Behavioural and reflex responses of mottled mojarra *Eucinostomus lefroyi* (Gerreidae) to cold shock exposure

Emma Samson, Jacob W. Brownscombe*, Steven J. Cooke

Fish Ecology and Conservation Physiology Laboratory, Ottawa-Carleton Institute for Biology, Carleton University, 1125 Colonel By Dr., Ottawa, ON K1S 5B6, Canada

ABSTRACT: Global climate change is predicted to increase incidences of abrupt declines in oceanic temperatures due to storms or upwelling. Fish occupying shallow, near-shore marine habitats may be vulnerable to mortality or sub-lethal fitness effects due to cold shock. Mottled mojarra *Eucinostomus lefroyi* are a ubiquitous prey species associated with subtropical and tropical coastal habitats and thus serve as a model for evaluating the consequences of cold shock events. Here, we conducted one of the first studies of cold shock on a tropical–subtropical fish species. Fish acclimated to 24°C were exposed to acute temperature drops to 16, 18, or 20°C for 1 h. Ventilation rates were assessed every 15 min during exposure, and equilibrium loss every 5 min. Thirty minutes after cold shock exposure, fish were placed in a circular swim flume and chased by hand until exhaustion to measure chase time and distance chased as proxies for swimming capabilities. Fish exposed to 18 and 20°C had significantly higher ventilation rates than those exposed to 16°C or controls held at 24°C. Exposure to 16 and 18°C caused reflex impairment (e.g. no response to tail grabbing), while exposure to 20°C caused no impairment. After 30 min of recovery at ambient temperature, no reflex impairments were detected in any of the treatments, and the swimming capabilities of fish exposed to cold shock were similar to, or better than control fish. Our findings suggest that abrupt changes in water temperature >4°C below ambient can cause behavioural impairments that may lead to mortality in this species.

KEY WORDS: Cold shock · Thermal biology · Subtropics · Mottled mojarra · Reflex Action Mortality Predictors · RAMP

INTRODUCTION

Temperature has been described as the ‘master’ abiotic factor for fish (Brett 1971) given its manifold effects on behaviour, biochemical processes, locomotion, bioenergetics, growth, life history, and cardio-respiratory physiology. In subtropical and tropical habitats, fish are adapted to a more narrow temperature range in comparison to their temperate counterparts (Larsen et al. 2011), which suggests that tropical fish species may be more sensitive to large fluctuations in ambient water temperature. Further, climate change is predicted to increase the intensity of extreme weather conditions such as tropical cyclones and heavy precipitation events (Michener et al. 1997), which will likely cause sudden declines in ambient water temperature, particularly in shallow waters (IPCC 2007). Moreover, coastal upwelling may become more intense, transporting cooler, deep waters to coastal areas (Bakun 1990). In the context of climate change, most efforts have been devoted to considering the biological consequences of warming waters (Roessig et al. 2004), but sudden decreases in water temperature are also relevant. For example, in Butternut Cay, Florida, USA, in January 2010 water temperatures declined 11°C, from 19 to 8°C, resulting in a large fish kill for many near-shore dwelling species (FWCC 2010, A. Adams pers. comm.).

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Publisher: Inter-Research · www.int-res.com
There are numerous studies examining the effects of an increase in temperature on tropical marine fish (e.g. Munday et al. 2008, Nilsson et al. 2009, Donelson et al. 2011), yet virtually no research on the effects of decreases in water temperature, including the phenomenon known as cold shock (Lamadrid-Rose & Boehlert 1988, Donaldson et al. 2008, Meyer-Rochow 2013). Studies focusing on temperate freshwater species, such as rainbow trout Oncorhynchus mykiss, have found that they can adapt physiologically to ecologically relevant acute or chronic cold water exposure (Shiels et al. 2003, Haverinen & Vornanen 2007). Yet for tropical marine fish, even basic information on thermal tolerances (especially lower) is rare (Fanque & Bennett 2003, Eme & Bennett 2009, Murchie et al. 2011). There is a need for more information on the effects of thermal change, including cold shock, on tropical marine fish (Wilson et al. 2010).

Cold shock in fish occurs when the temperature suddenly drops below a level the fish is acclimated to (Donaldson et al. 2008, Meyer-Rochow 2013). Previous research, largely on freshwater fish, has shown that cold shock may cause both behavioural and physiological consequences, in addition to fatalities (Fry 1947, reviewed in Donaldson et al. 2008). Inhibition of the spinal reflexes often occurs (Roots & Prosser 1962), which reduces the individuals’ swimming abilities, eventually leading to a loss of equilibrium (Lamadrid-Rose & Boehlert 1988). The cause of this inhibition appears to be a result of effects of cold temperatures on the hypothalamic–pituitary–interrenal axis and the brain (Donaldson et al. 2008). An experiment on the tropical species Mugil cephalus revealed that a cold shock 15°C below ambient resulted in many individuals losing equilibrium, and 75% mortality (Lamadrid-Rose & Boehlert 1988). In addition to spinal reflexes, other conditioned reflexes appear to become impaired. Such inhibition, however, is reversible if the fish survive the temperature change (Prosser & Farhi 1965, Montgomery & MacDonald 1990) and return to ambient water. The loss of equilibrium and reflex impairment is a particularly important factor for prey species. If the tolerance of prey species to cold shock is lower than that of their predators, it puts the prey species at risk due to reduced predator avoidance capabilities (Lamadrid-Rose & Boehlert 1988).

Mottled mojarra Eucinostomus lefroyi inhabit shallow subtropical and tropical marine waters on the east coast of North America, where they (along with congeners) are a major component of fish communities in estuaries and coastal habitats (Kerschner et al. 1985). They are a prey species for predators such as lemon sharks Negaprion brevirostris, for which mojarra species were found to compromise over 50% of the consumed prey biomass in a study carried out in 2003 at Bimini, The Bahamas (Newman et al. 2012). Since temperature fluctuations in shallow waters may be more extreme than in deeper waters (IPCC 2007), mottled mojarra may experience more frequent and more extreme temperature decreases in the future. Therefore, it is important to understand the effects of such decreases on the behaviour and physiological responses of the species, and whether their tolerance is within the threshold of the predicted decreases. Given their large distribution, the fact that mottled mojarra or other similar small-bodied fishes are so prominent in coastal tropical marine systems, and the apparent important role of such fish in marine food webs, research on the effects of cold shock on such fishes is warranted (Kerschner et al. 1985).

The objective of this study was to examine the sensitivity of mottled mojarra to cold shock exposure, by (1) determining the critical thermal (CT) minimum temperature for the species and (2) measuring the effects of a range of ecologically relevant, abrupt temperature declines on the behaviour, ventilation rate and reflex responses. We aimed to determine cold shock tolerance thresholds and relevant behavioural responses of an ecologically important nearshore marine fish, which may serve as a model for understanding the potential effects of this stressor on similar species.

**MATERIALS AND METHODS**

**Fish collection**

A total of 64 mottled mojarra (89 ± 7 mm total length, TL; range 75 to 100 mm) were collected by seine net from shallow sandy habitats in Kemps Creek (24° 48’ 54.29” N, 76° 18’ 03.09” W) and Page Creek (24° 49’ 4.7” N, 76° 18’ 51.6” W), and transported to a holding tank at the Cape Eleuthera Institute in Eleuthera, The Bahamas on 15 January 2013. Water temperature at time of capture ranged from 23 to 25°C. Fish were held for 24 h prior to examination in circular tanks (3.7 m diameter × 1.25 m height, 13 180 l) with 1 m water depths, supplied with constant flow of fresh seawater. The seawater intake was from 1.5 m of water such that the water temperature was stable (~24°C during the study period) despite more extreme diel thermal variation in the nearshore tidal creeks from which the fish were captured.
CT minimum

Mottled mojarra (n = 14) were exposed to a linear temperature decrease (~0.2°C min⁻¹) in individual aerated 1 l containers (containing 750 ml of seawater) from ambient temperature (24°C) until equilibrium was lost, following the methods of Murchie et al. (2011). Two separate trials were conducted with 7 fish in each. Fish were considered to have lost equilibrium when they were unable to maintain upright posture for a minimum of 5 s, at which point time to loss was recorded. Determination of CT minimum was needed to inform the design of subsequent experiments. That is, our interest was in sub-lethal outcomes, so we needed to identify the level of lower thermal tolerance of this species.

Cold shock

Cold shock trials were conducted from 09:00 to 18:00 h from 16 to 29 January 2013. Each trial consisted of 8 fish, all kept in water of the same temperature, with 2 trials for each treatment (16 fish in total). Trials alternated randomly amongst treatments, which consisted of 16, 18, and 20°C temperatures (all below ambient temperature), and controls that remained at ambient temperature (24°C).

To begin trials, individual opaque plastic containers (1 l volume) were filled with 750 ml of seawater at ambient temperature. A small air stone was placed in each container to provide oxygen and ensure that the water was well mixed. Containers were then partially submerged in an ice bath (100 l cooler), or in the case of the controls, ambient seawater. For cold shock treatments, the temperature in the containers was lowered to the desired temperature by managing the amount of ice in the cooler. Once treatment temperatures were attained, mottled mojarra were transferred by dip net from the holding tank into individual containers. Trials were then initiated and lasted for 1 h. Temperature was recorded every 15 min during trials using a YSI meter (EcoSense pH10 A, Xylem).

During cold shock trials, ventilation rates were recorded by the same observer every 15 min, by visually counting operculum beats for 30 s. Reflex Action Mortality Predictors (RAMP; Davis 2010) were also assessed at the beginning and end of each trial, or at the time of equilibrium loss. A total of 5 RAMP indices were used: tail grab, body flex, head complex, vestibular–ocular response (VOR), and equilibrium. These metrics were chosen because they are strong indicators of vitality in other fish species in the context of fisheries-related stress (Raby et al. 2012, Brownscombe et al. 2013, 2014, Cooke et al. 2014), as well as cold shock (Székeres et al. 2014). Tail grab was assessed by grabbing the fish’s tail by hand, and was considered impaired if the fish did not immediately attempt to swim away. Body flex was assessed by briefly holding the fish out of the water; a lack of attempt to struggle free indicated impairment. While held out of the water, head complex was considered impaired if regular opercular beats were not observed. VOR was assessed by rolling the fish from side to side to determine whether the eyes were able to track the handler. Equilibrium was assessed by flipping the fish upside down in the water, and was considered impaired if the fish failed to recover within 3 s. Higher RAMP scores indicated greater impairment.

Once trials were completed, mottled mojarra were transferred into recovery containers (1 l volume, containing 750 ml of water) at ambient temperature for 30 min. After this recovery period, fish were transferred individually to 5 l circular containers with 8 cm of water depth, which were divided into 4 equal quadrants by lines drawn on the bottom of the container. Fish were chased by hand until exhaustion by one researcher, while another researcher recorded the number of lines mottled mojarra crossed prior to exhaustion. Fish were considered exhausted when the chaser could touch the fish’s tail 3 times without an attempt to escape. RAMP was assessed both immediately before and after chase trials; ventilation rates were measured immediately after. Once completed, the fish were allowed to recover for a minimum of 1 h before being released into the wild.

Although this approach to evaluating swimming ability does not generate an actual swimming speed per se, it does provide a robust approach for making inter-treatment comparisons (Portz 2007).

Statistical analysis

The relationship between mottled mojarra TL and CT minimum was analysed with Pearson’s correlation coefficient. Mottled mojarra ventilation rates were compared between treatments and over time with a linear mixed effect (LME) model with individual fish as a random factor. Significant terms were determined using backwards model selection with log-ratio tests. A variance structure was used to correct for variance heterogeneity (Pinheiro & Bates 2000). Tukey’s HSD post hoc tests were used to deter-
mine significant differences between treatments. Mottled mojarra RAMP score frequencies were compared among treatments using a chi-squared test for independence. For the chase to exhaustion experiment, the effects of temperature on the number of lines crossed and time chased were analysed using 1-way ANOVA. Tukey’s HSD tests were used to determine significant differences. Assumptions of normality and homogeneity were tested prior to analysis. Results were considered significant at $p \leq 0.05$, and all analyses were conducted using R studio (R Core Team 2012).

RESULTS

CT minimum

The CT minimum (at which mottled mojarra lost equilibrium) was $12.29 \pm 0.75°C$ (mean ± SE). There was no significant association between TL and CT minimum within the size range of 75 to 100 mm ($r = 0.44$, $p = 0.56$).

Cold shock

During cold shock exposure, mottled mojarra ventilation rates were highly variable among treatments, with fish in both the 16 and 24°C treatments generally exhibiting lower ventilation rates than those in the 18 and 20°C treatments (Fig. 1). Comparing ventilation rates among treatments and over time, there was a significant effect of time (LME; $F_{1,254} = 79.3$, $p < 0.001$), and significant interaction between treatment and time ($F_{1,254} = 16.6$, $p < 0.001$). At the start of the exposure period, ventilation rates were significantly higher at 18 than at 24°C (Tukey’s HSD; $p = 0.01$). After 15 min of exposure, ventilation rates were significantly higher at 18 and 20°C than at 16°C ($p < 0.01$). For the remaining time periods (i.e. 30, 45 and 60 min) ventilation rates were significantly higher at 18 and 20°C than at 16 and 24°C ($p < 0.05$).

Prior to the cold shock exposure period, all mottled mojarra had RAMP scores of 0 (no impairment). During exposure, no fish lost equilibrium at 24 or 20°C, however, 3 (19%) and 9 (56%) fish lost equilibrium at 18 and 16°C respectively (Fig. 2). After the 60 min exposure period, there was a significant difference in the amount of reflex impairment between treatments ($\chi^2 = 44$, df = 12, $p < 0.001$). While mottled mojarra in the 20 and 24°C treatments exhibited no reflex impairment, fish in the 16 and 18°C treatments continued to experience reflex impairment (Fig. 3), which was more severe at 16°C (Table 1). After the cold shock exposure period, fish in all treatments had zero RAMP impairment, and those exposed to 16°C exhibited the best swimming performance, with the longest chase times and most lines crossed of any treatment (Fig. 4). There was a significant effect of
treatment on the amount of time chased ($F_{3,60} = 3.6, p = 0.02$); mottled mojarra in the 16°C treatment swam significantly longer than those in the 18 and 20°C treatments ($p < 0.05$; Fig. 4). There was also a significant effect of treatment on the number of lines crossed ($F_{3,60} = 4.1, p = 0.01$); fish in the 16°C treatment crossed significantly more lines than those in the 18°C treatment ($p = 0.01$). After the chase to exhaustion, all fish exhibited impaired tail grab response (integral to the chasing process), but no other RAMP impairment.

**DISCUSSION**

Abrupt declines in water temperature had significant impacts on the ventilation rates, reflexes, and swimming performance of mottled mojarra. Ventilation rates displayed some surprising patterns, where at relatively moderate decreases in temperature (18 and 20°C; 4 and 6°C below ambient), mottled mojarra exhibited higher ventilation rates than at ambient, while at the greatest decline in temperature (16°C; 8°C below ambient) fish had similar ventilation rates to ambient. Abrupt changes in temperature typically cause physiological disturbances that increase metabolic demands in ectotherms (Wendelaar Bonga 1997, Jentoft et al. 2005, Donaldson et al. 2008), which is likely why mottled mojarra exhibited increased ventilation rates at 18 and 20°C. However, some fish species can compensate physiologically to abrupt declines in temperature (Fry 1947, Shiels et al. 2003, Narum et al. 2013), which may explain why ventilation rates remained similar in fish exposed to 16°C to those at ambient temperature (Fig. 1). However, it is surprising that such compensation would not occur at lesser degrees of temperature change. Ventilation rates are often assumed to be reflective of...
cardio-respiratory demand, but there is growing evidence to suggest that when exposed to stressors, ventilation rates may not always be a strong predictor of physiological status (Barreto & Volpato 2004). A decline in temperature of 4°C caused significantly higher ventilation rates, but mottled mojarra exhibited no equilibrium loss during exposure, nor any other reflex impairment after the 1 h exposure period. Conversely, declines of 6 and 8°C caused rapid equilibrium loss and significant reflex impairment, particularly at 8°C below ambient temperature. Equilibrium loss is a common result of cold shock exposure in fish, and is an indication of physiological stress that may lead to mortality (Griffith 1978, Hyvärinen et al. 2004, Donaldson et al. 2008, Davis 2010, Raby et al. 2012). Loss of ability to swim upright also greatly reduces the capacity of fish to move into deeper water to escape further cold exposure (in the case of storm-induced perturbation of shallow waters) or avoid predators, further increasing the probability of mortality. Interestingly, tidal creek environments in Eleuthera (where fish were collected for this study) experience daily fluctuations of up to 11°C (Murchie et al. 2011), while the lower sub-lethal thermal limit for mottled mojarra in this study was 12°C on average. However, the cold shock scenarios tested here exposed fish to more abrupt declines in temperature that may be more similar to those resulting from storms or ocean upwelling than typical diel patterns associated with tidal cycles and solar radiation.

Post-exposure swimming capabilities yielded some surprising results: 30 min after 1 h of exposure to 4 and 6°C temperature declines, mojarra exhibited similar swimming capabilities to those maintained at ambient temperature, while those exposed to 8°C declines actually swam longer and crossed more lines than those in the other treatments. Despite the fact that mottled mojarra experienced significant reflex impairment at more severe temperature declines during exposure, swimming performance quickly returned after a 30 min recovery period at ambient temperature. Exposure to cold shock initiates a physiological stress response, a component of which is increased capacity to deliver oxygen to muscles, and an increase in available energy reserves (Wendelaar Bonga 1997, Shiels et al. 2003). This may explain why, when placed back into water at ambient temperature, mottled mojarra exposed to 8°C colder water had similar, if not greater swimming capabilities compared to other treatments. This is consistent with findings in another subtropical marine species, the bonefish (Albula vulpes; Szekeres et al. 2014), which collectively supports the concept that if fish are able to escape cold water during cold shock events, swimming capabilities quickly return and the chances of mortality due to predation are minimal. However, the ability of fish to escape near-shore areas affected by cold shock events (e.g. upwellings or storms) ultimately depends on the behaviour of the fish and the nature of the environment. In environments such as Kemps Creek in Eleuthera, with its expansive shallow flats, fish must travel long distances to reach deeper water.

In summary, exposing mottled mojarra to abrupt cold shock caused significant behavioural impairment; the level of impairment was generally associated with the magnitude of temperature change. Exposure to 16 and 18°C (8 and 6°C below ambient) caused significant reflex impairment, potentially reflecting a physiological stress response. Indeed, extended exposure to water >4°C below ambient may cause mortality in this species. However, if mottled mojarra are able to escape abrupt declines in temperature, swimming capabilities are quickly regained and therefore predation risk would likely be low. To date, very little research has focused on the consequences of cold shock on tropical or subtropical species. Future studies should extend our understanding of the impacts of cold shock to other species in these areas, including critical thermal thresholds and durations. Moreover, there is a need for research on the impacts of cold shock on a greater size range of individuals, especially smaller fish, which may be more sensitive to temperature variability (Donaldson et al. 2008). Such research would be timely, given that adverse weather conditions and changes in ocean currents driven by global climate change may expose near-shore dwelling tropical fish species to increasingly frequent cold shock events in the coming decades.

Acknowledgements. We thank the staff at Cape Eleuthera Institute for supporting our research. J.W.B. was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC). S.J.C. was supported by the Canada Research Chair’s program and NSERC. E.S. was supported by the University of Manchester with travel support from NSERC.

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Editorial responsibility: Victor Meyer-Rochow, Bremen, Germany

Submitted: June 25, 2014; Accepted: October 8, 2014
Proofs received from author(s): December 4, 2014