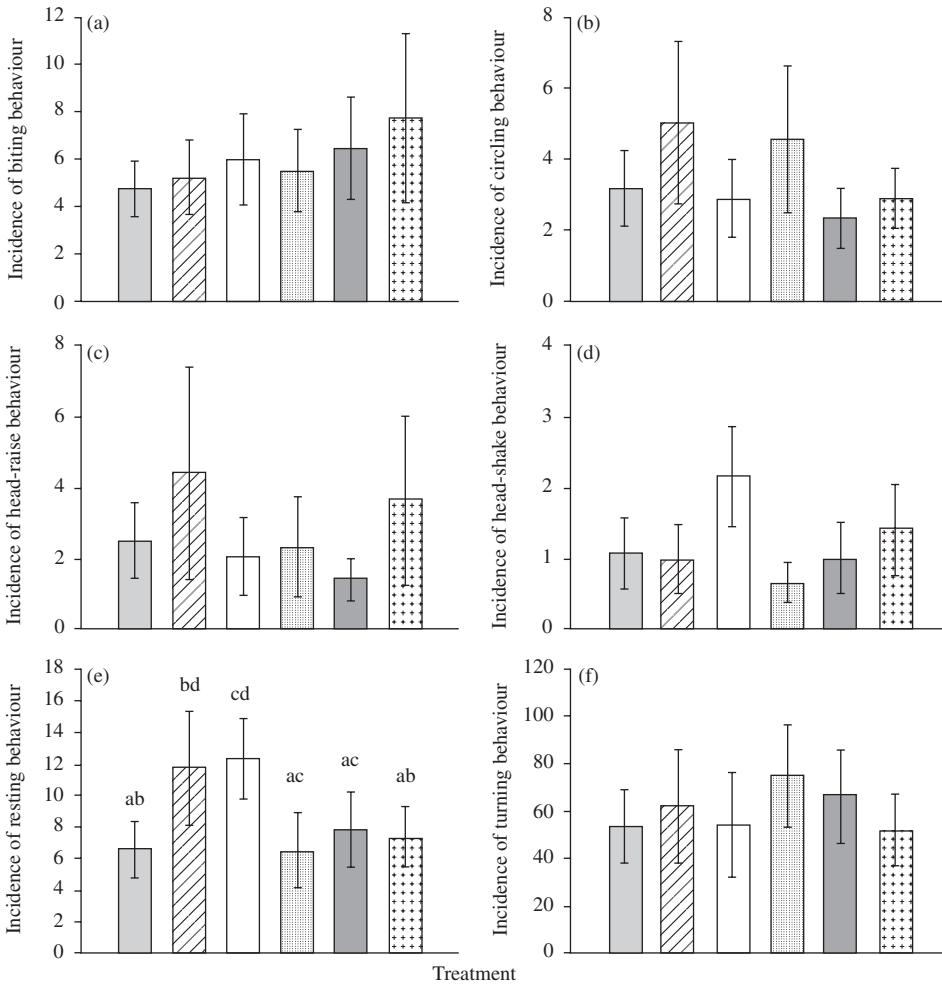
FIG. 3. Frequency of (a) biting, (b) circling, (c) head-raise, (d) head-shake, (e) resting and (f) turning behaviours exhibited by *Negaprion brevirostris* when exposed to different treatments: ammonia (▨), *Albula vulpes* water (▧), control (▢), cortisol (▨), lactate (▨) and urea (⊞). Values are for the entire 1 h test period and are means ± S.E. Sample sizes (*n*) were 12 for all tests except *A. vulpes* water (*n* = 11). Where significant differences occur they are indicated by mismatching lowercase letters.

of recovery from exercise but many other teleosts also display altered nitrogenous excretion in response to stress (Wood *et al.*, 1995; McDonald & Wood, 2004).

Albula vulpes do not adjust well to captivity and are easily stressed by human observation and the low stocking densities required for baseline stress levels (Murchie *et al.*, 2009) are unsuitable for detecting waterborne chemicals. As a result, baseline data on the excretion of nitrogenous waste in unstressed *A. vulpes* were not obtained. Therefore, while it can be concluded that *A. vulpes* release both ammonia and urea after an angling event it is difficult to determine the magnitude of this response. In the ureotelic gulf toadfish *Opsanus beta* (Goode & Bean), rates of ammonia and urea excretion in fish held in the laboratory have been recorded as 0·2 μmol N g^{-1} h^{-1}

and 1.0 μmol N g^{-1} h^{-1}, respectively (Barimo *et al.*, 2004), which is approximately five and one and a half times lower respectively than the rates found in the present study in angled *A. vulpes*. In *O. mykiss*, exhaustive exercise increases excretion of both ammonia and urea (Todgham *et al.*, 2001) with a threefold increase and twofold increase in ammonia and urea excretion respectively in *O. mykiss* exercised at high speed over a period of 2 days. It is, therefore, hypothesized that angling could cause a similar increase in ammonia and urea excretion in *A. vulpes*, although confirmation of this is dependent on the development of a method for obtaining baseline samples.

NEGAPRION BREVIROSTRIS BEHAVIOUR

When enclosed in circular tanks, sharks often show a persistent circling behaviour (Johnsen & Teeter, 1985; L. J. Dallas, pers. obs.), whereby they consistently swim around the tank's outer edge. This behaviour generates some water movement and is probably responsible for the rapid dispersal of inert dye during preliminary trials. Assuming that observation of dye under test conditions is representative of the dispersal of test substances, concentrated amounts of these substances would only have been present in the input quadrant for the first 5 min of the testing period. Johnsen & Teeter (1985), however, report that bonnethead sharks *Sphyrna tiburo* (L.) show continued response to chemical stimuli, even after dilution to below levels detectable analytically. This suggests that behaviour occurring after the initial 5 min period could still be the result of chemical exposure.

Sharks have highly developed sensory systems, and their olfactory system is particularly acute (Tester, 1963; Mathewson & Hodgson, 1972; Johnsen & Teeter, 1985). This enables them to locate the source of chemical signals [*e.g.* the nitrogenous compounds glutamate and trimethylamine (Mathewson & Hodgson, 1972)] and use these as stimuli when hunting (Johnsen & Teeter, 1985). Tester (1963) showed that multiple species of sharks became excited when exposed to water from tanks containing fishes; furthermore, these sharks became more excited and demonstrated a hunting response when exposed to water from tanks of agitated fishes. This suggests that hunting behaviour is triggered by chemicals that are excreted by fishes during both stressful and non-stressful situations, increasingly so in the former. The results of the current study give some indication of the chemicals that may elicit this behaviour in *N. brevirostris*.

Negaprion brevirostris exposed to ammonia and urea rested less frequently than when exposed to control water. This increased frequency of activity could be a searching response, as the fish attempt to locate the chemical source. Teleosts are the primary prey of *N. brevirostris* (Wetherbee *et al.*, 1990) and are mostly ammoniotelic (Wood, 1993). Therefore, the ability to detect traces of ammonia in water would be beneficial to *N. brevirostris* during hunting. While ammonia can be toxic at high concentrations, the change in resting behaviour detected here is unlikely to be an avoidance response due to the low concentrations of ammonia used. If the prey species of *N. brevirostris* are capable of ureotely, the ability to detect this chemical would provide a distinct advantage when searching for prey. The results presented here strongly suggests that *A. vulpes* can be ureotelic and another species known to be prey of *N. brevirostris*, the scarecrow toadfish *Opsanus phobetron* Walters & Robins (Newman *et al.*, 2004), belongs to the same family as the known ureotelic species *O. beta*. It is also possible, however, that detection of urea could indicate

the presence of other elasmobranchs as urea is their primary waste product (Wood, 1993). The possibility also exists that *N. brevirostris* in the present study altered their resting patterns in order to avoid predation or competition with larger sharks or to locate conspecifics.

For the behavioural experiment, 100 ml of a 500 mM ammonia and 3 mM urea solution elicited a behavioural change in *N. brevirostris*; addition of 100 ml *A. vulpes* water, however, did not. Given that levels of ammonia and urea excreted by angled *A. vulpes* in the present study were found to be *c*. 20 and 35 µM, the levels of ammonia and urea would be considerably lower in the *A. vulpes* water sample added to the behavioural arena. It is, therefore, perhaps not surprising that no response to *A. vulpes* water was seen. Nevertheless, when the dilution effect of adding the separate ammonia and urea solutions to a 9000 l tank is taken into account, the *N. brevirostris* would have been directly exposed to concentrations of ammonia and urea that were similar, or lower than, those found in the immediate vicinity of angled *A. vulpes*. Thus, the changes in resting behaviour seen in the present study in response to these compounds occur at environmentally realistic concentrations.

Inter-individual variation in behaviour of *N. brevirostris* in this study was very high, suggesting that external factors may play a role in its response to chemical stimuli. *Negaprion brevirostris* have a well-known capacity to learn in captivity (Clark, 1961) and presumably this is also the case for wild conspecifics; consequently the age of an animal and its previous experience of hunting, or indeed of stressed fishes, may influence its response to olfactory cues. While previous research certainly suggests that *N. brevirostris* use olfaction as a primary cue when hunting post-release *A. vulpes* (Danylchuk *et al*., 2007*c*), sharks also utilize an array of alternative senses when hunting, including vision (Gardiner & Atema, 2007), electroreception (Fields, 2007; Peters *et al*., 2007) and mechanoreception (Gardiner & Atema, 2007), and they are primarily rheotactic when localizing the source of a scent (Mathewson & Hodgson, 1972). The division of sensory employment that these *N. brevirostris* use to hunt *A. vulpes* is not known, but it is likely that this will vary across individuals.

This study indicates that angled *A. vulpes* do release chemicals into the water after angling and, therefore, it is possible that *N. brevirostris* may use these chemicals as cues when locating *A. vulpes*. Many other factors, however, also influence the post-release predation of *A. vulpes* by *N. brevirostris*, including predator density, handling, air exposure and loss of equilibrium (Cooke & Philipp, 2004; Danylchuk *et al*., 2007*c*; Suski *et al*., 2007*a*). To reduce the possibility of shark predation, anglers are currently advised to release *A. vulpes* into areas of lower predator density (Cooke & Philipp, 2004). This solution, however, is not ideal. It is impractical and costly (in both time and money) for anglers to transport fish any substantial distance, and there are currently no data on the ecological effect of relocating *A. vulpes*. The results of this study suggest that it might be possible to release *A. vulpes* in their capture location after allowing their metabolite excretion to return to basal levels. Until this has been tested experimentally, however, it is recommended that anglers continue to relocate fish and minimize fight time and air exposure to reduce stress (Ferguson & Tufts, 1992; Cooke & Philipp, 2007; Suski *et al*., 2007*a*). Recent advances in the ability to capture and hold *A. vulpes* in captivity for sustained periods of time (Murchie *et al*., 2009) will hopefully enable more data to be gathered on the baseline physiology of these important sportfish and allow for the experimental implementation of new handling and release techniques. This should ensure that future research continues

to inform the angling community of the best possible practices and improves the sustainability of *A. vulpes* angling in The Bahamas and further afield.

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