Refuge-Seeking Impairments Mirror Metabolic Recovery Following Fisheries-Related Stressors in the Spanish Flag Snapper (*Lutjanus carponotatus*) on the Great Barrier Reef*

Steven J. Cooke[1,†]
Vanessa Messmer[2]
Andrew J. Tobin[3]
Morgan S. Pratchett[2]
Timothy D. Clark[4]
[1]Fish Ecology and Conservation Physiology Laboratory, Department of Biology and Institute of Environmental Science, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S 5B6, Canada; [2]Australian Research Council Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, Queensland 4811, Australia; [3]Centre for Sustainable Tropical Fisheries and Aquaculture and School of Earth and Environmental Sciences, James Cook University, Townsville, Queensland 4811, Australia; [4]Australian Institute of Marine Science, PMB 3, Townsville MC, Queensland 4810, Australia

Accepted 3/29/2013; Electronically Published 6/26/2013

ABSTRACT

Fisheries and marine park management strategies for large predatory reef fish can mean that a large proportion of captured fish are released. Despite being released, these fish may experience high mortality while they traverse the water column to locate suitable refuge to avoid predators, all the while recovering from the stress of capture. The predatory reef fish Spanish flag snapper (*Lutjanus carponotatus*) is frequently released because of a minimum-size or bag limit or by fishers targeting more desirable species. Using *L. carponotatus* as a model, we tested whether simulated fishing stress (exercise and air exposure) resulted in impairments in reflexes (e.g., response to stimuli) and the ability to identify and use refuge in a laboratory arena and whether any impairments were associated with blood physiology or metabolic recovery. Control fish were consistently responsive to reflex tests and rapidly located and entered refugia in the arena within seconds. Conversely, treatment fish (exhausted and air exposed) were unresponsive to stimuli, took longer to search for refugia, and were more apprehensive to enter the refuge once it was located. Consequently, treatment fish took more than 70 times longer than control fish to enter the coral refuge (26.12 vs. 0.36 min, respectively). The finding that fish exposed to stress were hesitant to use refugia suggests that there was likely cognitive, visual, and/or physiological impairment. Blood lactate, glucose, and hematocrit measures were perturbed at 15 and 30 min after the stressor, relative to controls. However, measurements of oxygen consumption rate revealed that about 50% of metabolic recovery occurred within 30 min after the stressor, coinciding with apparent cognitive/visual/physiological recovery. Recovering the treatment fish in aerated, flow-through chambers for 30 min before introduction to the behavioral arena restored reflexes, and "recovered" fish behaved more similarly to controls. Therefore, we suggest that temporarily holding coral reef fish that have undergone an exhaustive fishing interaction and an air exposure episode should enable significant recovery of cognitive and metabolic attributes that would enable fish to more rapidly locate and utilize refugia to avoid postrelease predation. However, after nonexhaustive fishing interactions (i.e., minimal reflex impairment), it is likely that immediate release would be most beneficial.

Introduction

Marine ecosystems and associated fish communities provide many key services (Holmlund and Hammer 1999; Moberg and Folke 1999), including the provision of protein and fishing opportunities for humans. Recreational and commercial fishers harvest fish for food, although a large but unknown proportion of fish are released. Recreational fishers release nontarget species and individuals or species protected by harvest regulations (e.g., closed seasons, size limits, creel limits), or they can release target species because of a conservation ethic (Arlinghaus et al. 2007). Commercial fishers release nontarget species (typically those without a financially viable market, i.e., bycatch; Hall 1996) as well as undesirable or prohibited sizes of target fish. The fate of released fish is of interest to fisheries and marine park managers because the efficacy of management strategies may be compromised by "cryptic" postrelease mortality (Coggins et al. 2007), a topic poorly understood for reef-associated fish (Stephen and Harris 2010). Indeed, even low levels of fishing mortality (whether harvest or release mortality; e.g., 1%–5%) can be problematic for long-lived reef species with low reproductive

* This paper was submitted in response to a call for papers for a Focused Issue on "Conservation Physiology."

† Corresponding author; e-mail: steven_cooke@carleton.ca.

Physiological and Biochemical Zoology 87(1):136–147. 2014. 1522-2152/2014/8701-2143$15.00. DOI: 10.1086/671166

potential, such as giant sea bass (*Stereolepis gigas*; see Schroeder and Love 2002). Fisheries exploitation is regarded as one of the primary drivers of fish population declines directly via mortality (Wilson et al. 2008) and indirectly through depletion of functionally important predatory species, which influence coral reef ecosystem structure and function (Dulvy et al. 2004). Moreover, identification of cryptic mortality when and where it occurs can inform management strategies (e.g., harvest regulations; Bartholomew and Bohnsack 2005; Cooke and Schramm 2007) or educational initiatives (e.g., best-fishing-practices documents; Pelletier et al. 2007) to ensure long-term conservation of important fisheries species and sustainable use of fish resources.

Although marine protected areas are increasingly being used to protect fish from fishing (Agardy 1994; Gubbay 1995; Russ et al. 2008), some protected areas allow "nonextractive" or "limited-impact" activities such as catch-and-release fishing, under the assumption that postrelease mortality is negligible (see Laffoley 1995; Bartholomew and Bohnsack 2005; Cooke et al. 2006). However, for some fish these assumptions may be erroneous because of high levels of unreported or cryptic mortality. For example, within the northern region of Australia's Great Barrier Reef Marine Park (GBRMP), a number of "buffer" zones (3% of the entire marine park) exist to allow trollfishing for pelagic species, because this activity is deemed low impact (GBRMP Authority 2003). Buffer zones were introduced in 2004 to protect a historically and socioecologically important sport fishery that targets spawning aggregations of black marlin (*Istiompax indica*; Domeier and Speare 2012). Unfortunately, marlin catch-and-release fishing may be characterized by unrecorded and cryptic mortality, with anglers in the GBRMP black marlin fishery routinely losing hooked fish to sharks, and fish released in weakened condition are likely to be preyed upon (Domeier et al. 2003). In fact, there are a growing number of examples where spatial management does not prevent exploitation of fishes, as inferred from continued fish population declines (Westera et al. 2003; Denny and Babcock 2004), possibly associated with cryptic mortality.

Given that reef fish captured by hook and line may often be caught at depth, barotrauma is regarded as a significant driver of release mortality, such that standard practices involve venting fish that are released so that they can return to depth (Sumpton et al. 2008, 2010; Brown et al. 2010). However, the capture and handling process (landing, hook removal, venting, photographs/admiration—and associated air exposure) induces physiological disturbance associated with exercise and hypoxia (e.g., air exposure; Cooke and Suski 2005; Arlinghaus et al. 2007; Davis 2010). Consequently, released reef fish must regain their bearings, return to depth, and avoid predators as they recover from the fisheries encounter. Most fished habitats are comparatively predator rich (Hixon 1991; Hixon and Beets 1993), such that reduced performance of released fish may be a significant issue and contribute to postrelease mortality. Sharks, as an example, are often attracted to and prey on hooked fish (Domeier et al. 2003; Robbins et al. 2011) and may be a significant predator of released fish. Although few studies have examined the role of predators in postrelease mortality, there is evidence that exhausted fish are more likely to be preyed upon. For example, Danylchuk et al. (2007) revealed that angled bonefish that lost equilibrium because of exhaustion were 6.5 times more likely to be attacked by predators in the first 30 min following release.

Reef fish communities are increasingly being exploited by fishing. Release rates may be as high as 60% on the Great Barrier Reef (Sumpton et al. 2008), and in the Florida Keys members of the grouper/snapper complex are managed with various harvest regulations, such that release rates are high (e.g., in some regions, all snapper must be released; Coleman et al. 2000). Therefore, there is a need to understand the extent to which released fish may be subject to postrelease mortality via predation. Indeed, such knowledge is critical for better understanding of the efficacy of fisheries and marine park management strategies aimed at conserving species and sustaining fishing activities in the long term. To that end, we used Spanish flag snapper (*Lutjanus carponatatus*) as a model to determine the behavioral and physiological effects of catch-and-release practices, with an aim to determine whether physiological attributes could provide insight into the vulnerability of fish following their release. *Lutjanus carponatatus* are abundant in tropical and subtropical waters of the Indo-Pacific, where they are a frequent capture of commercial and recreational fisheries. They are often captured as bycatch by commercial fishers handlining to capture other more desirable species, and in many areas, such as the GBRMP, size and bag limits (Welch et al. 2008) and no-take zones are used to manage the recreational fishery, such that some component of the catch must be released (Sumpton et al. 2008). Moreover, a hooking mortality study for *L. carponatatus* revealed that short-term (48 h) mortality was low (2.6%) when fish were held in a laboratory (Diggles and Ernst 1997). While this provides some context by demonstrating high short-term survival, there is little understanding of whether the capture experience compromises individual performance such that cryptic postrelease mortality may occur once released.

We designed a study to test whether simulated fishing stress (exercise and air exposure) resulted in impairments in reflexes (e.g., response to stimuli) and the ability to identify and use refuge in a laboratory behavioral arena. Moreover, we characterized metabolic recovery and some aspects of blood physiology to test whether either was associated with any behavioral and/or reflex impairments. Finally, given our interest in not just documenting problems but identifying solutions, we also tested whether holding fish in a water-filled chamber for 30 min before release helped to rectify any impairments associated with the fishing stress, thus presumably putting them in a better position to avoid postrelease predation. In general, we sought to determine whether any physiological attributes could help to determine the overall state of fish after capture and therefore whether physiology could play some role in helping to minimize postrelease predation and conserve important fisheries species consistent with some of the general goals of conservation physiology (Cooke et al. 2013*b*).

Material and Methods

Fish Collection and Maintenance

Study animals (total length = 22.8–37.1 cm) were collected from the waters within 15 km of the Lizard Island Research Station (LIRS) on the Great Barrier Reef, Queensland, Australia (14°41′S, 145°27′E). Fish were caught with hand lines (24-kg test) baited with pilchards (*Sardinops neopilchardus*) on 8/0 Mustard Viking Hollow Point hooks on the bottom in 5–15 m depth adjacent to reefs. Fish were landed rapidly (<30 s), and target fish (i.e., Spanish flag snapper *Lutjanus carponotatus*) were immediately placed in 80-L plastic totes, after hooks were dislodged with a hook remover to minimize physical handling. Fish suffering barotrauma were vented with a 20-mm-long 16-gauge needle. Fish were held for up to 4 h in the 80-L totes, which were frequently refreshed with water, until transported back to LIRS.

Upon arrival at LIRS, all fish were exposed to a 2-min freshwater bath (as an antiparasite treatment) and tagged with a T-bar anchor tag (Hallprint, Hindmarsh Valley, Australia) before being introduced to mesh-covered flow-through, aerated 1,000-L seawater tanks with no bottom structure at densities of less than 2 kg m^3. Human activity around the tanks was restricted to minimize disturbance. Fish were held for 48–96 h before experimentation. Those fish held for the longest periods were fed daily to satiation (with cut pilchards) but not within 48 h of experimentation. Fish that were fed in captivity and those that did not have the opportunity to do so were randomly mixed among treatments and experimental components. Water temperatures were stable near 28°C at the time of capture and throughout experimentation. Salinity was 34 ppt, and dissolved oxygen in the holding tanks was maintained at 90%–100% air saturation.

Behavioral-Arena Experiments

To assess the effects of stress and recovery on fish behavior and risk taking, we constructed a behavioral arena with a refuge area. The arena consisted of a 2.5-m-long, 1.1-m-wide, and 51-cm-deep oval plastic tank, where water was maintained at a depth of 32 cm. A 15-cm-inner-diameter white PVC pipe (48 cm long) was placed widthways at the distal end of the arena and covered with staghorn coral rubble, such that the openings would be perceived as the only refuge in the arena. Shade cloth placed above the tank was used to minimize shadows and light gradients. A black plastic blind containing a small viewing slit surrounded the proximal end of the arena to enable fish observation without disturbance. Above the arena, a pulley enabled a 15 × 10-cm (length × diameter) piece of white PVC pipe to be dropped from 140 cm above at a standardized location (centered 55 cm from the proximal end of the tank), which acted as a "mock predator" to startle the fish. The flow-through water supply and aeration were removed before each trial to minimize stimuli. Between trials, the water was flushed to eliminate olfactory cues, remove metabolic wastes, and maintain water temperature and dissolved oxygen.

The experiment consisted of assessing the ability of replicate fish to seek refuge in the shelter provided according to three experimental groups: "treatment" ($N = 8$), "recovery" ($N = 8$), and "control" ($N = 9$). For the treatment group, fish were individually scooped from their holding tank in a water-filled bucket and exposed to a simulated fisheries encounter in a round plastic chase tank (110-cm diameter, 20 cm of water in a 42-cm-deep tank). Once fish were introduced to the chase tank, they were manually chased (by hand, usually tail tapping) to exhaustion for 3 min. Exhaustive exercise associated with chasing is deemed to be a suitable proxy for fisheries stressors (e.g., Kieffer 2000; Cooke et al. 2013*a*). Fish that failed to respond to chasing or did not swim well were excluded from analysis (~10% excluded). The tank was refreshed with ambient seawater between chase sessions. After the chase, fish were placed in a wetted Hypalon bag for 5 min of air exposure to simulate excessive, but relatively common, fisheries handling times (e.g., Thompson et al. 2008; Raby et al. 2013). Fish were measured and weighed during the air exposure period before being placed in a water-filled bucket (where reflex action mortality predictor [RAMP] was measured; see below) and slowly introduced to the proximal end of the behavioral arena by submerging and then tilting the bucket.

The recovery group underwent the same protocol as the treatment group, except that following air exposure, fish were placed individually in a 40-L flow-through, aerated tote for 30 min before being transferred in a water-filled bucket to the behavioral arena. The recovery environment was not hyperoxic, nor was the flow such that it promoted ram ventilation.

A third, control group was gently guided into a submerged bucket in the holding tank and transferred directly to the behavioral arena without being netted, chased, or air exposed. The control group was measured and weighed following experimentation. Introduction of the fish from the bucket to the behavioral arena occurred in a standardized way for all treatments. The order of treatments was randomized, and no fish was used more than once.

Fish were placed into the arena at the end farthest from the coral refuge (i.e., the proximal end) while a researcher viewed the fish through the blind at the proximal end. Using a stopwatch, we recorded (to the nearest 1 s) several endpoints. First, we recorded the time until the first movement of the fish (which required a movement of one body length from initial release site). Second, we recorded the time required for the fish to cross a line traversing the midline of the tank (i.e., 125 cm from the initial release site). Third, we recorded the time until the fish inspected the coral refuge within close range (<15 cm; note that the entrances to the refuge could not be seen from the proximal end of the arena, since the coral-covered PVC pipe was positioned widthways at the distal end of the arena). Fourth, the time at which the fish entered the refuge (required entire body to enter) was recorded.

A piece of PVC pipe (see above) was dropped near the release site in the arena to startle the fish and encourage refuge seeking by eliciting noise, vibration, and splashing. The pipe was first dropped at 2 s, then at 30 s, and then at 30-s intervals until

10 min. From 10 min through 30 min the pipe drops occurred at 1-min intervals. If the fish still had not completed the trial (i.e., entered the refuge) after 30 min (this applied to four treatment fish and one recovery fish), the caudal peduncle region of the fish was tapped once with a long PVC rod at 31, 32, and 33 min. Beginning at 34 min, the same rod was used to continually stimulate the tail region until the fish entered the refuge (applied to only two treatment fish). For each attempt to startle the fish we noted whether the fish was actually startled, as noted by initiation of movement (in any direction) or a freezing behavior if fish were actively swimming. The escalation from dropping the pipe, to tapping the tail, to doing so continually was employed to keep the trials to a reasonable time period. All trials were conducted between 0800 and 1600 hours.

RAMP is a method that involves checking for the presence or absence of natural animal reflexes to generate a condition score in response to stressors and to predict fate (Davis 2010). We used five reflex indicators that have been previously demonstrated to be reflective of fish condition and predictive of mortality in the context of fisheries interaction (Davis 2005, 2007, 2010; Raby et al. 2012). Individual reflexes were assessed categorically (0 = unimpaired, 1 = impaired), as described by Davis (2010). Reflexes tested were tail grab (response of fish to tail grab, characterized by burst swimming), body flex (holding fish in air using two hands to determine whether they attempted to contract axial muscles in an attempt to secure freedom), head complex (regular pattern of ventilation while held in air during a 5-s period), vestibular-ocular response (VOR; fish turned on side to determine whether the eye of the fish rolled to maintain positive pitch), and orientation (fish placed in water upside down to determine whether the fish could right itself within 3 s). Similar to the procedure of Raby et al. (2012), if a fish was too vigorous to allow handling and assessment of reflexes, it was assigned an unimpaired status for all reflexes. This was the case for all control fish.

For the treatment and recovery groups, RAMP was assessed at the conclusion of the fishing simulation (i.e., after the air exposure period), immediately before a fish was placed in the arena (for treatment group) or the recovery tote (for recovery group). Fish in the recovery group were further assessed after the 30-min recovery period, immediately before being introduced to the arena. RAMP was not formally assessed for control fish, given that they clearly were not impaired when removed from the holding tank and that we did not want to impose any stress. Instead, RAMP was assessed in a separate group of control animals during blood-sampling experiments (see below), and we noted that all reflexes were consistently present.

Blood Physiology Experiments

To complement the behavioral-arena trials, we conducted a parallel study to characterize the blood physiological status of fish, using independent samples wherever possible. No fish that were bled were subsequently used in behavioral or respirometry trials, but some fish used in behavioral assays were later used for blood sampling. We had limited numbers of fish and had to select optimal sampling periods that maximized our ability to interpret the behavioral findings. To that end, we compared control fish (netted from the holding tank and sampled immediately; $N = 7$), treated fish (i.e., chased and air exposed as above) held in 40-L flow-through recovery totes for 15 min ($N = 7$), and treated fish held in recovery totes for 30 min ($N = 7$). The totes were identical to those described above. That design enabled us to understand the short-term stress response and physiological recovery dynamics. RAMP was evaluated immediately before blood sampling, and then fish were placed supine in a water-filled trough (Cooke et al. 2005) before a 21-gauge sodium-heparinized needle and a 1-mL syringe were used to withdraw 0.5 mL of blood nonlethally from the caudal vasculature. The entire procedure took less than 3 min, which should have provided a blood sample from the control fish that was uninfluenced by the netting event (see Turner 2004; Clark et al. 2011). Blood was placed in a water-ice slurry for up to 1 h. Blood glucose and lactate were measured with a portable diagnostic meter (Accutrend Plus, Roche) that has been previously validated for use on fish (Beecham et al. 2006). Hematocrit (Hct) was determined by spinning blood at 11,000 rpm (ZIPocrit, LW, Lawrenceville, GA) for 4 min in 75-mm capillary tubes. Hemoglobin concentration ([Hb]) was determined with a handheld hemoglobin analyzer (Hb201+, HemoCue, Ängelholm, Sweden) calibrated for fish blood according to Clark et al. (2008). The mean cell hemoglobin concentration (MCHC) was calculated as [Hb]/(Hct/100).

Metabolic Experiments

To provide insight into the metabolic perturbations and metabolic recovery times experienced by fish utilized in the behavioral-arena experiments, a separate group of fish ($N = 8$; mean mass of 549 ± 62 g) was exposed to the same protocol as the treatment group above (i.e., 3-min chase plus 5 min of air exposure) but immediately placed into respirometers rather than the behavioral arena following the air exposure period. Although we did not use a separate nonexercised control group in the experiments, metabolic rates initially peaked and then declined and stabilized within several hours of exercise, suggesting that metabolic recovery was complete. The respirometers were cylindrical, had an internal diameter of 25 cm and a length of 80 cm, and were submerged in a large tank to ensure thermal stability (~28°C). The functioning of the respirometers was similar to that described previously (Clark et al. 2011, 2012). Briefly, each respirometer was equipped with a closed-circuit recirculation loop to ensure appropriate water mixing, and an automated flush pump refreshed the respirometer water for 5 min in every 10-min period. Dissolved oxygen in the respirometer was measured continuously with fiber optic sensors (PyroScience, Aachen, Germany) incorporated into the closed-circuit recirculation loop, and measurements of oxygen consumption rate were determined for each 5-min period between flush cycles. Dissolved oxygen in the respirometers never fell below 80% saturation. Fish remained in the respirometers

for 20–23 h to record the entire period of metabolic recovery. Background respiration was routinely checked with empty respirometers but was negligible in all cases.

Statistical Analyses

The size of fish in each treatment group was normally distributed, and mean size (evaluated with one-way ANOVA) did not differ ($P > 0.05$) among groups for both the behavioral (control = 303 ± 14 mm; exercise = 301 ± 7 mm; recovery = 317 ± 12 mm) and blood physiology experiments (control fish = 304 ± 16 mm; 15 min after stressor = 303 ± 5 mm; 30 min after stressor = 291 ± 10 mm). Preliminary exploratory analysis failed to reveal any significant associations between any of the measured physiological variables and fish size. Therefore, size was not considered as a covariate in analyses. Timing data from the behavioral-arena experiments were log transformed before being assessed for significance with one-way ANOVA. Statistical analyses on the timing data were run twice, once with all fish included and again after removal of fish that required extra stimuli because they failed to enter the coral refuge within 30 min. These exclusions resulted in only minor alterations to values and did not have any influence on the statistical outcomes of any of the comparisons between groups. Therefore, all data have been included here. One-way ANOVA was used to compare blood physiological variables between time points (independent samples). The Holm-Sidak method was used to account for multiple comparisons in all tests. Statistical significance was considered at $P < 0.05$.

Results

Control Fish

Control fish were vigorous upon removal from the holding tank and did not have any RAMP impairments (mean RAMP score ± SE = 0.0 ± 0.00). Control fish had Hct of 27.4% ± 0.9%, [Hb] of 79 ± 5 g L^{-1}, MCHC of 366 ± 23 g L^{-1}, blood lactate of 1.0 ± 0.1 mmol L^{-1}, and blood glucose of 1.4 ± 0.0 mmol L^{-1} (fig. 1). Upon entry into the behavioral arena, control fish reacted immediately to the drop of the PVC pipe, making their first movement in 2.0 ± 0.3 s, crossing the arena midline in 4.4 ± 0.7 s, and entering the coral refuge in 21.6 ± 9.9 s (fig. 2). The fish were clearly very aware, and they were quick to recognize the coral refuge as a safe haven.

Treatment and Recovery Fish

All fish exhibited powerful burst swimming for the first 1.5–2 min of the chase protocol, but became largely unresponsive to touch in the final 1–1.5 min of the chase before the commencement of the air exposure period. The RAMP score immediately following the chase and air exposure protocol was 0.6 ± 0.05, indicating substantial reflex impairment in comparison with control fish. This increase in RAMP score was primarily driven by a lack of response to the body flex and tail grab stimuli. Head complex and orientation were absent on occasion, while VOR was never impaired.

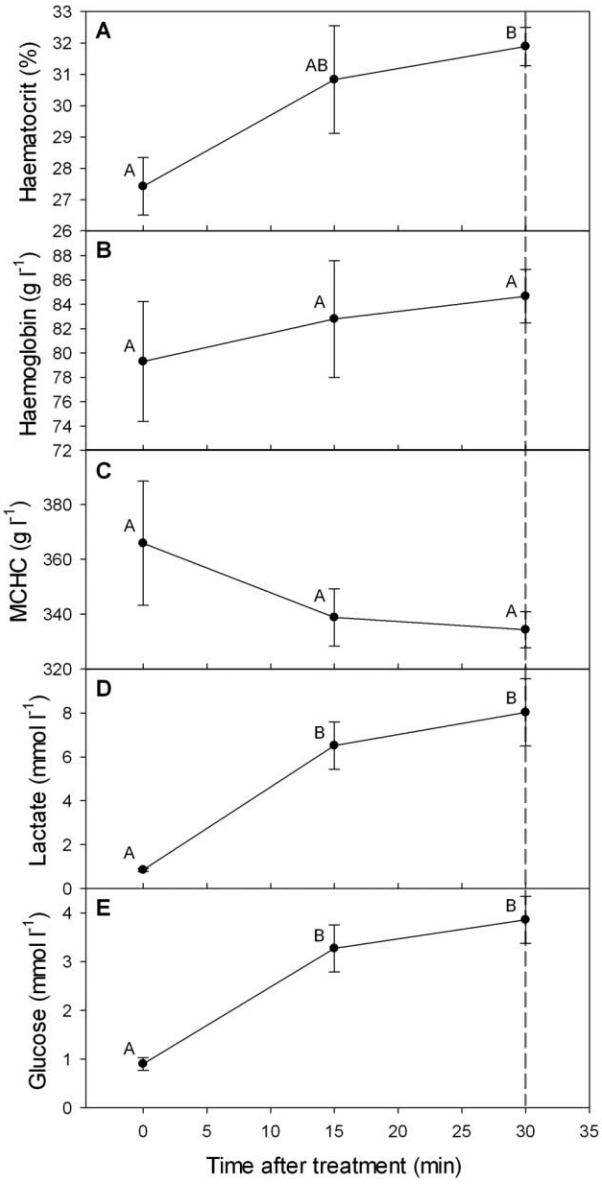

Figure 1. Responses of hematocrit (*A*), hemoglobin concentration (*B*), mean cell hemoglobin concentration (MCHC; *C*), blood lactate concentration (*D*), and blood glucose concentration (*E*) in *Lutjanus carponotatus* under control conditions (time = 0) and at 15 and 30 min following an exhaustive fisheries capture simulation (3-min chase plus 5 min of air exposure). Water temperature remained at 28°C. $N = 7$ for each time point, and each fish was used at only one time point (i.e., total $N = 21$). One-way ANOVA was used to compare between time points; dissimilar letters indicate significant differences where $P < 0.05$. The vertical dashed line at 30 min highlights the blood physiological conditions that would have been experienced by the "recovery" group at the time of introduction to the behavioral arena (see text for details). A color version of this figure is available online.

In comparison with control fish, fish placed immediately into the behavioral arena after the chase and air exposure treatment

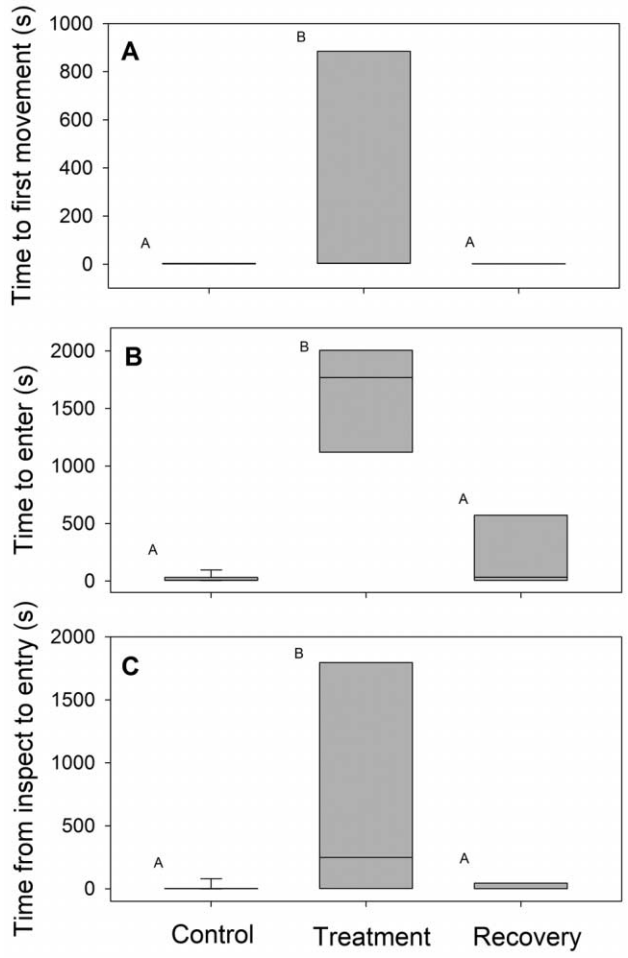

Figure 2. Responses of *Lutjanus carponotatus* in the behavioral arena, including control fish (transferred from holding tank directly to behavioral arena; $N = 9$), treatment fish (chased for 3 min, air exposed for 5 min, and then placed directly into behavioral arena; $N = 8$), and recovery fish (treated the same as treatment fish but given 30 min recovery in a flow-through tote before being placed in behavioral arena; $N = 8$). Variables shown are time of first movement (considered to be when the fish moved at least 1 body length from its starting position; *A*), time to enter the coral refuge (*B*), and time between inspecting the coral refuge and fully entering it (*C*). Water temperature remained at 28°C. One-way ANOVA was used to compare between groups; dissimilar letters indicate significant differences where $P < 0.05$.

took 177 times as long to make their first movement (354.5 ± 178.1 s), 135 times as long to cross the arena midline (598.9 ± 261.0 s), and 73 times as long to enter the coral refuge (1,567.0 ± 179.3 s; fig. 2). Moreover, the time that lapsed between inspecting the coral refuge and actually entering it was 74 times as long for the treatment fish (743.0 ± 321.3 s) than for the control fish (10.0 ± 10.6 s), suggesting an impairment of cognitive, visual, and/or physiological capacity of the treatment fish when assessing the refuge.

When fish were given 30 min to recover from the exhaustive-exercise treatment before being introduced into the behavioral arena, the RAMP score returned to 0.0 ± 0.00 and the refuge-seeking ability improved toward control values; time to make their first movement was 2.6 ± 1.3 s, time to cross the arena midline was 8.6 ± 3.5 s, time to enter the coral refuge was 350.4 ± 232.4 s, and time between inspecting the coral refuge and entering it was 97.1 ± 327.0 s (fig. 2). The improved cognitive/visual ability of fish after 30 min of recovery compares favorably with the finding above that treatment fish placed immediately into the behavioral arena took 1,567 s (26.1 ± 3.0 min) to enter the coral refuge.

The blood physiology measurements did not uncover any obvious variable(s) that helped to explain the regained capacity of fish to recognize the coral refuge as a safe haven at 26–30 min after treatment (i.e., no measured variables recovered in this time period). In comparison with control fish, the fish exposed to the chase and air exposure protocol were characterized by significant increases and no recovery in Hct, lactate, and glucose by 30 min after treatment, while [Hb] and MCHC displayed obvious trends but did not change significantly (fig. 1). In contrast, an examination of the metabolic recovery of treated fish yielded some insight into the behavioral differences between treatment groups. The highest oxygen consumption rates, 6.9 ± 0.7 mg min^{-1} kg^{-1}, were recorded immediately after the treatment (i.e., the first measurement once fish were placed into respirometers; fig. 3). While metabolic recovery took more than 6 h, approximately half of this recovery occurred in the first 30 min after treatment (fig. 3), suggesting the possible involvement of metabolism and oxygen transport in regulating the cognitive/visual ability and refuge-seeking capacity of the fish.

Discussion

In marine environments, particularly in and around reefs, predator burdens can be high, such that they provide a significant threat to fish that are released with behavioral and physiological impairments. Few studies have examined the fate of reef fishes that are captured and released, presumably because of the challenges of examining such questions in the wild, particularly in complex habitats such as reefs (Donaldson et al. 2008). For that reason, controlled laboratory experiments can be used to explore mechanisms that would potentially be associated with mortality as well as to test strategies to improve the condition and fate of released fish. Here, we used an experimental laboratory approach combining physiological and behavioral metrics to assess the consequences of fisheries interactions on a common reef fish. We also tested the potential benefits of temporarily holding fish to enable physiological recovery in an attempt to reduce behavioral impairments. We revealed that compared to control fish, those fish exposed to simulated fishing events exhibited severe behavioral and cognitive/visual/physiologic impairments that impeded their ability to reach, recognize, and enter refugia. Alterations in blood physiology, impairments in reflexes, and elevated metabolic rates were evident after fishing simulations. However, when exhausted fish were provided with 30 min of recovery, behavioral and cognitive/visual/physiologic impairments were largely eliminated

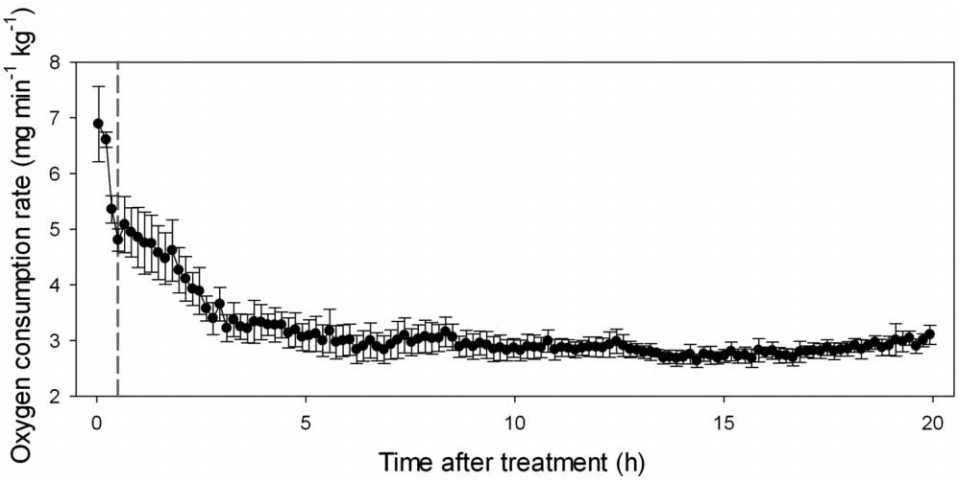

Figure 3. Recovery of oxygen consumption rate in *Lutjanus carponotatus* following an exhaustive fisheries capture simulation (3-min chase plus 5 min of air exposure; $N = 8$). The vertical dashed line at 30 min highlights the oxygen consumption rate that would have been experienced by the "recovery" group at the time of introduction to the behavioral arena (see text for details). Water temperature remained at 28°C. A color version of this figure is available online.

and ~50% of metabolic recovery occurred. We discuss these findings in the context of disturbance and recovery dynamics in reef fish as well as relative to the potential to use this information to inform management and conservation of important fisheries species.

Behavioral impairments are regarded as sensitive indicators of stress (Schreck et al. 1997), although in the context of fisheries interactions they are rarely studied, given the challenges of doing so in the wild (see Arlinghaus et al. 2007). It is notable that biotelemetry tools are helping to overcome some of these challenges (Donaldson et al. 2008). We used a laboratory-based arena to study behavior in a controlled environment. Control fish exhibited remarkably consistent behavior when introduced into the arena; they rapidly swam away from the release site and entered the refugia, on average, in 22 s. Control fish also consistently responded to the startle stimuli. In stark contrast, the fish that were placed into the arena immediately following the fishing simulation took 177 times as long to make their first movement and 73 times as long to enter the coral refuge (on average, 26 min). In the wild, during that period fish would be highly susceptible to predators. Also of interest was the fact that the time that lapsed between inspecting the coral refuge and actually entering it was 74 times that for the treatment fish, suggesting an impairment of visual, cognitive, and/or physiological capacity of the treatment fish when assessing the refuge. When fish were provided with 30 min to recover, there were remarkable improvements in behavior (including responses to startle stimuli), such that recovered fish were more similar to controls than to treated fish. On average, recovered fish entered the refuge after 6 min. Unlike the situation immediately after treatment, after recovery the time between inspection and entry was dramatically reduced, suggesting a recovery from the visual/cognitive/physiologic impairment.

The notion that fishes exhibit visual or cognitive impairments following stress has previously been studied, but rarely in the context of fisheries interactions. One of the only physiologically oriented studies of *Lutjanus carponotatus* tested whether visual performance is influenced by metabolic stress and the development of an oxygen-concentrating apparatus in the eye (i.e., the choroid *rete mirabile*; Herbert et al. 2002). When Herbert et al. (2002) exercised *L. carponotatus* to elicit lactate levels of ~8 mmol L^{-1} (the same lactate level achieved after treatment in our study), he failed to detect impairments in visual acuity. In our study fish were exposed to air, which presumably led to hypoxemic conditions more extreme than those experienced by the fish studied by Herbert et al. (2002), even though blood lactate levels were similar. Therefore, for *L. carponotatus* it is feasible that visual impairments could be associated with extreme fishing stressors and thus could be an explanation for our behavioral observation. Another possibility is that there were cognitive impairments. The cognitive abilities of fish are not as well studied as those of birds or mammals (Brown et al. 2011), but they are known to play an important role in mediating behaviors, especially as it relates to predator avoidance (and thus survival; Braithwaite and de Perera 2006). For example, relevant to predator avoidance, cognition includes perception, attention, memory formation, and executive functions related to information processing, such as learning and problem solving (Brown et al. 2011). There are many factors that affect cognition and decision making in fishes, and stress is believed to be one of them. Interaction with predators (or perceived predation risk) is inherently stressful (Lima 1998; Abrahams et al. 2007), just as organisms that are in a stressed state may be unable to efficiently make decisions (e.g., ability to assess what is a risk and what to do about it). In humans, a meta-analysis revealed that stress affects decision making, although whether it confers an advantage or a disadvantage is context specific (Starcke and Brand 2012). To our knowledge

there are no studies that have explicitly studied the effect of stress or physiological exhaustion on cognition in fish, but this would appear to be a worthwhile research tack, given the findings of our study. It is also worth noting that it is possible that disorientation was not cognitive in nature but rather a function of some physiological perturbation (other than the parameters measured here) that manifested in a behavioral malaise. In addition, the energy mobilization required for recovery may also have shifted priority away from seeking shelter and toward homeostatic recovery.

When undergoing exhaustive exercise and air exposure to simulate fishing interactions, *L. carponatatus* exhibited significant alterations in blood physiological status when sampled 15 min after the stressor. Relative to control fish, posttreatment fish had elevated lactate, a by-product of anaerobic metabolism. Poststressor lactate elevations have been observed in the subtropical coral trout (*Plectropomus leopardus*) following exposure to shallow-water stress (Frisch and Anderson 2000) and in bonefish and coral trout following exposure to simulated fishing stressors, including exercise and air exposure (Turner 2004; Suski et al. 2007; Cooke et al. 2008). Only one study has examined postexercise lactate levels in *L. carponotatus* (i.e., Herbert et al. 2002), and that study considered 8 mmol L^{-1}, which is consistent with our poststress values (~8 mmol L^{-1}), to indicate a severe disturbance. Hyperglycemia was also noted in our study of *L. carponotatus* following the fishing simulation, as has been well documented in a variety of fish (Barton 2002), including other subtropical marine fish (coral trout exposed to shallow-water stress [Frisch and Anderson 2000]; damselfish *Acanthochromis polyacanthus* exposed to handling stress [Begg and Pankhurst 2004]). Hematological parameters (i.e., Hct, [Hb], and MCHC) responded relatively subtly to the treatment in our study. The increase in Hct and the trend for decreasing MCHC are consistent with erythrocyte swelling, which is a classic stress response in teleosts associated with maintaining intracellular pH and Hb-binding affinity (Wood 1991 and references within).

Given our interest in exploring the potential to facilitate recovery by temporarily holding fish in flow-through totes for 30 min, we sampled blood at the end of the 30-min retention period and compared those values to control and 15-min-after-treatment values. When *L. carponotatus* were placed in a recovery tote for 30 min, neither blood lactate nor glucose exhibited signs of recovery. In fact, although not significantly different, mean values tended to be higher for both parameters at 30 min than at 15 min after treatment. That finding is not unexpected, given that when a fish is exposed to a stressor, maximal values of blood lactate and glucose are not achieved instantaneously; rather, they can increase for 15 to 60 or more minutes after the stressor, with the actual time depending on a variety of factors, such as fish size, species, nutrition, intensity of the stressor, and water temperature (reviewed in Kieffer 2000; Barton 2002).

Our desire in this study was to test a simple and inexpensive recovery method that could be used by fishers without significant effort or expense. Most fishers would have some kind of tote on board that could be filled with water, refreshed as needed, and supplemented with air using an aerator (powered by the boat's electrical system or battery). Much can also be gleaned from freshwater black bass tournaments, where fish are commonly retained in live wells during the events. Some fishers carry dissolved-oxygen meters to evaluate the need to change water (common in black bass tournaments), while others use behavioral indicators, such as gasping, to identify when water needs to be changed, although water quality is likely to be poor by the initiation of such behavioral indicators. There is a need to develop guidelines for retention based on fish oxygen demands (based on size of fish), water volume, and water temperature that can be used to emphasize to fishers how quickly fish can remove oxygen from water and thus how frequently they must be tended (e.g., Cooke et al. 2002 for a black bass example). Emphasizing the importance of good water quality during retention is critical, or the retention itself could become a stressor, as opposed to facilitating recovery (see Suski et al. 2004 for an example of the trade-offs between good and poor water quality during retention of black bass). Moreover, there is likely an optimal recovery duration beyond which retention may actually increase stress and injury (e.g., Donaldson et al. 2013). Beyond totes, there are a variety of inexpensive recovery tools available that provide flow-through water, including an inflatable tube that is advertised as a "portable livewell" (http://www.youtube.com/watch?v=3A4KPiLIvTs) and inexpensive bags (e.g., Brownscombe et al. 2013; Donaldson et al. 2013). Additional research specific to reef fish, coupled with educational efforts, is needed before such techniques could be broadly encouraged and adopted. We also note that minimizing fight duration and air exposure could negate the need for such recovery practices and thus should also be encouraged, given that retention does have the possibility of increasing stress and injury of fish if not done properly (Donaldson et al. 2013).

Indicators of reflex impairment were reasonably sensitive to fisheries stressors. Control fish had all reflexes intact, whereas after fish were chased and exposed to air, multiple reflexes were typically absent. For *L. carponotatus*, the body flex and tail grab responses were typically the reflexes that were absent after fisheries simulations. Those responses are relevant, in that fish that lack body tone or the ability to respond to a physical stimulus may be susceptible to postrelease predation. Following 30 min of recovery in the totes, the reflexes of *L. carponotatus* were uniformly present. In fact, it was difficult to handle fish to do the reflex test, in stark contrast to the fish that were evaluated for reflex impairment immediately after the stressor. Reflex indicators have gained popularity in recent years, given their simplicity but most importantly the fact that they have been shown to be predictive of mortality (e.g., Davis 2007; Raby et al. 2012), unlike many blood physiology metrics, for which predictive relationships have been elusive (Cooke et al. 2013*a*). Indeed, in our study we failed to document any evidence of recovery in blood physiology parameters at 30 min after treatment (which may be simply a reflection of the time course for response and recovery in glucose and lactate and the limited variables measured here), whereas reflex indicators were fully

recovered concurrently with a renewed ability to recognize the coral refuge as a safe haven. For that reason, RAMP (or a subset of reflexes, such as tail grab and body flex) could be used by fishers to determine whether fish require temporary holding to enable recovery and to gauge when fish are ready for release. RAMP is easy and rapid to do, such that it could be used by fishers as an objective indicator of fish condition (Raby et al. 2012).

To assess metabolic recovery, we used respirometry to quantify oxygen consumption rates, an approach that has been used previously to quantify fish metabolic stress and recovery (e.g., Barton and Schreck 1987; Clark et al. 2012). Similar to the blood physiology metrics, such as lactate and glucose, as well as RAMP scores, oxygen consumption rate was elevated immediately after the fishing stressor. However, after 30 min under conditions similar to those experienced in the recovery tote, there was evidence of significant metabolic recovery. Although complete metabolic recovery was not achieved until more than 6 h after the stressor, ~50% of recovery was achieved in the first 30 min. In other words, RAMP and oxygen consumption rate seemed to be associated with and reflective of the behavioral impairments we observed, while the measured blood parameters were not. The rapid initial recovery is important, since it means that fish have scope to respond to subsequent stressors, including the locomotor capacity to potentially escape predators or swim to (and recognize) refugia. When oxygen consumption rates are maximal (i.e., immediately after the stressor), there is negligible scope for such predator-avoidance activities, and indeed additional stressors can lead to mortality through metabolic exhaustion (Priede 1977). To date there are few studies that have considered fishing impacts in the context of metabolic recovery and scope, but they appear to have much promise (see several cardiac studies by Schreer et al. 2001 and Cooke et al. 2004 and metabolic studies, such as those of Shultz et al. 2011 and Clark et al. 2012).

Unlike most studies of fisheries interactions, we used behavioral and physiological endpoints that elucidated potential mechanisms that could promote postrelease predation as well as opportunities for reducing impairments. We used *L. carponotatus* as a model, but we expect that the general premise of this work would apply to other reef fishes. One point to note is that we elicited a rather severe level of stress (i.e., exhaustive exercise followed by 5 min of air exposure). For that reason it is possible that our results may not apply to responsible and efficient fishing practices, where fight time and air exposure are minimal. Nonetheless, there is immense variation in fisher behavior and attention to best handling practices (Arlinghaus et al. 2007), such that the level of air exposure used here is not unreasonable if one considers hook removal, venting, and an admiration period (i.e., air exposure associated with photography and fish viewing). In addition, even moderate exercise and air exposure or impairment from barotrauma (e.g., fish inability to return to depth) would have the potential to impair behavior, and in those cases retention for shorter periods may be effective. Of note in this study was that body flex and tail grab can be used to make an assessment of the condition of the fish before release. If those reflexes are present, then our findings suggest that it would be prudent to release the fish immediately. However, if the reflexes are absent, then there would appear to be merit in holding them for 30 min in a flow-through tank with well-aerated water and high water quality to enable fish to recover. The apparent visual, cognitive, and/or physiological impairments noted here could be a potential explanation for postrelease mortality. Interestingly, it was the metabolic recovery that seemed to be associated with impairments in behavior.

Reef fish communities subjected to fishing where a component of the catch is released would benefit from studies of the fate of released fish so that mortality can be incorporated into management models (Coggins et al. 2007). In addition, identification of problems can provide opportunities to develop strategies to reduce stress, behavioral alterations, and presumably mortality, thus improving conservation outcomes (Cooke and Schramm 2007) and addressing fish welfare concerns (Cooke and Sneddon 2007; Diggles et al. 2011). To explicitly test whether the 30-min recovery period provides sufficient recovery in physiology and behavior to mitigate postrelease mortality, we are seeking to undertake tag-and-release trials of *L. carponotatus*, comparing the proportion of fishes that are resighted or recaptured following immediate release with that following a 30-min recovery period. These studies are important to validate results of the present study.

Acknowledgments

Funding for this work was provided by the Peter Teakle Sustainable Fishing Research Grant from the Lizard Island Research Station (LIRS) of the Australian Museum. We thank the LIRS directors, Drs. Lyle Vail and Anne Hoggett, for research support. We also thank Bob and Tania Lamb for technical and nutritional assistance. S.J.C. was further supported by the Canada Research Chairs Program, the Ontario Ministry of Research and Innovation, and the Natural Sciences and Engineering Research Council of Canada; further support for T.D.C. was from the Australian Institute of Marine Science.

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