North American Journal of Fisheries Management 41:639–649, 2021

ISSN: 0275-5947 print / 1548-8675 online
DOI: 10.1002/nafm.10571

ARTICLE

Do Carbonated Beverages Reduce Bleeding from Gill Injuries in Angled Northern Pike?

Alexandria T. Trahan* **and Auston D. Chhor**
Fish Ecology and Conservation Physiology Laboratory, Department of Biology and Institute of Environmental and Interdisciplinary Science, Carleton University, Ottawa, Ontario K1S 5B6, Canada

Michael J. Lawrence
Department of Biological Sciences, University of Manitoba, 50 Sifton Road, Winnipeg, Manitoba R3T 2N2, Canada

Jacob W. Brownscombe, Daniel M. Glassman, Connor H. Reid, **and Alice E. I. Abrams**
Fish Ecology and Conservation Physiology Laboratory, Department of Biology and Institute of Environmental and Interdisciplinary Science, Carleton University, Ottawa, Ontario K1S 5B6, Canada

Andy J. Danylchuk
Department of Environmental Conservation, University of Massachusetts Amherst, 160 Holdsworth Way, Room 304, Amherst, Massachusetts 01003, USA

Steven J. Cooke
Fish Ecology and Conservation Physiology Laboratory, Department of Biology and Institute of Environmental and Interdisciplinary Science, Carleton University, Ottawa, Ontario K1S 5B6, Canada

Abstract
The premise of catch-and-release recreational angling is that postrelease survival is high. Therefore, it is common for anglers, management agencies, and conservation organizations to share information on handling practices and other strategies that are believed to improve the welfare and survival of fish that are released. A recent surge in popularity has sensationalized the use of carbonated beverages to treat bleeding fish—an intervention that is purported to stop bleeding but has yet to be validated scientifically. We captured Northern Pike *Esox lucius* via hook and line and experimentally injured their gills in a standardized manner. Gill injuries were treated with Mountain Dew, Coca-Cola, or carbonated lake water. The duration and intensity of bleeding as well as overall blood loss (using gill color as a proxy) were observed while the fish were held in a lake water bath. As a control, we used a group of experimentally injured fish that did not have liquid poured over their gills before the observation period. All treatments and the control were conducted at two different water temperatures (11–18°C and 24–27°C) to determine whether the effects of pouring carbonated beverages over injured gills are seasonally dependent. When compared to the control, we found that the duration and intensity of bleeding increased regardless of the type of carbonated beverage used in this study, and there was no effect of season. Use of chilled versus ambient-temperature beverages similarly had no influence on outcomes. As such, there is no scientific evidence to support the use of carbonated beverages for reducing or stopping blood loss in fish that receive gill injuries during recreational angling based on the context studied here. Our study reinforces the need to scientifically test angler anecdotes and theories regarding best practices for catch-and-release fishing.

*Corresponding author: atrahan9@gmail.com
Received June 13, 2020; accepted December 14, 2020

Recreational angling is a common practice around the globe. Although some fish are harvested, a greater percentage of them are released (Cooke and Cowx 2004). Catch-and-release (C&R) angling occurs when recreational anglers comply with local harvest regulations or when they adopt this practice voluntarily based on their conservation ethic (Arlinghaus et al. 2007). Regardless of the reason, the general premise with C&R angling is that most of the released fish will survive angling-related stress and physical injuries (Wydoski 1977). Hooking injury is the most important factor influencing whether a fish survives a C&R event, with hook injury to critical areas, such as the gills or deeply in the esophagus, yielding comparatively higher mortality than when fish are hooked in areas such as the corner of the jaw (Muoneke and Childress 1994; Bartholomew and Bohnsack 2005; Arlinghaus et al. 2007; Cooke and Schramm 2007). Consequently, there has been considerable effort to develop techniques that reduce hooking injuries in critical areas or that mitigate the bleeding that can occur where the hook penetrates the fish (reviewed by Brownscombe et al. 2017).

Pouring carbonated beverages (e.g., cola or citrus beverages) over the gills of a fish to reduce or stop bleeding caused by hooking injury is gaining in popularity within the recreational angling community as a best practice for C&R. For example, there is a Facebook page entitled "Save a Million Fish," where recreational anglers share videos and stories of how they have saved Muskellunge *Esox masquinongy* with deep-hooking injuries (Anderson 2018), including pouring carbonated beverages over the gills. Popular media articles in support of this practice suggest that the carbon dioxide (CO_2) in carbonated beverages causes vasoconstriction of the blood vessels to slow or stop bleeding (Pyzer 2015, 2019) or that the phosphoric acid in these beverages causes coagulation (Green 2015; Bardin 2019). However, the aforementioned perspectives are anecdotal and there is no empirical research on the effectiveness of carbonated beverages for impeding blood loss in injured fish.

It is well known that CO_2 has many effects on homeostasis in teleost fish. As a metabolic byproduct of aerobic respiration, the accumulation of CO_2 in the blood (i.e., hypercarbia) can produce a state of respiratory acidosis (reviewed by Brauner and Baker 2009). On the branchial epithelium, the expression of CO_2 receptors has been thought to occur in teleosts (reviewed by Gilmour and Milsom 2009) and exposures to a high environmental partial pressure of CO_2 (pCO_2) have been shown to induce bradycardia (slowing of the heart rate), heightened ventilatory rates, and hypertension in fish (Sundin et al. 2000; Gilmour et al. 2005; Tuong et al. 2018; reviewed by Gilmour 2001). Together, these effects would conceivably increase perfusion of the gills, which could exacerbate the effects of gill injury after angling if the animal's gills are doused with a carbonated solution. Furthermore, blood coagulation in teleosts is believed to be primarily stimulated by tissue injury, which also confers vasoconstriction to the affected area (Tavares-Dias and Oliveira 2009). It is possible that further injury/damage to the gill through the addition of carbonated beverages may confer a greater coagulation response and appear to be beneficial to the fish (i.e., cessation of bleeding) despite causing higher degrees of tissue damage to the animal. As such, the addition of soft drinks to fish gills may cause harm to the animal rather than achieving the purported benefits of these substances.

Gills are multifunctional organs for fish, playing critical roles in gas exchange, ion and water balance, ammonia excretion, and acid–base balance (Evans et al. 2005). Carbonated beverages have low pH coupled with high levels of CO_2 in aqueous solution, various sugars, caffeine (if not caffeine free), phosphoric acid (H_3PO_4; Coca-Cola), and citric acid ($C_6H_8O_7$; Mountain Dew). Several of these compounds could have an effect on gill injuries. There is a rich literature describing the effects of low-pH water on ion regulation, ammonia excretion, and metabolic acid (H^+) excretion (Wood and McDonough 1988; Evans et al. 2005; Kwong et al. 2014). The elevation of water pCO_2 levels when carbonated beverages are poured over the gills may drive CO_2 into the fish's body by reversing the normal gradient for CO_2 excretion from blood to water (Gilmour 2001, 2010; Gilmour and Perry 2009). This will cause a decrease in pH and an accumulation of HCO_3^- to compensate for acidosis, followed by a new steady state with an increased pCO_2, a normal pH, and an elevated HCO_3^- level (Perry and Gilmour 2002). In addition, chemoreceptors that detect changes in CO_2 are located in the gills and may activate cardiorespiratory reflexes, such as increased breathing, bradycardia, and peripheral vasoconstriction (Reid et al. 2000; Brauner et al. 2019; Tresguerres et al. 2019). Bradycardia may transiently reduce bleeding (Perry and Desforges 2006). Furthermore, caffeine is an nonspecific adenosine receptor antagonist that may alter chemoreceptor signaling (Coe et al. 2017). The exact effects of a combination of these substances on gill function are unclear, but it is possible that the individual effects may all occur when these substances are applied together.

The technique of pouring carbonated beverages over bleeding hook injuries in fish continues to be promoted, but debates as to the efficacy of its use have also arisen (Neuharth 2019). Air exposure also occurs when carbonated beverages are poured over injured gills, which could have negative physiological effects (Cooke and Sneddon 2007). Given the growing popularity of this technique, we set out to provide the first scientific evidence of whether carbonated beverages should be considered a best practice for C&R angling. We aimed to investigate whether carbonated beverages reduce or stop bleeding from the gills of injured fish. For this study, we angled Northern Pike *E. lucius* and simulated a hooking injury to a standardized section of gill filaments. We then poured a range of carbonated beverages (i.e., cola, citrus beverage, or carbonated lake water) over the gill injury, and we quantified the duration and intensity of bleeding as well as overall blood loss in comparison to a control. Because metabolism and related blood flow in fish are positively correlated with water temperature, we also tested the effects of carbonated beverages on bleeding in Northern Pike at two different temperature regimes. Furthermore, at the warmer water temperature, we tested whether the use of chilled beverages influenced outcomes relative to beverages at ambient temperatures.

METHODS

Animal welfare.— All experiments were conducted in accordance with regulations and guidelines set by the Canadian Council on Animal Care (Carleton University Animal Use Protocol 110558). Northern Pike were collected under Scientific Collection Permit 08577 from the Ontario Ministry of Natural Resources and Forestry.

Fish capture.— Northern Pike were selected for study owing to their relevance in addressing the question of interest and their popularity as a target species for recreational angling (Paukert et al. 2001). The study was conducted on wild Northern Pike from Lake Opinicon, Ontario, Canada (44.5590°, 76.3280°), and all fish were captured from a boat using conventional medium-heavy rod and reel and a variety of crankbaits, chatter baits, and spinner baits. Once hooked, fish were retrieved in under 1 min, brought into the boat using a rubberized landing net, and immediately transferred to a water-filled trough for hook removal (underwater). Only fish that were hooked in the jaw were used in experiments to avoid confounding effects between lure-induced gill damage and experimental gill injury. In addition, fish that were bleeding from the hooking site or the gills upon capture (<10% of fish) were not used in the study and were immediately released. After hook removal, the TL (mm) of the fish was recorded.

Experimental injury and postinjury monitoring.— Gill injuries were simulated by using end-cutting pliers to remove a 0.9-cm-long section of gill from the right middle gill arch (Figure 1). A 0.9-cm section reflects a typical wound size based on observations of naturally hooked fish that were injured in the gills during pilot studies. The small end-cutting pliers were able to remove the same length of gill filaments every time to standardize the cut, which was important for this experiment. Once the gills were clipped, one of three carbonated beverage treatments or the lake water control was applied (details below). The procedure was conducted while fish were held in the water-filled trough to eliminate air exposure; however, the anterior third of the body was removed from the water while the treatment (e.g., cola) was applied. When pouring carbonated beverages over the gills, a standardized volume of 150 mL was used. After the procedure, fish were transferred to a white-bottomed cooler (52.0 × 26.5 cm) containing nonaerated lake water (~25 L) for observations. We also included a reference or baseline group of fish that were not subjected to gill clipping or to the pouring of liquid over the gills before being transferred to the cooler.

FIGURE 1. Image of the standardized 0.9- × 0.9-cm section of gill filaments removed from Northern Pike to simulate hooking injury. [Color figure can viewed at afsjournals.org.]

Experiments were conducted at two different time periods. Experiment 1 was conducted in May 2019, when water temperature ranged between 11°C and 18°C, whereas experiment 2 took place in August 2019, when water temperature ranged from 24°C to 27°C. Experiment 1 had five treatment groups: (1) the gill was not injured and no carbonated beverages were used (baseline group); (2) after injury, fish were held in lake water (pH 6.71) without any other treatment; (3) after injury, carbonated lake water (pH 4.37) was poured over the gills; (4) after injury, Mountain Dew (pH 3.27) was poured over the gills; and (5) after injury, Coca-Cola (pH 2.56) was poured over the gills. During experiment 1, beverage temperature ranged from 11°C to 18°C according to ambient air temperature. Experiment 2 included the same baseline and control treatments (lake water, pH 6.71) as experiment 1 and compared Mountain Dew (pH 3.27) at ambient temperature (24–27°C) with Mountain Dew (pH 3.27) kept on ice (4–8°C); these treatments mimicked situations in which anglers either did not use a cooler or did use a cooler to hold beverages.

After treatment, fish were individually held in a cooler and visually monitored for 20 min to quantify (1) the time to bleeding cessation; (2) gill color, which served as a proxy for blood loss; and (3) bleeding intensity. Bleeding cessation time was recorded as the time from gill injury until noticeable bleeding from the gill area stopped. Gill color was assessed against a 20-point color gradient, with bright red (gill color = 20) at one end of the scale representing gills that were well perfused with blood (most common), through progressively lighter shades of red to pink and to nearly white (gill color = 1; Booth 1978). Gill color values were recorded at 10 and 20 min postinjury as well as immediately before the gill injury occurred, serving as a reference. A relative bleeding intensity value (BINV) was assigned based on the following scale: 0 represented no bleeding, 1 indicated minor bleeding (i.e., not obvious), 2 indicated obvious bleeding (i.e., easily observed), and 3 represented intense bleeding with pulsatile blood flow. For all treatment groups other than the baseline group, we recorded the BINV immediately before and after liquid was poured directly onto the wound while the fish was held in the water-filled trough. Additional BINVs were recorded at 3 and 5 min postinjury. After 20 min in the cooler, the vigor and condition of the fish were recorded using reflex action mortality predictors (RAMPs), and fish that were not moribund were released. Fish that were moribund were euthanized by cerebral percussion (three fish in total; see below).

Survival and reflexes.— The presence or absence of basic reflexes can be used to predict postrelease mortality of fish (Davis 2007; Raby et al. 2015). Our RAMP scoring system evaluated the (1) ability to maintain equilibrium, (2) reaction to grasping of the tail (burst swimming), and (3) vestibular–ocular response (i.e., eye tracking) to determine whether a fish was impaired posttreatment and should be euthanized or released.

Data analysis.— RStudio (R version 1.1.447; R Core Team 2019) was used to conduct all statistical analyses. For experiments 1 and 2, bleeding time was compared among treatments using linear regression models and significant effects were assessed using type I sums of squares. For the BINV (ordinal scale from 0 to 3) and gill color (ordinal scale from 1 to 20), ordinal logistic regression was applied to each time point at which the BINV or gill color was assessed. Full models included treatment, time, and their interaction as a fixed effect and individual as a random effect to account for repeated measures. Backward model selection was used to determine final model structure using Akaike's information criterion. When the best-fitting models included interactions, ordinal logistic regression models were fitted within each sampling time period to assess differences among treatments. Statistical significance was accepted at $\alpha = 0.05$; unless otherwise noted, all values are presented as means ± SE.

RESULTS

For experiment 1, 118 Northern Pike (50.9 ± 6.6 cm TL) were captured, while 38 Northern Pike (52.6 ± 6.7 cm TL) were captured for experiment 2. There were no significant differences in TL among the treatments for either experiment (experiment 1: $F_4 = 0.641$, $P = 0.634$; experiment 2: $F_3 = 0.583$, $P = 0.629$).

Time to Bleeding Cessation

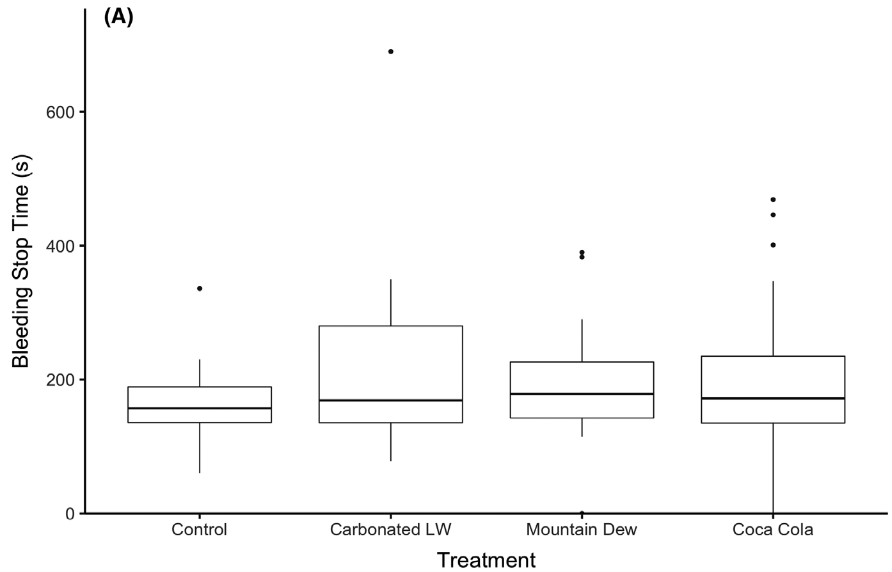
Time to bleeding cessation in experiment 1 ranged from 0 to 690 s (mean = 193 ± 95 s) and was not significantly different among treatments (Figure 2A; $F_3 = 0.83$, $P = 0.48$). For experiment 2, time to bleeding cessation ranged from 0 to 193 s (mean = 87 ± 40 s) and also was not different among treatments (Figure 2B; $F_2 = 2.47$, $P = 0.10$).

Gill Color Index

For experiment 1, the best-fitting model for the gill color index included a significant interaction between treatment and time (Table 1). The gill color index did not differ among treatment groups prior to gill injury. Postinjury, the baseline treatment (no injury) group exhibited significantly darker color (higher score; Figure 3) than all other treatment groups at both 10 min ($t_{126} = 3.60$, $P < 0.001$) and 20 min ($t_{127} = 5.03$, $P < 0.001$). However, no significant differences were detected among the control group (immersion in lake water) and any group treated with a carbonated beverage ($t < 5.03$, $P > 0.05$; Table 2). In experiment 2, there was also a significant interaction between treatment and time (Table 1). The baseline treatment (no injury) had a significantly higher gill color score (darker color) than all other groups at both 10 min ($t_{31} = 1.78$, $P < 0.001$) and 20 min ($t_{31} = 3.62$, $P < 0.001$) postinjury (Figure 4). No significant differences were detected between the control group and the group treated with chilled Mountain Dew ($t < -0.206$, $P > 0.05$). Fish had a significantly lighter gill color at 10 min when ambient-temperature Mountain Dew was used in comparison with the control group ($t_{31} = -2.309$, $P = 0.021$; Table 2).

Bleeding Intensity Values

For experiment 1, the BINV was not different among treatments (Figure 5A), as the best-fitting model included time as a fixed effect and individual as a random effect to account for repeated measures (Table 3). Similarly, treatment was not a significant contributing factor to variation in the BINV for experiment 2, as the best-fitting model included time and individual as independent predictors (Table 3).



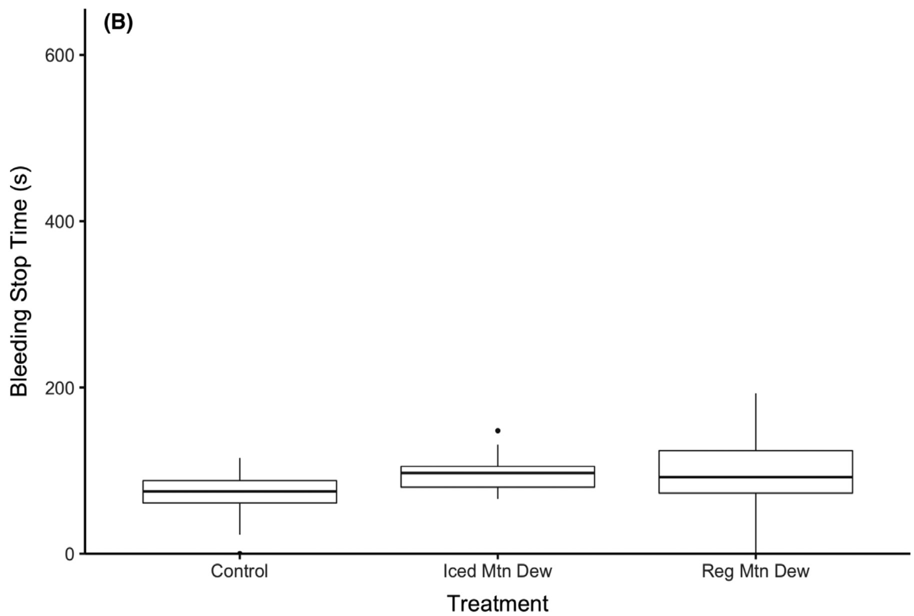


FIGURE 2. Bleeding cessation time after a standardized gill injury in Northern Pike: **(A)** in experiment 1, the control was compared against treatments with carbonated lake water (LW), Mountain Dew, and Coca-Cola; and **(B)** in experiment 2, the control was compared against treatments with Mountain Dew chilled to 4–8°C (Iced Mtn Dew) and ambient-temperature (24–27°C) Mountain Dew (Reg Mtn Dew).

TABLE 1. Model selection outputs for ordinal logistic regression models. Full models included gill color of Northern Pike as the response, the interaction between treatment and time as a fixed effect, and individual as a random effect. Backward model selection was used to determine final model structure (AIC = Akaike's information criterion). Significant differences in model fit are highlighted by bold italic font.

Experiment	Model specification	AIC	Residual deviation	Log likelihood	*P*-value	df
1 ($n = 118$)	***Treatment × Time***	***2,014.924***	***1,954.924***	***25.575***	***<0.001***	***8***
	Treatment	2,086.274	2,046.274	−29.198	1.000	2
	Time	***2,053.076***	***2,017.075***	***59.600***	***<0.001***	***2***
2 ($n = 38$)	***Treatment × Time***	***626.000***	***572.000***	***13.7312436***	***0.033***	***6***
	Treatment	652.3781	614.378	−0.0592199	1.000	1
	Time	***650.3189***	***614.318***	***21.8639438***	***<0.001***	***2***

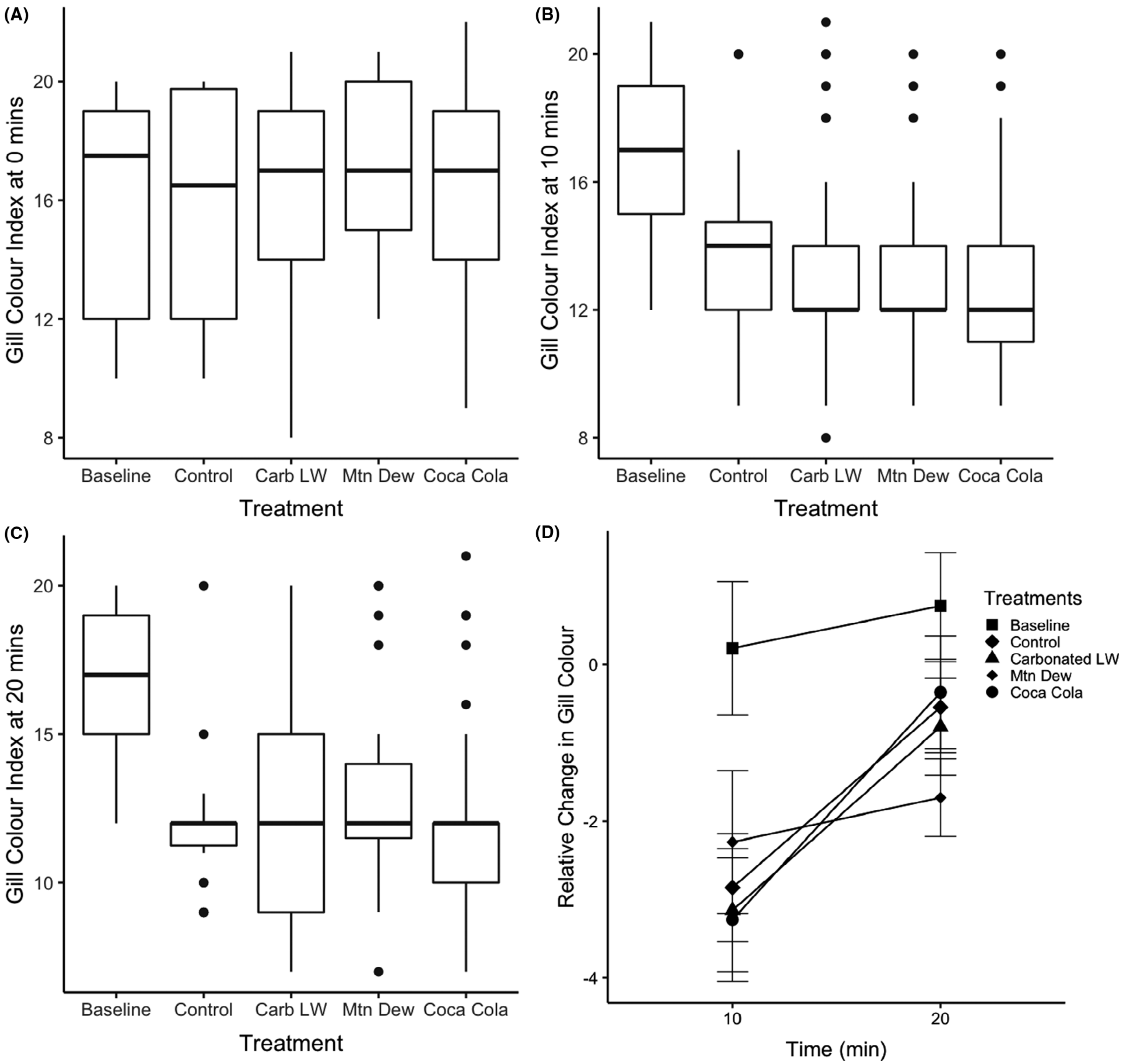


FIGURE 3. Gill color index for Northern Pike at **(A)** 0 min, **(B)** 10 min, and **(C)** 20 min in experiment 1. **(D)** The relative change in gill color from 0 to 10 min and from 10 to 20 min is shown. In all graphs, the control is compared against treatments with carbonated lake water (LW), Mountain Dew (Mtn Dew), and Coca-Cola.

Survival and Reflexes

In experiment 1, no significant effect of treatment group on RAMP score was detected ($F_4 = 1.008$, $P =$ 0.405). In experiment 2, a significant treatment effect was detected ($F_3 = 4.002$, $P = 0.013$), with fish subjected to the chilled Mountain Dew treatment exhibiting significantly higher impairment than the baseline group ($t_{46} = -3.43$, $P = 0.001$). Only three fish were euthanized owing to low RAMP scores (two fish in the carbonated lake water group and one fish in the Coca-Cola group during experiment 1).

DISCUSSION

The main claim of those in the recreational angling community who promote the use of carbonated beverages

TABLE 2. Ordinal logistic regression model outputs for gill color index of Northern Pike, with treatment as a predictor using control values as the reference group for comparisons at individual time periods (0, 10, and 20 min). Significant differences are highlighted by bold italic font.

Time (min)	Comparison	Value	SE	*t*-value	*P*-value
		Experiment 1 (*n* = 118, df = 439)			
0	Carbonated lake water	0.14346940	0.4523524	0.31716292	0.751
	Mountain Dew	0.61190184	0.4527499	1.35152284	0.176
	Coca-Cola	0.14213540	0.4630298	0.30696813	0.758
	Baseline	0.00598101	0.4948112	0.01208746	0.990
10	Carbonated lake water	−0.40226948	0.4550232	−0.88406369	0.376
	Mountain Dew	−0.19233292	0.4416917	−0.43544612	0.663
	Coca-Cola	−0.50898612	0.4646763	−1.09535621	0.273
	Baseline	***1.78152855***	***0.4945991***	***3.60196492***	***<0.001***
20	Carbonated lake water	0.1578421	0.4628604	0.3410145	0.733
	Mountain Dew	0.6228802	0.4417568	1.4100071	0.158
	Coca-Cola	−0.05273126	0.4547337	−0.1159608	0.907
	Baseline	***2.500492***	***0.4969529***	***5.0316475***	***<0.001***
		Experiment 2 (*n* = 38, df = 146)			
0	Chilled Mountain Dew	−0.1457	0.7063	−0.2063	0.8366
	Regular Mountain Dew	−0.4886	0.6783	−0.7204	0.4713
	Baseline	0.0071	0.7534	0.0094	0.9925
10	Chilled Mountain Dew	−0.8734	0.7416	−1.1777	0.2389
	Regular Mountain Dew	***−1.6813***	***0.7281***	***−2.3093***	***0.0209***
	Baseline	***1.3084***	***0.7342***	***1.7821***	***0.0747***
20	Chilled Mountain Dew	−1.2230	0.7538	−1.6225	0.1047
	Regular Mountain Dew	−0.2734	0.7305	−0.3742	0.7082
	Baseline	***3.3882***	***0.9348***	***3.6243***	***<0.001***

is that this practice decreases the duration of bleeding related to hooking injury, particularly in the gills (Pyzer 2019). However, our study found no differences in the time to cessation of bleeding, gill color (as an index of blood loss), or bleeding intensity among treatments in which three carbonated beverages were poured over bleeding gills or between the carbonated beverage treatments and a control group. Differences were only detected between the baseline group, in which no simulated hooking injury occurred, and all other groups, which were subjected to a standardized injury and exhibited bleeding. Overall, our study provided no evidence that pouring carbonated liquids on gill injuries of Northern Pike is beneficial. Both carbonated water and carbonated beverages appeared to result in similar bleeding scores, gill coloration, and impairment scores.

The presumed benefit of pouring CO_2-rich drinks on the gills of fish is that the combination of hypoxia- and CO_2-induced bradycardia may exert a positive effect on gills that are bleeding. Many anglers lift fish out of the water once landed, exposing them to air and promoting hypoxia (Cooke and Sneddon 2007), which can induce bradycardia (Randall 1982; Cooke et al. 2002, 2003; Furimsky et al. 2003; Farrell 2007). Therefore, when fish are removed from the water so that carbonated beverages can be used on the gills, the perception that bleeding has ceased is possible, but the effect is largely driven by hypoxia-induced decreases in cardiovascular output (Reid and Perry 2003; Perry and Desforges 2006). For our study, fish were held horizontally in a water-filled trough such that the gills were constantly submerged in well-oxygenated water. This approach should have limited hypoxia-induced bradycardia, allowing effects of the carbonated beverages to be detected. Because we used white coolers, CO_2-induced bradycardia effects were apparent. Fish would not bleed for over 30 s, followed by blood spurting from the gills. If a fish were to be released by an angler or held in the water, this bleeding might not be apparent and could account for reports of carbonated beverages stopping blood loss (for a short period).

Independent of the effects of carbonated beverages on bleeding, acidic solutions may have damaging effects on fish gill tissues. Low-pH water has been shown to cause a general inflammatory response, an increase in mucus production, and alterations in the structural morphology of the gills in teleost fish (Meyer et al. 2009). Although many of these studies have used more chronic exposures (i.e., >24 h) and different study species than used in our

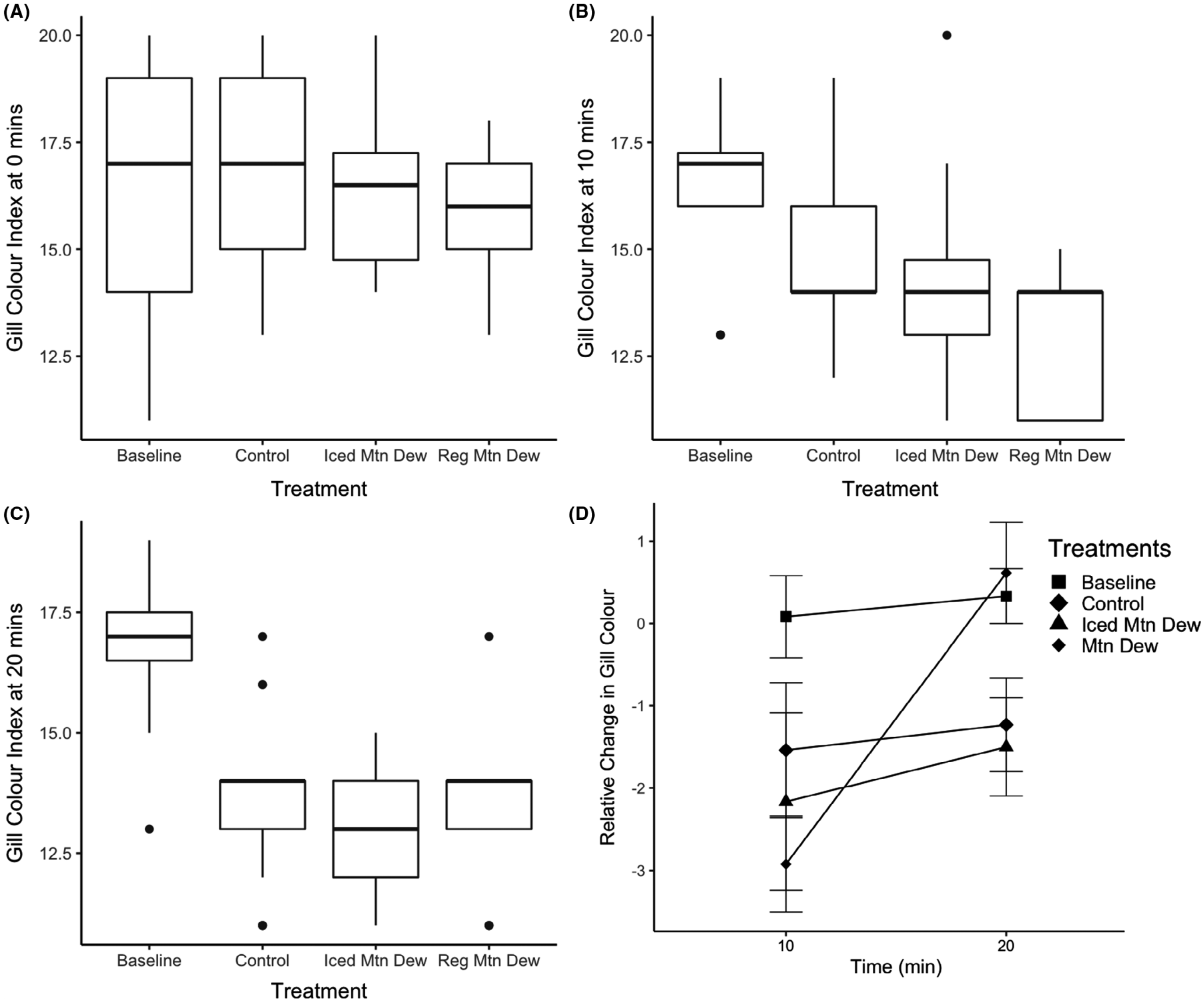


FIGURE 4. Gill color index for Northern Pike at **(A)** 0 min, **(B)** 10 min, and **(C)** 20 min in experiment 2. **(D)** The relative change in gill color from 0 to 10 min and from 10 to 20 min is shown. In all graphs, the control is compared against treatments with Mountain Dew chilled to 4–8°C (Iced Mtn Dew) and ambient-temperature (24–27°C) Mountain Dew (Reg Mtn Dew).

study, acute exposure may still have negative effects on fish (Meyer et al. 2009), particularly with regard to ion and acid–base regulation (Wright and Wood 2009). Interestingly, Northern Pike are relatively tolerant of environmental acidification and have been reported to survive in water with a pH of 4.2–5.5 (Beamish 1976; Haines 1981), which may allow them to cope with an acute acid exposure.

Further work should address how acute instances of branchial acid exposure can affect ion and acid–base status in this context to fully appreciate the biological consequences of using carbonated beverages in an angling setting. Additionally, further experiments should investigate the mechanistic physiology of the potential changes caused by carbonated beverages (i.e., gill structure histology, ionoregulatory flux, and blood physiology changes [pH, pCO_2, HCO_3^-, etc.]). Given that we limited our observations to 20 min during this study, we did not assess more chronic physiological and behavioral effects or postrelease mortality. Northern Pike are regarded as being relatively robust to hooking injury (Arlinghaus et al. 2008), and we observed little immediate or short-term mortality. Assessing survival would be a metric of interest to fisheries managers and should be explored in future studies. In Northern Pike that were actually injured during capture for this study, the longer-term consequences of

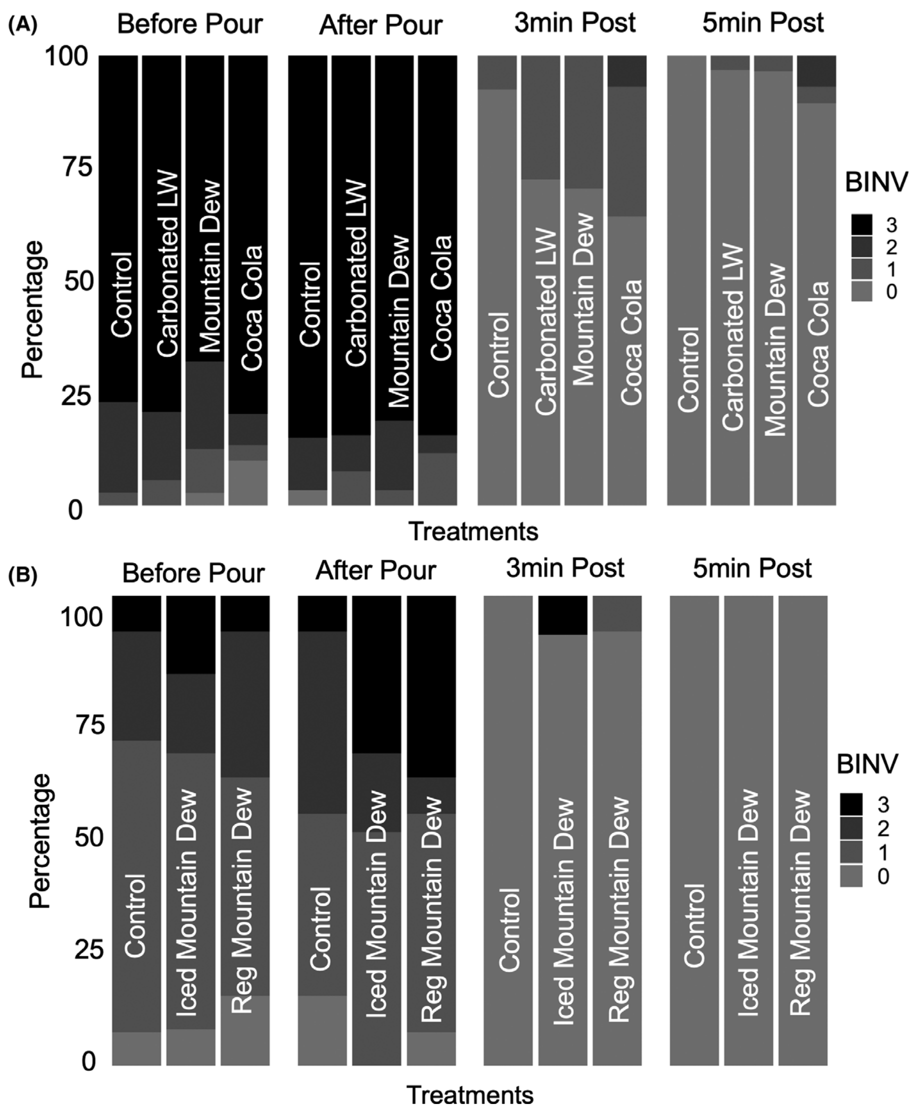


FIGURE 5. Bleeding intensity values (BINVs) after a standardized gill injury in Northern Pike: **(A)** in experiment 1, the control was compared against treatments with carbonated lake water (LW), Mountain Dew, and Coca-Cola; and **(B)** in experiment 2, the control was compared against treatments with Mountain Dew chilled to 4–8°C (Iced Mountain Dew) and ambient-temperature (24–27°C) Mountain Dew (Reg Mountain Dew). Panels show the BINVs after the gill injury but before the pour of any substance (Before Pour), after a substance was poured over the gills (After Pour), and 3 min (3min Post) or 5 min (5min Post) after the substance was poured over the gills.

TABLE 3. Model selection outputs for ordinal logistic regression models. Full models included bleeding intensity of Northern Pike as the response, the interaction between treatment and time as a fixed effect, and individual as a random effect. Backward model selection was used to determine final model structure (AIC = Akaike's information criterion). Significant differences in model fit are highlighted by bold italic font.

Experiment	Model specification	AIC	Residual deviation	Log likelihood	*P*-value	df
1 (*n* = 118)	Treatment × Time	531.4823	495.4823	9.473	0.394	9
	Treatment	1,030.8998	1,018.8998	0.059	0.996	3
	Time	***520.6760***	***508.6760***	***510.223***	***<0.001***	***1***
2 (*n* = 38)	Treatment × Time	230.3625	202.3625	1.7381	0.942	6
	Treatment	361.4302	351.4302	0.600	0.741	2
	Time	***217.4912***	***205.4912***	***145.939***	***<0.001***	***1***

pouring chemicals associated with carbonated sodas remain unknown. Future work involving telemetry or net-pens would be useful for understanding the longer-term consequences of this carbonated beverage technique. Lastly, our study used a simulated gill injury of a standardized size in a single species of fish, so further investigation is needed to determine whether the magnitude of injury or species-specific differences would produce different results by adding control experimental series. Instead of having to deal with hook injuries and bleeding, measures should be taken to prevent injuries that result in severe bleeding. Possible solutions include employing hook styles that may prevent deep hooking/gill damage and using proper hook sets, barbless hooks, and single hooks instead of treble hooks.

Overall, we provide the first evidence that counters the growing popularity of using carbonated beverages to stop bleeding in angled fish. Our observations shed light on the perception among anglers that carbonated beverages curtail bleeding, which may in fact be an artefact of bradycardia induced by air exposure and CO_2. We found no significant benefit or disbenefit from pouring carbonated beverages over the gills of Northern Pike, but it is possible that there are longer-term impacts (e.g., gill tissue damage). Importantly, our findings are specific to the context studied here. As such, it is possible that carbonated beverages could provide benefit or harm when used in other contexts, such as with other species (e.g., salmonids or Muskellunge), when using other beverages, or when applying the beverages in other ways (e.g., holding the fish in air for longer after applying the beverage). This study contributes to the growing body of literature that emphasizes the need for anglers and fisheries scientists to work collaboratively to ensure that the best practices employed by anglers provide a benefit to the fish (Brownscombe et al. 2017).

ACKNOWLEDGMENTS

We acknowledge John Anderson (Ottawa Musky Factory) for encouraging us to pursue this research. We extend our gratitude to Queen's University Biological Station for serving as a base for this research. Adam Williamson, Jon Kulbeka, Brooke Etherington, and many other researchers helped to make this project possible. Ben Hlina provided input on statistical analysis and figures. The document was kindly reviewed by Kathleen Gilmour. Funding was provided by Muskies Canada and The Anderson Family Foundation, with additional support from the Natural Sciences and Engineering Research Council of Canada and the Canada Research Chairs Program. All research was conducted in accordance with the guidelines of the Canadian Council on Animal Care as administered by Carleton University (Animal Use Protocol 110558). There is no conflict of interest declared in this article.

ORCID

Alexandria T. Trahan https://orcid.org/0000-0003-3556-0895
Auston D. Chhor https://orcid.org/0000-0003-1785-4902
Michael J. Lawrence https://orcid.org/0000-0002-4801-1580
Connor H. Reid,? https://orcid.org/0000-0001-9431-9044

REFERENCES

Anderson, J. 2018. Save a million fish. Available: https://www.facebook.com/pages/category/Environmental-Conservation-Organization/Save-a-Million-Fish-752045115140863/. (March 2020).

Arlinghaus, R., S. J. Cooke, J. Lyman, D. Policansky, A. Schwab, C. Suski, S. G. Sutton, and E. B. Thorstad. 2007. Understanding the complexity of catch-and-release in recreational fishing: an integrative synthesis of global knowledge from historical, ethical, social, and biological perspectives. Reviews in Fisheries Science 15:75–167.

Arlinghaus, R., T. Klefoth, A. Kobler, and S. J. Cooke. 2008. Size selectivity, injury, handling time, and determinants of initial hooking mortality in recreational angling for Northern Pike: the influence of type and size of bait. North American Journal of Fisheries Management 28:123–134.

Bardin, S. 2019. Pouring soda on fish gills: does it actually work? Wired2Fish (April 2). Available: https://www.wired2fish.com/biology/pouring-soda-on-fish-gills-does-it-actually-work/#slide_3. (May 2020).

Bartholomew, A., and J. A. Bohnsack. 2005. A review of catch-and-release angling mortality with implications for no-take reserves. Reviews in Fish Biology and Fisheries 15:129–154.

Beamish, R. J. 1976. Acidification of lakes in Canada by acidic precipitation and the effects on fishes. Water, Air, and Soil Pollution 6:501–504.

Booth, J. H. 1978. The distribution of blood flow in the gills of fish: application of a new technique to Rainbow Trout (*Salmo gairdneri*). Journal of Experimental Biology 73:119–129.

Brauner, C. J., and D. W. Baker. 2009. Patterns of acid–base regulation during exposure to hypercarbia in fishes. Pages 43–63 *in* M. L. Glass and S. C. Wood, editors. Cardio-respiratory control in vertebrates: comparative and evolutionary aspects. Springer, Berlin.

Brauner, C. J., R. B. Shartau, C. Damsgaard, A. J. Esbaugh, R. W. Wilson, and M. Grosell. 2019. Acid–base physiology and CO_2 homeostasis: regulation and compensation in response to elevated environmental CO_2. Fish Physiology 37:69–132.

Brownscombe, J. W., A. J. Danylchuk, J. M. Chapman, L. F. G. Gutowsky, and S. J. Cooke. 2017. Best practices for catch-and-release recreational fisheries—angling tools and tactics. Fisheries Research 186:693–705.

Coe, A. J., A. J. Picard, and M. G. Jonz. 2017. Purinergic and adenosine receptors contribute to hypoxic hyperventilation in Zebrafish (*Danio rerio*). Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 214:50–57.

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

Cooke, S. J., K. G. Ostrand, C. M. Bunt, J. F. Schreer, D. H. Wahl, and D. P. Philipp. 2003. Cardiovascular responses of Largemouth Bass to exhaustive exercise and brief air exposure over a range of water temperatures. Transactions of the American Fisheries Society 132:1154–1165.

Cooke, S. J., and H. L. Schramm. 2007. Catch-and-release science and its application to conservation and management of recreational fisheries. Fisheries Management and Ecology 14(2):73–79.

Cooke, S. J., J. F. Schreer, D. H. Wahl, and D. P. Philipp. 2002. Physiological impacts of catch-and-release angling practices on Largemouth Bass and Smallmouth Bass. Pages 489–512 *in* D. P. Phillip and M. S. Ridgway, editors. Black bass: ecology, conservation, and management. American Fisheries Society, Symposium 31, Bethesda, Maryland.

Cooke, S. J., and L. U. Sneddon. 2007. Animal welfare perspectives on recreational angling. Applied Animal Behaviour Science 104(3–4):176–198.

Davis, M. W. 2007. Simulated fishing experiments for predicting delayed mortality rates using reflex impairment in restrained fish. ICES Journal of Marine Science 64:1535–1542.

Evans, D. H., P. M. Piermarini, and K. P. Choe. 2005. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid–base regulation, and excretion of nitrogenous waste. Physiological Reviews 85:97–177.

Farrell, A. P. 2007. Tribute to P. L. Lutz: a message from the heart—why hypoxic bradycardia in fishes? Journal of Experimental Biology 210:1715–1725.

Furimsky, M., S. J. Cooke, C. D. Suski, Y. Wang, and B. L. Tufts. 2003. Respiratory and circulatory responses to hypoxia in Largemouth Bass and Smallmouth Bass: implications for "live-release" angling tournaments. Transactions of the American Fisheries Society 132:1065–1075.

Gilmour, K. M. 2001. The CO_2/pH ventilatory drive in fish. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 130:219–240.

Gilmour, K. M. 2010. Perspectives on carbonic anhydrase. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 157(3):193–197.

Gilmour, K. M., and W. K. Milsom. 2009. CO_2/H+ chemoreceptors in the respiratory passages of vertebrates. Pages 403–406 *in* G. Zaccone, E. Cutz, D. Adriaensen, C. A. Nurse, and A. Mauceri, editors. Airway chemoreceptors in the vertebrates: structure, evolution and function. Science Publishers, Enfield, New Hampshire.

Gilmour, K. M., W. K. Milsom, F. T. Rantin, S. G. Reid, and S. F. Perry. 2005. Cardiorespiratory responses to hypercarbia in Tambaqui *Colossoma macropomum*: chemoreceptor orientation and specificity. Journal of Experimental Biology 208(6):1095–1107.

Gilmour, K. M., and S. F. Perry. 2009. New insights into the many functions of carbonic anhydrase in fish gills. Respiratory Physiology and Neurobiology 184:223–230.

Green, J. 2015. Can you stop a fish bleeding from the gills with Coke or Sprite? Nodak Angler. Available: https://nodakangler.com/forums/content/373-Can-You-Stop-A-Fish-Bleeding-From-The-Gills-With-Coke-Or-Sprite. (March 2020).

Haines, T. A. 1981. Acidic precipitation and its consequences for aquatic ecosystems: a review. Transactions of the American Fisheries Society 110:669–707.

Kwong, R. W. M., Y. Kumai, and S. F. Perry. 2014. The physiology of fish at low pH: the Zebrafish as a model system. Journal of Experimental Biology 217:651–662.

Meyer, E. A., R. L. Cramp, and C. E. Franklin. 2009. Damage to the gills and integument of *Litoria fallax* larvae (Amphibia: Anura) associated with ionoregulatory disturbance at low pH. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 155:164–171.

Muoneke, M. I., and W. M. Childress. 1994. Hooking mortality: a review for recreational fisheries. Reviews in Fisheries Science 2: 123–156.

Neuharth, S. 2019. Stop pouring soda on fish gills. MeatEater (March 15). Available: http://themeateater.com/fish/bass/stop-pouring-soda-on-fish-gills. (June 2019).

Paukert, C. P., J. A. Klammer, R. B. Pierce, and T. D. Simonson. 2001. An overview of Northern Pike regulations in North America. Fisheries 26(6):6–13.

Perry, S. F., and P. R. Desforges. 2006. Does bradycardia or hypertension enhance gas transfer in Rainbow Trout (*Oncorhynchus mykiss*)? Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 144:163–172.

Perry, S. F., and K. M. Gilmour. 2002. Sensing and transfer of respiratory gases at the fish gill. Journal of Experimental Zoology 293:249–263.

Pyzer, G. 2015. Coke: it's the real thing to stop fish bleeding. Outdoor Canada (July 5). Available: https://www.outdoorcanada.ca/coke-its-the-real-thing-to-stop-fish-bleeding/. (May 2019).

Pyzer, G. 2019. Stop the bleeding: how soda water can save an injured fish. Outdoor Canada (March 13). Available: https://www.outdoorcanada.ca/stop-the-bleeding-how-soda-water-can-save-an-injured-fish/. (January 2020).

R Core Team. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available: https://www.R-project.org/. (February 2021).

Raby, G. D., S. G. Hinch, D. A. Patterson, J. A. Hills, L. A. Thompson, and S. J. Cooke. 2015. Mechanisms to explain purse seine bycatch mortality of Coho Salmon. Ecological Applications 25:1757–1775.

Randall, D. 1982. The control of respiration and circulation in fish during exercise and hypoxia. Journal of Experimental Biology 100:275–288.

Reid, S. G., and S. F. Perry. 2003. Peripheral O_2 chemoreceptors mediate humoral catecholamine secretion from fish chromaffin cells. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 284:R990–R999.

Reid, S. G., L. Sundin, A. L. Kalinin, F. T. Rantin, and W. K. Milsom. 2000. Cardiovascular and respiratory reflexes in the tropical fish, Traira (*Hoplias malabaricus*): CO_2/pH chemoresponses. Respiration Physiology 120:47–59.

Sundin, L., S. G. Reid, F. T. Rantin, and W. K. Milsom. 2000. Branchial receptors and cardiorespiratory reflexes in a neotropical fish, the Tambaqui (*Colossoma macropomum*). Journal of Experimental Biology 203:1225–1239.

Tavares-Dias, M., and S. R. Oliveira. 2009. A review of the blood coagulation system of fish. Brazilian Journal of Biosciences 7:205–224.

Tresguerres, M., W. K. Milsom, and S. F. Perry. 2019. CO_2 and acid–base sensing. Fish Physiology 37:33–68.

Tuong, D. D., B. Borowiec, A. M. Clifford, R. Filogonio, D. Somo, D. T. T. Huong, N. T. Phuong, T. Wang, M. Bayley, and W. K. Milsom. 2018. Ventilatory responses of the Clown Knifefish, *Chitala ornata*, to hypercarbia and hypercapnia. Journal of Comparative Physiology B 188:581–589.

Wood, C. M., and P. G. McDonough. 1988. Impact of environmental acidification on gill function in fish. Pages 162–182 *in* R. C. Ryans, editor. Fish physiology, fish toxicology, and fisheries management: proceedings of an international symposium. U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, Georgia.

Wright, P. A., and C. M. Wood. 2009. A new paradigm for ammonia excretion in aquatic animals: role of rhesus (RH) glycoproteins. Journal of Experimental Biology 212:2303–2312.

Wydoski, R. S. 1977. Relation of hooking mortality and sublethal hooking stress to quality fishery management. Pages 43–87 *in* R. A. Barnhart and T. D. Roelofs, editors. Catch-and-release fishing as a management tool. Humboldt State University, Arcata, California.