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Evaluating the effects of catch-and-release angling on Cape stumpnose *Rhabdosargus holubi* in a South African estuary

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Fisheries managers are increasingly promoting catch-and-release (C&R) to manage recreationally angled fish stocks. Despite this, there is a scarcity of information on the effects of C&R on estuarine-dependent species. Cape stumpnose Rhabdosargus holubi dominates the recreational fisheries catch and provides an important source of food for subsistence fishers in some temperate South African estuaries. The health and survival of R. holubi exposed to a C&R event was investigated by examining their physiological stress response (blood glucose and lactate), reflex impairment (reflex action mortality predictors [RAMP]) and short-term (12-hour) survival. Fish were captured and exposed to one of three air-exposure treatments: 0 s, 30 s or 90 s. Stress and health were measured either immediately (immediate) or one hour after (delayed) the C&R event. There was no significant difference in blood glucose between air-exposure treatments, but there was a significant difference between the mean immediate and delayed glucose levels within each treatment ($F_{(2,143)}$ = 81.8, p < 0.01). In contrast, blood lactate level was significantly higher in the 90-s treatment (p < 0.05). Immediate blood lactate levels were significantly lower than the delayed samples for each treatment (F = 4.29, p = 0.02; n = 169). Although all fish exhibited at least one reflex impairment, the RAMP score was significantly higher in the 90-s air-exposure treatment ($H_{(2.86)}$ = 9.73, p = 0.007). Also, RAMP scores were significantly lower in the delayed samples (p < 0.01). Although short-term mortality was relatively low (2.3%) for this species, it was highest in the 90-s treatment (7%). These results suggest that physiological stress is higher when R. holubi are exposed to longer periods of air exposure and that the physiological stress of fish subject to a C&R event is best measured after a delay.

Keywords: air-exposure, blood glucose, blood lactate, estuarine species, recreational fisheries, reflex impairment, West Kleinemonde Estuary

Introduction

Fisheries managers are increasingly promoting catchand-release (C&R) as a means to manage recreational fish stocks. Several commonly used output regulations. including bag and size limits, require the mandatory release of captured fishes by anglers. In addition to mandatory C&R, conservation-conscious anglers have adopted voluntary C&R behaviour as a result of the noticeable declines in the populations of many fishery species and this behaviour is becoming increasingly popular among recreational anglers (Cooke et al. 2013a). The combination of mandatory and voluntary C&R behaviour is substantial, as Raby et al. (2014) estimated that 60% of fish captured in global recreational fisheries are released. In South Africa, Cowley et al. (2013) found that a large proportion (mean 74% [SD 7.3]) of the five most-dominant fishery species captured in the Sundays Estuary recreational fishery were released.

The promotion of C&R as a sustainable angling practice is based on the assumption that released fish survive (Bower et al. 2016). However, several studies have shown that C&R practices can result in considerable mortality (e.g. Bartholomew and Bohnsack 2005; Danylchuk et al. 2014; Bower et al. 2016). Mortality can occur at several stages during, or after, the C&R event. During angling, hooking causes tissue damage resulting in injury and, in cases of

foul hooking and swallowed hooks, potential mortality (Albin and Karpov 1998; Arlinghaus et al. 2007). The 'fight' of the fish might lead to exhaustion and predation attempts, causing it further injury, sub-lethal stress and potential mortality (Arlinghaus et al. 2007; Suski et al. 2007; Raby et al. 2014). Once landed, the fish will be exposed to air and to direct handling during hook removal, which can result in slime and scale removal (Arlinghaus et al. 2007). Handling can also lead to disease or fungal infections, stress, injury and potential death (Arlinghaus et al. 2007). Finally, once the fish is released it will be vulnerable to predation because stressed and injured fish often attract predators due to the sensory cues that are released into the water (Jenkins et al. 2004; Arlinghaus et al. 2007). Mortality is not always immediate and, since it is often hidden, can lead to underestimates of fish mortality (Raby et al. 2012, 2014).

The rate of survival after C&R has been found to be both species- and habitat-specific (Bartholomew and Bohnsack 2005; Cooke et al. 2013b). Research has been conducted over a range of habitats, including freshwater environments (e.g. Cooke et al. 2001; Gingerich et al. 2007; Brownscombe et al. 2014; Bower et al. 2016) and marine habitats, both nearshore (Cooke and Philipp 2004; Danylchuk et al. 2007; Suski et al. 2007) and offshore (Skomal 2007; Ferter et al. 2015). Within habitats, authors have found that environmental factors such as temperature have an influence on survival (Brownscombe et al. 2014: Danylchuk et al 2014). Comparatively little research (except Lennox et al. 2015) has been done on estuarine fishes. The unique attributes of estuaries, which include rapidly fluctuating environmental conditions (e.g. temperature, salinity and turbidity) at a range of time-scales (Wallace et al. 1984; Whitfield 1998; Fulford et al. 2014), suggest that the outcomes of C&R might differ from more stable environments. The additional stress imposed by C&R might reduce the capacity for physiological adaptation to these dynamic environments. Alternatively, the broad physiological tolerance of estuarine fishes to their dynamic environment might make them more resilient to the stress imposed by C&R angling.

There are some 300 estuaries in South Africa, which provide vital habitats for 155 species of fish, of which 66% (103 species) are fully or partially dependent on estuaries (Whitfield and Cowley 2010). These habitats represent important nursery grounds for many popular recreational fishery species, including spotted grunter Pomadasys commersonnii, dusky kob Argyrosomus japonicus and Cape stumpnose Rhabdosargus holubi, and are subject to high fishing pressure (Cowley et al. 2013). Recreational angling in estuaries has increased in popularity in South Africa over the last few decades and now has a substantial economic value providing an important source of income to many sectors (Mann et al. 2002; Cowley et al. 2013). In their studies on trends in catch-per-unit-effort (CPUE), Whitfield and Cowley (2010) and Cowley et al. (2013) found that the stocks of many estuarine-dependent fishes have been overexploited. Since a substantial proportion of fish landed in South African estuaries are undersized (approximately 90% on the west coast, 50% on the south coast, and 60% on the east coast: Whitfield and Cowley 2010), exploitation in these habitats places these species at risk of growth overfishing (Cowley et al. 2013). Surprisingly, despite the large proportion of fish that are released in some South African estuaries (Cowley et al. 2013), there has been no research on the impacts of C&R.

The Cape stumpnose Rhabdosargus holubi is one of the dominant (most prolific) estuarine fishery species in South Africa and is captured by both the recreational and subsistence sectors (Pradervand and Baird 2002; Whitfield and Cowley 2010). This endemic sparid has a distribution extending from Maputo in Mozambique to St Helena Bay in the Western Cape Province of South Africa (Whitfield 1998; Götz and Cowley 2013). It has been categorised as an estuarine-dependent species with marine spawning, post-larval recruitment (at ~10 mm fork length [FL]) into estuarine systems, and a return to the marine environment at ~150 mm FL (Whitfield 1998; Cowley et al. 2001; James et al. 2007), which is thought to be a size just prior to maturation (Whitfield 1998). Because of this life history, the majority of *R. holubi* captured in estuaries are immature. This means that *R. holubi* in estuaries are highly vulnerable to growth overfishing (Pradervand and Baird 2002; Whitfield and Cowley 2010), which may have negative consequences for the subsistence sector that is heavily dependent on this species (Pradervand and Baird 2002; Cowley et al. 2013).

Since the minimum size limits for South African recreational fishery species are generally set around the size-at-50%-maturity, which is at 150 mm FL for *R. holubi* (Götz and Cowley 2013), the majority of individuals captured in estuarine fisheries will be subject to mandatory release. Thus, understanding the effects of C&R on *R. holubi* is important for the management of the stock and the conservation of this species.

There are multiple methods that are used to assess the health and potential for survival of a released fish (Bartholomew and Bohnsack 2005; Arlinghaus et al. 2007; Bower et al. 2016). These include measurements of reflex impairment, physiological stress, behavioural responses of released fish, and post-release mortality over a defined period (Lennox et al. 2015; Bower et al. 2016). Reflex impairment tests, such as the presence of the startle response and the ability of the fish to regain equilibrium (Davis 2010), are useful for assessing the condition of an angled fish, because they can be conducted non-invasively and quickly (Bower et al. 2016). Reflex impairment has been shown to be a reliable predictor of post-release mortality and an indicator of the general health of the fish (Davis 2010; Brownscombe et al. 2014).

The collection and analysis of blood samples taken prior to (if fish is in captivity), during, and after a C&R event is the most common physiological method used to evaluate stress in C&R science. Cortisol (primary stress response) or lactate and glucose (secondary stress response) are useful in evaluating the extent of a physiological disturbance after C&R (Cooke et al. 2013b). Prolonged air exposure, confinement, handling and exhaustion are known to increase glucose and lactate concentrations in the blood (Arends et al. 1999; Kieffer 2000). Sampling time after the C&R event must be optimised to ensure, if possible, that the blood measurements are taken at the peak of disturbance. This peak depends on the time-course of the physiological processes. Previous studies on teleosts have suggested that peak levels do not occur until one hour after the capture event (Cooke et al. 2013b; Brownscombe et al. 2015). There are many other physiological alterations associated with a stress response, such as changes in haemoglobin characteristics or ionic and acid-base status (Cooke et al. 2013b). Nevertheless, measuring the blood glucose and lactate levels represents the simplest and most efficient means of quantifying a stress disturbance as it can be conducted easily in the field using point-of-contact devices (Bower et al. 2016).

Measuring post-release mortality is often done by placing the angled fish into a recovery pen for an extended period, often between 24 and 48 hours, and observing the number of deaths that occur (Cooke and Schramm 2007). Although this is an effective way to measure mortality, its major drawback is that it cannot take post-release predation into account (Raby et al. 2014). All of these methods are often used when measuring C&R health and survival because they are simple, cheap and reliable. Biotelemetry has been used in some studies (e.g. Danylchuk et al. 2014) to measure post-release mortality; however, this method is prohibitively expensive and thus not feasible in many cases.

The aim of this study was to examine the health and survival of *R. holubi* subjected to C&R using traditional

hook-and-line techniques employed in the estuarine recreational fishery. Reflex impairment, physiological stress response and short-term survival were assessed for fish caught during experimental angling. We hypothesised that angling and increased air exposure would have a significant negative influence on the health of the fish, as indicated by the physiological stress response, reflex impairment and short-term survival of angled fish. Understanding the 'hidden,' or undetected, mortality rates of this species has application for the development of best-practice guidelines for C&R in South African estuarine recreational fisheries.

Materials and methods

Study site

The study took place on the intermittently open West Kleinemonde Estuary, situated approximately 15 km east of Port Alfred in the Eastern Cape Province, South Africa (33°33'0" S, 27°3'0" E) (Figure 1). The estuary is relatively shallow (2-3 m depth) and wide (30-200 m width), and extends 6 km inland (Cowley et al. 2004). Under normal conditions (closed) the estuary has a longitudinal salinity gradient, with the upper reaches experiencing salinities between 5 and 10 and the lower reaches being between 25 and 30 (Cowley et al. 2004). Surface water temperatures show diel and seasonal fluctuation, with summer temperatures between 22 °C and 29 °C and winter temperatures between 12 °C and 15 °C (Cowley et al. 2004). The estuary is situated in a coastal village and has experienced increased fishing pressure over the past 15 years, with most fishing taking place during weekends and holidays (Cowley et al. 2004).

Research approach

Sampling was conducted on two consecutive days during May 2017. On each day of sampling, weather conditions, water temperature, air temperature, salinity, dissolved oxygen and turbidity were recorded every three hours. Angling began at approximately 07:00 each day and continued to approximately 17:00. Rhabdosargus holubi were captured using traditional estuary fishing tackle: graphite rods ranging from 1.8 to 2.0 m in length, and fixedspool reels loaded with nylon or braided lines (4.5-10.0-kg breaking strain). The hook size (no. 1) and type (Mustad 92247 J hook) was standardised throughout the experiment. Sandprawn Callichirus kraussi was used as bait. Fight time (time from hooking to landing) was recorded, and a fish was considered landed once it was placed in an opaque, rectangular plastic bucket (38 × 28 × 27 cm) filled with fresh estuary water. Fish were measured (mm FL) underwater to limit air exposure. Hook placement (either corner, upper jaw, lower jaw, swallowed or foul) and extent of hooking injury (1 = superficial, 2 = somebleeding, 3 = excessive bleeding) were recorded. Fish were then exposed to one of three treatments: no air exposure, ~30 s air exposure, or ~90 s air exposure (Bower et al. 2016). Fish were held using wet bare hands for the ~30-s and ~90-s air-exposure treatments as this most accurately simulated a real-life angling event.

Three methods, namely blood chemistry, reflex action mortality predictors (RAMP) (Davis 2010) and shortterm survival were used to estimate health and survival of *R. holubi*. RAMP indicators were tested and blood samples were taken (see descriptions below) immediately after



Figure 1: Location of West Kleinemonde Estuary (star) in the Eastern Cape Province, South Africa

capture and exposure treatment on the first angling day (n = 86) (immediate group) (Figure 2). However, fish caught on the second day (n = 83) (delayed group) were first placed into perforated rectangular bins ($46 \times 32 \times 34$ cm), which were submerged in the estuary, for one hour (Figure 2). This time-frame was selected as this is generally accepted to be the peak time-delay for physiological disturbance (Suski et al. 2007; Cooke et al. 2013b; Brownscombe et al. 2015). Fish were then retrieved by hand before the RAMP indicators were tested and the blood sampling was conducted, in order to examine the short-term reflex recovery and peak physiological response, respectively (Brownscombe et al. 2014, 2015; Bower et al. 2016).

Rapid assessment

Reflex action mortality predictor (RAMP) tests (Davis 2010) were performed on all fish (n = 169). Reflex indicators that were used to generate RAMP scores were: 'head complex' (the presence of steady opercula beats), 'tail grab' (the ability of a fish to employ burst swimming action when its tail is grabbed), 'equilibrium' (the ability of a fish to right itself within three seconds of being inverted), and 'body flex' (the ability of a fish to flex its torso when held flat on the hand out of water) (Davis 2010). These indicators were used due to their simplicity and their previous validation by Raby et al. (2012). A binary system was used for RAMP scores: a score of zero was given if the reflex was present, and a score of one if the reflex was absent.

Blood samples

Blood samples (<0.5 ml, 24-gauge needle) were taken from the caudal vasculature of all fish (n = 169) and levels of blood lactate (mmol I⁻¹; Lactate-Pro 2, Arkray Inc., Kyoto, Japan) and glucose (mmol I⁻¹; Accu-Chek Active, Roche Diagnostics, Basel, Switzerland) were measured using point-of-contact devices. All blood samples that took longer than 40 seconds to extract were excluded from further analyses, which was well within the time-frame limits recommended by Lawrence et al. (2018).

Short-term survival

The rate of short-term (~12-h) survival was calculated for fish captured on the first angling day, using three survival tanks (diameter 220 cm, depth 90 cm, volume 3 425 l) situated on the edge of the estuary, adjacent to the capture site. The tanks were set up using a flow-through system with water from the estuary, pumped in by means of a BVP5000 submersible water pump (Leader Pumps, Italy) powered by a 6 kVA petrol generator, at a flow rate of 3 450 l h⁻¹ (>1 replacement per hour). The fish were transferred to the holding tanks immediately after RAMP tests were conducted and blood extracted, and then kept for 12 hours to monitor short-term survival. After 12 hours, any mortality was recorded before the fish were released back into the estuary.

Statistical analysis

Student's t-tests were used to determine whether there was a significant difference in fight time and fish size between the immediate and delayed groups. A one-way ANOVA and Tukey HSD post hoc test was used to determine if there was a difference in fight time and fish size among air-exposure treatments. Blood chemistry was tested using a factorial ANOVA and Tukey HSD post hoc test to determine the effect of air exposure on blood lactate and glucose concentration and between the immediate and delayed groups. Given that a RAMP score is an ordinal categorical multinomial response variable (Agresti 2002) which is not normally distributed, nonparametric Kruskal-Wallis tests were used to determine the effect of air exposure on reflex impairment of fish. Mann-Whitney U-tests were conducted to compare the mean RAMP ordinal scores between the immediate and delayed groups. The Shapiro-Wilk test and Levene's test were used to check model assumptions of normality and homogeneity of variance, respectively, and if assumptions were not met, nonparametric analyses were conducted. All data were analysed using STATISTICA 12 (StatSoft Inc.).



Figure 2: Schematic diagram illustrating the research approach of this study. RAMP = tests of reflex action mortality predictors

Results

Weather patterns on each of the sampling days were similar, with minimal cloud cover and wind on day one, and no cloud cover and moderate winds reaching a maximum of 20 km h⁻¹ on day two. Water temperatures ranged between 21 and 22 °C on day one, and between 19.5 and 20.5 °C on day two. Salinities ranged from 21 to 25 on day one but remained stable at 25 on day two. Dissolved oxygen remained constant at 6.9 mg l⁻¹ on day one, and at 6.5 mg l⁻¹ on day two.

The average size of fish caught during the study was 142 mm FL (SD 1.5, range 110-290 mm). There were no significant differences in the size of fish caught between the immediate (142.6 mm [SD 20.1]; n = 86) and delayed (140.8 mm [SD 8.8]; n = 83) groups (t = 0.75, p = 0.45) and between the three air-exposure treatments ($F_{(2,28)} = 0.08$, p = 0.92; Table 1). Average fight time during the study was 25.07 s (SD 17.31, range 10-170 s) and there were no significant differences in fight time between the immediate (25.01 s [SD 23.10]) and delayed groups (25.89 s [SD 8.3]) (t = 0.86, p = 0.38) or between the three air-exposure treatments ($F_{(2,80)} = 0.84$, p = 0.43; Table 1). The majority of fish were hooked in the corner of the mouth (48%) or lower lip (38%), with only 4% hooked in the upper lip and 10% foul-hooked; no fish swallowed the hook. Bleeding was observed in 15 (8%) of the fish (Table 1). The average duration of blood extraction was 22.45 s (SD 7.04, range 5-37 s). There were no significant differences in the duration of blood extraction between the immediate and delayed groups (t = 1.83, p = 0.07) and between the three air-exposure treatments ($F_{(2.79)} = 0.57$, p = 0.59; Table 1).

Blood chemistry

Blood extraction was successful (within a 40-s window) for 77 and 74 fish in the immediate group and delayed group, respectively. The average blood glucose concentration ranged from 2.31 mmol ml⁻¹ (SD 0.42) to 4.13 mmol ml⁻¹ (SD 1.13) and was not significantly different between the three air-exposure treatments in the immediate group or the delayed group ($F_{(2,140)} = 0.294$, p = 0.75) (Figure 3). There was, however, a significant difference in the blood glucose concentrations for all three treatments between the

immediate and delayed groups ($F_{(1,140)} = 81.8$, p < 0.001), with blood glucose concentration significantly higher in the delayed group for all air-exposure treatments (Figure 3).

The average blood lactate concentration ranged from 4.31 mmol ml⁻¹ (SD 1.42) to 15.49 mmol ml⁻¹ (SD 5.05). There was a significant difference between air-exposure treatments and the immediate and delayed groups, with the delayed group mean blood lactate concentration in the 90-s treatment (15.49 mmol ml⁻¹ [SD 5.05]) being significantly higher than in the 30-s (4.84 mmol ml⁻¹ [SD 1.14]) and 0-s treatments (4.31 mmol ml⁻¹ [SD 1.14], $F_{(2,145)} = 7.95$, p > 0.001; Figure 4). There was also a significant difference in mean blood lactate concentration between the immediate and delayed groups for all three treatments ($F_{(2,145)} = 4.29$, p = 0.015; Figure 4).

RAMP indicators

For the immediate group, reflex impairment was observed for 80% (n = 24) of the individuals in the 0-s treatment, 86% (n = 25) in the 30-s treatment, and 100% (n = 27) in the 90-s treatment (Table 2). Of the RAMP indicators, body flex



Figure 3: Mean blood glucose concentration in *Rhabdosargus holubi* subjected to different air-exposure treatments (0 s, 30 s or 90 s), sampled immediately (immediate group) or one hour (delayed group) after a catch-and-release event. Error bars denote standard error

Table 1: Mean fish size (mm FL), fight time, duration of blood extraction, and percentage of bleeding (from hooking injury) for *Rhabdosargus holubi* captured using conventional estuary fishing tackle in the West Kleinemonde Estuary, South Africa, and then exposed to one of three air-exposure treatments (0 s, 30 s or 90 s), in both the immediate (blood taken immediately after capture) and delayed (blood taken one hour after) groups

	General metrics												
Group	Air-exposure treatment	n	Fish siz	e (mm)	Fight tim	ne (s)	Bleeding (%)	Blood extraction duration (s)					
	(s)		Mean (SD)	Range	Mean (SD)	Range		Mean (SD)	Range				
Immediate	0	30	141 (2.96)	110–290	24.37 (27.93)	10–170	10	24.28 (6.38)	8–33				
	30	29	143 (0.75)	125–160	23.89 (13.39)	12–75	10.3	23.31 (7.41)	9–34				
	90	27	143 (1.68)	130–220	27.16 (26.34)	10–140	7.4	22.36 (6.81)	13–37				
Delayed	0	28	140 (0.82)	120–159	26.48 (9.77)	15–55	7.1	21 (7.14)	10–30				
-	30	28	138 (0.73)	117–155	26.96 (8.6)	17–60	3.6	20.93 (6.4)	10–30				
	90	27	143 (1.01)	125–165	24.25 (6.25)	11–37	14.8	22 (8.06)	5–30				

was the most sensitive, with 88% of individuals exhibiting an impaired response, followed by the equilibrium indicator (7.2% of fish impaired), head-complex indicator (4.9% of fish impaired) and tail-grab indicator (2.5% of fish impaired) (Table 2; Figure 5a). Reflex impairment was only observed for 10.7% (n = 3) of the individuals in each of the 0-s and 30-s groups, and for 23.1% (n = 6) of the individuals in the 90-s treatment in the delayed group (Table 2; Figure 5b). The body-flex indicator was again the most sensitive indicator and contributed most to the overall RAMP score (14.5% of fish impaired), followed equally by head complex (3.7% of fish impaired), equilibrium (3.7% of fish impaired)

and tail grab (3.7% of fish impaired) (Figure 5b). The mean RAMP score for the 90-s air-exposure treatment in the immediate group was significantly higher (0.34 [SD 0.22]) than for the other air-exposure treatments (0 s: 0.21 [SD 0.12]; 30 s: 0.22 [SD 0.1]) ($H_{(2,86)} = 9.73$, p = 0.007) (Table 2; Figure 6). In contrast, there was no significant difference in mean RAMP scores between the air-exposure treatments in the delayed group ($H_{(2, 82)} = 1.65$, p = 0.45) (Table 2; Figure 6). However, there was a significant difference in RAMP score between the immediate and delayed groups for the 0-s (U = 104.5, p < 0.01), 30-s (U = 112.0, p < 0.01) and 90-s (U = 115.5, p < 0.01) air-exposure treatments (Table 2; Figure 6).



Figure 4: Mean blood lactate concentration in *Rhabdosargus holubi* subjected to different air-exposure treatments (0 s, 30 s or 90 s), sampled immediately (immediate group) or one hour (delayed group) after a catch-and-release event. Error bars denote standard error

Short-term survival

Overall, post-release mortality was low (2.3%), but was highest for the 90-s air-exposure treatment (7%, n = 2), followed by the 0-s (3.7%, n = 1) and 30-s (3.4%, n = 1) treatments. The fish that died all showed signs of hooking injuries, but these were also observed in fish that survived, with the total number of fish exhibiting hooking injury similar among the 0-s (n = 3), 30-s (n = 3) and 90-s (n = 4) treatments.

Discussion

Despite the low short-term mortality observed, the results of this study suggest that all fish, regardless of air-exposure



Figure 5: Mean reflex action mortality predictor (RAMP) scores, per indicator, for *Rhabdosargus holubi* subjected to different air-exposure treatments (0 s, 30 s or 90 s) and sampled either (a) immediately (immediate group) or (b) one hour (delayed group) after a catch-and-release event

Table	2:	Mean	reflex	actior	n morta	lity p	redictor	(RAMF	P) ordina	l scores	and	percentage	impa	irment	per	RAMF	' indica	tor foi	Rha	abdosargus
holub	i, ca	aptured	with	hook	and line	e and	d subjed	ted to	different	air-exp	osure	e treatments	(0 s,	30 s (or 90) s) ei	ther im	media	itely	(immediate
group) or	one ho	our (de	elayed	group)	after	capture	. HC =	head co	mplex; ⁻	ΓG = 1	tail grab; EQ) = equ	uilibriu	n; Bl	F = bo	dy flex			

	RAMP										
Group	Air-exposure treatment (s)	n	HC%	TG%	EQ%	BF%	Impaired%	RAMP Mean (SD)			
Immediate	0	30	0	0	3.3	80	80	0.21 (0.12)			
	30	29	0	0	3.4	86	86	0.22 (0.10)			
	90	27	14.8	7.4	14.8	100	100	0.34 (0.22)			
Delayed	0	28	0	0	0	14.3	10.7	0.03 (0.08)			
-	30	28	0	0	0	7.1	10.7	0.03 (0.08)			
	90	27	11.5	11.5	11.5	23	23.1	0.14 (0.33)			



Figure 6: Mean reflex action mortality predictor (RAMP) scores for *Rhabdosargus holubi* subjected to different air-exposure treatments (0 s, 30 s or 90 s), sampled immediately (immediate group) or one hour (delayed group) after a catch-and-release event. Error bars denote standard deviation.

treatment, experienced considerable physiological and motor impairment from the catch-and-release (C&R) event. This suggests that capture has a negative impact on the health and survival of Rhabdosargus holubi, particularly if they are released into estuaries that are rich in avian and teleost predators (Cowley et al. 2017). Survival appears even less likely if fish are exposed to air for extended periods, as fish in the 90-s treatment exhibited considerable physiological stress and physical impairment. The significantly greater physiological stress response displayed by fish in the delayed group not only suggested that the peak physiological response to C&R is delayed, but also showed that the combined impact of the angling and air exposure resulted in a considerable negative physiological response (blood lactate concentration), despite apparent physical recovery (decreased RAMP score) in the same time-frame.

The significant positive relationship between air exposure and blood lactate concentration is not uncommon in the family Sparidae. In a controlled experiment for simulating aquaculture practices, Arends et al. (1999) found that the blood lactate levels of gilthead seabream Sparus aurata tripled after three minutes of air exposure, reaching an average concentration of 2.7 mmol ml-1 one hour after the intervention. The maximum mean lactate levels in our study were more than five-times higher (15.49 mmol ml⁻¹ [SD 5.05]), suggesting that the cumulative factors that contribute to angling stress (i.e. hooking, handling, hook removal) may have also played a role in this study. Air exposure has been identified as a key factor influencing blood lactate levels (Suski et al. 2007: Lennox et al. 2015; Bower et al. 2016; Butler et al. 2017), possibly through the mechanism of hypoxic conditions reducing mitochondrion capacity to produce adenosine triphosphate (ATP). Oxygen acts as the terminal electron receptor in ATP production and the lack of oxygen in hypoxic conditions inhibits this process (Wang and Richards 2011). Therefore, anaerobic metabolism must be initiated in order to fulfil the energy requirements of the fish, and the end product of this process is lactate (Butler et al. 2017).

No significant difference in blood lactate levels between the 0-s and 30-s air-exposure treatments were found during this experiment, suggesting that 30 s of air exposure is not sufficient to promote 'hypoxic conditions' and stimulate the production of lactate. Other studies have also found that conditions of no air exposure or reduced air exposure result in a reduced physiological stress response in fishes (Suski et al. 2007; Lennox et al. 2015; Bower et al. 2016).

Blood lactate levels were significantly higher in the delayed group than in the immediate group. This is unsurprising since many studies have found that lactate levels peak up to an hour after a stressor has occurred (Arends et al. 1999: Arlinghaus et al. 2007: Suski et al. 2007; Brownscombe et al. 2014). This may also explain why there was no significant difference in lactate levels between treatments in the immediate group. The increase in blood lactate levels from immediate to one hour later in all air-exposure treatments showed that not only air exposure but the angling event itself likely contributed to the increase in blood lactate concentration in R. holubi. Suski et al. (2007) found that after exercising (chasing by tail grab) bonefish Albula vulpes for one minute, an almost three-fold increase occurred in lactate concentrations as compared with the level in the control (non-exercised fish). It is likely that the cumulative effect of air exposure and high activity while hooked increases the blood lactate levels in R. holubi during C&R.

In contrast to the blood lactate results, there was no relationship between air exposure and blood glucose levels. Suski et al. (2007) similarly found no significant difference in blood glucose levels between bonefish that experienced one minute or three minutes of air exposure. It is likely that air exposure does not directly influence glucose levels because teleosts switch from aerobic respiration, where glucose is used, to anaerobic respiration during hypoxic conditions (Butler et al. 2017). There was, however, a significant increase in glucose levels in fish from the immediate to the delayed group, which is consistent with several studies showing that glucose levels peaked an hour after a stressor (Arlinghaus et al. 2007: Suski et al. 2007: Brownscombe et al. 2014). We can surmise from the elevated glucose levels observed that the angling event, and probably the energy expended during the fight, rather than air exposure, affects blood glucose levels in R. holubi. During a stress event, such as angling, glucose is released into the bloodstream of fish to provide energy for the muscles (Martinez-Porchas et al. 2009; Wendelaar Bonga 2011). However, adrenalin and cortisol, which are released during stress, regulate the production of glucose. Adrenalin stimulates glycogenolysis in the liver and causes blood glucose concentrations in the blood to increase rapidly, and cortisol stimulates both glycogenolysis and gluconeogenesis, also causing an increase in blood glucose (Reid et al. 1998; Wendelaar Bonga 2011). Brownscombe et al. (2014) found that fight intensity was positively correlated with blood glucose levels in largemouth bass Micropterus salmoides. Furthermore, Suski et al. (2007) found a two-fold increase in blood glucose levels in A. vulpes that were exercised for four minutes as compared with fish exercised for one minute.

The results of the RAMP tests indicate that *R. holubi* experience significant reflex impairment due to C&R. Most fish, and even all the fish in some treatments, exhibited some form of reflex impairment during this study. However,

mean RAMP scores were low, indicating that despite the high proportion of reflex impairment observed, the severity of impairment was low. Similarly, Bower et al. (2016) found that peacock bass Cichla ocellaris exhibited low RAMP scores despite high rates of impairment after a C&R event. Previous studies (e.g. Brownscombe et al. 2015) have found that air exposure causes a significant increase in reflex impairment. In our study, fish which underwent 90-s air exposure, compared with the 0-s and 30-s air-exposure treatment groups, had significantly higher mean RAMP scores. This finding mirrors the results seen in the blood lactate levels of fish that underwent 90-s air exposure, and further confirms our hypothesis that extended air exposure affects both the physical and physiological stress of this species. No significant difference in the reflex impairment of fish between the 0-s and 30-s air-exposure treatments indicates that there is a 30-s window for anglers to handle their fish without significant deleterious impacts on the individual. Significantly lower RAMP scores for the delayed group as compared with the immediate group (Table 2) suggests that the physical recovery of R. holubi from a C&R event is rapid. Furthermore, the lack of difference in RAMP score among treatments in the delayed group shows that, despite the greater reflex impairment elicited by fish in the 90-s air-exposure treatment, extended air exposure does not hinder the physical recovery of R. holubi, within the time-frame used in this study.

Our results suggest that the angling event itself contributes most to the RAMP score in this species, while extended air exposure will significantly increase reflex impairment. This is evidenced by the fact that fish in the 0-s and 30-s air-exposure treatments, while significantly lower than the levels in the 90-s air-exposure treatment, still exhibited high levels of reflex impairment (80% and 86% of the fish were impaired, respectively). It is possible that the 'fight' following being hooked causes teleost fish to become exhausted. This is supported by the study of Butler et al. (2017) who found that fight time was a significant predictor of the RAMP score in the marine shore-based fishery in South Africa.

The body-flex indicator was responsible for a high percentage of impairment (up to 100%). Raby et al. (2012) suggested that this indicator is linked to physical exhaustion, with the white muscles becoming exhausted due to exertion. Body flex is indicative of the fish's ability to escape predation (Brownscombe et al. 2017); hence, the high impairment for this indicator suggests a reduced ability for R. holubi to take evasive action from a predator after C&R. Some have questioned the validity of the body-flex indicator, and Raby et al. (2012) found that body flex in coho salmon Oncorhynchus kisutch was impaired for both control and treatment groups. However, the recovery we observed and the subsequent reduction in body-flex impairment (14.5% impaired after one-hour recovery) indicates that this indicator is useful and highly sensitive. Similarly, Butler et al. (2017) found that after a 24-hour recovery period, teleost fish that had experienced a C&R event were not impaired for body flex. Brownscombe et al. (2017) state that 'body flex' and 'tail grab' are usually the first indicators to reflect impairment. Impairment in both cases is associated with exhaustion; however, the low levels of tail-grab

impairment observed in our study suggest that body flex may provide an exaggerated estimate of exhaustion. Up to 14.8% and 11.5% of the *R. holubi* in the immediate and delayed groups, respectively, lost equilibrium. Previous studies have found that a loss of equilibrium in *A. vulpes* resulted in increased post-release predation within the first 30 minutes after C&R (Danylchuk et al. 2007). Although the percentages showing impaired equilibrium maintenance were low, our results suggest that *R. holubi* are likely to be vulnerable to predation after a C&R event.

Although post-release mortality was not measured, the short-term survival of R. holubi after a C&R event was high (97.7%). However, there was a higher mortality rate in the 90-s air-exposure treatment (7%) than in the other treatments, suggesting that although R. holubi appear able to survive a C&R event, extended air exposure reduces the likelihood of their short-term survival. Lennox et al. (2015) reported similar findings in their study of fat snook Centropomus parallelus in the Iguape Estuary, Brazil; although the short-term mortality of the fish was low, the authors suggested that, because of the increased reflex impairment and significantly elevated blood glucose and lactate levels, post-release mortality might be considerable over the longer term. Ten percent of fish in our study were foul-hooked and all fish that died showed signs of serious hooking injury, suggesting that hooking injury may be a concern for this species. Hooking injury also played a major role in the post-release mortality of juvenile lemon sharks Negaprion brevirostris (Danylchuk et al. 2014). Although R. holubi are important in the subsistence sector, they are generally not targeted by recreational anglers (except young anglers) in estuaries and tend to be caught as bycatch. Hence, the fishing tackle traditionally used, which includes large hook sizes, may be unsuitable and harmful to this species.

A number of changes to experimental design may improve future studies of this nature. A limitation of our study was that post-release predation was not considered, because acoustic telemetry fell outside the scope of this study. However, Raby et al. (2014) argued that released fish are often injured and have impaired physiological capacity and altered behaviour and that this greatly increases their risk of predation. Studies have found that risk of predation is more likely in fish that are stressed (Raby et al. 2014) and have impaired reflexes (Mesa 1994). Total mortality rates of R. holubi are likely to be underestimated in this study as predation post-release could not be determined. Future studies should aim to include a determination of post-release predation into mortality estimates, either through the use of acoustic telemetry or by placing angled fish into artificial environments containing natural predators. However, as artificial environments are never completely analogous with the natural environment, a study such as this would require great care to avoid confounding environmental factors. Another potential limitation in our study is that the immediate and delayed tests were performed on separate, yet consecutive days; this was done to minimise confounding factors within the two treatment classes. As the weather conditions were similar on the two days, we did not consider weather a critical limitation. However,

future studies might consider carrying out immediate and delayed tests on the same day. Despite these limitations we consider that the experimental design gave valid results and would be useful in evaluating the health and survival of other species after C&R events.

The results of this study have important implications for the management of South African estuarine fisheries because R. holubi accounts for a substantial proportion of the recreational and subsistence catch in estuaries (Cowley et al. 2004). Furthermore, the fact that the majority of R. holubi caught in estuaries are undersized (i.e. <200 mm TL) means that most fish caught will have to be released by law. The significantly greater physiological and physical impacts in the 90-s air-exposure treatment suggests that air exposure should be reduced to around 30 s to improve survival. To do this, the mandatory use of buckets filled with fresh estuarine water would limit air exposure if fish were immediately placed into these after capture (see Butler et al. 2017). The removal of the barb regardless of hook type is encouraged: however, the use of barbless circle hooks instead of 'J' hooks is recommended, as studies have shown that circle hooks decrease the likelihood of serious hooking injuries (Prince et al. 2002). It is possible that recovery buckets (Brownscombe et al. 2013) may substantially reduce post-release predation in predatorrich environments, as R. holubi appeared to exhibit rapid physical recovery from the effects of C&R. Although these suggestions may be helpful in minimising the negative effects of C&R on R. holubi, success will be limited if the lack of awareness of the negative effects of C&R and the poor knowledge of best practices are not improved. Thus, we would do well to raise awareness of the problem through educational drives, particularly focused on learners in schools, and disseminating knowledge of best practice for C&R handling as widely as possible through the publication of both scientific and popular articles, as well as through contributions (video and text) on social media platforms. Ultimately, these steps will contribute to the protection of the biodiversity and ecological integrity of South African estuaries in addition to protecting the food security of impoverished South Africans.

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