Temperature effects on the physiological status and reflex impairment in European grayling *Thymallus thymallus* from catch-and release angling

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Abstract

There is a growing body of research communicating how angler behaviour can be adjusted and optimised to reduce fish injury and impairment resulting from the capture of recreationally angled fishes. However, few studies have focused on how individual and interacting abiotic variables influence the outcomes of catch and release (C&R) angling. A population of European grayling *Thymallus thymallus* at their upper thermal limit of their geographic distribution provided a model cold-water species that was representative of other fishes sensitive to climate warming impacts and that are subjected to C&R across different seasons. Here, C&R angling for *T. thymallus* was conducted during summer (>15 ^{o}C) and winter (<10 ^{o}C), with 97 fish captured (220 - 490 mm). Measurement of tertiary stress responses (reflex impairment, as time to body equilibrium, an important predictor of post-release mortality) revealed that at >15 ^{o}C, fish took significantly longer to regain equilibrium (178 ± 44 s) than at <10 ^{o}C (70 ± 40 s). Multivariate testing revealed air exposure had a stronger effect on reflex impairment than fight time. Testing of post-capture, pre-release blood chemistry on sub-samples of captured fish revealed fish had significantly elevated levels of both glucose and lactate at >15 ^{o}C versus <10 ^{o}C, In entirety, these results suggest that stress responses and post-release mortality risk in cold-water fishes subjected to C&R could be reduced via temperature-informed fishery management practises, and by minimising, or ideally eliminating, air exposure.

1. Introduction

Global participation in recreational fisheries (the practice of catching fish with rod and line for non-commercial gain) has been estimated at C.700 million (Cooke and Cowx, 2004). The activity represents the primary use of wild freshwater fish stocks in all industrialised countries (Arlinghaus et al., 2017; FAO, 2012). With participatory growth also now evident in many developing countries (FAO, 2012; Freire et al., 2012), the sustainability of this global fishery resource is highly dependent on how resilient individual species and their populations are to angling exploitation. Accordingly, catch-and-release (C&R) angling is being increasingly implemented as a management strategy to promote conservation goals.

Estimates suggest that more than 60 % of the global recreational catch of 47 billion fish per annum are purposely released alive following capture (Cooke and Cowx, 2004). The C&R process is based on the assumption that a high proportion of released fish not only survive, but are also not compromised regarding their future health (Post-Release Mortality, PRM) (Bartholomew and Bohnsack, 2005; Cooke et al., 2013; Cook et al., 2015; Pollock and Pine, 2007) or their reproductive potential (Pinder et al., 2016; Richard et al., 2013). In recent years, there has been considerable attention directed towards understanding the stress response and subsequent survival and performance of a broad range of C&R angled marine, freshwater and transitional sport fishes (e.g. bonefish (*Albula vulpes*) Danylchuk et al., 2007; Atlantic cod (*Gadus morhua*) Weltersbach and Strehlow, 2013; peackock bass (*Chichla ocellaris*) Bower et al., 2016a; mahseer (*Tor khudree*), Bower et al., 2016b; Atlantic salmon (*Salmo salar*) Lennox et al., 2017). There has been specific emphasis on the effects of angling on disruptions to baseline blood chemistry as a secondary (physiological) stress response (Cooke et al., 2013), and the application of simple reflex action impairment indicators as a tertiary (whole body) stress response (Davis and Ottmar, 2006; Davis, 2007, 2010). In combination, these secondary and tertiary stress responses provide rapid assessment of individual vitality and relative PRM risk of the C&R process (Cooke et al., 2013).

In general, studies using rapid assessment tools have demonstrated that individual species, and even populations (Cooke and Suski, 2005), exhibit different vulnerabilities and tolerances to the various mechanics of C&R (e.g. fight time, hook damage, air exposure, etc.). However, the additive and interacting impacts of these C&R mechanics with abiotic factors (e.g. water temperature) have received relatively little attention (Arlinghaus et al., 2007). In particular, there is a paucity of sufficiently robust field data pertaining to temperature impacts on the recovery of individual fish from C&R that could be used to inform fishery management best practice (Gale et al., 2013). Consequently, the aim of this study was to apply secondary and tertiary stress response principles to quantify how natural seasonal variations in water temperature affect C&R impacts.

The fish species used in the study was a temperature sensitive species, the cold-water European grayling (*Thymallus thymallus*), using a population at the edge of its upper thermal range (Southern England) (Bašić et al., 2018). Observations from the current study, therefore, may have implications for other species that either currently experience periods of thermal stress (e.g. *Salmo salar*, Corey et al., 2017) or will do so in future due to climate change (Ruiz-Navarro et al., 2016). Objectives were, thus, to examine the influence of seasonal water temperatures on reflex impairment and blood chemistry disruption as indicators of C&R stress and PRM, complement previous observations of behavioural and physiological response to C&R associated stressors for *T. thymallus* (e.g. air exposure *cf*. Lennox et al., 2016), and identify best practice to assist both conservation based fishery management decisions and angler behaviour.

2. Methods

2.1 Study population and sampling methodology

The study river was the River Frome, a chalk stream in Dorset, Southern England. The stretch used was at East Stoke (N: 50°40'49", W: 2°11' 37" to N: 50°40'45", W: 2°10'38"). Here, the river widths were approximately 10 to 12 m and maximum depth was 1.5 m. Within the stretch, the long-term mean daily flow was 6.67 m^3s^{-1} (Q_{95} 2.51 m^3s^{-1}; Q_{10} 12.69 m^3s^{-1}) (CEH, 2018). In addition to *T. thymallus*, the fish assemblage in this river included Atlantic salmon *Salmo salar*, brown trout *Salmo trutta*, dace *Leuciscus leuciscus* and Northern pike *Esox lucius*, the last of these species being abundant and an important predator of *T. thymallus*.

To represent typical angler behaviours, angling was undertaken by a team consisting of the authors and a further five anglers of mixed experience accompanied by one of the authors. Data were collected between June and December 2016 and covered two primary periods of water temperature. The first angling period was conducted when water temperatures were between 15 and 20.9 °C (hereafter referred to as >15 °C) and the second when the water temperatures were between 5 and 10 °C (hereafter referred to as <10 ^{o}C). A continuous record of water temperature was recorded at 30 minute intervals throughout 2016 using a TinyTag data logger (measuring to 0.01 ^{o}C) in the main river channel. On each angling occasion, water temperatures were measured using a combination of a TinyTag logger within the water column coupled with hand-held thermometers that recorded water temperatures in the river margins following the capture of each individual fish. On each angling occasion, the angling style employed was either float-fished or bottom-fished (legered) maggots (*Calliphora vomitoria*), with hook choice (size 18 micro-barbed) and line strength (1.4 kg breaking strain) appropriate for the methods employed, i.e. the time taken to play fish to near exhaustion (the point they could be safely landed and unhooked) would be representative of normal angling activity.

2.2 Sampling protocol

The sampling protocol for each individual fish commenced when the angler initially hooked the fish. Using a neck-lanyard mounted stopwatch, individual anglers (or assisting observers') recorded two time related angling metrics for each individual fish. The first was the time between hooking and the fish being removed from the water, referred to as 'fight time' (to nearest s). The second was the time between the removal of the fish from the water and its return, referred to as 'air exposure time' (to nearest s). Air exposure included the unhooking period of the fish. As per Bower et al. (2016a), the anatomical hook position (HP) was recorded using a simple scoring system (1: hooked in lip; 2: inside buccal cavity; 3: oesophagus). Scores were also applied to hook damage (HD) and ease of unhooking (EU). For hook damage, the scoring was 1: minor injury (a small tissue tear < 3 mm in length, including any visible tissue tear or abrasion resulting from hooking); 2: moderate injury such as the presence of bleeding, bruising or a tissue tear > 3 mm in length; and 3: major injury that occurred from the hook position, including ocular or gill damage with significant pulsatile bleeding that resulted from the fish being hooked in the oesophagus. For ease of unhooking, the scoring was 1: a hook that was removed easily and in less than 10 s; 2: a hook requiring between 10 and 20 s for removal; and 3: a hook requiring > 20 s to remove or requiring the line to be cut and the hook left *in situ* (Cooke et al., 2001). The period of air exposure also included the time taken to measure each individual fish (fork length, nearest 0.1 cm).

When individual fish were returned to the water, they were observed within the landing net (minimum surface area 0.25 m^2, minimum depth 0.3 m). Following initial trials, the inhibition of reflex behaviours was not measured using the full suite of reflex action mortality predictors of ‘RAMP’ (‘Equilibrium’, ‘Tail grab’, ‘Body flex’ and ‘Head complex’) (Davis, 2010). This was for two reasons. Firstly, access to the water from the riparian zone was often limited and whilst fish could be easily observed in the landing net, they could not always be monitored for all RAMP responses. Secondly, where RAMP responses were able to be recorded, failures always included ‘Equilibrium’, the response that measures whether the fish is able to right itself within three seconds after being placed upside-down in water, and all passed ‘Head complex’, where the fish exhibits steady operculum beats during handling (Davis, 2010). Thus, throughout the study, ‘Equilibrium’ was the sole RAMP response recorded. Rather than using Equilibrium as a pass (reflex present) and fail (reflex absent) metric, the time taken to regain equilibrium was recorded. This was based on the assumption that an increased time to regain equilibrium represented greater impairment to the fish from the C&R process. Once equilibrium was regained, the fish was released.

Blood samples were also obtained for a minimum sub-set of 15 captured fish per water temperature group. However, for welfare reasons, blood was only sampled from fish for which the total time of air exposure plus the time to regain equilibrium was ≤10 minutes. Fish for which this time was >10 minutes were released once they regained equilibrium. For those fish that were appropriate for blood sampling, after the 10 minute period had elapsed, the fish was removed from the landing net and anaesthetised (MS-222). Approximately 1 ml of blood was then drawn from the caudal vasculature (Barton, 2002) using a 22G needle and 5.0 ml collection tube and analysed immediately for its lactate and glucose concentrations using pre-calibrated portable point-of-care (PoC) devices. As per Pinder et al. (2016), lactate concentration was determined using a Nova Lactate Plus Meter (Nova Biomedical, Massachusetts, USA) that had a detection range of 0.3 to 25.0 mmol/L; its accuracy had been pre-demonstrated to be consistent with other PoC devices and plasma-based laboratory methods (Karon et al., 2007). Similarly, glucose concentration was determined using a SD CodeFree™ Blood Glucose Monitoring System (SD Biosensor, Inc, GyeongGi-do, Korea) with a detection range of 0.6 to 33.3 mmol/L. Although designed for human use, the PoC devices used to measure glucose and lactate have been previously shown to produce valid and accurate data in fisheries studies (Stoot et al., 2014). Immediately following blood collection, fish were returned to the landing net in the river margin for recovery. Once equilibrium was regained the fish were released.

All procedures completed on the fish were approved by the Animal Welfare and Ethical Review Committee of Bournemouth University and licenced by the UK Home Office (project licence 70/8063).

2.3 Statistical analyses

Initial analyses used linear regression to determine the significance of relationships between fish length and the angling variables of fight time and air exposure. A general linear model then tested differences in these variables between the two temperature groups, where fight time or air exposure was the dependent variable, temperature group was the independent variable and fish length was the covariate. Outputs were mean fight time and mean air exposure for each temperature group as estimated marginal means, and the significance of their differences according to linearly independent pairwise comparisons with Bonferroni adjustment for multiple comparisons. Testing of the proportions of captured fish in the different categories of ease of unhooking (EU), hook position (HP) and hook damage (HD) used a chi-square test of independence. The expected distribution was a 50:50 split between fish easy to unhook with no damage (scores of 1) and fish more difficult to unhook with some damage (score of 2 and above). Scores of ease of unhooking were then used as groups in a generalised linear model to test their effects on air exposure, where fight time, fish length and water temperature were covariates. Model outputs were the significance of ease of unhooking on air exposure and the significance of the effects of each covariate.

The time taken for fish to regain 'Equilibrium' was then tested between the two temperature groups using a general linear model, where time was the dependent variable, temperature group was the independent variable, and fight time, air exposure and fish length were covariates. Outputs were the mean time to equilibrium for each temperature group as estimated marginal means, and the significance of their differences according to linearly independent pairwise comparisons with Bonferroni adjustment for multiple comparisons. The same tests were then used to test differences between the temperature groups for blood lactate and glucose concentrations.

Throughout all analyses, compliance of data with assumptions of homogeneity of variance and normality of distribution were tested using Levene and Shapiro–Wilk tests on each variable prior to analysis. Where assumptions were met, testing used general linear models as described above. Only where they were not met were the data tested using the non-parametric tests outlined. All data are presented as mean ± 95 % confidence intervals unless stated. All research procedures were conducted under licence as granted by the UK Home Office.

3. Results

3.1 Ambient temperature regime

Throughout 2016 water temperature at the study site ranged between 6.2 and 20.8 ^{o}C (Fig. 1), with the lower temperature group (< 10 ^{o}C) being typical of the winter season (October to April) and the higher temperature group (> 15 ^{o}C) representative of the summer months (June – September). Excluding the coarse fishing closed season (15 March – 15 June inclusive) for *T. thymallus* in England and Wales, totals of 123 and

93 days fell within the lower (< 10 ^{o}C) and upper (> 15 ^{o}C) temperature groups respectively.

3.2 Angling related metrics

Over the course of the study a total of 97 *T. thymallus* were captured and released, with 52 at <10 ^{o}C and 45 >15 ^{o}C. Mean fish length at >15 ^{o}C was 314 ± 19 mm and at <10 ^{o}C was 323 ± 21 mm. In both temperature groups, fight time significantly increased as fish length increased (<10 ^{o}C: R^2 = 0.53, $F_{1,50}$ = 56.85, P < 0.01; >15 ^{o}C: R^2 = 0.57, $F_{1,43}$ = 57.87, P < 0.01; Fig. 2a). The GLM testing fight times between the temperatures was significant ($F_{1,94}$ = 6.60, P < 0.01), where the effect of fish length as covariate was significant in the model (P < 0.01). The model revealed mean fight times (adjusted for fish length) were significantly longer in the higher temperature group (<10 ^{o}C: 69 ± 12 s; >15 ^{o}C: 91 ± 11 s; P < 0.01). Air exposure also increased significantly as fish length increased (<10 ^{o}C: R^2 = 0.30, $F_{1,50}$ = 21.35, P < 0.01; >15 ^{o}C: R^2 = 0.29, $F_{1,43}$ = 17.43, P < 0.01; Fig. 2b). However, differences in mean air exposure between the temperature groups were not significant (<10 ^{o}C: 48 ± 9 s; >15 ^{o}C: 55 ± 10 s; $F_{1,94}$ = 1.25, P = 0.27).

For ease of unhooking (EU), hook damage (HD) and hook position (HP), a minimum of 86 % of captured fish in each temperature group were scored at either 1 or 2 (Table 1).

Table 1. Proportions of fish per score for ‘ease of unhooking’ (EU), ‘hook damage’ (HD) and ‘hook position’ (HP) in each water temperature group. For HP, any fish that were foul hooked were omitted from the numbers.

			Score		
	Metric	N	1	2	3
< 10 °C	EU	52	79	17	4
	HD	52	81	17	2
	HP	51	82	4	14
	Metric	N	1	2	3
> 15 °C	EU	45	60	29	11
	HD	45	80	13	7
	HP	44	75	11	14

Irrespective of temperature, there were significantly higher proportions of fish scored at 1 than at 2 and 3 for each of these metrics (EU: χ^2 = 15.68; P < 0.01; HD: χ^2 = 34.33; P < 0.01; HP: χ^2 = 27.29; P < 0.01) (Table 1). Fish that were difficult to unhook either had scores indicating higher hook damage and hook positions in the buccal cavity or oesophagus, or unhooking was complicated due to the hook having been dislodged but passing through and threading line through the lip and becoming embedded within the net material (*cf.* discussion). Moreover, a GLM revealed fish that were more difficult to unhook had increased air exposure (Wald χ^2 = 5.03 P = 0.03), where covariates of fight time, fish length and temperature were not significant (P = 0.43, 0.94 and 0.71 respectively). Thus, as these hooking related metrics were all related to each other and significantly influenced the duration of air exposure, then only air exposure was used subsequently in tests on body equilibrium and blood chemistry.

3.3 Time to body equilibrium

All of the fish captured by angling during the study subsequently regained body equilibrium and were successfully released. Across all temperatures, the mean time to equilibrium was 120 ± 37 s. In the fish captured at water temperatures >15 °C, the time to equilibrium ranged between 0 and 1440 s (mean 187 ± 70 s), whilst at <10 °C, they ranged between 0 and 300 s (mean: 62 ± 25 s). The univariate relationships between time to body equilibrium versus fight time and air exposure were all significant (fight time, >15 °C: R^2 = 0.26, $F_{1,43}$ = 15.29, P < 0.01; fight time <10 °C: R^2 = 0.37, $F_{1,50}$ = 7.91, P < 0.01; air exposure >15 °C: R^2 = 0.27, $F_{1,43}$ = 15.90, P < 0.01; air exposure <10 °C: R^2 = 0.43, $F_{1,50}$ = 38.18, P < 0.01) (Fig. 3).

In the multivariate testing of the effect of the water temperature groups on the time to body equilibrium, the GLM was significant overall ($F_{1,92}$ = 12.27; P < 0.01). In the GLM, air exposure was a significant covariate (P < 0.01), but fish length (P = 0.54) and fight time (P = 0.09) were not. The model revealed that the mean time to equilibrium (when adjusted for the effects of all covariates) was significantly higher at >15°C (178 ± 44 s) than < 10 °C (70 ± 40 s) (P < 0.01; Fig. 4a).

3.4 Blood chemistry

The GLM testing the effect of water temperature on blood lactate concentration was significant ($F_{1,30}$ = 7.21; P = 0.01). In this model, none of the covariates had significant effects on lactate concentration (fight time: P = 0.65; air exposure P = 0.57; fish length: P = 0.60). At <10 °C, mean lactate concentration was 4.96 ± 0.59 mmol l^{-1} versus 6.18 ± 0.69 at >15 °C, with this difference significant (P = 0.01; Fig. 4b). For blood glucose concentration, the GLM was also significant $F_{1,30}$ = 9.74; P <

0.01); the effect of fight time was a significant covariate in the model (P = 0.01), whereas air exposure and fish length were not significant covariates (P = 0.99 and P = 0.06 respectively). At < 10 ^{o}C, mean glucose concentration was 4.15 ± 0.44 mmol l^{-1} versus 5.22 ± 0.52 at > 15 ^{o}C, with this difference significant (P < 0.01; Fig. 4c).

4. Discussion

There has been general acknowledgement that water temperature at the time of capture represents a pertinent abiotic factor in determining the post-release performance of C&R angled fishes (Arlinghaus et al., 2007; Cooke et al., 2013; Gale et al., 2013). However specific attention to cold-water fishes has, to date, been largely restricted to examining the effects of extreme high temperatures on Atlantic salmon *Salmo salar* (e.g. Boyd et al., 2010; Havn et al., 2015; Lennox et al., 2017; Wilkie et al., 1997). Using *T. thymallus* as a representative species for cold-water fishes more generally, this study demonstrates that natural variations in seasonal temperatures can have substantial influences on the secondary and tertiary stress responses of fish subjected to C&R angling.

Temperature effects were evident from the onset of the angling event, as fish captured in the higher temperature group (> 15 ^{o}C) maintained longer fight times and were thus subject to significantly greater exercise. Despite playing all fish to near exhaustion, fish in the higher temperature group were generally observed to be more problematic to handle and unhook due to exhibiting powerful body flexing in the landing net (Personal observations, the authors). There were also often cases where lip hooked fish had the point of the hook snagging the mesh of the landing net. This caused the line to thread through the lip, resulting in delayed unhooking. In addition to contributing to increased air exposure, this may have also caused additive physiological stress through increased handling and mechanical abrasion, coupled with epithelial damage. This represents a potentially important additive stressor that was also highlighted in a C&R study completed in a Norwegian *T. thymallus* fishery at water temperatures of 17 to 18 ^{o}C (Lennox et al., 2016) and also elevating risk of fungal, bacterial or viral infection, and PRM (Colotelo and Cooke, 2011; Brownscombe et al., 2017).

Independent in design but consistent with the methods applied to examine the effect of air exposure on *T. thymallus* (Lennox et al., 2016), the standard suite of RAMP tests (Davis, 2010) was simplified here to a single test of 'Equilibrium'. Other species of recreationally targeted fishes which have been subject to full RAMP assessment (e.g. tail grab, body flex, head complex and equilibrium) have typically applied a three second threshold to define pass or failure of the equilibrium test (e.g. coho salmon *Oncorhynchus kisutch* Raby et al., 2012; peacock bass *Cichla ocellaris* Bower et al, 2016a; mahseer *Tor* sp., Bower et al, 2016b; black bream *Spondyliosoma cantharus*, Pinder et al., 2016). In comparison, *T. thymallus* has been shown to exhibit substantially increased sensitivity to C&R angling through extended durations required to recover equilibrium in the present study overall (mean 120 ± 37 s), with Lennox et al (2016) reporting incomplete recovery in some individuals 30 minutes after being subjected to 120 s air exposure treatment). Thus, the significantly longer recovery times observed in the higher temperature group (>15 ^{o}C) corresponds with the results of previous studies (Barton, 2002; Wendelaar Bonga, 1997). They are also consistent with results from a meta-analysis of the impacts of temperature on C&R

angling more generally, which indicated that sub-lethal stress and/ or mortality increased with temperature in 70 % of existing studies (Gale et al., 2013).

Glucose and lactate concentrations in blood have been widely used as a rapid qualification of normal physiological disruption in C&R angled fishes (e.g. Barton, 2002; Bower et al., 2016a,b; Pinder et al., 2016). However, it is apparent that throughout the C&R literature, there is an issue around the timing of blood sampling. This is potentially important, given that the manifestation of blood chemistry alteration may differ between species and abiotic conditions. This lack of standardisation in the time between fish captures and blood sample collection thus makes inter-species comparisons difficult. Typically, the timing of blood sampling has been either from the end of timed air exposure treatments (e.g. Bower et al., 2016b; Lennox et al., 2016) or immediately following reflex impairment tests (e.g. Bower et al., 2016b; Pinder et al., 2016). In considering the limitations of available tools and their utility in C&R studies, Cooke et al. (2013) concluded there is no optimal protocol for blood sampling in the field and, instead, recommended that studies should focus on the comparison of treatment groups as opposed to placing inappropriate confidence in absolute values. Consequently, the approach applied here was to standardise the sampling time to 10 minutes from the onset of the stressor (i.e. the fish being hooked). This then allowed for blood chemistry sampling using a standard protocol within the study that allowed consistent testing for differences between the two water temperature groups. Although designed for human use, the PoC devices used to measure glucose and lactate have been previously shown to produce valid and accurate data in fisheries studies (Stoot et al., 2014).

Temperature was not found to influence hooking mechanics, with 14 % of fish in each temperature group being 'deep hooked' and, in most cases, requiring the line to be cut leaving the small (#18 micro-barbed) hooks *in-situ*. Damage caused by deep hooking has been described as the most important factor affecting PRM (Alos et al., 2008; Bartholomew and Bohnsack, 2005; Muoneke and Childress, 1994). The use of small hooks and live invertebrate baits typically result in higher incidence of deep hooking over artificial baits (lures) and flies (*cf*. Brownscombe et al., 2017). While *T. thymallus* is popularly targeted using fly-fishing, this tends not to result in deep hooking (R. Lennox, pers. comm.). However, the species is also widely targeted using invertebrate baits, where the use of low diameter and low breaking strain lines and relatively small hooks are necessary for fish capture. Fisheries often mandate the use of 'barbless' hooks to minimise hook damage and enable easier unhooking (Alós et al., 2008; Cooke and Sneddon, 2007; DuBois and Dubielzig, 2004; Schaeffer and Hoffman, 2002). Although air exposure may be reduced via aided ease of unhooking, there is no evidence suggesting their use reduces PRM risk when a fish is hooked in the oesophagus (Bartholomew and Bohnsack, 2005; Brownscombe et al., 2017; Muoneke and Childress, 1994).

A growing body of science on C&R fisheries has led to the recent development of generic best practice guidance for anglers (Brownscombe et al., 2017). Emerging initiatives such as 'Keepemwet Fishing' (KWF, https://www.keepemwet.org) have assisted the communication of relevant scientific information to the global recreational angling community via popular social media platforms (Danylchuk et al., 2018). In the UK, the 'Grayling Society', a body promoting *T. thymallus* angling and conservation, have already adopted KWF principles. The Society states that KWF principles should be applied to all *T. thymallus* fisheries (Grayling Society, 2018). A feature of this guidance is the minimisation of *T. thymallus* fight times through the use of appropriate gear. Here, fight time was a positive influence on elevated blood glucose levels and its univariate relationship with time to body equilibrium was significant. These results suggest some potential benefits in minimising fight times. However, delaying the landing of a fish until it is close to exhaustion not only reduces handling times but also reduces the risk of light lines breaking, so preventing hooks being left in lost fish (with hook damage generally being an important PRM predictor; Alos et al., 2008). There is thus a trade-off between the risk of line breakage and long-term hook damage versus causing elevated stress via prolonged fight times and the associated extended time required to unhook lively fish. Moreover, in the GLM testing the effects of water temperature on time to body equilibrium, air exposure was a significant co-variate but fight time was not. This suggests that minimising air exposure through reducing fish handling times will have a stronger influence on reducing reflex impairment than reducing fight times, and it is suggested fishery best practice schemes initially focus on this. For example, it has been discussed that hook entanglement in landing nets is a period of additional air exposure. This can potentially be eliminated by the use of rubberised, large mesh nets, as these can reduce the risk of hook entanglement and consequent abrasive damage (Lizée et al., 2018). Their mandated use would thus provide benefits to fish welfare and angler experiences. Best practice measures should also dictate that the angling position must facilitate quick and easy access for water entry by the angler. This would enable fish to be unhooked and released with minimal air exposure (e.g. not exceeding an upper threshold of air exposure of 10 s; Lennox et al., 2016).

Although minimising air exposure of captured *T. thymallus* would provide substantial welfare benefits via reduced secondary and tertiary stress responses, the effects of high temperature on these responses was highly significant. While C&R fisheries are often temporarily closed during perceived periods of elevated risk to fish welfare from C&R, these tend to be mandated closed seasons for protecting spawning populations (Kubacki et al., 2002), however, temporary closures for high water temperatures are also mandated in some high value C&R salmonid fisheries (Cooke and Suski, 2005). The outputs of the current study demonstrate the importance of raising the awareness of the recreational angling community and fishery managers to the elevated sensitivity of *T. thymallus* to C&R derived PRM risk during periods of elevated water temperature.

Quantification of PRM in natural fishery scenarios is problematic due to the multiple challenges associated with tracking the fate of released fish without fish incurring additional non-capture related stressors (e.g. telemetry tag attachment) (Cooke et al., 2013). Nevertheless, this study successfully applied reflex impairment and physiological disruption to demonstrate the elevated sensitivity of *T. thymallus* to C&R angling during water temperatures typically experienced at the upper limits of the species thermal tolerance. These results highlight the potential for future climate change projections (e.g. elevated summer temperatures that are concomitant with low flows; Ruiz-Navarro et al., 2016) to exacerbate C&R mediated risks to cold-water sport fishes more generally. Despite all fish here demonstrating sufficient recovery of equilibrium to swim away strongly, this was only after the provision of adequate and extensive post-capture and pre-release care (up to 1440 s duration). While a proportion of these fish may still have been at risk of delayed PRM through either hook retention and/or delayed physiological normalisation, fish released prematurely by anglers would be subject to considerably elevated risk of more immediate PRM. This risk would be through both an inability to orientate into a recovery position and through increased susceptibility to predation. The practise of discouraging anglers to target *T. thymallus* during periods of high water temperature, combined with minimisation or elimination of air exposure, have the potential to increase the resilience of populations to C&R angling exploitation and support the conservation of this popular sport species.

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References

Alos, J., Palmer, M., Grau, A.M., Deudero, S., 2008. Effects of hook size and barbless hooks on hooking injury, catch per unit effort, and fish size in a mixed-species recreational fishery in the western Mediterranean Sea. *ICES J. Mar. Sci.* 65, 899–905.

Arlinghaus, R., Cooke, S.J., Lyman, J., Policansky, D., Schwab, A., Suski, C., Sutton, S.G., Thorstad, E.B., 2007. Understanding the Complexity of Catch-and-Release in Recreational Fishing: An Integrative Synthesis of Global Knowledge from

Historical, Ethical, Social, and Biological Perspectives. *Rev. Fish. Sci.* 15, 75-167. DOI: 10.1080/10641260601149432

Arlinghaus, R., Alós, J., Beardmore, B., Daedlow, K., Dorow, M., Fujitani, M., Huhn, D., Haider, W., Hunt, L.M., Johnson, B.B., Johnston, F., Klefoth, T., Matsumura, s., Monk, C., Pagel, T., Post, J.R., Rapp, T., Riepe, C., Ward, H., Wolter, C., 2017. Understanding and managing freshwater recreational fisheries as complex adaptive social-ecological systems. *Rev. Fish. Sci. Aquac*. 25, 1-41.

Bašić, T., Britton, J.R., Cove, R.J., Ibbotson, A.T., Gregory, S.D., 2018. Roles of discharge and temperature in recruitment of a cold-water fish, the European grayling *Thymallus thymallus*, near its southern range limit. *Ecol. Fresh. Fish*. DOI: 10.1111/eff.12405.

Bartholomew, A., Bohnsack, J.A., 2005. A review of catch-and-release angling mortality with implications for no-take reserves. *Rev. Fish. Biol. Fish.* 15, 129-154.

Barton, B.A., 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integr. Comp. Biol*. 42, 517–525.

Boyd, J.W., Guy, C.S., Horton, T.B., Leathe, S.A., 2010. Effects of catch-and-release angling on salmonids at elevated water temperatures. *N. Am. J. Fish. Manage.* 30, 898-907.

Bower, S.D., Danylchuk, A.J., Brownscombe, J.W., Thiem, J.D., Cooke, S.J., 2016a. Evaluating effects of catch-and-release angling on peacock bass (*Cichla ocellaris*) in a Puerto Rican reservoir: a rapid assessment approach. *Fish. Res.*175, 95-102.

Bower, S.D., Danylchuk, A.J., Raghavan, R., Clark-Danylchuk, S.E., Pinder, A.C., Cooke, S.J., 2016b. Rapid assessment of the physiological impacts caused by catch-and-release angling on blue-finned mahseer (*Tor* sp.) of the Cauvery River, India. *Fish. Manag. Ecol.* 23, 208-217.

Brownscombe, J.W., Danylchuk, A.J., Chapman, J.M., Gutowsky, L.F.G., Cooke, S.J., 2017. Best practices for catch-and-release recreational fisheries – angling tools and tactics. *Fish. Res*. 186, 693-705. http://dx.doi.org/10.1016/j.fishres.2016.04.018

CEH, 2018. National River Flow Archive. *NERC Centre for Ecology & Hydrology*. https://nrfa.ceh.ac.uk/data/station/info/44001. Last accessed 05 July 2018

Colotelo, A.H., Cooke, S.J., 2011. Evaluation of common angling-induced sources of epithelial damage for popular freshwater sport fish using fluorescein. *Fish. Res.* 109, 217-224.

Cooke, S.J., Cowx, I.G., 2004. The role of recreational fishing in global fish crises. *BioScience.* 54, 857-859.

Cook, K.V., Lennox, R.J., Hinch, S.G., Cooke, S.J., 2015. Fish out of water: how much air is too much? *Fisheries*. 40, 452–461.

Cooke, S.J., Suski, C.D., 2005. Do we need species-specific guidelines for catch-and-release recreational angling to effectively conserve fishery resources? *Biodivers. Conserv*. 14, 1195-1209. DOI 10.1007/s10531-004-7845-0

Cooke, S.J., Sneddon, L.U., 2007. Animal welfare perspectives on recreational angling. *Appl. Anim. Behav. Sci*. 104, 176-198.

Cooke, S.J., Philipp, D.P., Dunmall, K.M., Schreer J.F., 2001. The influence of terminal tackle on injury, handling time and cardiac disturbance of rock bass. *N. Am. J. Fish. Manag*. 21, 333–342.

Cooke, S.J., Donaldson, M.R., O'Connor, C.M., Raby, G.D., Arlinghaus, R., Danylchuk, A.J., Hanson, K.C., Hinch, S.G., Clark, T.D., Patterson, D.A., Suski, C.D., 2013. The physiological consequences of catch-and-release angling:

perspectives on experimental design, interpretation, extrapolation and relevance to stakeholders. *Fish. Manag. Ecol*. 20, 258–287.

Corey, E., Linnansaari, T., Cunjak, R.A., Currie, S., 2017. Physiological effects of environmentally relevant, multi-day thermal stress on wild juvenile Atlantic salmon (*Salmo salar*). *Conserv. Physiol. 5*(1). https://doi.org/10.1093/conphys/cox014

Danylchuk, A.J., Danylchuk, S.E., Cooke, S.J., Goldberg, T.L., Koppelman, J.B., Philipp, D.P., 2007. Post-release mortality of bonefish, *Albula vulpes*, exposed to different handling practices during catch-and-release angling in Eleuthera, The Bahamas. *Fish. Manag. Ecol.* 14, 149-154.

Danylchuk, A.J., Danylchuk, S.C., Kosiarski, A., Cooke, S.J., Huskey, B., 2018. Keepemwet Fishing—An emerging social brand for disseminating best practices for catch-and-release in recreational fisheries. *Fish. Res.* 205, 52-56.

Davis, M.W., Ottmar, M.L., 2006. Wounding and reflex impairment may be predictors for mortality in discarded or escaped fish. *Fish. Res.* 82, 1–6.

Davis, M.W., 2007. Simulated fishing experiments for predicting delayed mortality rates using reflex impairment in restrained fish. *ICES J. Mar. Sci*. 64, 1535–1542.

Davis, M.W., 2010. Fish stress and mortality can be predicted using reflex impairment. *Fish. Fish.* 11, 1–11.

DuBois, R.B., Dubielzig, R.R., 2004. Effect of hook type on mortality, trauma, and capture efficiency of wild stream trout caught by angling with spinners. *N, Am. J. Fish. Manage*. 24, 609-616.

FAO, 2012. *Technical Guidelines for Responsible Recreational Fisheries*. Food and Agriculture Organization, Rome. 176 pp.

Freire, K.M., Machado, M.L., Crepaldi, D., 2012. Overview of inland recreational fisheries in Brazil. *Fisheries*. 37, 484-494.

Gale, M.K., Hinch, S.G., Donaldson, M. R., 2013. The role of temperature in the capture and release of fish. *Fish Fish.* 14, 1-33.

Grayling Society, 2018. Grayling Angling Code. http://www.graylingsociety.net/angling/ Last accessed 12/06/2018.

Havn, T.B., Uglem, I., Solem, Ø., Cooke, S.J., Whoriskey, F.G., Thorstad, E.B., 2015. The effect of catch-and-release angling at high water temperatures on behaviour and survival of Atlantic salmon *Salmo salar* during spawning migration. *J. Fish. Biol.* 87, 342-359.

Karon, B.S., Scott, R., Burritt, M.F., Santrach, P.J., 2007. Comparison of lactate values between point-of-care and central laboratory analyzers. *Am. J. Clin. Pathol*. 128, 168–171.

Kubacki, M.F., Phelan, F.J.S., Claussen, J.E., Philipp, D.P., 2002. How well does a closed season protect spawning bass in Ontario?. In *American Fisheries Society Symposium* (pp. 379-386). American Fisheries Society.

Lizée, T.W., Lennox, R.J., Ward, T.D., Brownscombe, J.W., Chapman, J.M., Danylchuk, A.J., Nowell, L.B., Cooke, S.J., 2018. Influence of Landing Net Mesh Type on Handling Time and Tissue Damage of Angled Brook Trout. *North American Journal of Fisheries Management*, (just-accepted).

Lennox, R.J., Mayer, I., Havn, T.B., Johansen, M.R., Whoriskey, K., Cooke, S.J., Thorstad, E.V., Uglem, I., 2016. Effects of recreational angling and air exposure on the physiological status and reflex impairment of European grayling (*Thymallus thymallus*). *Boreal Environ. Res*. 21, 461–470.

Lennox, R.J., Cooke, S.J., Davis, C.R., Gargan, P., Hawkins, L.A., Havn, T.B., Johansen, M.R., Kennedy, R.J., Richard, A., Svenning, M., Uglem, I., Webb, J., Whoriskey, G.G., Thorstad, E.B., 2017. Pan-Holarctic assessment of post-release mortality of angled Atlantic salmon *Salmo salar*. *Biol. Cons.* 209, 150-158.

Muoneke, M.I., Childress, W.M., 1994. Hooking mortality: a review for recreational fisheries. *Rev. Fish. Sci.* 2, 123-156.

Pinder, A.C., Velterop, R., Cooke, S.J., Britton, J.R., 2016. Consequences of catch-and-release angling for black bream *Spondyliosoma cantharus*, during the parental care period: implications for management. *ICES J. Mar. Sci.* 74, 254-262.

Pollock, K.H., Pine, W.E., 2007. The design and analysis of field studies to estimate catch-and-release mortality. *Fish. Manag. Ecol.* 14, 123-130.

Raby, G.D., Donaldson, M.R., Hinch, S.G., Patterson, D.A., Lotto, A.G., Robichaud, D., English, K.K., Willmore, W.G., Farrell, A.P., Davis, M.W., Cooke, S.J., 2012. Validation of reflex indicators for measuring vitality and predicting the delayed mortality of wild coho salmon bycatch released from fishing gears. *J. Appl. Ecol.* 49, 90–98. doi:10.1111/j.1365-2664.2011.02073.x

Stoot, L.J., Cairns, N.A., Cull, F., Taylor, J.J., Jeffrey, J.D., Morin, F., Mandelman, J. W., Clark, T.D., Cooke, S.J., 2014. Use of portable blood physiology point-of-care devices for basic and applied research on vertebrates: a review. *Cons. Physiol*. 2, 1–21.

Richard, A., Dionne, M., Wang, J., Bernatchez, L., 2013. Does catch and release affect the mating systems and individual reproductive success of wild Atlantic salmon (*Salmo salar* L.)? *Mol. Ecol*. 22, 187–200.

Ruiz Navarro, A., Gillingham, P., Britton, J.R., 2016. Predicting shifts in the climate space of freshwater fishes in Great Britain due to climate change. *Biol. Cons.* 203, 33-42.

Schaeffer, J.S., Hoffman, E.M., 2002. Performance of barbed and barbless hooks in a marine recreational fishery. *N. Am. J. Fish. Manage.* 22, 229-235.

Weltersbach, M.S., Strehlow, H.V., 2013. Dead or alive - estimating post-release mortality of Atlantic cod in the recreational fishery. *ICES J. Mar. Sci.* 70, 864-872.

Wendelaar Bonga, S.E., 1997. The stress response in fish. *Physiol. Rev.* 77, 591-625.

Wilkie, M.P., Brobbel, M.A., Davidson, K.G., Forsyth, L., Tufts, B.L., 1997. Influences of temperature upon the post exercise physiology of Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* 54, 503-511.

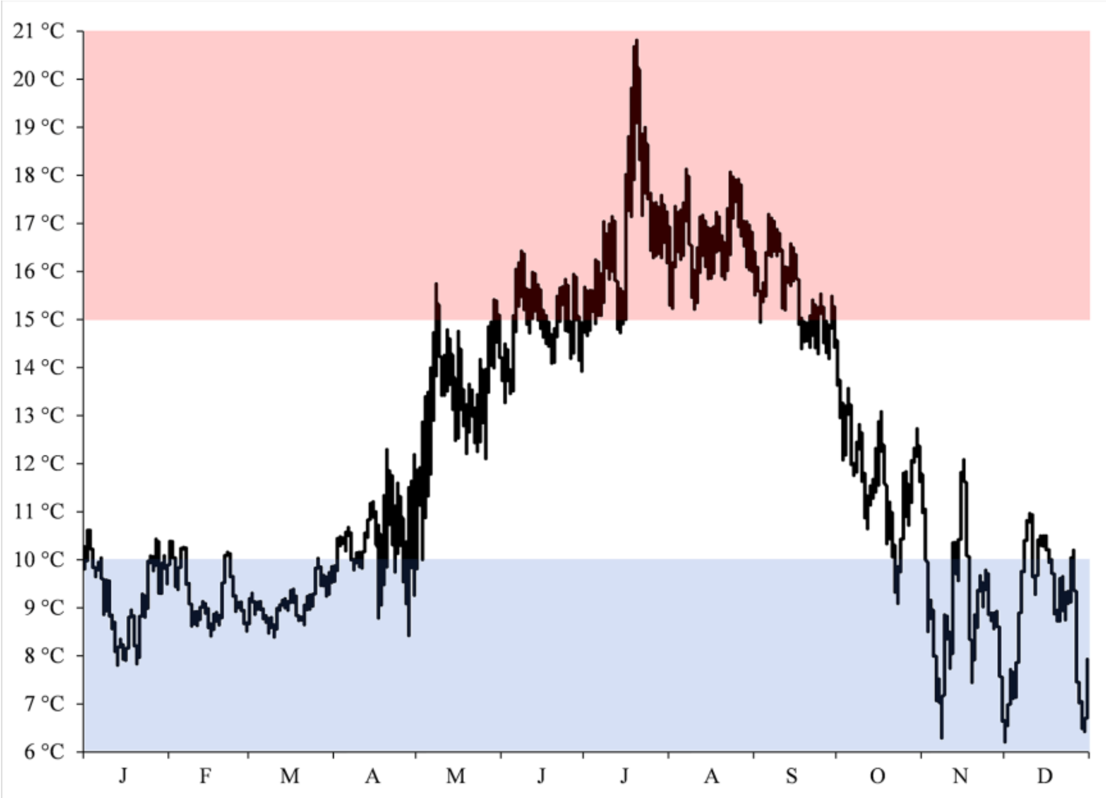
Figure captions

Figure 1. Ambient water temperature recorded in the main channel of the River Frome during 2016. Red and blue shaded areas represent upper (>15 ^{o}C) and lower (<10 ^{o}C) temperature groups respectively.

Figure 2. Relationships of fish length versus fight time (a) and air exposure (b) at water temperatures below 10 ^{o}C (clear circles) and above 15 ^{o}C (filled circles). Lines are significant relationships between variables according to linear regression, where straight line represents the relationship above 15 ^{o}C and dashed line below 10 ^{o}C.

Figure 3. Relationships of fight time (a) and air exposure (b) versus time to body equilibrium at water temperatures below 10 ^{o}C (clear circles) and above 15 ^{o}C (filled circles). Lines are significant relationships between variables according to linear regression, where straight line represents the relationship above 15 ^{o}C and dashed line below 10 ^{o}C. Note a single outlying time to body equilibrium of 1440 s recorded at above 15 ^{o}C is not included on plots, but was used to construct regression lines.

Figure 4. Mean time to body equilibrium (a), mean blood lactate concentration (b) and mean blood glucose concentration (c) per water temperature group, where mean values are adjusted for the effect of fish length, fight time and air exposure as covariates in general linear models and * difference between the temperature groups is significant at $P < 0.01$.



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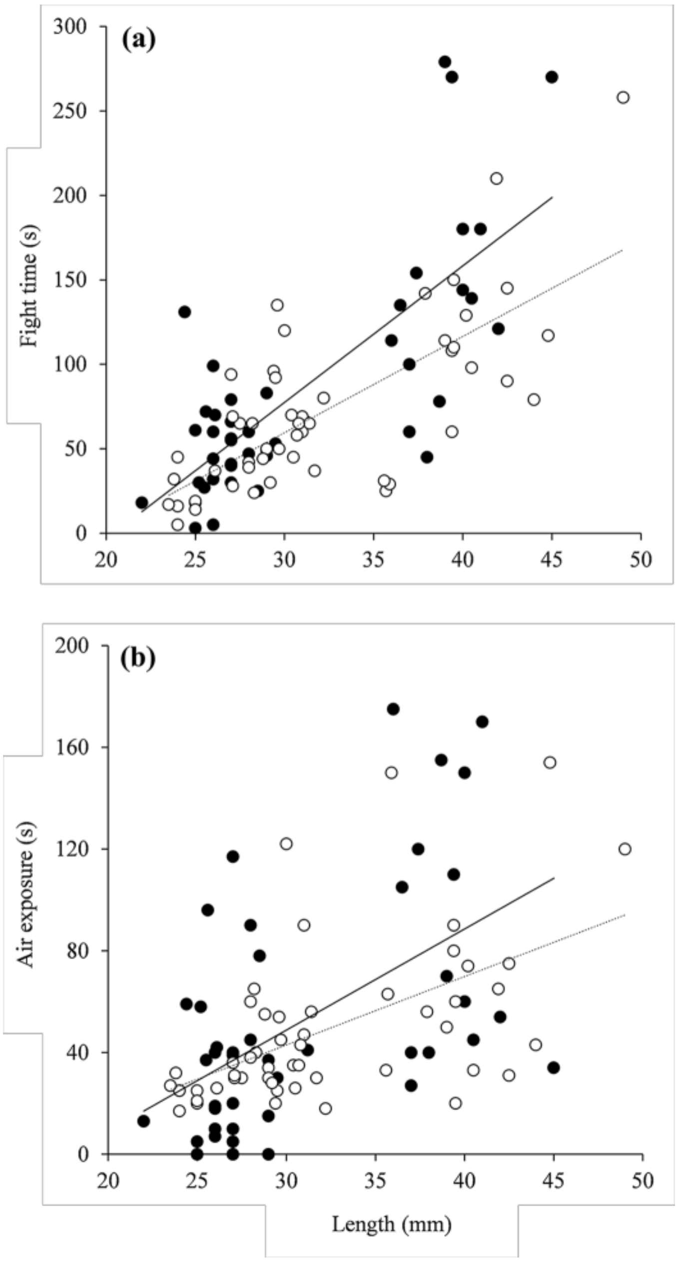

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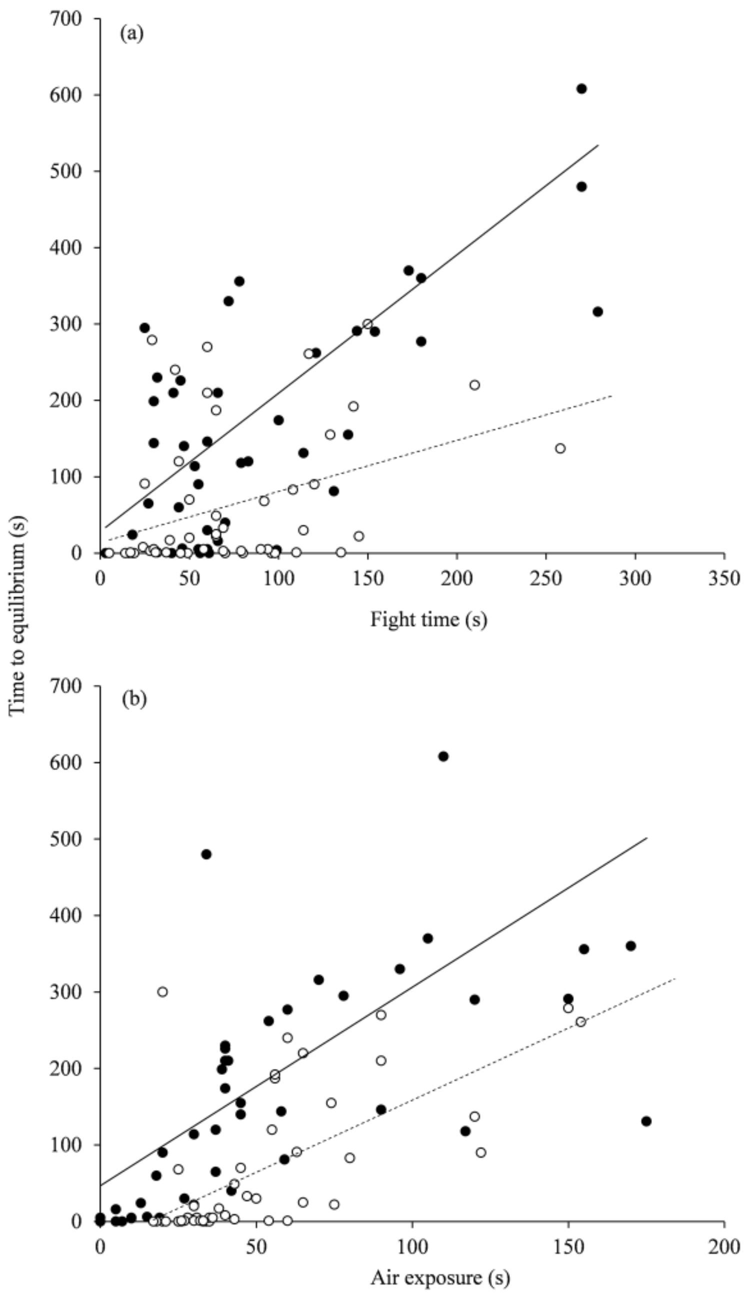

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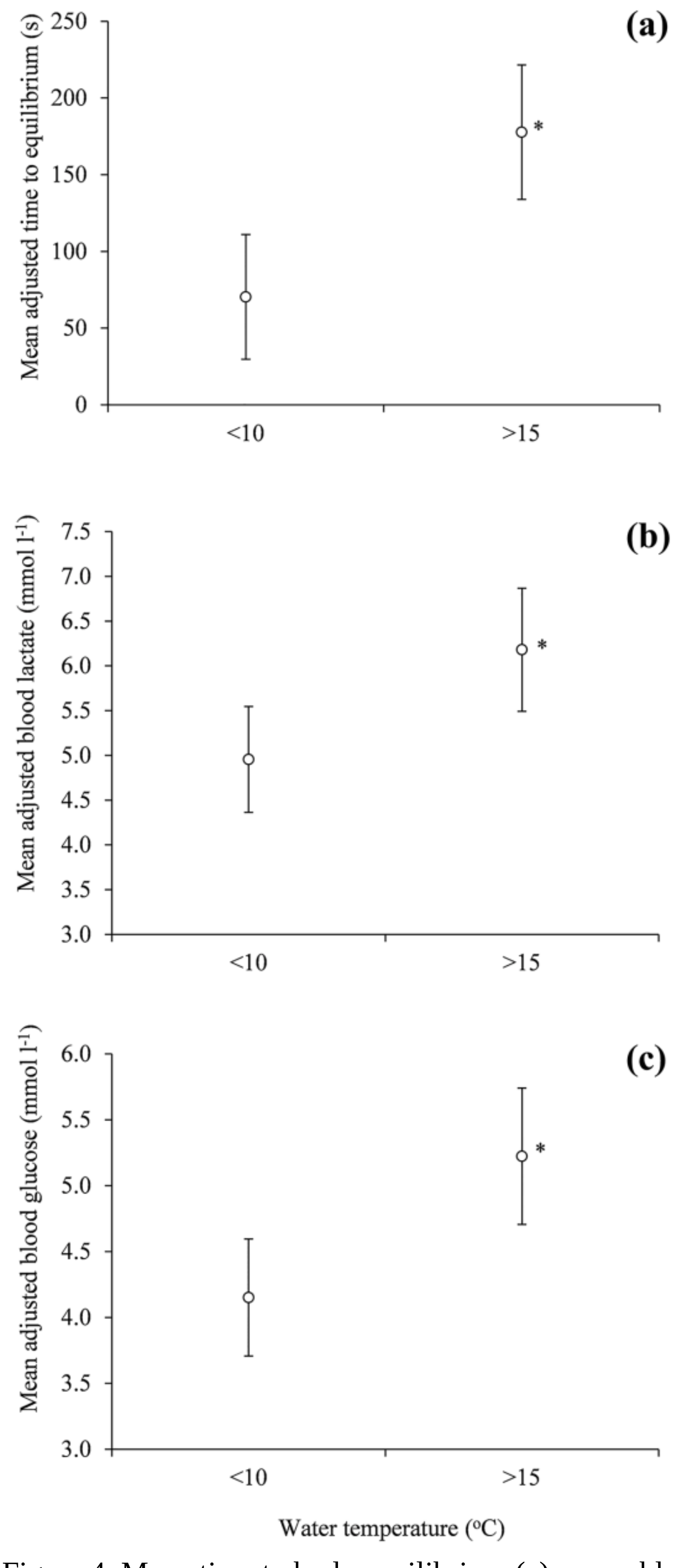

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