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Consequences of simulated multiple catch-and-release events and different handling procedures on reflex impairment, ventilation rate, and body condition in Tor khudree

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ABSTRACT

As catch-and-release becomes more popular and angling pressure increases, it is likely that fish in popular fisheries will experience catch-and-release events on more than one occasion (i.e., "multiple captures"). Anecdotal reports from anglers suggest that blue-finned mahseer (*Tor khudree*) in WASI Lake, Karnataka, India, commonly experience multiple capture events that are closely timed together (e.g., often on successive days). To determine whether multiple capture events were likely to result in cumulative physiological effects compared to single capture events, we quickly angled (i.e., landed in < 2.5 min) 124 blue-finned mahseer and placed them in one of four simulated capture treatment groups: control, air exposure, chase, or a combination of air exposure and chase. Blue-finned mahseer were held overnight in a net pen and subjected to a second (n = 91) or third (n = 60) instance of simulated capture. Our results showed increasing ventilation rates and condition score but no pattern in reflex impairment score in blue-finned mahseer for each treatment group and across each day. Ventilation rates were lowest in the air exposure and combination treatments than for other treatment groups, possibly indicating a masking effect from air exposure. The findings of our study indicate blue-finned mahseer are relatively robust to capture-related stressors, even when experienced with short intervals between fisheries encounters; however, we urge further research for improved understanding of the manner in which these stressors interact, particularly in tropical, freshwater environments.

1. Introduction

Catch-and-release (C&R) angling refers to the capture and subsequent release of a fish back into the water, presumably unharmed (Arlinghaus et al., 2007). C&R has been applied as a voluntary or mandatory management tool for recreational fishing of numerous threatened species (e.g., *Hucho taimen*, Jensen et al., 2009; and see Cooke et al., 2016 for additional case studies) and an estimated 60% of all fish captured globally during recreational fishing activities are released (Cooke and Cowx, 2004). During the C&R process, fish can experience a range of physiological alterations related to angling activities ranging from minor temporary increases in blood lactate and glucose to post-release mortality if physiological stress exceeds biological thresholds (Arlinghaus et al., 2007). Fish may experience behavioural alterations (such as reduced movement or feeding behaviour) that can result in increased rates of post-release predation (e.g., *Albula vulpes*, Danylchuk et al., 2007) or decreased success in crucial life history behaviours (such as migration and reproductive success, Thorstad et al., 2007). This variability in potential response has led to calls for research into species-specific responses to C&R (Cooke and Suski, 2005) and improved understanding of how angler behaviours and environmental conditions contribute to these responses (Cooke et al., 2013), particularly as stress may be cumulative (Barton et al., 1986). Also among these knowledge gaps is the question of how many fish experience capture on

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multiple occasions ('multiple capture') and whether any negative physiological or behavioural effects arise as a result of multiple capture that are not evident after single instances of capture (e.g., recommended by Bartholomew and Bohnsack, 2005; Patterson et al., 2017).

Instances of multiple capture occur over short (e.g., Nelson et al., 2005) and long (e.g., Cline et al., 2012) time frames and can result in numerous physiological and behavioural changes in individual fish and potentially fish populations (e.g., changes to habitat selectivity for *Esox lucius* populations were noted in single capture event study, Klefoth et al., 2011). Some studies have demonstrated that multiple capture events can influence individual fish growth (e.g., Cline et al., 2012), while others have found no apparent effects of multiple capture on mortality rates (e.g., in *Thymallus arcticus*, Clark et al., 1991). Nelson et al. (2005) found that while as many as three instances of multiple capture did not impact mortality rates or spawning success in steelhead (*Oncorhynchus mykiss*), hatchery fish were more likely to be recaptured than wild fish. Similarly, Garrett (2002) found that angling vulnerability in *Micropterus salmoides* could be selectively bred for and that individuals vulnerable to angling were more likely to be caught on multiple occasions. Given the increasing use of C&R as a management tool and the selective pressures exerted on fish by angling activities, the impact of multiple capture events on mortality rates and sublethal effects should be considered a priority for C&R populations particularly those targeting threatened species and populations subject to high angling pressure.

Measuring the effects of multiple capture in C&R scenarios involves several challenges inherent to C&R research, including difficulties establishing true controls (such as for mortality estimates, Pollock and Pine, 2007) and a reliance on tools such as holding pens and capture simulation to enable data-gathering during the recapture period. Holding pens have been found to result in elevated plasma stress (e.g., elevated plasma cortisol and elevated plasma glucose, Donaldson et al., 2011), and their use can involve extensive and repeated handling (Cooke et al., 2013), suggesting that it is difficult to disentangle effects of C&R from effects of holding. Thus, simulation studies can result in a disconnect between simulated and genuine outcomes (Cooke et al., 2013, 2017). Yet, as ensuring a high rate of genuine instances of multiple capture can be challenging in a field study scenario, simulation studies can be used to ensure adequate data points over a short term. Despite these challenges, the use of simulated angling events and holding pens are beneficial to the study of C&R impacts by allowing researchers to control variables and gain visual access to 'released' fish and may be unavoidable when attempting to quantify the effects of closely timed multiple capture events.

2. The blue-finned mahseer C&R recreational fishery of the Kaveri River

The blue-finned mahseer (*Tor khudree*) C&R recreational fishery of the Kaveri (also known as the Cauvery) River in South India is among the best-known recreational fisheries in India. Mahseer species throughout India are currently under threat from anthropogenic stressors including agricultural pollution, hydropower development, invasive species, and overfishing (Everard and Kataria, 2011). The blue-finned mahseer is not currently threatened, however, and may not be native to the Kaveri River basin (Pinder et al., 2019a).

The Wildlife Association of South India (WASI) is a conservation-oriented organization that manages a leased stretch on the Kaveri River where C&R of blue-finned mahseer is mandatory. Their main lodging and angling area consist of two man-made lakes (WASI Lake and Forbes Sagar) situated along a canal that diverts water from the Kaveri. WASI members are able to rent two cottages on site and pay daily license fees to fish from WASI Lake and Forbes Sagar. Formal permission to conduct this study was granted from WASI and the Karnataka Fisheries Department.

Previous studies of blue-finned mahseer responses to C&R have indicated that *T. khudree* are physiologically (Bower et al., 2016a) and behaviourally (Bower et al., 2019) resilient to common C&R practices but have noted that prolonged angling times and air exposure may result in increased impairment, particularly in larger fish. Blue-finned mahseer caught in WASI Lake tend to be smaller than those caught elsewhere on the Kaveri River and much easier to catch (personal observation, S.D. Bower), possibly because of their high numbers. Anecdotal reports of angling activity at the WASI site have suggested blue-finned mahseer are likely to experience multiple capture events over a short term (i.e., a few days to a week), indicating that a study of multiple capture events would be of value. Thus, we simulated multiple capture events at this location to test the hypothesis that the number of capture events would influence post-release physiology in blue-finned mahseer. Our previous findings supported a prediction that *T. khudree* were unlikely to experience significant reflex impairment as a result of closely timed simulated multiple capture events. We further predicted that there would be no significant differences in reflex impairment or ventilation rates across treatment or days. However, as blue-finned mahseer have demonstrated a sensitivity to air exposure and extended angling times (Bower et al., 2016a), we predicted that fish in air exposure and chase treatment groups would have significantly different reflex impairment and significantly different ventilation rates than those in the control group, and that blue-finned mahseer in the combination treatment group would have significantly different reflex impairment and ventilation rates than those in air exposure and chase treatment groups.

3. Methods

3.1. Study site and angling practices

Angling and sampling took place in a small, man-made impoundment along the Kaveri River, called Malligemaradahalla Lake but more commonly known as WASI Lake (12.288979ºN, 77.155393ºE; Fig. 1), from May 6 to June 7, 2016. This timeframe represents the pre-monsoon angling season, characterized by low flow and high water temperatures. Angling activities took place from shore, during daylight hours (ranging from 0830 h to 1900 h). Fish were angled using lightweight spinning gear and method feeder rigs packed with busa (a mixture of flours, oils, cow feed, and corn), the most commonly used method for catching blue-finned mahseer in this area (Fig. 2). To ensure that initial angling processes resulted in as little added physiological stress as possible, blue-finned mahseer were landed in under 2.5 min (maximum time from hooking to unhooking in processing pool) in an attempt to minimize physiological disturbance prior to treatment (Kieffer, 2000). Thus, any fish not landed within this timeframe ($n = 3$) were excluded from analysis and released. Water temperatures during the multiple capture simulation study averaged 30 ± 0.6 ℃ (range: 28 ℃ – 32 ℃).

It is important to note that this study does not account for potential mortality risk related to deep hooking, which would be expected to increase with multiple recaptures, nor did this study mimic multiple hooking sites in any way. Yet, rates of deep hooking are believed to be very low for this species (i.e., fewer than one per 100 caught, personal observation, S.D. Bower), thus we find this omission reasonable for reasons of relevance and animal care.

3.2. Simulated multiple capture procedures

On landing, each individual fish was placed in a processing pool. After placement in the processing pool, each blue-finned mahseer was measured for total body length (TL, mm), and a temporary uniquely numbered identification tag (5 cm length, with a 1 cm × 1 cm fine fabric tag; Avery Dennison, Westboro, MA, USA) was inserted into the dorsal musculature behind the dorsal fin using a Mark III Fine Fabric Pistol Grip tagging gun (Floy Tag, Seattle, WA, USA). Each blue-finned mahseer then underwent one of four treatments: control, air exposure, chase, or combination (chase and air exposure). Fish were pre-assigned

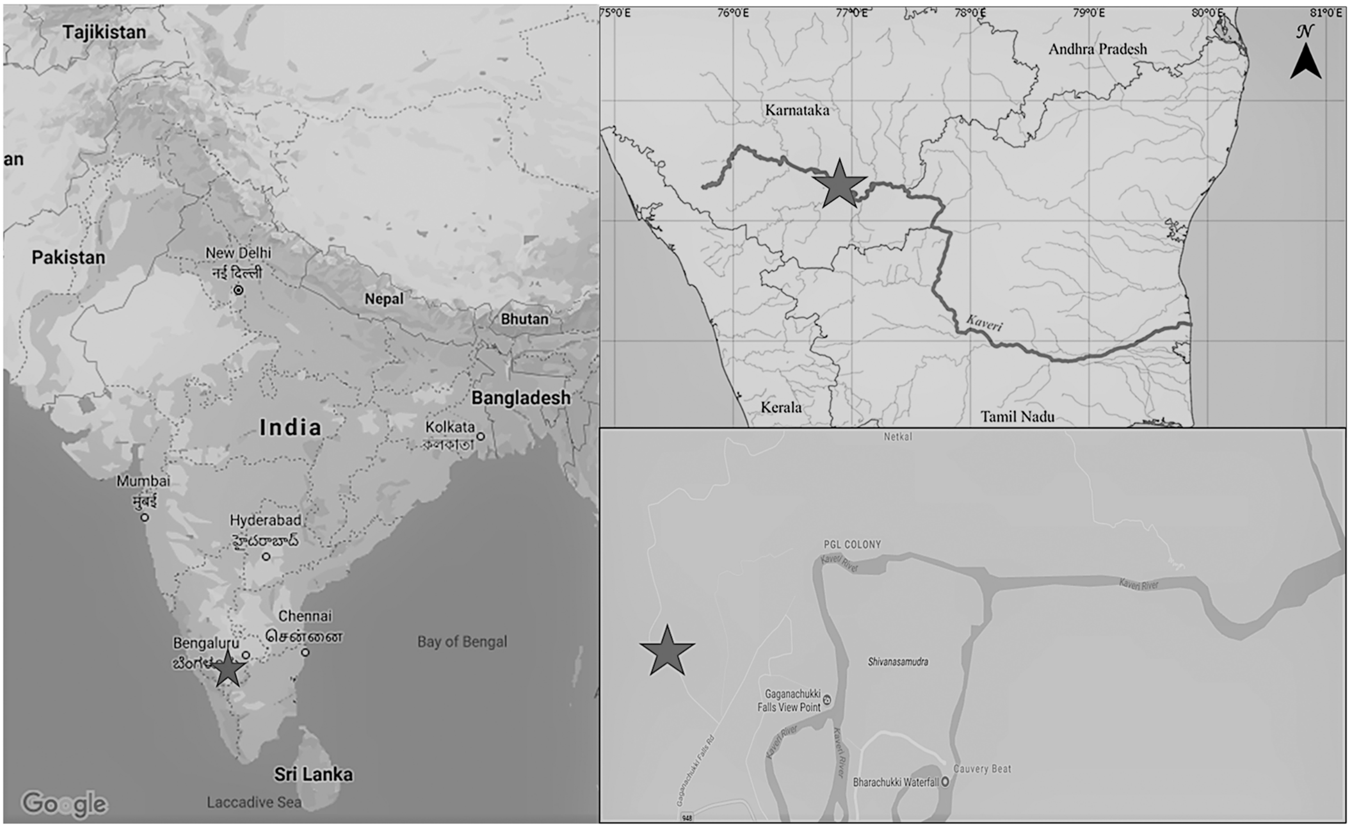

Fig. 1. The location of the field site shown as a series of related maps. A map of India, left,with the location of the Kaveri (Cauvery) River, Karnataka shown as a star, the Kaveri River(upper right) with the WASI Lake and Forbes Sagar location shown as a star, and the location ofWASI Lake (lower right) at a diversion of the Kaveri River near Shivanasamudra Falls shown as a star.

Fig. 2. Blue-finned mahseer (*Tor khudree*) caught at the WASI Lake study site (left) are typically caught using method feeder rigs (top right), packed with a grain and corn-based bait known as busa (bottom right).

to treatment groups (i.e., before angling began) on a daily basis in an effort to maintain evenness of treatment group numbers. Duration of the treatments was based on angling times common to the location:

- Fish placed in the control group were left undisturbed in a mesh-ended, black holding bag (75 cm in length; Dynamic Aqua Ltd., Vancouver, BC, Canada) in the processing pool for 5 min
- Fish placed in the air exposure treatment group were left undisturbed in the holding bag for 4 min, then suspended out of water (with all water drained out of the holding bag) for 1 min
- Fish in the chase treatment group were "chased' by hand (fluttering and grabbing behind them without making contact) to encourage them to swim vigorously around the processing pool for 5 min
- Fish in the combination treatment group were made to swim vigorously (as above) around the processing pool for 4 min before being placed in the holding bag, then suspended out of water (with all water drained out of the holding bag) for 1 min

After treatment, each blue-finned mahseer was measured for reflex impairment using Reflex Action Mortality Predictors (RAMP; Davis, 2010) and ventilation rate (number of operculum beats/10 s) while in the holding bag. RAMP measurements were scored on a binary basis (0, 1) according to whether the fish exhibited the behaviour (a score of 0, or unimpaired) or did not exhibit the behaviour (a score of 1, or impaired). Scores from each impairment measurement were then summed to develop an overall impairment score ranging from 0 to 1 for each fish (for e.g., see Brownscombe et al., 2013). The reflex impairment measurements applied in this study were 'tail grab', the presence of burst swimming reflex activity within 3 s of being grabbed by the caudal peduncle; 'orientation', the presence of re-orienting capability within 3 s of being placed upside down in water; and 'operculum beats', the regularity and steadiness of operculum beats (as opposed to irregular or unsteady beats; Davis, 2010). In this study, we noted that many blue-finned mahseer released air bubbles from the gills or demonstrated a brief hiccup in operculum beats during processing, however, if the rate of operculum beats was steady, we did not consider this a demonstration of impairment.

Ventilation rate is typically used as a non-invasive measurement of energy consumption in fish (e.g., Millidine et al., 2008), however it is also used as a measurement of sympathetic response to stress (Barreto and Volpato, 2011). In this study, we used ventilation rate as a complementary whole-body stress indicator to compare and supplement impairment scores measured with RAMP (Sopinka et al., 2016 recommend the use of multiple whole-body stress indicators). Measurements of ventilation rate were later transformed from beats/10 s to beats/min for analysis.

After treatment and measurements, each fish was brought from the processing pool to a net pen (1.5 m x 2 m x 3 m) in the holding bag (i.e., kept under water) and left in the net pen overnight. The net pen was secured at the top with a mesh lid covered with an 8 cm foam layer to prevent injury to fish attempting to jump free. Blue-finned mahseer held overnight were supplied with food in the form of busa.

Fish held overnight in the net pen were processed the following morning from 0730 h to 0830 h. Day 2 and Day 3 processing excluded the initial angling event, body size measurement, and temporary tag placement. Blue-finned mahseer were removed from the net pen, placed into the holding bag and brought to the processing pool. Fish were kept in the same assigned treatment group throughout the experiment, and ventilation rates and impairment measurements were performed in the same manner on both Day 2 and Day 3. All fish had their temporary tags removed and were released after measurements on Day 3. All experimental manipulations performed during this study were conducted in accordance with Canadian Council of Animal Care regulations under Carleton University Protocol #101005.

3.3. Adjustments made due to escape frequency and holding condition

A high rate of overnight escapes over the first week of the study led the team to further secure the net pen at night by loop stitching the net pen shut with oiled twine. The observation of small blue-finned mahseer, such as the ones sampled during this study, congregating in large groups (Bower et al., 2016a) led us to believe holding effects from a group of 15 small fish held overnight in the net pen would be insignificant, but this proved not to be the case. On effectively securing the net pen and preventing a high rate of overnight escapes, we noted that blue-finned mahseer held overnight exhibited severe signs of holding effects, including clouded eyes and complete slime loss. All of these fish were immediately released (May 18), and excluded from further analysis, i.e., only data from the previous day or days were used. A new maximum of 10 blue-finned mahseer per day were retained in the pen. To account for holding effects, we also instituted (based on our field notes) a condition score (e.g., see Campbell et al., 2009). Our condition score ranged from 0 to 3, where 0 indicated a fish in excellent body condition (no external damage). A score of 1 – 3 was given to blue-finned mahseer exhibiting one or more of the following:

- Score 1 (minimal external damage): 1 – 2 fins tattered and/or frayed, $<$ 3 scales abraded, 1 or fewer abrasions at nares or along dorsal ridge, and minor slime loss.
- Score 2 (moderate external damage): 3 – 4 fins tattered and/or frayed, 3 – 5 scales abraded, $>$ 1 abrasion at nares or along dorsal ridge, eyes showing early signs of clouding, and moderate slime loss.
- Score 3 (major external damage): $\geq$ 5 fins tattered and/or frayed, $>$ 5 scales abraded, distinct abrasions at nares or along dorsal ridge, eyes cloudy, and major slime loss.

Blue-finned mahseer captured prior to development of the condition score for whom field notes did not adequately describe body condition were assigned a score of 'n/a' and were excluded from analysis. While these changes eliminated overnight escapes and improved the condition of fish held in the net pen, it did not prevent the escape of fish during treatments. Unfortunately, this resulted in uneven sample sizes across days and treatments.

3.4. Statistical analyses

Results were compared both across days and treatment groups. Treatment was used as a between-subjects factor and day was treated as a within-subjects factor, and all linear models included treatment x day as a crossed factor. After removing three extreme outliers from Day 1 (combination treatment), ventilation rate was normally distributed and so a two-way repeated measures ANOVA (rstatix and tidyverse packages, Kassambara, 2020) was used to determine whether ventilation rate differed significantly for blue-finned mahseer among Days 1, 2, and 3 and across treatment groups. Significant differences found in the model were explored first using individual ANOVAS and then Tukey's HSD (via emmeans package, Lenth, 2020). In order to avoid condensing variables to binary distributions, reflex impairment score analysis was conducted using ordinal regression models (MASS, polr package, Venables and Ripley, 2002) to determine whether reflex impairment scores differed significantly for blue-finned mahseer among Days 1, 2, and 3 and across treatment groups. The chosen models can perform in the same manner as traditional repeated measures models but are more robust to non-normally distributed variables, imbalanced variables, and allows for unequal variance in within-subjects variables (Clark, 2017), all attributes that were relevant to this analysis. Condition score data proved unsuitable for comparable models, however. Thus, condition score was analyzed using a Friedman Rank Sum test (stats package, R Core Team 2016) to determine whether condition scores differed significantly for blue-finned mahseer among Days 1, 2, and 3 and across treatment groups. Significant differences in the Friedman Rank Sum test were followed by Wilcoxon Signed Rank test. All analyses were conducted in R Studio (version 1.3.959, R Core Team, 2019).

4. Results

All 124 blue-finned mahseer caught were subjected to the first simulated capture event (Day 1). Of these, 33 escaped either from the net pen or during processing events. Four of these escaped fish were later caught again during angling activities but were released on identification as a recapture. There were 91 fish in total subjected to simulated multiple capture processes: all 91 blue-finned mahseer were held overnight for a single night and subjected to a second simulated angling process (Day 2) and 60 of these blue-finned mahseer were held overnight for a second night and subjected to a third simulated angling process prior to release (Day 3).

Due to the number of escaped fish during measurements, we ended up with uneven numbers of fish per treatment group: 27 in the air exposure treatment group, 30 in the chase treatment group, 43 in the combination treatment group, and 24 in the control group.

Blue-finned mahseer included in the study ranged from 300 mm to 586 mm (TL). The mean TL decreased very slightly over the course of the holding period but this decrease was 3 mm throughout the study period and did not differ significantly among treatment groups (F= 0.468, df = 119, p = 0.468; Table 1). Despite exposing fish to various treatments and multiple simulated capture events over a short time, no blue-finned mahseer died during experimentation and all were released following the conclusion of the study.

Mean ventilation rates increased throughout the holding period, rising from 90 beats/min on Day 1–98 beats/min on Day 2, and 108 beats/min on Day 3 (Table 1). Mean ventilation rate was lowest in the air exposure group, followed by combination, control, and chase groups (Table 2). Mean ventilation rate rose in the air exposure group (84 beats/min to 92 beats/min to 96 beats/min), the combination group (82 beats/min to 93 beats/min to 108 beats/min), and the control group (98 beats/min to 104 beats/min to 109 beats/min). Mean ventilation rate was highest overall for blue-finned mahseer in the chase treatment group (98 beats/min to 107 beats/min to 117 beats/min).

The two-way repeated measures ANOVA model indicated that ventilation rate was significantly different among treatment groups and days but was not different when interactions between treatment and day were included in the model (Table 3). Sources of the differences among treatment groups were determined to be between ventilation rate values of chase and air exposure treatments (p < 0.0001), between control and air exposure treatments (p = 0.0002), between combination and chase treatments (p < 0.0001), and between control and combination treatments (p = 0.0002; see also Table 3). Differences were found to occur across all days but were more significant between Days 1 and 3 (p < 0.0001) than between Days 1 and 2 (p = 0.0007) or Days 2 and 3 (p = 0.002; Table 3).

Table 1
Mean total length, impairment score, ventilation rate, and condition score across all treatment groups (control, air exposure, chase, combination [chase + air exposure]) by day. Mean length and ventilation rate values are presented ± standard deviation. Impairment (0, 0.33, 0.66, 1) and condition scores (0, 1, 2, 3) are presented as counts for each level, with associated percentage of mahseer represented to enhance count meaning. As described in the methods section, n/a refers to those fish captured prior to development of the condition score.

	Mean TL (mm)	Impairment Score (0, 0.33, 0.66, 1)	Ventilation Rate (beats/min)	Condition Score (0, 1, 2, 3)
Day 1 (n = 124)	369 ± 50	0: 112 (91%) 0.33: 12 (9%) 0.66: 0 1: 0	90 ± 16	0: 118 (95%) 1: 6 (5%) 2: 0 3: 0
Day 2 (n = 91)	368 ± 48	0: 84 (92%) 0.33: 5 (6%) 0.66: 2 (2%) 1: 0	98 ± 18	0: 21 (26%) 1: 30 (37%) 2: 22 (27%) 3: 8 (10%) n/a: 10
Day 3 (n = 60)	366 ± 47	0: 50 (83%) 0.33: 8 (13%) 0.66: 1 (2%) 1: 1 (2%)	108 ± 18	0: 8 (15%) 1: 15 (27%) 2: 17 (31%) 3: 15 (27%) n/a: 5

Table 2
Mean total length, impairment score, ventilation rate, and condition score per treatment group (control, air exposure, chase, combination [chase + air exposure]) by day. Mean total length and ventilation rate values are presented ± standard deviation. Impairment (0, 0.33, 0.66, 1) and condition scores (0, 1, 2, 3) are presented as counts for each level, with associated percentage of mahseer represented to enhance count meaning. As described in the methods section, n/a refers to those fish captured prior to development of the condition score.

Treatment Group	Mean TL (mm)	Ventilation Rate (beats/min)	Impairment Score (0, 0.33, 0.66, 1)	Condition Score (0, 1, 2, 3)
Control Day 1 (*n* = 24)	372 ± 37	98 ± 19	0: 22 (92%) 0.33: 2 (8%) 0.66: 0 1: 0	0: 23 (96%) 1: 1 (4%) 2: 0 3: 0
Control Day 2 (*n* = 19)	374 ± 39	104 ± 16	0: 19 (100%) 0.33: 0 0.66: 0 1: 0	0: 9 (60%) 1: 6 (40%) 2: 0 3: 0 n/a: 4
Control Day 3 (*n* = 15)	375 ± 42	109 ± 19	0: 12 (80%) 0.33: 3 (20%) 0.66: 0 1: 0	0: 5 (41%) 1: 2 (17%) 2: 3 (25%) 3: 2 (17%) n/a: 3
Air Exposure Day 1 (*n* = 27)	376 ± 51	84 ± 13	0: 25 (93%) 0.33: 2 (7%) 0.66: 0 1: 0	0: 27 (100%) 1: 0 2: 0 3: 0
Air Exposure Day 2 (*n* = 20)	376 ± 51	92 ± 14	0: 18 (90%) 0.33: 2 (10%) 0.66: 0 1: 0	0: 2 (11%) 1: 8 (42%) 2: 8 (42%) 3: 1 (5%) n/a: 1
Air Exposure Day 3 (*n* = 14)	378 ± 58	96 ± 13	0: 14 (100%) 0.33: 0 0.66: 0 1: 0	0: 0 1: 5 (36%) 2: 4 (28%) 3: 5 (36%) n/a: 0
Chase Day 1 (*n* = 30)	374 ± 71	98 ± 15	0: 27 (90%) 0.33: 3 (10%) 0.66: 0 1: 0	0: 27 (90%) 1: 3 (10%) 2: 0 3: 0
Chase Day 2 (*n* = 20)	369 ± 67	107 ± 17	0: 19 (95%) 0.33: 1 (5%) 0.66: 0 1: 0	0: 5 (30%) 1: 6 (35%) 2: 6 (35%) 3: 0 n/a: 3
Chase Day 3 (*n* = 16)	356 ± 46	117 ± 20	0: 11 (69%) 0.33: 4 (25%) 0.66: 1 (6%) 1: 0	0: 1 (6%) 1: 5 (33%) 2: 5 (33%) 3: 4 (27%) n/a: 1
Combination Day 1 (*n* = 43)	359 ± 36	82 ± 10	0: 38 (88%) 0.33: 5 (12%) 0.66: 0 1: 0	0: 41 (95%) 1: 2 (5%) 2: 0 3: 0
Combination Day 2 (*n* = 32)	359 ± 37	93 ± 18	0: 28 (88%) 0.33: 2 (6%) 0.66: 2 (6%) 1: 0	0: 5 (17%) 1: 10 (33%) 2: 8 (27%) 3: 7 (23%) n/a: 2
Combination Day 3 (*n* = 15)	353 ± 39	108 ± 13	0: 13 (87%) 0.33: 1 (6%) 0.66: 0 1: 1 (6%)	0: 2 (14%) 1: 3 (21%) 2: 5 (36%) 3: 4 (29%) n/a: 1

Table 3
Output resulting from mixed-effects model testing of differences in ventilation rate among treatment groups and across days of capture, ordinal regression model testing of differences in impairment cores among treatment groups and across days of capture, and Friedman testing outputs of differences in condition scores among treatment groups and across days of capture. *Post hoc* testing results are presented where statistically significant results occurred. SE refers to 'standard error'.

Ventilation Rate (beats/min) Model Outputs					
	df	Sum Sq.	Mean Sq.	*F* value	p value
Treatment	3	13165	4428	11.949	< 0.001
Day	2	8678	4549	11.815	< 0.001
Treatment x Day	6	1688	379	0.766	0.598
Residuals	111	40763			
	Tukey's HSD				
Treatment		Difference	Lower CL	Upper CL	p adjusted
Chase-Air		15.63	7.83	23.45	p < 0.0001
Combination-Air		1.08	-6.26	8.41	p = 0.98
Control-Air		13.31	5.28	21.35	p = 0.0002
Combination-Chase		-14.56	-21.80	-7.36	p < 0.0001
Control-Chase		-2.32	-10.24	5.59	p = 0.87
Control-Combination		12.24	4.79	19.68	p = 0.0002
Day					
2–1		8.74	3.22	14.25	p = 0.0007
3–1		18.29	11.94	24.64	p < 0.0001
3–2		9.55	2.87	16.23	p = 0.002
Reflex Impairment Score Model Outputs					
Residual Deviance: 179.1372					
AIC: 193.1372					
Coefficients	Odds Ratio		SE	t value	p value
Chase	2.1		0.6	1.1	0.3
Combination	1.8		0.6	0.9	0.4
Control	1.5		0.7	0.6	0.6
Day 2	0.8		0.5	-0.5	0.6
Day 3	1.7		0.5	1.1	0.3
Condition Score Model Outputs					
Friedman Rank Sum Test, Condition Score by Treatment Group and Day					
χ^2		df		p value	
711.25		3		< 0.0001	
Wilcoxon Signed Rank Test, Condition Score by Treatment Group					
	Air	Chase		Combination (Chase + Air)	
Chase	1.0	–		–	
Combination (Chase + Air)	1.0	1.0		–	
Control	0.03	0.11		0.10	
Wilcoxon Signed Rank Test, Condition Score by Day					
		Day 1	Day 2		
Day 2		< 0.0001	–		
Day 3		< 0.0001	0.005		

Scores indicating no reflex impairment (score 0) were the most common for all treatment groups, though fish in each group demonstrated impairment of one or more reflexes across all days (Tables 1 and 2). Twelve blue-finned mahseer (9%) demonstrated reflex impairment after the first simulation (Day 1; Table 1). Of these, twelve demonstrated impairment in a single reflex (five lost tail grab, one lost orientation, and six lost regular operculum beats). Seven fish (8%) demonstrated impairment after the second simulation (Day 2; Table 1). Of these, five showed impairment in a single reflex (two lost tail grab, one lost orientation, and two lost regular operculum beats). The two fish that showed impairment in two reflexes lost tail grab and orientation, and tail grab and regular operculum beats. Ten blue-finned mahseer (17%) demonstrated impairment after the third simulation (Day 3; Table 1). Eight fish (13%) demonstrated impairment in a single reflex (one lost tail grab and seven lost regular operculum beats), one fish lost orientation and operculum beats, and another fish lost all three reflexes measured.

Blue-finned mahseer in control and air exposure groups registered impairment in none or in a single reflex on all three days. Blue-finned mahseer in the chase group registered impairment in none and one reflex on Day 1 and Day 2, and in none, one, and two reflexes on Day 3. Fish in the combination group registered impairment in none or one reflex on Day 1, in none, one, or two reflexes on Day 2, and in one or three reflexes on Day 3 (Table 2). Ordinal regression model testing showed no significant differences in impairment scores between treatment groups or across days (Table 3).

Trends in condition scores showed changing values among treatment groups and days (Table 1). Unlike reflex impairment score, condition scores on Day 3 were clearly higher than on Day 2 which were also higher than on Day 1 (Table 1), though 0 score remained the most common Day 3 condition score for the control group. The majority of blue-finned mahseer had low condition scores (0, 1), but higher condition scores (2, 3) were more common in treatment groups other than control (Table 2). Treatments involving air exposure (air exposure, combination) had the largest number of condition 3 scores (Table 2). Condition score was significantly different between air exposure and control treatment groups but not among other treatment groups (Table 3). Condition score was also significantly different from Day 1 to Days 2 and 3, and from Day 2 to Day 3 (Table 3).

5. Discussion

Our initial predictions that blue-finned mahseer would demonstrate robustness to simulated multiple capture events but sensitivity to treatment types were partially correct. The results of our study indicate that C&R-induced stressors such as exhaustive exercise and air exposure result in responses in blue-finned mahseer that were cumulative across instances of multiple capture over a short time period (as indicated by ventilation rate model results) but are unlikely to result in significant reflex impairment that influences post-release mortality rates (as indicated by reflex impairment model results). Measurements of ventilation rate suggested that blue-finned mahseer do experience significant physiological effects when subject to closely timed (e.g., within 24 h) instances of simulated multiple capture. The ventilation rate model results further indicate that blue-finned mahseer that have experienced air exposure (air exposure and combination treatments) are significantly more likely to have lower ventilation rates than fish subjected to vigorous chase or control.

The findings of our study may also reflect masked effects of C&R-induced stressors that were more clearly defined by measurements of ventilation rate than by reflex impairment. For example, it would typically be expected that fish in the combination treatment would exhibit the most severe impairment because air exposure is known to exacerbate stress responses (reviewed in Cook et al., 2015). However, air exposure has been found to result in lowered ventilation rate in fish (in studies examining interactive effects with water temperature, e.g., Gingerich et al., 2007; Gale et al., 2014; Pinder et al., 2019b), while ventilation rate has been shown to increase in relation to numerous other stressors, such as exercise (e.g., in Nile tilapia [*Oreochromis niloticus*], Barreto and Volpato, 2011). In this study, blue-finned mahseer in the combination treatment had slightly higher ventilation rates than those in the air exposure group, and significantly lower ventilation rates than blue-finned mahseer in the chase group. This unexpected result may indicate that these two stressors interact in blue-finned mahseer such that these opposing effects are masked when measured by whole body indicators. Alternatively, Barreto and Volpato (2004) suggested that ventilation rate measurements may be less reliable for measuring the severity of a stressor than for identifying the presence of a stressor; however, given the clear trends indicated by ventilation rate measurements in this study we believe this caveat does not apply. Additionally, there may be an unstudied interaction occurring in blue-finned mahseer in the form of escape response. Barreto et al. (2003) mentioned that increases in ventilation rate may be linked to imminent escape response, which is noteworthy in this context due to the high rates of escapes in blue-finned mahseer subject to combination treatments relative to other treatments. We recommend further research examining the physiology of escape response in blue-finned mahseer behaviour.

Holding effects are not universal among C&R studies. Taylor et al. (2001) used control trials to anticipate the impact of holding in a post-release mortality study of common snook (*Centropomus undecimalis*) and found no impact of holding. In contrast, Donaldson et al. (2011) noted that net pen recovery resulted in greater physiological impairment to *Oncorhynchus nerka* than study treatments. The modelled outcomes of the condition scores used to account for holding effects in analysis showed statistically significant differences in condition score across days, indicating that condition score worsened from Day 1 to Day 3. Condition score measurements aligned closely with those of ventilation rate measurements in that blue-finned mahseer in the air exposure group demonstrated the clearest trend of worsening condition across days, followed by blue-finned mahseer in the combination group and chase group. This suggests that the combined results of this multiple capture study (physiological and holding effects) can be viewed as a 'worst case scenario' prediction of immediate impacts arising from multiple capture events. Additionally, these results suggest that fish subjected to different stressors may respond differently to holding, and in particular that blue-finned mahseer exposed to air may respond more poorly to holding than to those exposed to exercise alone.

Findings in studies examining cumulative and interactive effects of combined stressors have described varied comparative, additive, and multiplicative processes. Meta-analysis by Crain et al. (2008) described overall interactions among two or more stressors in marine ecological communities as typically synergistic (i.e., combined stressors result in more severe responses than single stressors) but noted that additive and antagonistic effects were also common. While several studies have examined interactive effects among C&R-induced stressors and water temperature in fish inhabiting temperate waters (e.g., Gingerich et al., 2007; Gale et al., 2014), few have examined interactive effects among C&R stressors themselves, and even fewer of these studies occur in tropical or subtropical fresh waters or examine the issue of multiple capture. White et al. (2008) examined combined effects of air exposure and exercise in *Micropterus salmoides* and *M. dolomieu* (temperate water species) but did not observe significant impacts of both stressors in a single species and concluded that the two stressors were acting in a separate manner.

Studies in subtropical and tropical waters have found similar negative effects of air exposure and exercise on post-release mortality in studies examining the same effects in temperate freshwater species (e.g., on *Salmelinus brasiliensis*, Gagne et al., 2017) and even compared the likelihood of mortality relative to stressor combination and duration (e.g., *Cichla ocellaris*, Bower et al., 2016b), but no known studies have examined the interactions among these stressors in multiple capture scenarios. Similarly, most multiple capture studies occur using mark-recapture methodologies over a longer time period (i.e., years), though some recaptures occur within short time frames also (e.g., in a day; Cline et al., 2012). To our knowledge, this is the only study examining multiple capture within such a short time period (i.e., multiple captures over three days), but this issue is increasing in importance in high volume C&R fisheries, such as the white sturgeon (*Acipenser transmontanus*) fishery on the Fraser River, British Columbia, Canada. McLean et al. (2016) found physiological stress responses were seen in each angled sturgeon studied, and stress responses were found to be heightened in summer temperatures, the most popular season for angling sturgeon.

A confounding finding in this study was the prevalence of irregular operculum beats (RAMP indicator) as a sign of minor reflex impairment in blue-finned mahseer. In previous studies, irregular operculum beats were almost universally a sign of extreme reflex impairment (Bower et al., 2016a, 2019), leading to recommendations that anglers could use measurements of this reflex as an indication that blue-finned mahseer were likely to be highly impaired and require assisted recovery. In this study, irregularity of operculum beats was the most commonly impaired reflex across all three days.

6. Conclusions

The findings of our study indicate blue-finned mahseer are relatively robust to capture-related stressors, even when experienced with short intervals between fisheries encounters. Holding effects, here measured as condition score, were present in this study and thus our results should be considered a conservative measurement of potential physiological effects of multiple capture on blue-finned mahseer. While holding is a necessary component for many C&R studies, future such studies for many species would benefit greatly from alternative methods where possible. Ventilation rates in this study were significantly lower for air exposure and combination treatment groups compared to control and chase groups, suggesting that air exposure may have a masking effect on ventilation rate in blue-finned mahseer This finding supports the call for improved operational definitions for the combined effects of multiple stressors (Folt et al., 1999; Crain et al., 2008) such that antagonistic, cumulative, synergistic interactions, and other modes of interaction may be accounted for in the responses of fish to C&R. We urge further research for improved understanding of the manner in which these stressors interact, particularly in tropical, freshwater environments.

CRediT authorship contribution statement

Shannon D. Bower: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – review & editing. **Petra Szekeres:** Funding acquisition, Investigation, Methodology, Editing. **Rajeev Raghavan**: Conceptualization, Funding acquisition, Methodology, Project administration, Editing. **Andy J. Danylchuk:** Conceptualization, Methodology, Writing – review & editing. **Steven J. Cooke:** Conceptualization, Funding acquisition, Methodology, Project administration, Editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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