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Impacts of sun protection on feeding behavior and mucus removal of bonefish, *Albula vulpes*

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Abstract Catch-and-release angling is growing as a tool for the conservation of fish stocks because it assumes that the impacts of angling are negligible. However, many studies have shown that catch-and-release can be stressful to the fish and even result in mortality. Bonefishing represents a popular catch-and-release fishery in the tropics and subtropics, with most anglers spending 6+ hours per day in full sunlight. To protect themselves, anglers typically employ sun protection in the form of liquid sunscreen and UV-blocking clothing. Exposure to chemicals contained in sunscreens may impose additional stressors on fish that are handled and subsequently released. In this study we conducted two separate experiments in the lab facilities in Cape Eleuthera, Bahamas. The first examined bonefish feeding behaviors in response to bait handled with zinc-based sunscreen, oxybenzone-based sunscreen, and no coating on the researcher's hands. The second experiment quantified the effects of sunscreens and UV blocking gloves on the removal of fish's protective mucus layer as a result of handling. We did not observe evidence of a change in feeding behavior when bait was handled with hands covered in sunscreen compared to wet hands. However, there was an increase in removal of protective mucus of bonefish when researcher's hands were coated in oxybenzone containing sunscreen compared to researchers handling fish with wet hands. The results of this study indicate wet hands are the best way to handle fish when participating in catch-and-release angling.

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Introduction

Bonefishing is a popular recreational fishery in the Bahamas, and anglers voluntarily partake in catch-and-release practices. Catch-and-release angling is often practiced in an effort to increase the longevity and sustainability of a fishery. This type of angling assumes the costs of landing a fish are negligible and that fish survival will not be negatively impacted by capture (Muoneke and Childress 1994; Cooke and Suski 2005; Cooke and Schramm 2007; Danylchuk et al. 2007b); however, recent research has shown that catch-and-release practices can result in greater susceptibility to predation and thus can lead to mortality (Broadhurst et al. 2005; Danylchuk et al. 2007a). Sub-lethal consequences of catch-and-release angling such as changes in

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physiology, reproductive behavior, swimming performance, and physical injury due to hooking can also result in delayed mortality or reduced fitness (Suski et al. 2003; Schreer et al. 2005; Arlinghaus et al. 2007; Stein et al. 2012).

The physiological and behavioral consequences of catch-and-release angling have been attributed to stressors such as exhaustive exercise, injury, air exposure, and handling (Suski et al. 2007; Danylchuk et al. 2007a). In particular, injuries incurred as a result of handling (e.g., mucus removal, abrasions, scale loss, and fin fraying) increase a fish's susceptibility to diseases/pathogens, cause dehydration due to passive water loss, and increase the energetic demand of swimming due to reduced lubrication, all of which can lead to delayed mortality (Bernadsky et al. 1993; Barthel et al. 2003). Kostecki et al. (1987), for example, found that mucus and scale removal in Atlantic salmon smolts resulted in mortality directly from the injuries, but more often from secondary problems such as predation or disease. Typically, handling fish with dry hands results in the greatest loss of mucus and scales with most catch-and-release guidelines indicating it is best to handle fish with wet hands or wet gloves (Pelletier et al. 2007), yet very little is known about how sunscreen applied to hands might influence mucus loss.

Anglers are often concerned with the negative health issues associated with prolonged exposure to the sun (Kricker et al. 1994). This is particularly true in the tropics, where the glare/reflection of the sun off the surface of the water increases sun exposure (Woolley et al. 2002). To protect themselves, anglers typically apply sun protection in the form of conventional cream or spray sunscreen and/or wear UV blocking clothing (e.g., gloves, shirts, buffs) (Neale et al. 2002). UV blocking apparel has become more popular due to the fact that sunscreen can wash off over time. There are many different types of sunscreens available to the average angler; however, these can generally be broken up into oxybenzone containing sunscreens, and zinc-based sunscreens (Cross et al. 2007).

Many anglers believe that fish can detect certain odors on artificial flies such as, gasoline, insect repellent and sunscreen (Brown 2008), which will make them less likely to consume bait. A common response to environmental contaminants is reduced feeding which can lead to reduced energy intake (Maltby 1999). Research by Brown et al. (1987) found that for largemouth bass the addition of the chemical pentachlorophenol to the water caused fish to perform fewer strikes. Additionally, Morgan and Kiceniuk (1990) found that low levels of fenitrothion decrease the efficiency of Atlantic salmon's attack sequence as well as the number of prey ingested. However, Little et al. (1990) tested 6 different agricultural chemicals, carbaryl, chlordane, dimethylamine salt of 2,4-dichlorophenoxyacetic acid (2,4-DMA), tributyl phosphorotrithioate (DEF1), methyl parathion, and pentachlorophenol, and found that for rainbow trout the frequency of strikes is less sensitive to certain toxicants compared to others. These studies have focused on what adding chemicals to the water will do to feeding behavior, and there is a lack of literature focusing on how directly contaminated bait will affect feeding response.

The efficacy of catch-and-release angling in preserving fish stocks may be reduced if sun sunscreens result in injury or altered feeding behaviors. The goals of this study were two-fold. First, we quantified the effects of oxybenzone sunscreen and zinc-oxide sunblock on the feeding behavior of bonefish. Second, we assessed how sunscreens, UV-blocking gloves, and wet hands affected mucus layer removal and recovery time of bonefish following a simulated angling event. The outcomes of this study will improve best handling practices for catch-and-release angling of bonefish.

Materials and methods

Bonefish collection and husbandry

This study was conducted at the Cape Eleuthera Institute (CEI), Eleuthera, the Bahamas between January 24th and July 18th, 2014. Adult bonefish $N=77$ [average size=396.3 mm fork length, standard error (SE)= 5.8 mm, range=326–505 mm] were collected from nearby saltwater tidal creeks (see Danylchuk et al. 2007a for a description of sampling sites) by seine net, then transported in 142 L plastic coolers by boat to CEI's wetlab. The duration of transport was typically 15–30 min and water was exchanged every 5 min (Murchie et al. 2009). Prior to experimentation, bonefish were acclimated to continuously aerated 13,180 L flow-through (1800 L/h) seawater holding tanks for at least 3 days or until they resumed feeding. During acclimation, water temperature (range 21–28.1 °C), dissolved oxygen (range 5.16–8.3 mg/L), and salinity (range

34.7–39.4 ppt) were monitored on a daily basis. Bonefish were fed standard sized, 1–2 g, pieces of commercially available raw shrimp (Sea Best, Beaver Street Fisheries Inc., Jacksonville, FL, USA).

Feeding preference

Following acclimation, fish (N=31) were transferred to 53 L totes filled with seawater and transported to an individual 13,180 L tank. Bonefish were acclimated to their tank for 24 h (Murchie et al. 2009), then continually fed raw shrimp for a 10 min observation period, or until fish became satiated (Grove et al. 1978). Any shrimp remaining in the tank following the 10-min period was collected and weighed. During this observational period, feeding behaviors of the bonefish were recorded, and the mass of shrimp consumed was calculated as the difference between the wet weight of shrimp placed in the tank, and the wet weight of shrimp left uneaten. Four different feeding behaviors were recorded along with the time they were first exhibited: investigation, pick-up, expel, and mouth (Bardach et al. 1980). Investigation was classified as visual examination of the shrimp, indicated by bonefish coming into close proximity to the raw shrimp. Pick-up was recorded when a bonefish drew a piece of shrimp into its mouth, and expel was noted when a fish dropped a piece of shrimp. Mouthing was recorded when the bonefish mechanically processed (i.e., broke the food item into smaller pieces) the shrimp, exhibited by simultaneous rapid opening and closing of the mouth, head thrusts, and operculum flares.

The influence of sunscreen on the feeding behavior of bonefish was assessed after fasting the fish for 24 h. Researchers applied oxybenzone based sunscreen (Banana Boat, broad spectrum SPF 30 2012 Energizer Personal Care, LLC, Shelton, CT, U.S.A.) (N=10) or zinc-based sunscreen (Solar Sense, broad spectrum SPF 50, CCA Industries, Inc., Rutherford, NJ, U.S.A.) (N=11) to their hands before handling the same quantity of shrimp consumed on the previous day. Bonefish were continually fed raw shrimp for a 10 min observation period, or until fish became satiated. Feeding behavior during this period was recorded as described above. Shrimp handled with wet, clean hands were fed to bonefish (N=10) and served as controls for this experiment. All researcher's hands were washed prior to handling food or applying sunscreen. The suggested amount of sunscreen by the U.S. FDA is 2 mg for every cm^2 of skin, so 0.25–0.30 g of sunscreen was applied (Danovaro et al. 2008).

Handling and recovery

Throughout acclimation bonefish (N=46) were fed Spanish sardines (*Sardinella aurita,* McRoberts Sales Co., Gainesville, FL, USA). Once acclimated, bonefish were transferred using rubber dip nets to individual 1400 L tanks (Casselman 2005). Anglers typically fight a fish to exhaustion (4 min) and then handle the fish to remove the hook (Cooke and Philipp 2004). To simulate a typical angling event, bonefish were exercised by lightly grabbing the tail in an effort to make the fish swim continuously for 4 min, (Cooke and Philipp 2004; Danylchuk et al. 2007a). Following exercise, bonefish were handled with wet hands (N=9), wet hands coated with 0.25–0.30 g oxybenzone based sunscreen (N=9), wet hands coated with 0.25–0.30 g zinc based sunscreen (N=10), and wet fishing gloves (N=9) (Abaco Bay Fingerless Sun Glove, Glacier Glove, Reno, NV, U.S.A.). These fish were also air exposed for 30 s to simulate the typical amount of time needed for anglers to take a photo of their fish (Cooke and Philipp 2004; Danylchuk et al. 2007a). Control fish (N=9) were exercised and transferred into a holding tank using a rubber dip net without being handled. All fish were transferred to tanks using a rubber dip net, in an attempt to standardize treatments. Mucus lost by bonefish as a result of handling was collected using a plastic scraper to remove mucus from the experimenters' hands, and a sieve (No. 35, 32 mesh, 0.500 mm, #3076 Hubbard) was used to collect any additional mucus remaining in the water of the 53L handling tote. Bonefish mucus is generally easily identifiable on the experimenters' hands and the surface of the water due to its "clumping" nature (Fig. 1). Mucus was air dried for 48 h and the dry weight measured to the nearest 0.001 g (Gemini-20, Portable milligram scale, AWS, Norcross, GA).

Following the 30 s handling protocol, bonefish were transferred to a 53 L tote filled with seawater, dart tagged for identification (80 mm, PDS small plastic tipped dart tag, Hallprint, Hindmarsh Valley, South Australia, Australia), and then transferred to a separate 13,180 L flow-through tank for recovery. Prior to tag insertion, the ends of the dart tags were dipped in different colored Plasti Dip (Performix, Plasti Dip International, Blaine, MN) to aid in visual identification of individual fish in tanks. Injury caused by handling

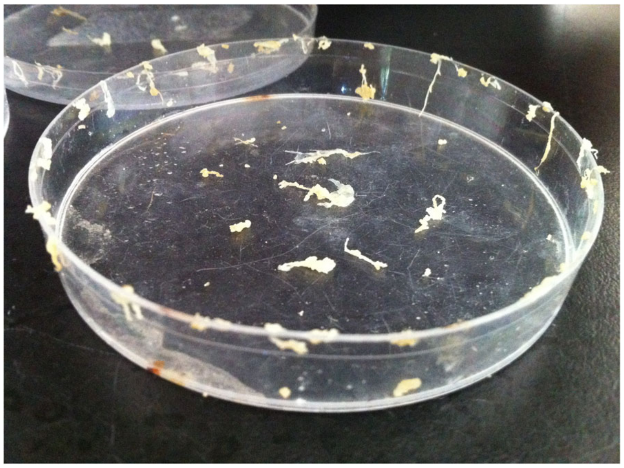

Fig. 1 Image of slime collected from a bonefish depicting its "clumping" nature

treatment was quantified daily by visually estimating the percent of the body covered by bacterial growth and bruising. Recovery was monitored daily for 2–3 weeks after handling, or until the bonefish exhibited normal feeding behavior and showed no sign of bacterial growth or bruising.

Analyses

A visual analysis of fitted residuals, using a normal probability plot (Anscombe and Tukey 1963), was used to assess normality, while Hartley's F_{max} test (Hartley 1950), combined with visual inspection of the distribution of fitted residuals, were used to assess homogeneity of variance for all tests. Ranked data were used if either normality or homogeneity of variance assumptions were violated (Siegel and Castellan 1988). Time until initial pickup in the control, zinc and oxybenzone treatments was compared with a one-way ANOVA. The difference in the amount of shrimp consumed between uncoated and sunscreen coated treatments was quantified with a one-way, repeated-measures ANOVA, where the main effects were mass of shrimp consumed, sunscreen treatment (control, zinc, or oxybenzone), and their interaction, with fish ID as a repeated random variable. A repeated measures design was necessary because multiple measurements were taken from each animal on sequential days meaning that each measurement might not be independent and could potentially be correlated within an individual (Laird and Ware 1982; Lindstrom and Bates 1990). Time until first expulsion minus time until first pick-up of shrimp in control, zinc and oxybenzone treatments as well as weight of mucus removed across treatments were assessed using one-way analysis of variance (ANOVA) followed by a Tukey-Kramer HSD test when appropriate. All analyses were performed using JMP 7.0.1 (SAS Institute Inc., Cary, NC, USA), all means reported as±SE where appropriate. Significance was accepted at probabilities of 0.05 or less.

Results

Feeding preference

Fork length (346.5±3.7 mm; mean±SE) of bonefish did not vary across treatments (ANOVA, $F=1.45$, $P=0.27$). The time it took for a fish to pick-up shrimp did not vary for the zinc-based, oxybenzone-based or control treatments (repeated measures ANOVA, $F=1.49$, $P=0.24$) (Fig. 2a). Similarly, bonefish did not expel shrimp covered in oxybenzone or zinc-based sunscreen faster relative to uncoated shrimp (repeated measures ANOVA, $F=0.34$, $P=0.71$) (Fig. 2b). The amount of uncoated shrimp consumed compared to the amount of shrimp consumed the following day (when shrimp was coated in sunscreen) did not vary across treatments (RMANOVA, $F=2.07$, $P=0.15$) (Fig. 3).

Handling and recovery

The fork length did not vary across handling treatments (380.2±5.0 mm, mean±SE) ($F=0.94$, $P=0.45$). No mucus loss was observed when transporting bonefish in the rubber-bagged dip net. Bonefish handled with wet hands lost 50 % less mucus (0.05±0.01 SE g) than the bonefish that were handled with oxybenzone-based sunscreen (0.10±0.01 SE g) (ANOVA, $F=3.38$, P 0.03; Fig. 4). The zinc and glove treatments resulted in an intermediate loss of mucous, but no significant difference between these treatments and the mass of mucuos lost in either the wet hands or oxybenzone-coated hands treatments was observed (Fig. 4). Despite this, recovery time did not vary across treatments (ANOVA, $F=1.6$, $P=0.19$). Two of the bonefish handled with zinc sunscreen-coated hands developed a bacterial infection, which lasted the duration of the 2-week recovery period. Bacterial infection was not observed in the control, oxybenzone-based sunscreen, or UV glove handling treatments.

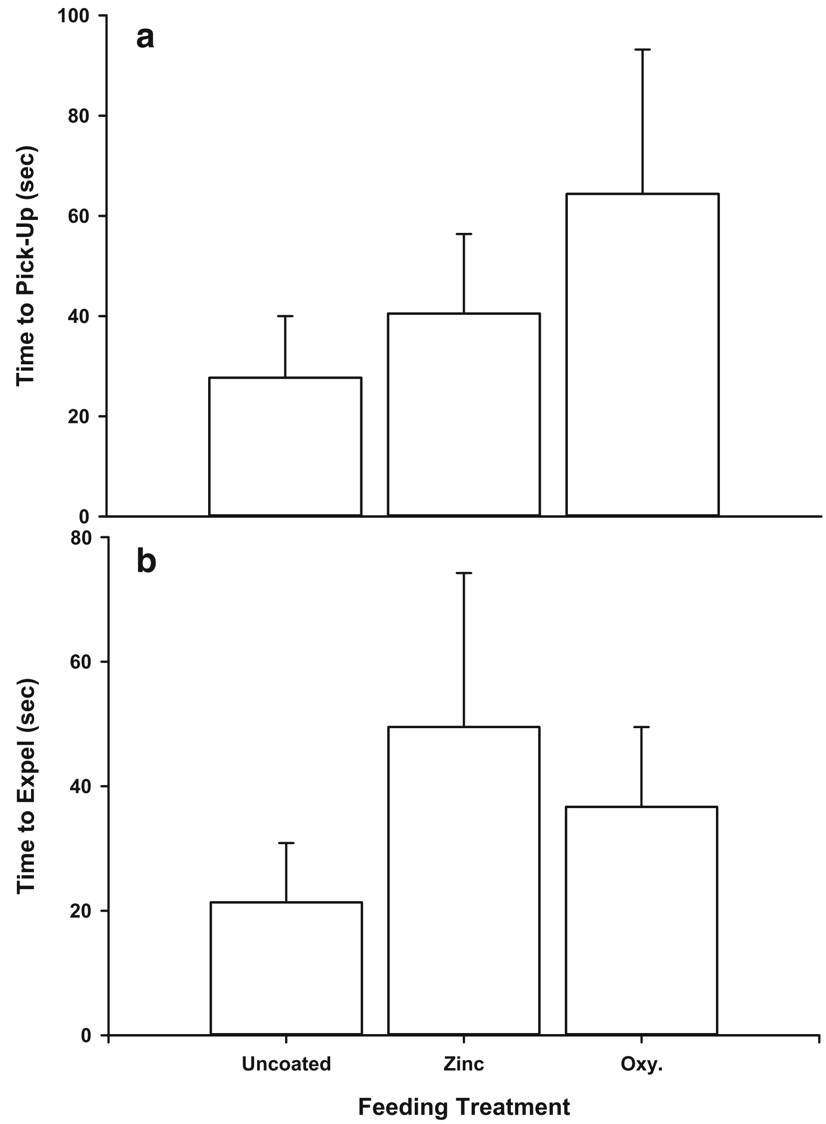

Fig. 2 **a** Mean time for bonefish to pick-up raw shrimp, shrimp handled with zinc-coated hands, and shrimp handled with oxybenzone-containing sunscreen coated hands. *Whiskers* indicate standard error

Discussion

A handling event is stressful for fish because it often involves air exposure, the removal of protective mucus, abrasions, loss of scales, and fin fraying (Ashley 2007; Cooke and Schramm 2007). This study shows that the amount of protective mucus layer removed increased by 50 % when fish were handled with hands coated in oxybenzone-based sunscreen relative to fish handled with wet hands. The zinc and gloves treatments removed intermediate amounts of mucus, but were not significantly different from either wet hands or the oxybenzone treatment. Mucus acts as a physical barrier between a fish and its environment, and contains chemical properties to minimize viral, bacterial, or parasitic infection (Hellio et al. 2002; Ashley 2007). Removal of the mucus layer due to handling may result in delayed mortality, and heightened energetic demands to cope with infection and physiological stress (Kostecki et al. 1987). Dry hands were not chosen as a treatment in this study because it is well documented that dry hands are the most detrimental way to handle a fish due to the removal of the slime layer (Ashley 2007; Butcher et al. 2009; Thomson 2011; Schwabe et al. 2014). Not handling a fish removed the least amount of mucus followed by handling fish with wet hands, where as handling bonefish with hands coated in oxybenzone-based sunscreen resulted in the greatest loss of mucus. Thus, if possible fish should not be handled, but if handling is unavoidable, then fish should be handled with wet uncontaminated hands.

Although recovery time did not vary across treatments, the only two fish to exhibit bacterial infections

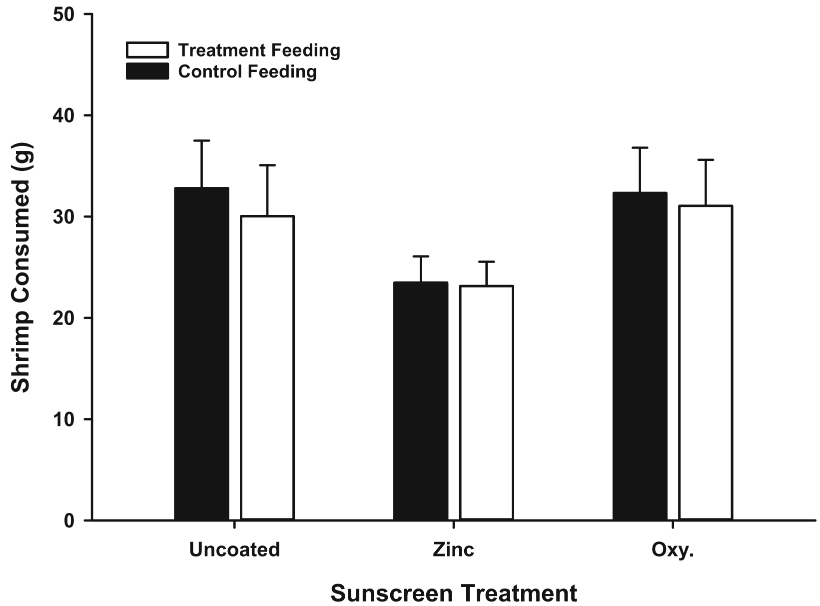

Fig. 3 Mean amount of shrimp consumed by fish of each treatment (control, zinc and oxybenzone) before treatment (*black bars*: control feeding of uncoated shrimp) and 24 h later with treatment (*white bars*: shrimp handled with zinc, oxybenzone or control) in grams

after handling were those in the zinc-sunscreen treatment group. Zinc-based sunscreens are thicker and can clog pores (Moloney et al. 2002). Although clogging pores has only been observed in humans, it may have contributed to the infection on the fish handled with zinc. The risk of infection may be exacerbated because of the additional stressors (e.g., exhaustion, air exposure, injury) that occur during a catch-and-release angling event (Meka 2004). Even though a difference in mucus removal was observed, all the fish were able to recover after 2 weeks, and most showed no visible signs of bruising or abrasions. Stable laboratory conditions may have contributed to an underestimation of recovery time as bonefish were not exposed to typical stressors after release such as predators and increased energetic cost of foraging. Thus, the fish could have used their energy to heal rather than spend energy foraging or avoiding predators.

Although time to pick-up was slightly shorter for uncoated shrimp than for shrimp exposed to sunblock, there was no significant difference across treatments,

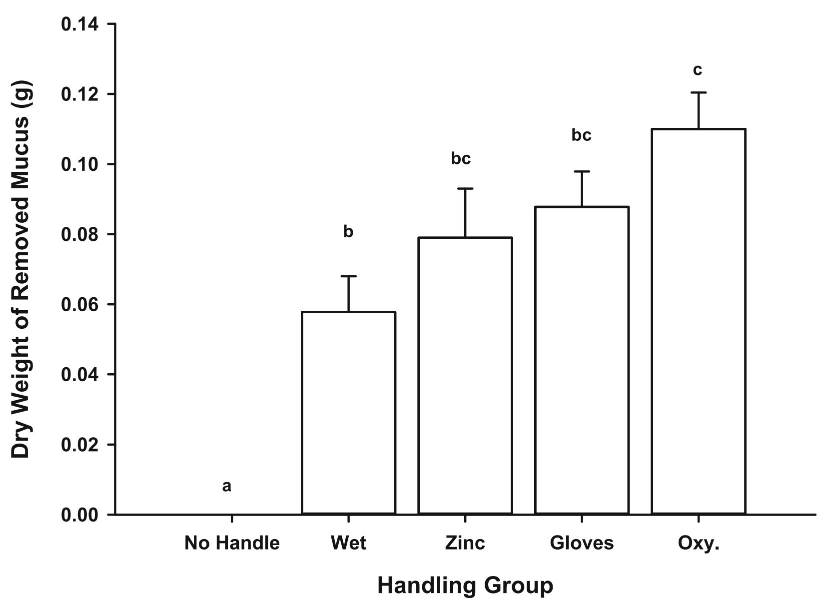

Fig. 4 Dry mass of protective mucus layer removed from fish in each treatment reported in means ±SE grams. Dissimilar *letters* indicate statistically significant differences ($p<0.05$)

nor was there a difference between the time it took for a fish to expel the shrimp across treatments. Similarly, bonefish did not consume less zinc or oxybenzone-based sunscreen-coated shrimp when compared to un-coated shrimp. In teleosts, the identification of a forage item via olfactory, visual, or vibratory cues elicits a feeding response indicated by changes in fish behavior (e.g., gill flaring, initiating a search pattern) to best maximize the likelihood of encountering the prey item (Jones 1992; Hara 2006). Primary foraging habitat of bonefish is characterized by shallow (<2 m) sand, mud, or sea grass flats, with generally good visibility (Crabtree et al. 1998). The non-discriminate foraging by bonefish of food contaminated by sunscreen in this experiment suggests that other cues such as sight may play a more important role in identifying prey than olfactory cues.

Catch-and-release angling is often promoted as a way conserve fish stocks and a wealth of information exists on the best practices when handling bonefish (Cooke and Philipp 2004; Danylchuk et al. 2007b). Recommendations include limiting fight time, avoiding air exposure, using barbless hooks, avoiding locations with high predator burdens, and handling fish with wet hands (Cooke et al. 2006). Here we show that some, but not all UV protection techniques have the potential to injure bonefish by removing mucus. However, UV protection remains a concern for anglers, a population of people that spend a disproportionate amount of time exposed to the elements (Kricker et al. 1994). To limit mucus loss and provide sun protection for anglers, we recommend the following options; remove hooks without handling the fish or, if the previous option is not feasible, then handle the fish with wet hands that have not been coated with sunscreen. By applying these recommendations in conjunction with other best handling practices, anglers will minimize disturbances associated with catch-and-release angling.

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Compliance with Ethical Standards These authors would like to report no conflicts on interest. This article does not contain any studies with human participants performed by any of the authors. All applicable international and institutional guidelines for the care and use of animals were followed.

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