

Metabolic response of bluegill to exercise at low water temperature: implications for angling conservation

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Received: 7 April 2018 / Accepted: 14 September 2018 / Published online: 20 September 2018
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Abstract The metabolic response of fish to exercise is highly dependent on environmental factors such as temperature. In addition to natural challenges that force exercise (foraging, avoiding predators, etc.), sportfish species are also subjected to exercise when they are hooked by anglers, leading to metabolic energy costs that may impact fitness. While several studies have examined the physiological response of fish to capture in warm conditions, little work has examined this response under cold winter conditions when fish are targeted by ice-anglers. To fill this gap, we examined the metabolic impacts of exercise duration and air exposure on bluegill, *Lepomis macrochirus*, at a temperature typical for ice angling. Thirty-two bluegill were subjected to a simulated angling session which included either a light (30 s) or exhaustive exercise procedure, followed by either 30 s or 4 min of air exposure. Fish were then assessed at 5 °C for the following metabolic metrics using intermittent-flow respirometry: standard metabolic rate (SMR), maximum metabolic rate (MMR), aerobic scope (AS), recovery time, and excess post-exercise oxygen consumption (EPOC). Fish exercised to exhaustion had higher EPOC compared to lightly exercised fish, however EPOC was not affected

by air exposure time. No other metrics were impacted by air exposure or exercise duration. These results are directly applicable to physiological outcomes for fish captured by ice-anglers during the winter and suggest that both low temperatures and low durations of exercise serve to keep metabolic costs low for fish angled during the winter months.

Keywords Metabolic phenotype · Ice-angling · Respirometry · EPOC · Aerobic scope · Recovery

Introduction

Winter represents a critical time in the life cycle of freshwater fish in temperate regions. Fish populations often face a recruitment bottleneck in winter due to a variety of unique threats, including winterkill as a result of a collapse in oxygen availability underneath winter ice (Tonn et al. 2004; Clement et al. 2015), as well as starvation in individuals that fail to accumulate sufficient energy reserves to persist as food availability declines (Garvey et al. 1998, 2004). Winter at high latitudes is also a time where fish undergo temperature-driven reductions in foraging activity, resulting in a concomitant drop in growth rate (Fullerton et al. 2000; Xu et al. 2010) underpinned by a reduction in metabolic rate (Suski and Ridgway 2009). This can lead to a general reduction in overall performance that can reduce both the ability to capture prey and the ability to escape predatory threats (Sidell and Moerland 1989; Hasler et al. 2009). To optimize performance in winter, fish

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must therefore be able to overcome extremes in their physical environment to respond adequately to the challenges they present.

Freshwater fish are often subjected to periods of intense exercise in their natural environment, both in the winter and in warmer conditions. Burst swimming is often required when pursuing prey, avoiding being eaten by predators, and engaging in social behavior such as shoaling and territory defense (Wood 1991; Wakefield et al. 2004; Killen et al. 2015). This exhaustive exercise consumes oxygen and exhausts energetic reserves in the form of adenosine triphosphate (ATP) and phosphocreatine (PCr) (Wood 1991; Kieffer et al. 1994; Kieffer 2000). During activity, metabolic rate rises from the standard metabolic rate (SMR) to a maximum metabolic rate (MMR), with the difference between them termed aerobic scope (AS) (Fry 1971). Higher AS is typically associated with a greater capacity for prolonged exercise, as well as greater overall performance (Killen et al. 2014; Binder et al. 2016). Anaerobic metabolism is also activated during burst swimming, which leads to the accumulation of metabolites such as lactate as well as metabolic ions in the blood and tissues (Reidy et al. 2000; Svendsen et al. 2010; McKenzie 2011). Following exercise, elevated oxygen consumption continues as homeostasis is restored. This excess post-exercise oxygen consumption (EPOC) represents the metabolic “costs” above and beyond the energy used during the exercise bout itself. When these costs, and/or the costs incurred during exercise are high enough, energy reserves can be depleted which can impact performance during subsequent challenges (Nedrow et al. 2001; Lee et al. 2003). Collectively, the physiological disturbances resulting from exercise can not only lead to a short-term risk of mortality (Suski et al. 2007; Brownscombe et al. 2015), but can impact fish performance over a longer time scale.

Abiotic conditions, particularly ambient temperature, are known to affect performance during both anaerobic and aerobic exercise, ultimately impacting the degree of physiological disturbance (Kieffer et al. 1994; Suski et al. 2006). During aerobic exercise at low temperatures AS is significantly reduced in fish, which can lead to a concomitant reduction in sustained swimming performance (Brett 1964; Portner and Knust 2007; Pang et al. 2010). This reduction in AS can also cause fish to recruit anaerobic metabolism to power swimming relatively early in an exercise bout (Sidell and Moerland 1989). While anaerobic metabolism is indeed utilized by burst-swimming fish in winter, the overall anaerobic capacity

of fish for exercise at low temperatures is still reduced, though to a lesser degree than aerobic capacity (Brett 1964; Wieser et al. 1986). This, coupled with reductions in both muscle contractile properties and the activity of enzymes dedicated to mobilizing energy resources, leads to reduced overall swimming performance in fish at low temperatures (Sidell and Moerland 1989). While this reduction may negatively impact the ability of a fish to perform life-sustaining tasks such as foraging and predator avoidance, it may also cause the degree of physiological disturbance following exercise to be reduced as well (Davis 2004; Gale et al. 2011). For temperate freshwater fish species, the physiological impacts of intense exercise may therefore vary seasonally, as dictated by fluctuating water temperatures.

One situation in which fish are forced to vigorously exercise at winter temperatures is found in the case of sportfish that are hooked and captured by winter ice-anglers. While swimming performance and metabolic rates are lower in winter for many fish species, some species maintain physical activity at low temperatures, which leaves them vulnerable to capture by ice-anglers utilizing baits suspended through the surface of ice-covered lakes (Cooke et al. 2003). Just as in summer, capture during winter involves a period of burst exercise, followed by a period of air exposure while the fish is handled by the angler after capture (Cooke et al. 2002; Arlinghaus et al. 2009). A significant proportion of captured fish are released by ice-anglers (Dubois et al. 1994; Margenau et al. 2003), making physiological disturbance after capture relevant to post-release mortality. Catch-and release angling presumes that released fish will survive to be captured later (Policansky 2008); however, a large body of work shows that fish can experience a host of physiological disturbances following release (Cooke and Suski 2005; Bartholomew and Bohnsack 2005; Gingerich et al. 2007). These disturbances can be exacerbated by prolonged angling fight times, as well as long periods of air exposure following landing (Meka and McCormick 2005; Cook et al. 2015; Suski et al. 2007). In all cases, these disturbances trigger excess post-exercise oxygen consumption EPOC during recovery, with abiotic conditions such as temperature possibly impacting both the magnitude of disturbance and the course of recovery (Wilkie et al. 1997; Louison et al. 2017b). While disturbance and recovery from an angling event has been well-studied at warm conditions, far less research has examined the impacts of angling at cold, winter conditions.

The goals of this study were to quantify metabolic phenotype in fish at winter temperatures, and to evaluate how the duration of exercise and subsequent air exposure during a simulated angling bout could impact post-exercise metabolic costs as well as recovery rates in fish. To accomplish these goals, bluegill *Lepomis macrochirus* were chosen as a focal species. Bluegill were chosen because they are a very common target species for ice-anglers throughout much of the Eastern and Central United States and Canada, and also are often frequently released following capture (Gaeta et al. 2013; Louison et al. 2017b). The metabolic response of bluegill to simulated angling was assessed using intermittent flow respirometry, a method that allows for a continuous evaluation of metabolic cost over the course of several hours, as opposed to tests for blood-based stress indicators (such as cortisol or glucose) that generate only a snapshot of disturbance levels at the point of sampling (Arlinghaus et al. 2009). We hypothesized that capture and handling would result in differences in the metabolic costs incurred by the simulated angling, specifically that longer periods of both exercise and air exposure would increase metabolic costs and prolong recovery time in bluegill. Results from this study will be useful in guiding recommendations as to best handling practices to reduce physiological disturbance in caught-and-released fish at winter temperatures.

Methods

Fish holding and husbandry

Bluegill used in this study were acquired in the fall of 2015 from Jake Wolf Fish Hatchery in Topeka, IL, and transported to the Illinois Natural History Survey's Aquatic Research Facility in Champaign, IL. At the research facility, bluegill were held in a single 0.04 ha earthen-bottom pond. The pond featured naturally occurring macroinvertebrate food items, and was also stocked periodically with fathead minnows *Pimephales promelas* for additional forage. On 19 January 2017, the pond was drained and 32 bluegill (total weight = 151.3 ± 5 g standard error of the mean, SEM) were retrieved. After draining, all fish were held for five days in a single, common 1175 L circular holding tank in an indoor wet lab, which provided time for the fish to recover from pond draining and handling. The common

holding tank contained an air stone for aeration, along with heater-chillers (Teco®, Ravenna, Italy) that maintained water temperatures between 5 ° and 6 °C, a temperature that matched the ambient conditions for fish held in the earthen-bottom ponds. Air temperatures in the lab varied with outdoor ambient temperatures, ranging from 3 ° to 8 °C. During holding, fish were fed daily with frozen bloodworms (Chironomidae) acquired from a local pet store.

Study treatments and metabolic rate

Respirometry to quantify metabolic phenotype took place from 24 January to 4 February 2017. On the afternoon prior to respirometry, sets of four fish were collected from the common holding tank, weighed to the nearest 0.01 g, and then isolated in 30 L opaque plastic containers perforated with small holes immersed within the holding tank, which allowed for water exchange while preventing food items from entering. Daily feeding of the remaining fish in the holding tank took place after the set of 4 was first isolated, thus ensuring that isolated fish had not been fed within 48 h of respirometry (the 24 h between their last feeding and being isolated, followed by the 24 h in isolation), minimizing the impact of digestion or specific dynamic action on metabolic rate (Castanheira et al. 2011; Nelson and Chabot 2011). Water temperature in all tanks was kept near 5 °C using the same heater chillers described above (daily mean temperature = 5.1 ± 0.3 °C). This temperature was chosen as it is near the temperature at which water under ice frequently stabilizes, making it representative of ice-angling conditions (Mishra et al. 2011). Additionally, during pilot trials, bluegill were lethargic and showed minimal swimming ability when forced to exercise at temperatures below 5 °C.

After 24 h of isolation in the opaque plastic containers, each fish was removed and subjected to a combination of exercise and air exposure prior to loading into respirometry chambers. The exercise protocol consisted of first quickly netting a fish from its isolation container and placing it into a 190 L plastic tote filled with water identical to that of the common holding tank. The fish was then exercised via “tail pinching”, whereby an observer attempted to grab the tail of the fish forcing it to burst to escape, a protocol that has been used to simulate angling previously (Galloway and Kieffer 2003; Arlinghaus et al. 2009; McArley and Herbert

2014; Clark et al. 2017). Each fish was randomly assigned to either be “lightly” exercised for a period of 30 s, or exercised to exhaustion (as indicated by a lack of ability to continue burst swimming away from the observer). These exercise durations were chosen as they represent angling situations in which fish were either landed quickly or fought to exhaustion (Louison et al. 2017a, b). All fish in the exhaustive exercise treatment became exhausted in under 3 min. Following the exercise protocol, bluegill were netted from the exercise tank and subjected to air exposure for either a “short” period of 30 s, or a “long” period of 4 min. These air exposure treatments were based on air exposure times used in prior work on winter angling and are representative of situations in which an angler either handles a fish quickly, or holds the fish out of the water in order to take photographs or take additional time to decide whether or not to release it (Louison et al. 2017a). Together, these two exercise durations (light or exhaustive) and the two air exposure treatments (short and long) were employed in a full 2×2 factorial design, with $N=8$ bluegill for each experimental block. Fish weight did not vary across experimental blocks (one-way analysis of variance, ANOVA $F_{3,18}=0.41$; $P=0.74$, Fig. 1f). Following exercise and air exposure, fish were loaded into one of four 5.26 L respirometry chambers (Loligo Systems®, Tjele, Denmark).

Intermittent-flow respirometry was used to quantify metabolic metrics based on previously established protocols (Killen et al. 2015; Louison et al. 2018) with a few modifications. Respirometry chambers were immersed in an 1175 L rectangular stock tank, and dissolved oxygen concentration in each chamber was measured every 2 s using a fiber-optic dissolved oxygen dipping probe (100 mm probe tip length, Loligo Systems®, Tjele, Denmark). The measurement cycle was set for a 7 min flush phase, a 5 min wait (when the recirculation pump is running but no measurements are taken, this phase allows for water to be adequately mixed in the probe chamber before measurements begin) and a 45 min measurement phase, which allowed for a single measurement of mass-specific oxygen consumption (MO_2 , in $mg\ O_2$ consumed $kg\ fish^{-1}\ h^{-1}$) every 57 min (0.95 h). During the measurement phase, dissolved oxygen saturation remained above 80% at all times for all fish. This MO_2 value was based on a regression line between time (X axis) and dissolved oxygen concentration (Y axis), and calculated automatically (AutoResp software, Loligo Systems®, Tjele,

Denmark) using the following equation (Rosewarne et al. 2016):

$$MO_2 = k V \beta M^{-1}$$

where k is the rate of decline in oxygen concentration over time, V is the volume of the respirometry chamber minus the volume of the fish, β is the solubility of oxygen based on the temperature of the water, and M is the body mass of the fish. While the length of the measurement phase used in this study is long relative to prior studies, due to the low rates of oxygen consumption (see results) DO saturation in the chambers never declined below 80% during any cycle. Fish were left in the chambers overnight to recover from exercise, and then removed from chambers the following morning. To reduce potential background oxygen consumption between trials, chambers and equipment were soaked in a 10% bleach solution after each trial, and pilot trials indicated that background MO_2 was near zero.

Standard metabolic rate (SMR) was determined as the lowest tenth percentile of recorded MO_2 values, maximum metabolic rate (MMR) was defined as the highest single post-exercise MO_2 measurement, while aerobic scope (AS) was calculated as the difference between MMR and SMR (Killen et al. 2015). Routine metabolic rate (RMR) was calculated using the average of all MO_2 values, excluding the first two measurements (taken in the first 2.24 h) when MO_2 values were at their most elevated post treatment (Killen et al. 2014). The calculation of RMR then allowed for a determination of excess post-exercise oxygen consumption (EPOC) for each fish. For EPOC calculations, a 6th order polynomial regression curve (Killen et al. 2015) was plotted through all MO_2 measurements over the trial, and EPOC was calculated by integrating the area between the curve and RMR up until the point where the recovery curve intersected with RMR. Recovery time (T_s) was set as the length of time needed for each fish to recover 50% of its aerobic scope (Killen et al. 2015).

Statistical analysis

To quantify the effects of exercise and air exposure on MMR, SMR, AS, recovery time, and EPOC, separate two-way ANOVAs were run for each component of metabolic phenotype. For each ANOVA model, the metabolic metric of interest was set as the dependent

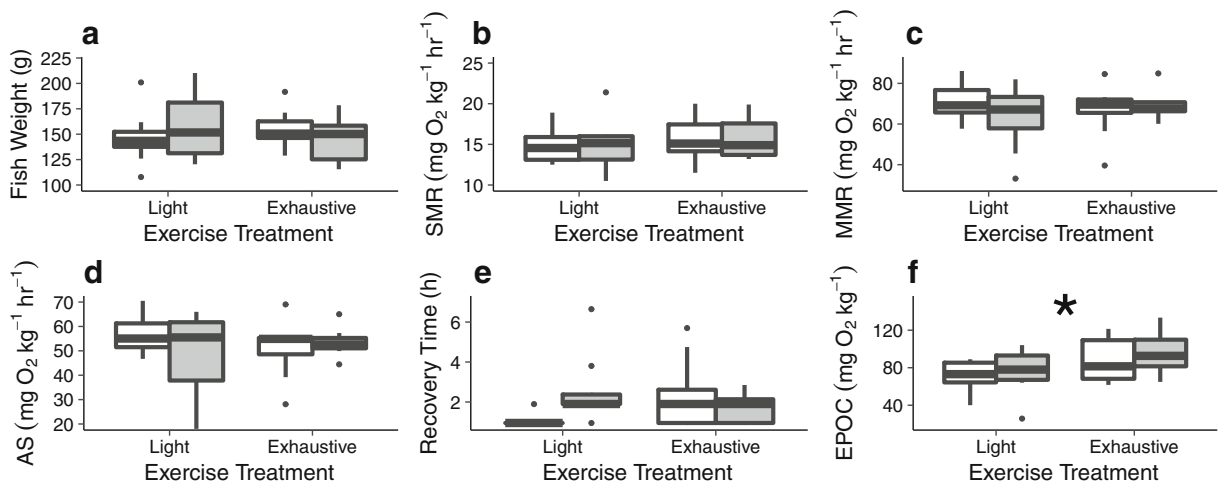


Fig. 1 Boxplots detailing means for (a) Fish Weight, b Standard Metabolic Rate (SMR), c Maximum Metabolic Rate (MMR), D) Aerobic Scope (AS), E) Recovery Time, and F) Excess Post-exercise Oxygen Consumption (EPOC), for bluegill following exercise and air exposure, using intermittent-flow respirometry. Data are broken down by exercise treatment (exhaustive vs. 30 s

light exercise) and air exposure treatment (open bars represent 30 s air exposure, gray bars represent 4 min air exposure). The asterisk over panel F indicates a significant difference between exercise treatments for EPOC, with exhaustively exercised fish having a significantly higher value

variable, with exercise time (light or exhaustive), air exposure time (short or long), and their interaction entered as fixed factors. Fish weight did not differ between treatment blocks (ANOVA, $F_{3,18} = 0.41$, $p = 0.74$; Fig. 1a), and did not significantly predict any metabolic metrics when included in the models ($p > 0.08$ for weight in all cases) and as such weight was not included as a covariate in our final models. In the event of a statistically significant interaction, main effects were ignored and between-block comparisons were made with Tukey Honest Significant Difference (HSD) tests. For all ANOVAS, normality of the data was assessed with Shapiro-Wilk tests, while homogeneity of variances was assessed with Levene’s tests. Significance (α) for each ANOVA was set at $P = 0.05$, and all values are shown ± 1 SEM where appropriate. All analyses were conducted in R version 3.3.2 (R Core Team, Vienna, Austria).

Results

The SMR of bluegill held at 5 °C averaged 15.1 mg O₂ kg⁻¹ h⁻¹, and, following exercise, MMR was measured at 67.6 mg O₂ kg⁻¹ h⁻¹ on average. Neither SMR nor MMR differed significantly across exercise or air exposure treatments, nor was the interaction between those factors significant (Fig. 1b, c). Similarly, AS, defined as the difference between MMR and SMR, ranged from

18.0 to 70.5 mg O₂ kg⁻¹ h⁻¹ across individuals, but did not vary across exercise or air exposure treatments, again with no significant interaction (Fig. 1d; Table 1). The time required for bluegill to recover 50% of aerobic scope following exercise and air exposure treatments ranged from 0.95 to 6.65 h across individuals, and, again, did not differ across any treatment group (Fig. 1e; Table 1).

Unlike other metabolic variables, EPOC was significantly influenced by exercise time (Fig. 1f, Table 1). On average, bluegill EPOC was found to be 83.24 mg O₂ kg⁻¹, and bluegill that were exhaustively exercised required 25.5% more oxygen during recovery than animals exercised for 30 s (Fig. 1f). This difference occurred independent of air exposure duration (Table 1). The difference in EPOC between exercise treatments, however, was not due to any specific differences in SMR, MMR, or recovery time (Fig. 2). While a significant interaction between air exposure and exercise time was found for recovery time (Table 1), post-hoc tests revealed no specific between-block differences (Tukey HSD, $p > 0.12$ for all pairwise comparisons).

Discussion

Exercise and air exposure treatments had no impact on the SMR, MMR, or aerobic scope of bluegill, indicating a degree of resilience to angling stress at cold

Table 1 Statistical outputs from two-way analyses of variance (ANOVAs) assessing the effects of exercise time, air exposure time, and their interaction on metabolic variables in bluegill assessed for metabolic rate using intermittent-flow respirometry

Metabolic Variable	Main Effect	Df	F	<i>p</i> value
SMR (mg O ₂ kg ⁻¹ h ⁻¹)	Exercise Time	1,28	1.37	0.25
	Air Exposure Duration	1,28	0.04	0.83
	Exercise × Air Exposure	1,28	0.002	0.96
MMR (mg O ₂ kg ⁻¹ h ⁻¹)	Exercise Time	1,28	0.03	0.84
	Air Exposure Duration	1,28	0.39	0.53
	Exercise × Air Exposure	1,28	1.56	0.22
AS (mg O ₂ kg ⁻¹ h ⁻¹)	Exercise Time	1,28	3.78	0.06
	Air Exposure Duration	1,28	1.21	0.28
	Exercise × Air Exposure	1,28	0.26	0.61
EPOC (mg O ₂ kg ⁻¹)	Exercise Time	1,28	5.55	0.02
	Air Exposure Duration	1,28	0.74	0.39
	Exercise × Air Exposure	1,28	0.14	0.70
	Recovery Time (h)	Exercise Time	1,28	0.24
Recovery Time (h)	Air Exposure Duration	1,28	0.97	0.37
	Exercise × Air Exposure	1,28	4.95	0.03

Significant main effects are given in **bold**

temperatures that could be due to reductions in metabolic rate at these temperatures. Indeed, a separate study on bluegill using virtually identical methodology at 24 °C recorded SMR for bluegill at 90 mg O₂ kg⁻¹ h⁻¹, and AS at 117 mg O₂ kg⁻¹ h⁻¹ (Louison et al. 2018), values far higher than what was recorded in the present study (mean SMR = 15.1 mg O₂ kg⁻¹ h⁻¹, mean AS = 52.5 mg O₂ kg⁻¹ h⁻¹). Further work on

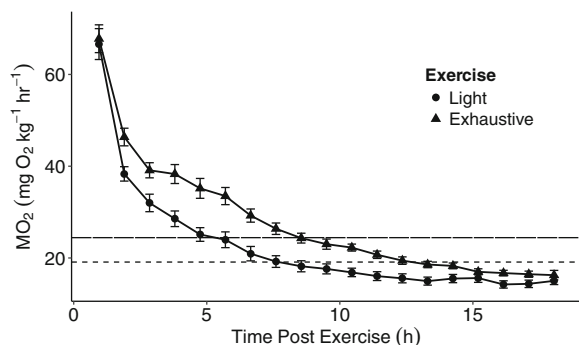


Fig. 2 Average MO₂ for bluegill either exercised lightly (30 s) or to exhaustion, as measured by respirometry following exercise and air exposure. The long dashed line (—) represents mean RMR for exhaustively exercised fish, while the short dashed line (---) represents mean RMR for lightly exercised fish. Both the curves indicating MO₂ recovery and the lines representing RMR are averaged across air exposure treatments within each respective exercise treatment. Post-exercise Oxygen Consumption (EPOC) was significantly higher for exhaustively exercised fish

bluegill, albeit on smaller individuals, has also recorded far higher values for aerobic metabolic metrics at 24 °C [SMR ~ 175 mg O₂ kg⁻¹ h⁻¹, AS ~ 300 mg O₂ kg⁻¹ h⁻¹; (Binder et al. 2016)]. Bluegill are a eurythermal, temperate species that inhabits waterbodies that experience ice cover in the winter, when they undergo concomitant reduction in SMR (Beitinger and Fitzpatrick 1979; McDermot and Rose 2000). While some eurythermal fishes (cyprinid fishes in particular) can undergo plastic changes to their physiology to maintain aerobic capacity in both red and white muscle at low temperatures (Sidell and Moerland 1989), many aspects of muscle performance are negatively impacted in temperate fish. These include anaerobic enzyme activity (e.g., lactate dehydrogenase, Thibault et al. 1997) as well as the contractile properties of muscle (Sidell and Moerland 1989; Guderley 2004). This in turn leads to a reduction in aerobic and anaerobic swimming capacity at cold temperatures (Sidell and Moerland 1989; Hasler et al. 2009; Pang et al. 2015), which contributes to a reduction in aerobic scope for cold-acclimated fish relative to warmer temperatures (Portner and Knust 2007). Indeed, pilot trials with bluegill at 2 °C could not be completed due to the inability of fish to burst swim away from an observer during exercise, indicating that at this temperature aerobic scope and capacity for exercise are eliminated. At 5 °C however, bluegill are still sufficiently metabolically active to escape

from chasing, and are also likely to be able to perform tasks such as foraging (Shoup and Wahl 2008). In sum, SMR and AS were both not impacted by exercise and air exposure in the temperate bluegill at 5 °C, reflecting a general dampened impact of angling stress when fish are captured at these temperatures.

At winter temperatures, exhaustive exercise led to 25% higher post-exercise metabolic costs than light exercise. Regardless of treatment however, EPOC in the present study was lower ($83.2 \text{ mg O}_2 \text{ kg}^{-1}$) than that recorded for bluegill at 24 °C ($171 \text{ mg O}_2 \text{ kg}^{-1}$, Louison et al. 2018). During exhaustive exercise, anaerobic energy stores such as ATP, PCr and glycogen are depleted to fuel swimming, while byproducts of anaerobic metabolism including lactate and metabolic protons are produced (Segal and Brooks 1979; Wood 1991; Kieffer 2000; Suski et al. 2006). The magnitude of the disturbances generated by this activity has been shown to correlate positively with the intensity and/or duration of exercise (Meka and McCormick 2005; Raby et al. 2015). Excess post-exercise oxygen consumption is dedicated, in part, to restoring homeostasis by returning levels of these anaerobic byproducts to pre-exercise levels (Scarabello et al. 1991; Wood 1991) that were likely greater for animals that experienced longer exercise durations (Scarabello et al. 1991), though in an experimental setting increased oxygen consumption after exercise could be partially due to the stress of handling and confinement rather than the exercise treatment per se. Interestingly, despite reductions in swimming performance and ATP generation (Sidell and Moerland 1989), it has been shown that lactate production is not reduced at cold temperatures in studies of rainbow trout *Oncorhynchus mykiss* (Kieffer et al. 1994) or Atlantic salmon *Salmo salar* (Wilkie et al. 1997) exercised from 5 to 18 ° and 12–23 °C, respectively. The maintenance of lactate production at cold temperature can likely be explained by the fact that white muscle fibers are recruited at lower swimming velocities in cold water compared to higher swimming velocities in warm water, likely resulting in increased lactate production for animals exercised at cold temperatures, even though overall anaerobic capacity is reduced (Rome et al. 1984; Sidell and Moerland 1989). At cold temperatures therefore, bluegill undergoing activities such as foraging, escaping predation, or being angled will likely experience a reduction in aerobic and anaerobic capacity, though anaerobic metabolism requiring post-exercise oxygen consumption will still be possible.

The recovery time required following exercise for bluegill was similar to prior results from bluegill exercised at warm temperatures, despite a lower degree of overall disturbance. Prior work on bluegill at 24 °C found that it took, on average, 1.85 h to recover 50% of AS (Louison et al. 2018), a value very similar to the 1.95 h it took on average for bluegill to recover in the present study. Recovery involves the return of numerous metrics (cortisol, lactate, oxygen consumption rates, glycogen stores, etc.) back to baseline levels following an exercise bout, and it typically takes 4–24 h for physiological metrics to return all the way to baseline, depending on the variable being examined (Milligan 1996). Notable in the comparison of the present results with Louison et al. (2018) was the far lower aerobic scope recorded in the present study (i.e. bluegill did not have as much aerobic scope to recover after treatment). As a result, while a similar duration of time was required for bluegill to recover at 5 °C, because the degree of metabolic disturbance was lower, that recovery rate (in terms of MO_2 recovered per unit time) was slower in the present study. This reduction in the rate of MO_2 recovery is likely related to reduced enzymatic and ion-channel activity at cold temperatures, which prevents levels of lactate and ions from being restored to baseline as quickly, and in turn requires oxygen consumption to remain elevated over RMR (Galloway and Kieffer 2003; Hyvarinen et al. 2004; Suski et al. 2006). As described above however, the slow recovery rate is made up for by the low degree of physiological disturbance overall. As a result, the duration of time for metabolic parameters to return to baseline following exercise appears to be similar for fish exercised at cold temperatures as compared to warm temperatures, likely due to a restriction in the amount of energy used by a fish during an exercise bout.

Exercise and air exposure did not influence any aspect of metabolic measures or recovery time for bluegill exercised at 5 °C. This result, while contrary to prior work documenting significant physiological disturbance at warmer temperatures, is in line with a newer body of work on winter captures that has shown physiological disturbance to be rather muted, even after extended air exposure. Work on ice-angled northern pike *Esox lucius*, for example, detected no difference in cortisol or lactate production between fish subjected to either 0, 2, or 4 min of post-capture air exposure, either 30 min or 4 h following capture (Louison et al. 2017a), a result that mirrors the lack of an effect of air exposure on metabolic

metrics measured in the present study. In addition, mortality rates for walleye *Sander vitreus* were very low following ice angling, regardless of hooking method (Twardek et al. 2018). While the mechanism explaining this muted response to capture is not immediately clear, it is possible that the slowing of metabolism as a result of cold temperature (O'Hara 1968; Evans 1984) rendered bluegill resilient to air exposure duration, a finding that has been reported in other work. For example, Meka and McCormick (2005) showed that the combined effects of fight and air exposure time on levels of plasma cortisol and lactate was reduced when rainbow trout were captured from relatively cool water (9.8 °C) relative to warmer water (13.2 °C). The results of the present study show indicate that this effect may extend to even colder temperatures, as longer air exposure duration did not lead to increased metabolic costs or a prolonged recovery duration.

Collectively, results from the current study have a number of implications for bluegill angled and released at cold, winter temperatures. Most notably, the current results indicate that the metabolic costs of capture may be low in ice-angled fish relative to summer-angled fish. Metabolic costs were reduced even further in fish that were lightly exercised compared to those that were exercised to exhaustion. Because freshwater fish captured through the ice are often subjected to shorter fight times compared to summer angling (Louison et al. 2017a), ice-angling practices appear to play a role in reducing physiological disturbance. In addition, ice-angled bluegill may be more physiologically resilient to air exposure compared to individuals captured at warmer temperatures, though several unanswered questions require further study before more liberal best practices for handling angling fish in winter conditions can be advanced. First, the degree of physiological disturbance caused by air exposure may vary depending on species in that other species may be more sensitive to air exposure when angled in winter (Cooke and Suski 2005; Pottinger 2010). Second, air exposure in sub-freezing conditions may leave a captured fish at greater risk of cold-related injury such as freezing of eyes, gills or other soft tissues (Warrenchuck and Shirley 2002). Because bluegill in the present study were air exposed at temperatures above freezing, these potentially negative consequences of air exposure may not have been realized. Until these unanswered questions are addressed, it is advised that ice-anglers utilize more conservative best practices of minimizing air exposure for captured fish in

a similar fashion to in warmer conditions (Cook et al. 2015). Regardless of the effects of air exposure, low metabolic costs, combined with similar recovery durations (Binder et al. 2016) indicates that physiological disturbance is low when bluegill are captured at cold, winter temperatures. Future studies are needed in order to determine if delayed recovery from disturbance at cold temperatures (Davis 2004; Louison et al. 2017b) is associated with a prolonged period of behavioral impairment in angled fish. In addition, future work will be needed to assess whether this lower level of disturbance correlates with lower levels of mortality, and to determine how capture and handling procedures may interact with environmental conditions to determine final outcomes for released fish.

Acknowledgements The authors would like to thank Justin Rondón for his assistance with all aspects of executing the experiments. This research was funded by Federal Aid in Sport Fish Restoration Project via the United States Fish and Wildlife Service, project F-69-R to J.A.S. All research was performed in accordance with guidelines established by the University of Illinois at Urbana Champaign Institutional Animal Care and Use Committee (IACUC), protocol # 15169.

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