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

L. J. Dallas*, A. D. Shultz†, A. J. Moody*, K. A. Sloman*‡ and A. J. Danylchuk§∥

*School of Marine Science and Engineering, University of Plymouth, Drake Circus, Plymouth PL4 8AA, U.K., †Flats Ecology and Conservation Program, Cape Eleuthera Institute, c/o Twin Air, 3406 SW 9th Avenue, Fort Lauderdale, FL 33315, U.S.A. and §Department of Natural Resources Conservation, University of Massachusetts Amherst, Amherst, MA 01003, U.S.A.

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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.

Key words: catch-and-release angling; nitrogenous waste; olfaction; recreational fisheries; stress; welfare.

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...
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., 2003a), reproductive behaviour (Suski et al., 2003b) 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., 2007a; 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., 2007b). 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., 2007a). The recreational angling industry presents an interesting model since most participants voluntarily practice catch-and-release (Cooke & Philipp, 2004; Danylchuk et al., 2007b). 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 (LT)
are considered possible predators of Albula spp. (Cooke & Philipp, 2004; Danylchuk et al., 2007a).

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., 2007a, 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., 2007c). 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., 2007c), 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. Philipp, 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 \([L_T \text{ and fork length } (L_F) \text{ to the nearest 5 mm}]\). Mass \((M, \text{g})\) was estimated from \(L_T \text{ (mm)}\) 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<sub>T</sub>) was obtained by adding total ammonia-nitrogen (N<sub>TA</sub>) to total urea-nitrogen (N<sub>TU</sub>) 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<sub>TA</sub> and N<sub>TU</sub> were calculated as percentages of N<sub>T</sub>.

**NEGAPRION BREVIROSTRIS BEHAVIOUR**

Juvenile *N. brevirostris* (*n* = 12; mean ± s.e. *L<sub>T</sub> = 679.0 ± 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<sup>−1</sup>) of filtered, aerated sea water. Tank volume was c. 9000 l of water (mean ± s.e. dissolved oxygen: 5.38 ± 0.07 mg l<sup>−1</sup>, 24.80 ± 0.15°C and salinity: 37.90 ± 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 siphorned 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<sup>−1</sup>), 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.*, 2007a, 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*<sub>T</sub> 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>
<thead>
<tr>
<th>Behaviour</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Resting</td>
<td>Complete cessation of forward movement, resting on the tank floor</td>
</tr>
<tr>
<td>Rolling</td>
<td>Slow lateral turn onto the side, while swimming in mid or surface water</td>
</tr>
<tr>
<td>Turning</td>
<td>Fast turn taking place within one body length&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Circling</td>
<td>Swimming in a tight circling motion, within one or two body lengths</td>
</tr>
<tr>
<td>Biting</td>
<td>Complete opening and closing of the mouth</td>
</tr>
<tr>
<td>Head shake</td>
<td>Vigorous lateral movement of the head, greater than that normally associated with swimming motion</td>
</tr>
<tr>
<td>Head raise</td>
<td>Elevation of the head, breaking the water surface</td>
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<sup>a</sup>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 $254.29 \pm 14.82$ s. Of the three fish that were used to produce *A. vulpes* water for *N. brevirostris* experiments ($L_T$ $443.33 \pm 28.91$ mm; $M$ $0.71 \pm 0.17$ 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 $0.52 \mu$mol 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 $0.77 \mu$mol 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
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*.

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Time of sampling (min)

Percentage of total NT

Fig. 2. Mean ± s.e. ammonia (■) and urea (□) as a percentage of total nitrogen (NT) 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, NTU exceeded NTA 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 uricolyis, 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 uricolyis) 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 (Barholomew & 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...
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., 2007c), sharks also utilize an array of alternative senses when hunting, including vision (Gardiner & Atema, 2007), electrosception (Fields, 2007; Peters et al., 2007) and mechanoresception (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., 2007c; Suski et al., 2007a). 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., 2007a). 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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