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Physiological response of one of South Africa's premier freshwater sport angling species, the Orange-Vaal smallmouth yellowfish *Labeobarbus aeneus*, to catch-and-release angling

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Abstract

The practice of catch and release fishing has been widely promoted by angling fraternities as a fisheries management tool. The aim of this investigation was to determine the physiological response of Orange-Vaal smallmouth yellowfish, *Labeobarbus aeneus*, to catch and release angling in the Vaal River, South Africa. Fish were collected using standard fly fishing techniques, anaesthetised in clove oil and blood drawn from the caudal vein; thereafter weighed, measured, revived and released. Blood plasma was analysed for concentrations of glucose, cortisol and lactate to determine the effects of angling duration, fish size, and water temperature. Larger fish were angled for a longer duration compared to smaller fish. Levels of glucose were affected by water temperature (influenced by time of year). Plasma glucose concentrations decreased with greater angling duration. Few individuals (n=12) showed increased plasma cortisol concentrations. In extended capture fish (angled for >1 min) lactate concentrations were found to increase significantly above values for rapid capture fish (angled for >30s). This data suggests

that catch and release causes physiological stress to fish, but nonetheless this practice can be a valuable fisheries management tool to ensure the sustainability of fish populations. Other factors beyond the 'angling' time are likely to contribute to physiological disruptions in homeostasis and therefore handling and air exposure of angled fish as well as to determine the longer-term impact of angling on fish health has yet to be determined.

Keywords: angling stress, biomarkers, blood physiology, fly-fishing

Introduction

A fish's response to a stressor can impact on numerous physiological levels of organisation from cellular, to individual organisms, to population structures (Barton 2002). To all living organisms the stress response is normal and of vital importance for survival. Three distinct categories can be distinguished from the general stress response of fish. Firstly, a neuroendocrine response such as the release of catecholamines and cortisol (Gamperl et al. 1994); secondly, perturbations in the organism's biochemistry and physiology could occur from adjustments to metabolism, respiration, acid-base balance and immune function and result in haematological changes, such as increased glucose and lactate levels (Barton 2002). Finally, the tertiary response, which represents changes to the whole organism and/or populations that diverts energy away from essential life processes such as feeding, growth and reproduction (Barton 2002). One sub-lethal stressor imposed on the fish is the practice of catch and release fishing by sport anglers (Cooke and Suski 2007; Cooke and Sneddon 2007).

The increased popularity for freshwater game fishing in southern Africa, and worldwide, highlights the importance in elucidating the response of these fishes to the stress of sport angling. To date, only a single study has examined the angling stress responses of a freshwater game fish, in this case the tigerfish, *Hydrocynus vittatus* (Castelnau, 1861), in southern Africa (Smit et al. 2009). However, another important species, *Labeobarbus aeneus* (Burchell, 1822) (commonly referred to as the Vaal-Orange smallmouth yellowfish; hereafter referred to as yellowfish), is widely recognized and promoted as a popular sport angling fish (Granek et al. 2008; O'Brien et al. 2013). Granek et al. (2008) reported that the value of yellowfish dependent angling in the Orange Vaal River was ZAR 1.2 billion (~US\$160 million) and Brand et al. (2009) estimated that it provided an economic value exceeding ZAR 133 million per annum. Consequently with the increased interest in inland fisheries in South Africa and the high value of recreational fishing, there is a growing need for greater knowledge on the target species to develop fisheries management recommendations (McCafferty et al. 2012). Therefore, the aim of this study was to examine the response of yellowfish to catch and release angling, using blood plasma glucose, cortisol and lactate as biomarkers of physiological stress.

Material and methods

Study site and fish capture methods

The University of Johannesburg's Institutional Research Ethics Committee ratified all experimental procedures prior to data collection. A total of 96 yellowfish were captured in 2008 from June (winter) to December (summer) by means of fly-fishing in the middle reaches of the Vaal River close to Potchefstroom (Figure 1). Fly anglers were encouraged to fish normally using a 5/6 weight rod fitted with floating line, a 2X monofilament tippet and artificial flies (barbless hook size 10 to 16). The fishing areas were dependent on the time of year; during the cooler months (June to mid September) deeper water pools were fished, whereas during warmer months (mid September to December) shallower parts of the river, mainly in riffles and rapids, were targeted. For each fish caught, the time to land the fish (time from hooking the fish to landing the fish at the boat in a landing net) and handling procedure (the time when the fish was netted until the hook was successfully removed) were recorded and subsequently the landing, handling and total angling times were calculated (Smit et al. 2009).

Anaesthesia and sampling procedure

Clove oil ($32 \text{ mg} \cdot \ell^{-1}$) in solution with ethanol (1:9 ratio of clove oil mixed with ethanol) was added to an insulated vessel containing 50 L of fresh river water (Anderson et al. 1997; Meka and McCormick, 2005). Following capture, fish were anaesthetised for 2 min in the container, which has been previously shown to have a negligible physiological effect (Wagner et al. 2002). Anaesthetised fish were removed from the container and $\sim 2 \text{ mL}$ of blood was drawn from the caudal vein using a 21 gauge needle, which was completed in $\leq 30 \text{ s}$. Blood was immediately transferred to heparinised vacutainers and kept cool until centrifugation, the plasma supernatant was snap frozen in liquid nitrogen in the field and then stored at -80°C on return to the laboratory until subsequent analysis. Following bloodletting, fish characteristics (mass; standard length, SL; fork length, FL; and total length, TL) were recorded. On completion of data collection, each fish was revived in fast flowing river water and released.

Plasma analysis

Plasma glucose and lactate were determined using a commercially available kit (model no. 11448668 216 [CV = 1.8%] and model no. 11822837 190 [CV = 1.0%], respectively; Roche/Hitachi, Mannheim, Germany). Plasma cortisol was determined through ELISA cortisol test kit (model no. 402710 [CV < 10.0%], Neogen Corporation, Lexington, Kentucky USA). All samples were analysed in duplicate.

Reference group

Sixteen randomly chosen fish caught during the August survey (moderate temperature range) were chosen to act as a reference group. Following the initial bloodletting, these fish were revived in fresh river water and kept in an aerated, insulated container filled with fresh river water. These fish were then transported to a field laboratory within 60 min and released into a 15,000 L aquarium containing fresh river water. A 10,000 L.h⁻¹ water pump was used to aerate the water and simulate flowing water. Approximately 20% of the water in the control pool was replaced daily to provide fish with fresh river water (Smit et al. 2009). Fish were left in a quiet condition for a 72 h period to allow any angling stress to subside in an attempt to emulate the conditions of free-swimming, unstressed fish (Gustaveson et al. 1991; Smit et al. 2009). Following the 72 h period, clove oil was added to the water to anaesthetise the fish and a further blood sample was taken and used as a reference value to examine the differences in dependent variables within those fish immediately after capture.

Data analysis

All data were analysed using SPSS for windows v. 14. Descriptive data are reported as mean \pm SD. Following previous methods described by Meka and McCormick (2005) and Smit et al. (2009) fish were grouped by landing time which was divided into minute intervals (< 1 min, 1-2 min, 2-3 min, etc) and grouped according to the water temperature, i.e. low (<12°C), moderate (16-22°C) and warm (>26°C). Inferential statistical analysis was conducted by using previously established methods for studies of this nature and also to allow for direct comparisons with the

existing body of literature. A linear regression analysis was also used to examine the influence of fish body mass on landing time. A one-way ANOVA was used to determine whether the water temperature groups differed significantly with regard to the landing times of the captured fish in each group; no significant differences were observed so, a further one-way ANOVA was used to examine the plasma cortisol, plasma glucose, and plasma lactate responses resulting from the different landing times and water temperatures. Where appropriate, significant effects were followed up using an LSD *post-hoc* test. Significance levels were set *a priori* at a level of 0.05.

Results

Water temperature ranged from 10°C to 27°C depending on the time of year of sampling. Mean total length (TL) and body mass of the fish were 504 ± 88 mm (range, 217 – 666 mm) and 1.47 ± 0.68 kg (range, 0.12 – 3.18 kg), respectively. Total angling time was $2 \text{ min } 40 \text{ s} \pm 1 \text{ min } 9 \text{ s}$ (range, 50 s – 7 min 27 s) with a landing time of $1 \text{ min } 30 \text{ s} \pm 55 \text{ s}$ (range, 20 s – 5 min 56 s). The linear regression (Figure 2) between body mass and landing time showed a positive correlation ($r^2 = 0.2248$, $p = 0.043$). However, there was a poor correlation for glucose ($r^2 = 0.0622$, $p > 0.05$), cortisol ($r^2 = 0.0685$, $p > 0.05$) and lactate ($r^2 = 0.0278$, $p > 0.05$) with body mass.

Following 72 h in the aquarium, the plasma lactate concentration for the reference fish was 4.68 ± 1.67 mMol. ℓ^{-1} (range, 2.07 – 7.93 mMol. ℓ^{-1}) and was lower ($p = 0.002$) than the values immediately following hook and line capture (5.74 mMol. ℓ^{-1}). Glucose and cortisol levels from reference fish were not significantly different to the post capture levels.

A one-way ANOVA revealed that plasma glucose and cortisol concentrations from fish captured at moderate temperatures were not different between groups (Figure 3a-b) when calculated using landing time ($p > 0.05$). Plasma lactate concentrations (Figure 3c) were found to be significantly different when grouped by landing time ($F = 3.6$, $p = 0.010$). LSD *post-hoc* analyses showed no difference between plasma lactate concentrations in the reference group ($n = 16$) and rapid capture fish (< 1 min) ($n = 31$); the reference group however, was lower than the following groups: 1-2 min ($p = 0.021$), 2-3 min ($p = 0.011$), and > 3 min ($p = 0.03$).

Fish were also grouped according to the water temperature they were caught at [low (n = 15), moderate (n = 64) and warm (n = 17)]. A one-way ANOVA revealed that the landing time of the fish captured at the varying water temperatures were not different ($F = 1.7, p > 0.05$). Based on this information we grouped the glucose, cortisol and lactate values attained at the three different temperature ranges. A one-way ANOVA revealed that plasma glucose and plasma lactate were different (glucose – $F = 12.6, p < 0.001$; lactate – $F = 11.9, p < 0.001$) when calculated using water temperature, while plasma cortisol was not ($p > 0.05$). The *post-hoc* analysis showed that glucose levels at moderate temperatures were higher ($p \leq 0.001$) than low and warm temperatures (Figure 4a). There were no significant correlations between cortisol and temperature (Figure 4b). Post-capture plasma lactate concentrations also rose with increasing water temperature (Figure 4c); *post-hoc* analysis showed that fish caught in warm water was higher than low and moderate temperature groups ($p < 0.001$).

Discussion

This study is the first to examine the physiological stress response to game catch and release angling in yellowfish and the first report of game angling responses on any fish in South Africa. Importantly, the blood parameters examined from these fish angled from the Vaal River exhibit no changes in glucose and cortisol levels, but plasma lactate concentrations increased significantly from reference and rapid capture values at moderate temperatures. Furthermore, the plasma lactate response to angling was greater in higher water ($>26^{\circ}\text{C}$) temperatures.

Effect of size (body mass) and angling period

Unsurprisingly, larger body mass of the fish was positively correlated with angling duration. This supports previous observations in other species, for example; Atlantic salmon (Thorstad et al. 2003), rainbow trout (Meka and McCormich 2005) and tigerfish (Smit et al. 2009). Glucose, cortisol and lactate levels showed no relationship with increased body mass. Interestingly, Smit et al. (2009) concluded that the lactate response was independent of body mass. Consequently we

conclude, like others, that the metabolic stress caused by angling duration was likely, the principle cause of elevated blood lactate and not body mass of the fish *per se*.

Fish that are angled for longer periods are generally larger individuals, and consequently result in larger increases in lactate, which concur with other studies using different species (Meka and McCormick 2005; Smit et al. 2009). This might suggest that the larger and older individuals of the population are placed under the greatest potential stress and future research should focus on the longer-term impact, if any, resulting from this type of stress especially since the larger individuals would most likely be repeat spawners and deliver larger contributions to the population (Gerber et al. 2012, Weyl et al. 2009). Based on previous observations in other species (Cooke et al. 2000, 2002; Thorstad et al. 2003) that examine the implications of angling on spawning success, it makes the expectation tenable that angling stress could affect spawning behaviour and breeding success of yellowfish.

According to Booth et al. (1995) the most physically demanding form of exercise stress for fish is capture by angling. Numerous studies have shown that the longer fish are angled, the greater the subsequent physiological stress response, which can result in elevated mortality rates (Pankhurst and Dedual 1994; Thorstad et al. 2003; Meka and McCormick 2005; Smit et al. 2009). Exhaustion from extended angling duration is characterised by increased lactate concentrations, which is further exacerbated when the fish is exposed to air where the gill lamellae might collapse. This series of events will negatively affect gas exchange and increase CO₂ production that is accompanied with a concomitant decrease in O₂ uptake (Casselmann 2005; Arlinghaus et al. 2009). Notwithstanding these factors, it is likely that the principal contributor to the physiological stress of yellowfish is the metabolic work done whilst hooked (Smit et al. 2009; Arlinghaus and Hallerman, 2007).

With the exception of individuals angled for <1 min, the plasma lactate response of yellowfish to angling duration was greater than reference values at every time interval. This indicates increased metabolic stress even after a relatively short angling duration (> 1 min). Comparable results were previously shown (Gustaveson et al. 1991; Smit et al. 2009) in the response of largemouth bass (*Micropterus salmoides*) and tigerfish (*H. vittatus*), respectively. Tigerfish showed a

rapid response with higher blood lactate concentrations observed in rapid capture fish (<1 min) whereas largemouth bass angled for ≥ 1 min also had higher lactate than the reference fish from the present study. Conversely, significant increases in lactate levels of angled rainbow trout were only found after 2-3 min of angling (Meka and McCormick 2005). Interestingly, Meka and McCormick (2005) compared these data to a control group that were considered 'rapid capture fish' (fish angled for < 2 min). However, based on the data from aforementioned studies the control fish from Meka and McCormick (2005) would likely have elevated lactate following rapid capture (<1 min); whereas the control yellowfish in the current study had a 72 h recovery period from initial capture and may account for the disparity in time course of lactate elevations.

Cortisol showed no increase with angling time or difference from reference fish. Nonetheless cortisol values were comparable to rapid capture rainbow trout (< 11 ng.ml⁻¹), which were considered to reflect those of free-swimming natural fish populations (Meka and McCormick 2005). Consequently, we propose that the cortisol values are also reflective of free-swimming yellowfish. Interestingly, a few individuals (n = 12) did show elevated cortisol concentrations of 18 – 160 ng.ml⁻¹. Conceivably cortisol release from the adrenal cortex is delayed and consequently may take some time before the response can be seen in blood and therefore not seen in the sample taken following capture (Bartone 2002). Glucose concentrations did not change with angling duration compared to reference fish, which supports previous work on rainbow trout (Meka and McCormick 2005), while Silbergeld (1974) and Wydoski et al. (1976) found an increase in stressed fish within 5 minutes of capture. However, other studies have found that glucose levels may take up to an hour to change (Ristori and Laurent 1985; Carey and McCormick 1998). Similarly to Meka and McCormick (2005), we propose that the post capture lactate and cortisol levels found in our study represent the initial stages of a stress response caused by angling, and that the levels will continue to rise and will only peak some time after the initial stressor is applied, but then subside after 72 h.

Water temperature

It has been suggested that temperature influences the physiological responses of fish during angling, specifically post-capture levels of glucose, lactate and cortisol (Meka and McCormick 2005; Gale et al. 2011; Landsman et al. 2011). The water temperatures in this study ranged between 10 and 27°C and are representative of seasonal temperatures. Post-capture glucose was shown to be lower at warm and low temperatures (extremes), and highest at the moderate temperatures; an identical trend which has also been shown with sockeye salmon (Gale et al. 2011). Conversely, Meka and McCormick (2005) found a significant positive relationship between glucose and temperature in rainbow trout. The glucose concentrations shown by Gale et al. (2011) and the current study are not likely to be indicative of the stress response, but rather illustrate the variation in the substrate/food availability during different times of the year.

Post-capture lactate levels reflected the range of water temperatures sampled. Fish angled at the coldest temperatures showed lactate values similar to reference fish, whereas fish angled at the higher temperatures showed lactate levels similar to extended capture fish (> 2 min). Rainbow trout (Meka and McCormick 2005) and largemouth bass (Gustaveson et al. 1991) and sockeye salmon (Gale et al. 2011) exhibited similar trends in lactate levels as the water temperature increased. Additionally, these studies (except Gale et al. 2011, 2013) showed that, regardless of temperature, lactate increased with angling duration and these were highest at warmer water temperatures. The lactate response found in this study showed that fish angled at lower and moderate temperatures have a smaller response, whilst fish angled at higher temperatures have the greatest response. Kieffer et al. (1994) observed similar results, in that post exercise fish acclimated to higher temperatures (18°C) showed lactate concentrations two-fold greater than fish acclimated to lower temperatures (5°C). Gale et al. (2011) showed that after simulated capture stress, respiration rates in sockeye salmon were 18 - 25% higher at moderate and high water temperatures, respectively; ultimately, for the fish, there is likely a greater excess post-exercise oxygen consumption (EPOC). Therefore fish angled at higher water temperatures are put under greater stress and as a result under greater risk of a wide range of sub-lethal impacts and even mortality, than fish angled at lower water temperatures. Recently Brownscombe et al. (2015) also found that water

temperature is one of the most important predictors of the stress response in largemouth bass, however their results showed that angling at colder temperatures (<15°C) caused a greater physiological stress than at warmer temperatures (>20°C). Their results again highlight the need for species specific data on the physiological effect of angling stress under different environmental conditions as proposed by Cooke and Suski (2007).

Conclusions

The results obtained in this study directly apply to yellowfish in the Vaal River, but given the agreement with other investigations, these data may be considered representative of other popular catch and release populations globally. Water temperature and angling times are clearly influential factors on the glucose, cortisol and especially the lactate response. Based on the results of this study by using blood glucose, cortisol and lactate levels as indicators of angling stress, it is clear that catch and release angling, when correctly and ethically conducted, could be a viable management strategy for yellowfish in the Vaal River. Nonetheless, anglers should be aware of the potential harmful effect of extended angling time on fish that are released, especially when angling at warmer water temperatures (> 25°C). Future work could consider examining the effect of the handling and air exposure of catch and release fish, the effect on fish health of different angling techniques as well as the long-term impact of the physiological responses of angled fish at the individual and population level. This will further aid fisheries managers in setting scientifically based guidelines for catch and release angling in South Africa. Especially, since Cooke et al (2013) argued in their extensive review on physiological response of catch and release angling that it is still relevant to use physiological endpoints as indicators of fish health, even if it is difficult to demonstrate causal links with mortality or population-level impacts.

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FIGURES

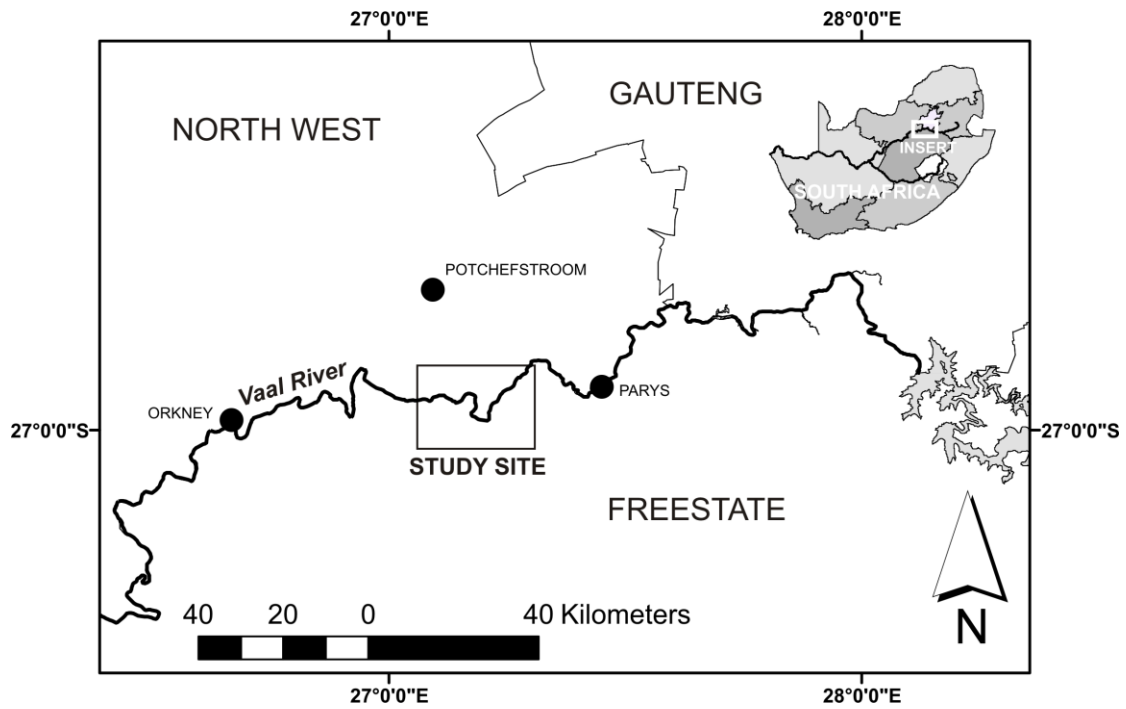


FIGURE 1: The study site in the Vaal River, South Africa.

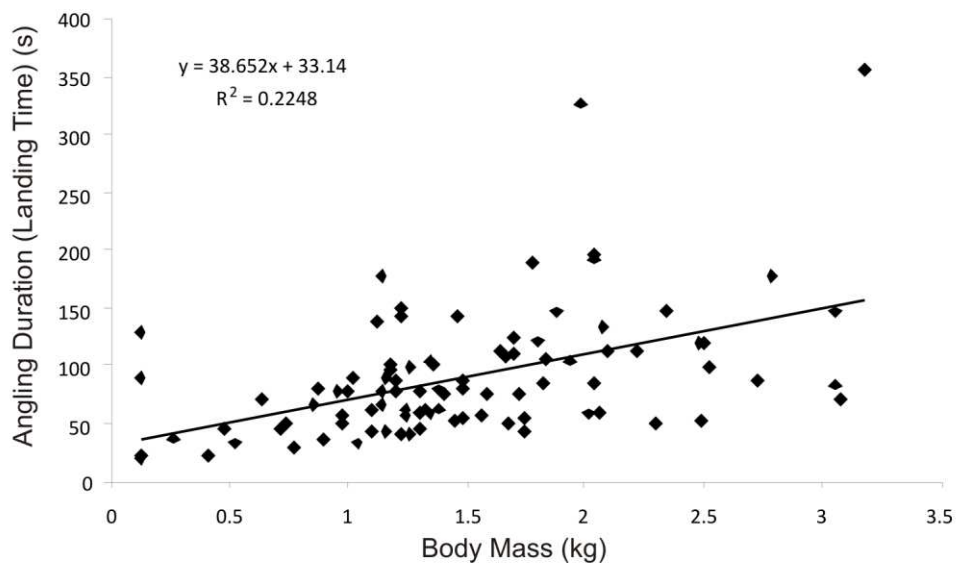


FIGURE 2: Linear regression of the landing time and body mass of yellowfish ($y = 38.652x + 33.14$) ($n = 96$). The variables were significantly related ($R^2 = 0.2248$; $P=0.043$).

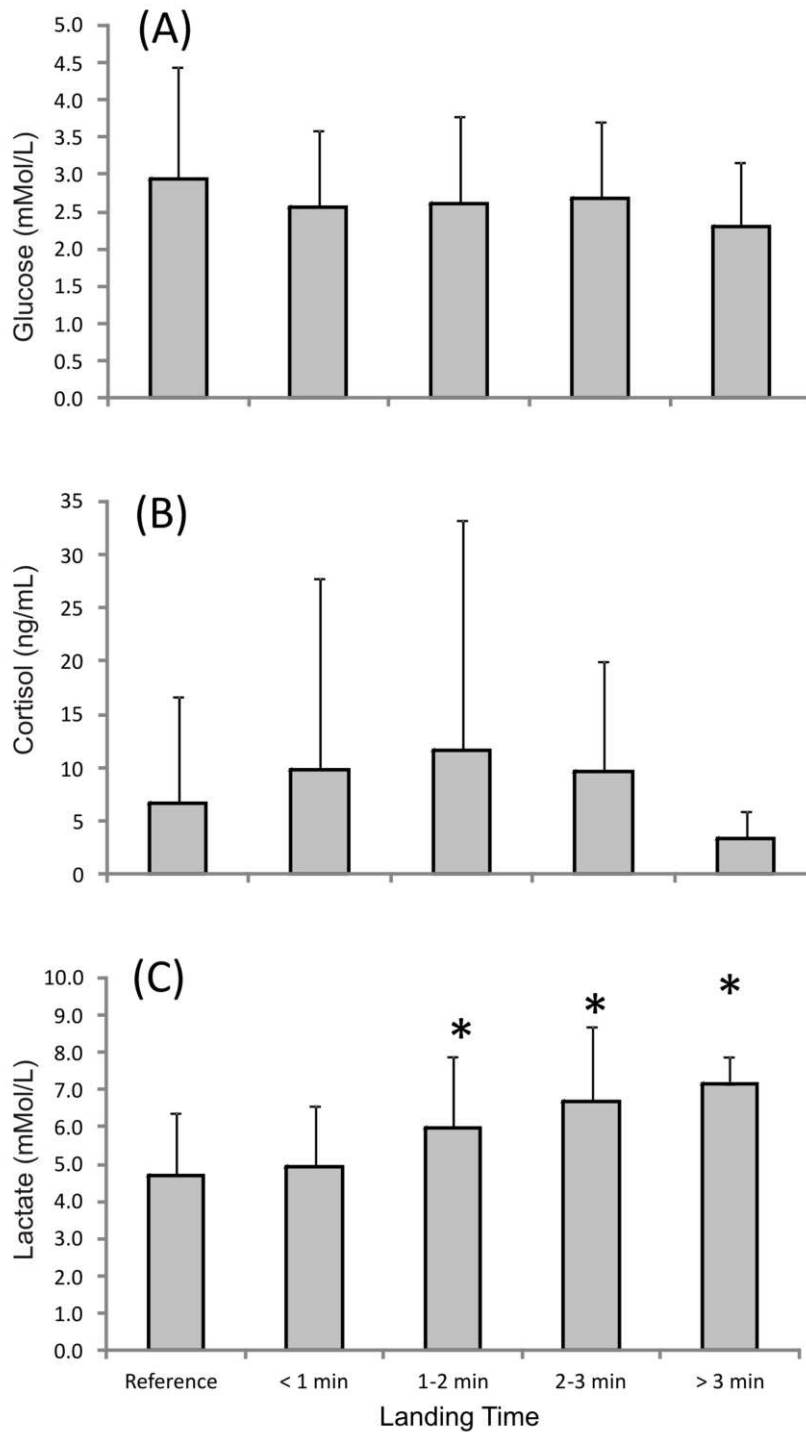


FIGURE 3: The glucose (A), cortisol (B) and lactate (C) response of smallmouth yellowfish to catch and release angling in the Vaal River, South Africa grouped by landing time and compared to the reference sample (n = 16); values are mean \pm SD. * denotes significantly greater lactate than the reference group and fish angled for less than 1 minute ($P \leq 0.044$); < 1 min (n = 31); 1-2 min (n = 44); 2-3 min (n = 16); > 3 min (n = 5).

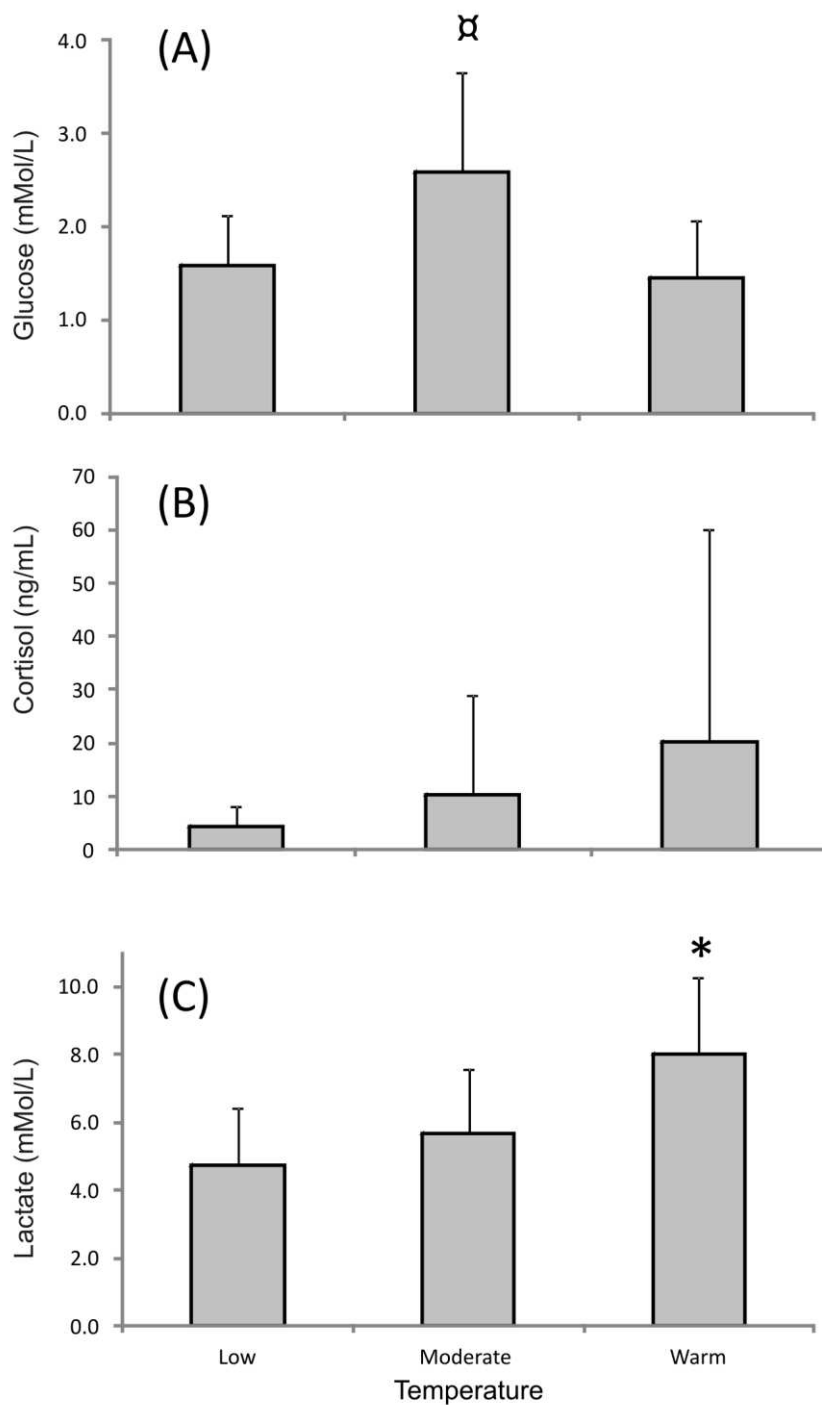


FIGURE 4. The glucose (A), cortisol (B) and lactate (C) response of smallmouth yellowfish to catch and release angling in the Vaal River, South Africa at low (n = 15), moderate (n = 64) and warm (n = 17) water temperatures. α denotes significantly greater glucose than low and warm water temperature ($P < 0.001$); * denotes significantly greater lactate than cool and moderate water temperatures ($P \leq 0.001$).