# Influence of water temperature and net tending frequency on the condition of fish bycatch in a small-scale inland commercial fyke net fishery 

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#### Abstract

To date, most studies of commercial fisheries bycatch have focused on mortality at time of capture as an endpoint. However, sub-lethal indicators of organismal condition have the potential to reveal mechanisms associated with mortality (both at time of capture and post-release) and opportunities for improving fish welfare. In this study, we simulated commercial fishing efforts in inland lakes with fyke nets during a typical fishing season (early April to late June) in southeastern Ontario, Canada, where bycatch of non-target fish species had previously been documented. Using non-target gamefish (i.e., largemouth bass [Micropterus salmoides, Lacépède], northern pike [Esox Lucius, L.]), as well as a target species (i.e., bluegill [Lepomis macrochirus, Rafinesque]), we examined the sub-lethal consequences of capture (e.g., blood physiology, reflex impairment, and injury) and compared the effects of being retained in the net for two different durations (i.e., two or six days) over a range of water temperatures (i.e., 3-28 ${ }^{\circ} \mathrm{C}$ ). Sublethal physiological disturbances (i.e., blood glucose and lactate) in largemouth bass and bluegill tended to be greater at higher water temperatures. However, fish retained for six days generally did not exhibit greater stress than those retained for two days, with the exception of plasma glucose in largemouth bass. Reflex impairment was similar among temperature and retention periods. Fish retained in nets experienced a range of injuries (including fin frays, scale loss, and mouth damage) that had the potential to facilitate the development of opportunistic pathogenic infections. Greater incidences of injury on fish bycatch tended to be associated with higher temperatures and longer retention. To reduce physiological disturbances and injury that could lead to delayed mortality, we suggest that regulations for inland commercial fishers require them to check their nets more frequently as water temperatures increase. We suggest that future studies of bycatch incorporate sub-lethal endpoints given that they serve as an objective measure of fish welfare and can provide quantitative mechanistic information to support management actions.


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## Introduction

In inland commercial fisheries, gill nets and fyke nets (and other similar passive entrapment gear such as trap nets and pot traps) are the most commonly used fishing gears (Hubert 1996). Because fish entangled in gill nets typically die or are injured upon removal, gill net collection is not recommended in instances where non-target organisms occur with target species (Hopkins \& Cech

[^0]1992). On the other hand, organisms (both target and bycatch) captured in fyke nets or other entrapment gears are often alive, enabling selective fisheries whereby bycatch is released back into the water. Bycatch can include sub-legal sized fish of the target species (e.g., paddlefish, Polyodon spathula, Walbaum; Dieterman et al., 2000), non-target fish species (e.g., black bass, Micropterus spp., and northern pike, Esox Lucius, L.; Larocque et al., 2012), and non-fish taxa (e.g., turtles; Barko et al. 2004; Larocque et al. 2012; platypus, Ornithorhynchus anatinus, Shaw; Grant \& Temple-Smith 2003). Although several studies have quantified the incidence of bycatch as well as mortality that occurs within the nets (e.g., Bettoli \& Scholten 2006; Larocque et al. 2012; Schorfhaar \& Peck 1993), little is known about the sub-lethal consequences of being captured and discarded. To our knowledge, there are no studies that
address this issue in inland commercial fisheries which is in contrast to the marine realm where issues related to the sub-lethal consequences of being captured and discarded are comparatively well studied (Raby et al. 2011). Knowing whether there are sublethal consequences to entrapment is important in a management context, because current harvest strategies assume that released bycatch survives with no fitness or welfare impacts (Diggles et al. 2011; Hall 1996). Sub-lethal endpoints can also be used to identify opportunities to improve fishing policy and practices (Wikelski \& Cooke, 2006; Young et al. 2011).

In southeastern Ontario, Canada, a small-scale inland commercial fyke net fishery operates on a variety of lakes and large river systems. Commercial fishers target a variety of species such as lepomid sunfish (Lepomis spp.), bullheads (Ameiurus spp.), yellow perch (Perca flavescens, Mitchell), and suckers (Catostomus spp.) (Burns 2007). Fishing regulations require that non-target gamefish such as largemouth bass (Micropterus salmoides, Lacépède), smallmouth bass (Micropterus dolomieu, Lacépède), walleye (Sander vitreus, Mitchell), northern pike, and muskellunge (Esox masquinongy, Mitchell) be released. In a recent study, Larocque et al. (2012) demonstrated that non-target fish represent up to $13 \%$ of the fish captured. In particular, largemouth bass (up to $11 \%$ of total catch) and northern pike (up to $3 \%$ of total catch) were identified as being particularly common bycatch species with up to $0.5 \%$ and $15.6 \%$ immediate mortality at time of net tending, respectively.

Current commercial fishery regulations in eastern Ontario (managed by the Ontario Ministry of Natural Resources) mandate that nets be checked and processed at least once per week (Larocque et al. 2012). Changing the net tending frequency seasonally (i.e., shorter sets during warmer periods and longer sets during cooler periods) is a possible bycatch management strategy, but until recently there has been little biological data to assist with decision making. Mortality rates of both target and non-target fish species are lower when nets are checked and processed after two days, when compared to nets set for six days (Larocque et al. 2012). While water temperature was not found to be predictive of immediate mortality by Larocque et al. (2012), it is a primary factor influencing the sub-lethal stress associated with recreational catch-and-release fishing events (Cooke \& Suski 2005), and may influence the behavior and mortality of fish released from the commercial fishery (Davis 2002). Moreover, duration of retention in the net and water temperature may influence the extent and severity of injury, as well as the consequences of such injury. For example, longer net retention would allow fish more time to interact with the net and other organisms in the net, and thus potentially sustain injuries. Water temperature is known to modulate fish activity levels (Fry 1967), so at warmer temperatures fish may be more active in the net, again leading to more interaction with potentially injurious mesh or net structures. Opportunistic diseases also tend to be more prevalent and severe in warmer water (Karvonen et al. 2010). Recently, researchers have used reflex impairment as a predictor of post-release mortality (Davis 2010). The approach was developed specifically for bycatch assessments of fish and has been applied in a wide range of marine and freshwater fisheries and provides the opportunity to evaluate fish condition and vigor at time of net tending (Raby et al. 2012).

Even in the absence of injury, evidence of significant sub-lethal physiological disturbance could provide information on the potential for delayed mortality or other negative consequences such as growth impairments or disease development. The objective of this study was to characterise the consequences of being captured by fyke net with a focus on sub-lethal metrics (blood chemistry, reflexes, injury) and how these varied with both the duration of net set and water temperature. We focused our research on several species including non-target gamefish (i.e., largemouth bass,
northern pike) as well as an abundant target species which thus served as a model (i.e., bluegill).

## Materials and methods

## Study area

Newboro Lake ( $44^{\circ} 38^{\prime} \mathrm{W} 76^{\circ} 20^{\prime} 0$ ) is a target lake for commercial fishers in southeastern Ontario, Canada, and thus was chosen for our simulated commercial fishing efforts. The lake is shallow (mean depth $=3 \mathrm{~m}$, maximum depth $=24 \mathrm{~m}$, surface area $=787$ hectares) and is characterised by submerged stumps and heavy macrophyte cover. The fish community of Newboro Lake is dominated by centrarchids.

## Study gear and deployment procedures

Fyke nets were set in Newboro Lake during the spring of 2009 after "ice-off" (April 4) until the end of the legal fishing season (June 20) to allow sampling over a range of water temperatures (see Larocque et al. (2012) for full description of nets including a diagram and deployment methods). Briefly, nets consisted of eight 0.8 m diameter wooden hoops positioned 0.5 m apart. There were three narrowing throats per net, on the first, third and fifth hoop of the net. Each net had two wings ( 2.9 m long and 0.8 m high ) and a lead ( 11 m long and 0.8 m high) attached to the front hoop. These throats funnel fish into the net where they become trapped and are unable to find their way out. All nets, wings and leads were constructed with 5.08 cm stretch nylon mesh. To emulate the commercial fishery, all nets were set in tandem by adjoining two hoop nets by their leads, with the net openings facing each other and extending the wings to a $45^{\circ}$ angle from the entrance of the net. Nets were deployed for either two or six days to examine the sublethal effects of fish captured under current regulations, as well as potential options for changes to the regulations, respectively. For nets deployed for two days the nets were open and fishing for two days. For nets deployed for six days, the nets were open for the first two days, after which the nets were sewn shut holding the fish for an additional four days. Therefore, two treatments of duration were used in this study; fish held up to two days and fish held for four to six days.

## Catch metrics and sub-lethal evaluations of fish condition

A variety of sub-lethal metrics (blood chemistry, reflexes, injury) were measured on largemouth bass (bycatch), northern pike (bycatch), and bluegill (commercial target species). While bluegill are a target species for the commercial fishery, they were included in sub-lethal analyses as we were confident that they would be captured over a wide range of water temperatures and in numbers that would ensure reasonable sample sizes. We regard them as a reasonable proxy for sub-adult largemouth bass given that they are confamilials and of a similar size. Moreover, although bluegill are legally one of the possible target species, some of the commercial fishers only retain some species or sizes of target fish and therefore some are discarded. Not all measures were obtained from each species based on availability of fish and in some cases because of existing validations of the techniques used in this study.

## Blood physiology

Within four minutes of pulling the fyke net, two largemouth bass and two bluegills were non-lethally sampled for blood. Blood samples were not obtained for northern pike due to low catch rates. To sample blood, fish were placed in a foam-lined trough filled with fresh lake water. Using the caudal puncture method,
blood was drawn into a heparinised container ( 3 cm , 21 gauge vacutainer syringe or a 1 ml syringe with a $3 \mathrm{~cm}, 25$ gauge needle, for largemouth bass and bluegill, respectively) to prevent blood coagulation. Approximately 1.5 ml of blood was collected for largemouth bass and approximately 0.5 ml of blood was collected for bluegill. All samples were immediately placed in an ice-water slurry until analysis (Clark et al. 2011). Whole blood was tested for glucose and lactate levels ( $\mathrm{mmol} / \mathrm{l}$ ) using field meters (Accu-Chek Compact Plus, Roche, Basal, Switzerland; Lactate Pro LT-1710 Analyzer, Arkay, Inc., Kyoto, Japan) within several minutes of blood sampling. The field physiology meters were recently calibrated and had previously been validated for use on fish (e.g., Morgan \& Iwama 1997; Cooke et al. 2008). Changes in blood glucose and lactate levels are secondary responses to stress for fishes and are commonly used to assess the stressed states in fishes (Barton 2002).

## Reflex assessment

Reflex impairment can be used as a rapid assessment of fish stress and for predicting delayed mortality (Davis 2010). RAMP (Reflex Action Mortality Predictor) involves assessing a variety of reflexes (e.g., orientation, dorsal fin erection, body flex upon restraint, gag response, response to mechanical stimuli) and recording them as either present or absent. Reflexes are recorded as absent unless they are clearly observed. The number of reflexes absent is divided by the number of reflexes tested yielding a score between zero and one, with " 0 " indicating no impairment and " 1 " indicating full impairment. The RAMP method has been previously validated for use on bluegill, demonstrating increased impairment with increased intensity of a stressor (Low 2008), and we therefore only used it on this species.

## Injury assessment

Injury following capture in the fyke nets was assessed for bluegill, northern pike, and largemouth bass using the two methodologies outlined below.

Fluorescein. On bluegill, we used flourescein, a non-toxic dye, to detect latent epithelial injuries. Flourescein produces a green color in the presence of blood under a UV light (see Noga \& Udomkusronsi 2002; Colotelo et al. 2009; Colotelo \& Cooke 2011). Bluegill were anesthetised in 50 ppm clove oil (clove oil emulsified in ethanol in a $1: 9$ ratio) until they reached stage- 4 anesthesia (Summerfelt \& Smith 1990). Anesthetised fish were then placed in a solution of $0.2 \mathrm{mg} / \mathrm{ml}$ flourescein in distilled water for 6 min , and then placed back into a 50 ppm clove oil bath for an additional 6 min . After a second round of anesthetic treatment, fish were placed on a black background and photographed on both sides with a digital SLR ELIXIM Pro EX-F1 camera (Casio Computer Co., Tokyo, Japan) positioned 42 cm above the fish. We used a 20 s exposure under UV illumination (Mineralight ${ }^{\circledR}$ UVGL-84, UVP Inc., Upland, CA). Photographs were analysed using ImageJ software (http://rsb.info.nih.gov/ij/, National Institute of Health, Bethesda, MD ) by tracing the areas of green and counting the number of pixels in the area. The proportion of injury on the entire body of the fish (on both sides) was determined by dividing the number of green pixels by the total number of pixels the fish represented. Colotelo et al. (in press) demonstrates that fluorescein is most effective for detecting epithelial damage that is less than 24 h old (i.e., recent damage).

Gross macroscopic evaluations. The level of injury on largemouth bass and northern pike captured in fyke nets was assessed by visually inspecting the fish for scale loss, caudal fin fraying, and damage to the mouth. Scale loss was scored on a 3-point scale with '0' indicating no scale loss, ' 1 ' indicating $1-15 \%$ scale loss over the body, and ' 2 ' indicating a loss of scales over $>15 \%$ of the body. Caudal fin


Fig. 1. Mouth damage on northern pike associated with fyke net capture.
fraying was also measured on a 3 -point scale with ' 0 ' indicating no observable fin fraying, ' 1 ' indicating $<50 \%$ of the fin was frayed, and ' 2 ' indicating $>50 \%$ of the fin was frayed. Mouth damage was recorded as either present or absent. For largemouth bass, mouth damage included redness and swelling around the mouth area, while for northern pike, mouth damage indicated that the flesh of the upper mouth had been torn (see Fig. 1).

## Statistical analyses

All data were analysed with two independent factors: temperature; and, net set duration. Temperatures were grouped into three categories: low ( $<10^{\circ} \mathrm{C}$ ); moderate $\left(10-20^{\circ} \mathrm{C}\right)$; and, high ( $>20^{\circ} \mathrm{C}$ ). Two-way analysis of variance (ANOVA) was used to assess blood physiology and reflex assessment data. Injury assessment data were rank transformed and compared using a non-parametric twoway ANOVA design (Conover \& Iman 1981). ANOVAs were followed by a Tukey's HSD post hoc test, where appropriate to assess the statistical significance of temperature and net set duration on the response variables. Transformations were completed as necessary to meet the assumptions of normality and homogeneity of variance required for parametric tests. JMP software (SAS Inc., Cary, NC) was used for all statistical tests and significance was assessed at $\alpha=0.05$.

## Results

## Blood physiology

There was no significant difference in the total lengths of largemouth bass among the different treatment groups (Table 1; Two-way ANOVA: $F=1.23$; d.f. $=5,152 ; p=0.298$ ). Plasma glucose values were significantly higher for largemouth bass captured and retained for 6 days at high water temperatures (Tables 1 and 2; Two-way ANOVA: $F=13.34$; d.f. $=5,152 ; p<0.0001$ ). Plasma lactate values increased significantly as water temperature increased; however, there was no effect of net set duration (Tables 1 and 2). For bluegill, although we selected fish at random for blood sampling, total lengths were significantly lower for bluegill captured in the high water temperature groups when compared with those captured at low and moderate temperatures (Table 1 ;Two-way ANOVA: $F=4.83$; d.f. $=5,156 ; p<0.001$ ). Plasma glucose values for bluegill did not show the same trend as largemouth bass. There was no significant difference among any of the treatment groups (Table 1; Two-way ANOVA: $F=1.21$; d.f. $=5$, 156; $p=0.309$ ). Plasma lactate values, however, increased with temperature (Tables 1 and 2; temperature: $F=8.38$; d.f. $=2,152 ; p<0.001$ ).

## Reflex assessment

When the reflexes (as described above) were tested for bluegill captured and retained in the nets during this study, there was no

Table 1
Comparison of total length evaluated for fish captured at three different groups of water temperatures (low ( $<10^{\circ} \mathrm{C}$ ), moderate ( $10-20^{\circ} \mathrm{C}$ ) and high ( $>20^{\circ} \mathrm{C}$ )) and retained in fyke nets for two days or six days. Data are means $\pm$ S.E.; $n=$ number of largemouth bass or bluegill. For each species and plasma parameter, means with different lowercase superscript letters indicated significant differences between treatment groups for that variable. Length is reported in mm and glucose and lactate concentrations are expressed as $\mathrm{mmol} / \mathrm{l}$.

| Species | Temperature group | Duration |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Two days |  |  | Six days |  |  |
|  |  | $\begin{aligned} & \text { Length } \pm \text { S.D. } \\ & (\mathrm{mm}) \end{aligned}$ | Plasma parameter value $\pm$ S.E. ( $\mathrm{mmol} / \mathrm{L}$ ) | $n$ | $\begin{aligned} & \text { Length } \pm \text { S.D. } \\ & (\mathrm{mm}) \end{aligned}$ | Plasma parameter value $\pm$ S.E. $(\mathrm{mmol} / \mathrm{L})$ | $n$ |
| Largemouth bass | Glucose |  |  |  |  |  |  |
|  | Low | $379 \pm 49$ | $3.01 \pm 0.21^{\text {a }}$ | 17 | $394 \pm 66$ | $3.09 \pm 0.25^{\text {a }}$ | 12 |
|  | Moderate | $367 \pm 38$ | $3.57 \pm 0.37^{\text {a }}$ | 29 | $384 \pm 60$ | $3.18 \pm 0.20^{\text {a }}$ | 45 |
|  | High | $384 \pm 50$ | $4.36 \pm 0.40^{\text {a }}$ | 21 | $403 \pm 45$ | $7.30 \pm 0.92^{\text {b }}$ | 17 |
|  | Lactate |  |  |  |  |  |  |
|  |  |  |  |  |  | $1.60 \pm 0.35^{\text {a }}$ |  |
|  | Moderate | $367 \pm 40$ | $3.92 \pm 0.51{ }^{\text {b }}$ | 25 | $381 \pm 66$ | $3.20 \pm 0.32^{\text {b }}$ | 36 |
|  | High | $398 \pm 44$ | $5.74 \pm 0.60^{\text {c }}$ | 19 | $401 \pm 45$ | $4.75 \pm 0.66^{\text {c }}$ | 14 |
| Bluegill | Glucose |  |  |  |  |  |  |
|  | Low | $182 \pm 27^{\text {a }}$ | $2.61 \pm 0.25$ | 15 | $179 \pm 15^{\text {a }}$ | $2.08 \pm 0.13$ | 9 |
|  | Moderate | $183 \pm 12^{\text {a }}$ | $2.70 \pm 0.38$ | 36 | $184 \pm 14^{\text {a }}$ | $2.25 \pm 0.09$ | 46 |
|  | High | $169 \pm 9^{\text {b }}$ | $2.74 \pm 0.12$ | 21 | $173 \pm 8^{\text {b }}$ | $2.91 \pm 0.14$ | 17 |
|  | Lactate |  |  |  |  |  |  |
|  | Low | $182 \pm 27^{\text {a }}$ | $1.11 \pm 0.16^{\text {a }}$ | 15 | $179 \pm 15^{\text {a }}$ | $1.77 \pm 0.45^{\text {a }}$ | 9 |
|  | Moderate | $182 \pm 10^{\text {a }}$ | $2.28 \pm 0.24^{\text {a }}$ | 27 | $183 \pm 17^{\text {a }}$ | $1.91 \pm 0.18^{\text {a }}$ | 32 |
|  | High | $171 \pm 13^{\text {b }}$ | $2.66 \pm 0.16^{\text {b }}$ | 18 | $173 \pm 11^{\text {b }}$ | $3.26 \pm 0.80^{\text {b }}$ | 14 |

Table 2
Results of a two-way ANOVA with temperature, duration and the temperature $\times$ duration as effects, comparing three water temperature groups (low [<10 ${ }^{\circ} \mathrm{C}$, moderate $\left[10-20^{\circ} \mathrm{C}\right]$ and high $\left[>20^{\circ} \mathrm{C}\right]$ ) with two durations (two days and six days) for plasma parameters for largemouth bass and bluegill sampled from fyke nets. Italicised values represent those that are significant $p<0.05$.

| Species | Plasma parameter | Temperature |  |  | Duration |  |  | Temperature $\times$ duration |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | F | df | $p$ | $F$ | df | $p$ | $F$ | df | $p$ |
| Largemouth bass | Glucose | 16.46 | 2 | <0.0001 | 6.47 | 1 | 0.015 | 8.38 | 2 | <0.0001 |
|  | Lactate | 19.8 | 2 | <0.0001 | 3.12 | 1 | 0.062 | 0.13 | 2 | 0.988 |
| Bluegill | Glucose | 1.42 | 2 | 0.246 | 1.05 | 1 | 0.307 | 0.80 | 2 | 0.451 |
|  | Lactate | 8.38 | 2 | 0.0004 | 1.15 | 1 | 0.286 | 1.84 | 2 | 0.164 |

significant difference in the overall RAMP scores among treatment groups (Two-way ANOVA: $F=2.20$; d.f. $=5,162 ; p=0.057$ )(Table 3). Although some reflex impairment was recorded for bluegill, the results do not suggest severe impairment which would lead to mortality. Nonetheless, the highest mean RAMP impairment score was for bluegill held for the maximum duration at the highest water temperature (Table 3).

## Injury assessment

## Fluorescein

Overall, bluegill captured at moderate water temperatures had significantly higher proportions of injury detected using fluorescein when compared to those captured at high water temperatures (Table 4; Two-way ANOVA on Ranks temperature: $F=64.44$, d.f. $=1$, 49; $p<0.0001$ ). There was no significant difference in the proportions of injury detected using fluorescein for bluegill captured and

Table 3
Summary of mean Reflex Action Mortality Predictor (RAMP) scores ( $\pm$ S.E.) for bluegill captured and held in fyke nets during the study. This score is between 0 and 1 ; with 0 indicating no impairment and 1 indicating complete impairment.

| Temperature group | Duration |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- |
|  | Two days |  |  | Six days |  |
|  | Mean $\pm$ S.E. | $n$ |  | Mean $\pm$ S.E. | $n$ |
| Low | $0.15 \pm 0.02$ | 22 |  | $0.07 \pm 0.03$ | 10 |
| Moderate | $0.15 \pm 0.02$ | 44 |  | $0.15 \pm 0.02$ | 56 |
| High | $0.07 \pm 0.03$ | 17 |  | $0.18 \pm 0.05$ | 14 |

retained in the nets for two and six days (Table 4; Two-way ANOVA on Ranks duration: $F=0.09$, d.f. $=1,55 ; p=0.766$ ).

## Gross macroscopic evaluations

There was a significant, positive interaction effect between temperature and net set duration for scale loss (Table 5 and Fig. 2a. Two-way ANOVA on Ranks: $F=4.22$; d.f. $=2,672 ; p<0.05$ ), caudal fin fray (Table 5 and Fig. 2b. Two-way ANOVA on Ranks: $F=9.25$; d.f. $=2,672 ; p<0.0001$ ) and mouth damage scores (Table 5 and Fig. 2c. Two-way ANOVA on Ranks: $F=69.58$; d.f. $=2$, 694; $p<0.0001$ ) for largemouth bass.

The interaction effect between water temperature and net set duration was significant for scale loss for northern pike (Table 5 and Fig. 3a.Two-way ANOVA on Ranks: $F=4.02$; d.f. $=1,601 ; p=0.046$ ). Caudal fin fray was not significantly influenced by either water temperature or net set duration, nor an interaction of these variables (Table 5 and Fig. 3b. Two-way ANOVA on Ranks: $F=1.91$; d.f. $=2$, 201; $p=0.1282$ ). Water temperature was a significant factor in determining mouth damage. Northern pike captured at moderate water temperatures had significantly higher levels of mouth damage than those captured at low water temperatures (Table 5 and Fig. 3c. Two-way ANOVA on Ranks Temperature: $F=5.86$, d.f. $=1$, 196; $p=0.016$ ). Lack of capture of pike at high water temperatures and retention for six days limited statistical analysis to only fish captured at low and moderate water temperatures. However, of those fish captured at high water temperatures and retained for two days in the net, $20.0 \%$ ( $n=1 / 5$ ) did not exhibit scale loss, $80.0 \%$ ( $n=4 / 5$ ) did not exhibit caudal fin fray, and $42.7 \%(n=3 / 7)$ did not exhibit mouth damage.

Table 4
Proportions of injury measured using fluorescein for bluegill captured and held in fyke nets for two or six days at low ( $<10^{\circ} \mathrm{C}$ ), moderate ( $10-20^{\circ} \mathrm{C}$ ) and high ( $>20^{\circ} \mathrm{C}$ ) water temperatures. Dissimilar letters indicate a significant difference ( $p<0.05$ ). Due to lack of flourescein injury data for bluegill captured at low water temperatures and held for two days, only bluegill captured at moderate and high water temperatures were included in statistical analysis.

| Temperature group | Duration |  |  |
| :--- | :--- | ---: | :--- |
|  | Two days |  |  |
|  | Proportion of injury $\pm$ S.E. | $n$ | Six days |
|  | n/a | 0 | $0.1581 \pm 0.0701$ |
| Low | $0.1270 \pm 0.0217^{\mathrm{a}}$ | 10 | $0.1882 \pm 0.0362^{\mathrm{a}}$ |
| Moderate | $0.0178 \pm 0.0038^{\mathrm{b}}$ | $0.0051 \pm 0.0024^{\mathrm{b}}$ |  |

## Discussion

The results of this study demonstrated that water temperature and net set duration influence sub-lethal effects observed for fish captured and retained in fyke nets, such as those employed by the commercial fishery in southeastern Ontario. For largemouth bass, plasma glucose levels, scale loss, caudal fin fray and mouth damage were all higher for six day net set durations and higher water temperatures. Scale loss was also observed at a higher rate for northern pike captured at warmer water temperatures and retained for six days. While these findings were not consistent among metrics and species, they are suggestive of the notion that net tending frequencies could and should be varied relative to water temperature (i.e., seasonally) to reduce stress and injury for fish bycatch.

The current study showed increases in plasma glucose levels for largemouth bass with increases in water temperature and net set duration, indicating that capture and confinement in a net is inherently stressful (Wendelaar Bonga 1997). Glucose is indicative of a glucocorticoid stress response and when elevated suggests that energy has been mobilised to provide the organism with the resources needed to deal with a challenge (Barton 2002). Deviations in physiological measures from baseline can influence the behavior and survival of discarded fish (Davis 2002). For both largemouth bass and bluegill, net set duration did not significantly affect plasma lactate levels, with increases being directly related to water temperature. Lactate is an indicator of anaerobic metabolism which can result from anaerobic exercise or hypoxic conditions (Kieffer 2000; Wendelaar Bonga 1997). In the recreational fisheries literature, water temperature has been shown to be a significant factor in mediating physiological disturbance (Suski et al. 2006; Thompson et al. 2008); and this appears to hold true in commercial fisheries as well. In the context of fyke nets, plasma lactate is likely indicative of variation in locomotory activity (e.g., such as struggling to escape) that would require anaerobic metabolism (Kieffer 2000), something noted previously for struggling fish in gill nets (e.g., Farrell et al. 2000). It is worth noting that the absolute level of physiological disturbance noted in this study was relatively modest. Although we did not collect control samples as part of this study, our team has done extensive physiological sampling of largemouth
bass and bluegill, and within the water temperature ranges studied here, baseline values of lactate and glucose are low and stable (i.e., $<2 \mathrm{mmol} / \mathrm{l}$ for both parameters; Cook et al. 2011; McConnachie et al. 2012; Suski et al. 2004; Thompson et al. 2008). The levels of disturbances observed in the current study were typically in the range of $2-5 \mathrm{mmol} / \mathrm{l}$ with few values exceeding $10 \mathrm{mmol} / \mathrm{l}$. For centrarchids, glucose and lactate can both exceed $20 \mathrm{mmol} / \mathrm{l}$ in the face of extreme stressors (e.g., Kieffer \& Cooke, 2009; McConnachie et al. 2012; Suski et al. 2004). Sampling occurred during the reproductive period for the three species studied which may have contributed to stress. Nonetheless, this is also the period during which some fishing occurs and is thus highly relevant.

Recently, reflex impairment has become a popular endpoint in bycatch studies given its simplicity and its ability to predict post release mortality (Davis 2010; Raby et al. 2012). We used bluegill as a model for reflex assessment given that reflexes have previously been validated for this species (Low 2008). Reflexes were largely intact upon capture and tended to be insensitive to changes in water temperature and net tending duration. The reflex that was most commonly impaired was the bursting escape response when stimulated by the research team. The bursting escape reflex would be influenced by the extent to which fish were exhausted from earlier anaerobic bursting activity. For bluegill, lactate increased with water temperature and was consistently above baseline levels (as per Cook et al. 2011 using bluegill from the same region) suggesting some level of anaerobic disturbance which is consistent with the reflex impairments. Although net tending frequency and water temperature do not seem to influence reflex impairment for bluegill, the modest and relatively consistent level of impairment among all treatments is suggestive that interactions with fishing nets do result in bursting which is presumably an attempt to escape or a result of interactions with conspecifics and other species that may be present in the net. Fish released with reflex impairments (especially the escape response reflex) could be subject to postrelease predation (e.g., see Danylchuk et al. 2007).

Epithelial damage associated with capture and retention in fyke nets during the current study did not differ among treatments for bluegill. Macroscopic injuries were more prevalent for largemouth bass and northern pike as water temperature and net

## Table 5



 held for six days, only northern pike captured at low and moderate water temperatures were included in statistical analysis.

| Species | Injury | Temperature |  |  | Duration |  |  | Temperature $\times$ duration |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | F | df | $p$ | F | df | $p$ | F | df | $p$ |
| Largemouth bass | Scale loss | 4.17 | 2 | 0.0158 | 10.33 | 1 | 0.0014 | 4.22 | 2 | 0.0151 |
|  | Caudal fin fray | 9.91 | 2 | <0.0001 | 6.07 | 1 | 0.014 | 9.25 | 2 | <0.0001 |
|  | Mouth damage | 143.58 | 2 | <0.0001 | 136.39 | 1 | <0.0001 | 69.58 | 2 | <0.0001 |
| Northern pike | Scale loss | 7.46 | 1 | 0.007 | 0.12 | 1 | 0.727 | 4.02 | 1 | 0.046 |
|  | Caudal fin fray | 0.05 | 1 | 0.824 | 4.82 | 1 | 0.029 | 1.28 | 1 | 0.259 |
|  | Mouth damage | 5.86 | 1 | 0.016 | 1.6 | 1 | 0.208 | 3.49 | 1 | 0.063 |



Fig. 2. Proportion of largemouth bass held in the net for either two days (left) or six days (right) exhibiting scale loss (a), caudal fin fray (b), and mouth damage (c). Dissimilar letters signify statistically significant differences between treatment groups.
set duration increased. Locomotor activity in the net may have been heightened at the warmer temperatures (i.e., reflecting basic temperature-activity metabolism relationships for fish; Fry 1967) such that potential for injury was heightened with longer periods in the net exacerbating those injuries. In general, higher proportions of individuals exhibited scale loss, fin fraying and mouth damage, associated with interaction with the net or other fish in the net. As well, the severity of scale loss and fin fraying increased as water temperature and net set duration increased. These injuries have the potential to result in infection of pathogens following release, which can influence post-release survival (e.g., Nguyen et al. 2012). Research is lacking in this area, as many studies examining injury associated with netting focus on gilling, despite the fact that scale loss has been documented in other areas of fisheries research. Gill
nets are believed to cause more injury to fish due to the direct interaction with the net (Chopin \& Arimoto 1995) however, the results presented here indicate that the use of fyke nets can also result in injuries for released fish. Injuries to pike mouths were quite common and have not been previously observed during extensive angling trials in this region by our team (e.g., Arlinghaus et al. 2008a, 2008b). Mouth injuries could potentially influence feeding or respiration but that supposition has not been tested.

## Management implications

The results of the current study demonstrate that both water temperature and net set duration need to be considered when defining regulations for the use of fyke nets. In inland commercial


Fig. 3. Proportion of northern pike held in the net for either two days (left) or six days (right) exhibiting scale loss (a), caudal fin fray (b), and mouth damage (c). Dissimilar letters signify statistically significant differences between treatment groups. Northern pike were not captured in a 6 day treatment and injury assessments were not conducted (labeled as $n / a$ ).
fisheries in north temperate regions, a wide range of water temperatures are experienced over the fishing season (from $\approx 0^{\circ} \mathrm{C}$ to $26^{\circ} \mathrm{C}$ surface water temperature in the region where this work was conducted). Given the manifold influence of water temperature on fish physiological processes (Fry 1967), and the fact that temperatures approaching thermal maxima are stressful (Beitinger et al. 2000), it is not surprising that it has commonly been studied as potential driver of bycatch stress and survival (Davis 2002). Moreover, water temperature can interact with other stressors (e.g., air exposure) that at low temperatures are not problematic but at higher temperatures become multiplicative (e.g., Gingerich et al. 2007). For that reason we would encourage the adoption of fishing practices that
reduce stress (and injury) to fish at all temperatures, but particularly at warmer temperatures. Specifically, to reduce physiological disturbances and injury that could lead to delayed mortality, we suggest that inland commercial fishers check their nets more frequently as water temperatures increase. Fisher adoption of these practices could be accomplished by the development of outreach and education materials (e.g., codes of practice) or through regulations (e.g., seasonal closures, seasonal gear restrictions, seasonal net tending restrictions). All efforts to reduce stress and injury of fish that will be released have the potential to reduce post-release mortality (and in-net mortality) and to improve the welfare status of bycatch (Diggles et al. 2011).

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## References

Arlinghaus, R., Klefoth, T., Gingerich, A. J., Donaldson, M. R., Hanson, K. C., \& Cooke, S. J. (2008). Behaviour and survival of pike, Esox lucius, with a retained lure in the lower jaw. Fisheries Management and Ecology, 15, 459-466.
Arlinghaus, R., Klefoth, T., Kobler, A., \& Cooke, S. J. (2008). Size-selectivity, capture efficiency, injury, handling time and initial hooking mortality of angled northern pike (Esox lucius) in Germany and Canada: The influence of bait size and type. North American Journal of Fisheries Management, 28, 123-134.
Barko, V.A., Briggler, J. T., \& Osendorf, D. E. (2004). Passive fishing techniques: A cause of turtle mortality in the Mississippi River. Journal of Wildlife Management, 68, 1145-1150.
Barton, B. A. (2002). Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology, 42, 517-525.
Beitinger, T. L., Bennett, W. A., \& McCauley, R. W. (2000). Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. Environmental Biology of Fishes, 4, 245-256.
Bettoli, P. W., \& Scholten, G. D. (2006). Bycatch rates and initial mortality of paddlefish in a commercial gillent fishery. Fisheries Research, 77, 343-347.
Burns, C. (2007). Biological sustainability of commercial fishing in the inland waters of Kemptville District. Kemptville: OMNR Technical Paper, OMNR.
Chopin, F. S., \& Arimoto, T. (1995). The condition of fish escaping from fishing gears - A review. Fisheries Research, 21, 315-327.

Clark, T. D., Donaldson, M. R., Drenner, S. M., Hinch, S. G., Patterson, D. A., Hills, J., et al. (2011). The efficacy of field techniques for obtaining and storing blood samples from fish. Journal of Fish Biology, 79, 1322-1333.
Colotelo, A. H., \& Cooke, S. J. (2011). Evaluation of common angling-induced sources of epithelial damage for popular freshwater sport fish using fluorescein. Fisheries Research, 109, 217-224.
Colotelo, A. H., Cooke, S. J., \& Smokorowski, K. E. (2009). Application of forensic techniques to enhance fish conservation and management: Injury detection using presumptive tests for blood. Endangered Species Research, 9, 169-178.
Colotelo, A.H., Smokorowski, K.E., Haxton, T., \& Cooke, S.J. Detection of epithelial injury on fish using presumptive tests for blood: A comparative field evaluation of four different tests. Journal of Fish and Wildlife Management, in press.
Conover, W. J., \& Iman, R. L. (1981). Rank transformations as a bridge between parametric and nonparametric statistics. The American Statistician, 35, 124-129.
Cook, K. V., O'Connor, C. M., Gilmour, K. M., \& Cooke, S. J. (2011). The glucocorticoid stress response is repeatable between years in a wild teleost fish. Journal of Comparative Physiology: Part A, 60(489), 497.
Cooke, S. J., \& Suski, C. D. (2005). Do we need species-specific guidelines for catch-and-release recreational angling to effectively conserve diverse fishery resources? Biodiversity and Conservation, 14, 1195-1209.
Cooke, S. J., Suski, C. D., Danylchuk, S. E., Donaldson, M. R., Pullen, C., Bulté, G., et al. (2008). Effects of different capture techniques on the physiological condition of bonefish Albula vulpes evaluated using field diagnostic tools. Journal of Fish Biology, 73, 1351-1375.
Danylchuk, S. E., Danylchuk, A. J., Cooke, S. J., Goldberg, T. L., Koppelman, J., \& Philipp, D. P. (2007). Effects of recreational angling on the post-release behavior and predation of bonefish (Albula vulpes): The role of equilibrium status at the time of release. Journal of Experimental Marine Biology and Ecology, 346, 127-133.
Davis, M. W. (2002). Key principles for understanding fish bycatch discard mortality. Canadian Journal of Fisheries and Aquatic Sciences, 59, 1834-1843.
Davis, M. W. (2010). Fish stress and mortality can be predicted using reflex impairment. Fish and Fisheries, 11, 1-11.
Dieterman, D. J., Baird, M. S., \& Galat, D. L. (2000). Mortality of paddlefish in hoop nets in the Lower Missouri River, Missouri. North American Journal of Fisheries Management, 20, 226-230.

Diggles, B. K., Cooke, S. J., Rose, J. D., \& Sawynok, W. (2011). Ecology and welfare of aquatic animals in wild capture fisheries. Reviews in Fish Biology and Fisheries, 21, 739-765.
Farrell, A. P., Gallaugher, P., Clarke, C., DeLury, N., Kreiberg, H., Parkhouse, W., et al. (2000). Physiological status of coho salmon (Oncorhynchus kisutch) captured in commercial nonretention fisheries. Canadian Journal of Fisheries and Aquatic Sciences, 57, 1668-1678.
Fry, F. E. J. (1967). Responses of vertebrate poikilotherms to temperature. In A. H. Rose (Ed.), Thermobiology (pp. 375-409). London: Academic Press.
Gingerich, A. J., Cooke, S. J., Hanson, K. C., Donaldson, M. R., Hasler, C. T., Suski, C. D., et al. (2007). Evaluation of the interactive effects of air exposure duration and water temperature on the condition and survival of angled and released fish. Fisheries Research, 86, 169-178.
Grant, T. R., \& Temple-Smith, P. D. (2003). Conservation of the platypus, Ornithorhynchus anatinus: Threats and challenges. Aquatic Health and Management, 6, 1-15.
Hall, M. A. (1996). On bycatches. Reviews in Fish Biology and Fisheries, 6, 319-352.
Hopkins, T. E., \& Cech, J.J. (1992). Physiological effects of capturing striped bass in gill nets and fyke traps. Transactions of the American Fisheries Society, 121, 819-822.
Hubert, W. A. (1996). Passive capture techniques. In L. A. Nielsen, \& D. L. Johnson (Eds.), Fisheries techniques (pp. 95-122). Bethesda: American Fisheries Society.
Karvonen, A., Rintamäki, P., Jokela, J., \& Valtonen, E. T. (2010). Increasing water temperature and disease risks in aquatic systems: Climate change increases the risk of some, but not all, diseases. International Journal for Parasitology, 40, 1483-1488.
Kieffer, J. D. (2000). Limits to exhaustive exercise in fish. Comparative Biochemistry and Physiology A, 126, 161-179.
Kieffer, J. D., \& Cooke, S. J. (2009). Physiology and organismal performance of centrarchids. In S. J. Cooke, \& D. P. Philipp (Eds.), Centrarchid fishes: Diversity, biology, and conservation (pp. 207-251). Oxford: Blackwell Publishing Ltd.
Larocque, S. M., Colotelo, A. H., Cooke, S. J., Blouin-Demers, G., Haxton, T., \& Smoko rowski, K. E. (2012). Seasonal patterns in bycatch composition and mortality associated with a freshwater hoop net fishery. Animal Conservation, 15, 53-60.
Low, S.Y. (2008). Validation of reflex action mortality predictors for use with Lepomid sunfish for the study of fisheries interactions. B.Sc. Biology thesis. Ottawa, Ontario: Carleton University.
McConnachie, S. H., O’Connor, C. M., Gilmour, K. M., Iwama, G. K., \& Cooke, S. J. (2012). Supraphysiological cortisol elevation alters the response of wild bluegill sunfish to subsequent stressors. Journal of Experimental Zoology: Part A, 317, 321-332.
Morgan, J. D., \& Iwama, G. K. (1997). Measurement of stressed states in the field. In G. K. Iwama, A. D. Pickering, J. P. Sumpter, \& C. B. Schreck (Eds.), Fish stress and health in aquaculture (pp. 247-268). Cambridge: Cambridge University Press.
Nguyen, V. M., Rudd, M., Cooke, S. J., \& Hinch, S. G. (2012). Differences in information use and preferences among recreational salmon anglers: Implications for management initiatives to promote responsible fishing. Human Dimensions of Wildlife, 17, 248-256.
Noga, E. J., \& Udomkusronsi, P. (2002). Fluorescein: A rapid, sensitive, nonlethal method for detecting skin ulceration in fish. Veterinary Pathology, 39, 726-731
Raby, G. D., Colotelo, A. H., Blouin-Demers, G., \& Cooke, S. J. (2011). Freshwater commercial bycatch: An understated conservation problem. BioScience, 61, 271-280.
Raby, G. D., Donaldson, M. R., Hinch, S. G., Patterson, D. A., Lotto, A. G., Robichaud, D., et al. (2012). Validation of reflex indicators for measuring vitality and predicting the delayed mortality of wild coho salmon bycatch released from fishing gears. Journal of Applied Ecology, 49, 90-98.
Schorfhaar, R.G., \& Peck, J.W., (1993). Catch and mortality of nontarget species in lake whitefish trap nets in Michigan waters of Lake Superior. Michigan Department of Natural Resources Fisheries Research Report 1974.
Summerfelt, R. C., \& Smith, L. S. (1990). Chapter 8: Anesthesia, surgery, and related techniques. In C. B. Schreck, \& P. B. Moyle (Eds.), Methods for fish biology (pp. 213-272). Bethesda: American Fisheries Society.
Suski, C. D., Killen, S. S., Cooke, S. J., Kieffer, J. D., Philipp, D. P., \& Tufts, B. L. (2004), Physiological significance of the weigh-in during live release angling tournaments for largemouth bass. Transaction of the American Fisheries Society, 133 1291-1303.
Suski, C. D., Killen, S. S., Kieffer, J. D., \& Tufts, B. L. (2006). The influence of environmental temperature and oxygen concentration on the recovery of largemouth bass from exercise: Implications for live-release angling tournaments. Journal of Fish Biology, 68, 120-136.
Thompson, L. A., Donaldson, M. R., Hanson, K. C., Arlinghaus, \& Cooke, S. J. (2008). Physiology, behavior and survival of angled and air exposed largemouth bass. North American Journal of Fisheries Management, 28, 1059-1068.
Wendelaar Bonga, S. E. (1997). The stress response in fish. Physiological Reviews, 77, 591-625.
Wikelski, M., \& Cooke, S. J. (2006). Conservation physiology. Trends in Ecology and Evolution, 21, 38-46.
Young, P. S., Cech, J. J., \& Thompson, L. C. (2011). Hydropower-related pulsed-flow impacts on stream fishes: A brief review, conceptual model, knowledge gaps, and research needs. Reviews in Fish Biology and Fisheries, 21, 713-731.


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