Testing potential mitigation strategies for injuries in angled fish

By

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General Abstract

Recreational angling is a common and enjoyable activity that many people consider a hobby, lifestyle and/or passion. It does however affect fish populations worldwide. In order to promote sustainable fisheries, anglers should use best practices when returning fish to the water. Possible interventions that could be implemented to enable long term survival and improve welfare outcomes include alterations to angling gear or the use of tactics that could reduce blood loss. The goal of my thesis was to identify strategies to mitigate injuries that arise during recreational fishing events. Chapter 2 investigated if replacing treble hooks on hard plastic lures with single hooks would reduce injuries and fish handling time in three common targeted gamefish species. My data suggested that making these alterations reduced unhooking time for Northern Pike, Smallmouth and Largemouth Bass. Shorter unhooking time was shown to decrease air exposure, fish handling time and reduce injuries associated with long unhooking and handling times. Chapter 3 explored the tactic of pouring carbonated beverages on bleeding fish injuries. I did not find any benefits of using Mountain Dew™, Coca Cola™ or carbonated lake water on bleeding injuries in Northern Pike. This study encourages anglers to return the fish to the water to recover instead of intervening. In both chapters, fish were caught with rod and reels with various lure types. Together, these studies recommend that anglers switch the treble hooks on their hard plastic lures to single hooks and return the fish to the water as soon as possible in order to mitigate injuries that arise during catch-and-release events.

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Chapter 1. General Introduction

1.1 Recreational Fishing

Recreational fishing is considered to be fishing done largely as leisure activity. This type of angling is practiced for reasons other than meeting basic nutritional needs or for selling/trading (Food and Agriculture Organisation of the United Nations 2017). Recreational fishing is a popular activity around the globe that generates numerous social and economic benefits (Cooke and Schramm 2007). Just over 10.5% of the global population participates in recreational fishing (Arlinghaus et al. 2015). Recreational fisheries generate billions of dollars in both developed, transitioning, and developing countries (Food and Agriculture Organization of the United Nation 2012). It is also considered a cultural ecosystem service due to it being relevant to human health and wellbeing (Hernández-Morcillo et al. 2013; Arlinghaus et al. 2019; Pousso et al. 2019). Recreational fishing can have a lower impact on fish stocks and ecosystems than industrial fisheries (Cooke and Cowx 2006).

To this day, there remain many unknowns about global recreational fisheries and their effects are underestimated (Cooke and Cowx 2006; Lewin et al. 2006; Pauly and Zeller 2016). Historically, captured fish were harvested but recently but there has been a tendency for recreational anglers to voluntarily release fish (Arlinghaus et al. 2017). Moreover, harvest regulations are commonly used by fisheries managers that require some fish to be released to be in compliance (Cowx 2002). There is a need for research to investigate an effective way to sustain recreational fishing but not harm fish populations (Arlinghaus et al. 2019).

Recreational fishing is sometimes regarded as less detrimental to fish populations than commercial fishing. However, its effects are not negligible, and limitations of size and number of fish harvested are still essential. These limitations need to be implemented to encourage healthy population numbers (Cooke and Cowx 2006; Policansky 2007). Recreational fishing is primarily practiced for pleasure but is also used to generate income and to supply food (Cooke and Cowx 2006). A single angler has less of an impact than a commercial fishing boat. However, millions of anglers can have a large impact on fish populations (Arlinghaus et al. 2002; Cooke and Cowx 2006).

1.2 Catch-and-Release Angling

The premise of catch-and-release (C&R) angling is that fish returned to the water survive and can be caught again (Cooke and Suski 2005). C&R is also practiced when anglers are trying to catch fish for harvest as they have to release some fish that do not meet the size requirements of the law (Arlinghaus et al. 2007). When a fish is caught, it is penetrated by a hook, which is a type of injury. Fish can be injured or experience physiological alterations via air exposure, angler handling, hooking removal, fight time, hooking location, and lures/hooks used (Brownscombe et al. 2017). To minimize these injuries, anglers may implement best practices to decrease the severity of injuries, reduce bleeding, and increase survival when releasing fish (Brownscombe et al. 2017).

1.3 Physiological Effects of Catch-and-Release Angling

There is no form of angling where there is zero risk of mortality for captured fish. Therefore, the notion that C&R has a 100% survival rate is not realistic (Arlinghaus et al. 2007). However, when implementing best practices during C&R angling it is possible to minimize mortality, injuries, and bleeding. Best practices vary by species and environment (Muoneke and Childress 1994; Bartholomew and Bohnsack 2005). These practices are further mediated by angler skill and gear choice (Muoneke and Childress 1994; Bartholomew and Bohnsack 2005). An underlying issue that is not always visible to the naked eye is the physiological effects on fish that are caught and released. When fish are fought on the end of a fishing line and then removed from the water, there are energetic disruptions that have negative impacts on them short term but could also result in longer-term impacts down the road, like reductions in fitness (Cooke et al. 2002). Although the initial survival rate of various species has been well studied in the long-term, sublethal effects of C&R events remain unclear (Pope et al. 2007). Studies have demonstrated that warmer water, long unhooking times, and air exposure increase stress, change in blood chemistry, and behaviour of fish within the first 72 hours (Gustaveson et al. 1991; reviewed in Arlinghaus et al. 2007). Researchers have employed various tools/techniques such as blood sampling and muscle physiology, cardiorespiratory tests, and reflex impairment assessment (Raby et al. 2012). Blood sampling and muscle physiology provide information to researchers such as lactate, glucose, osmolality, ions (sodium, potassium, chloride), hemoglobin levels in addition to hormone levels like cortisol (Gustaveson et al. 1991; Cooke et al. 2002; Meka and McCormick 2005; Pope et al. 2007; Cooke et al. 2013; Louison et al. 2017). A downside of taking blood samples is the manipulation that has to be done to the fish, such as the air exposure, confinement, possible sedation, and invasive nature of the extraction. On occasion, these changes in blood physiology cannot be linked to a specific type of manipulation (Hoffman and Lommel 1984; Lawrence et al. 2020). Cardiorespiratory tests include but are not limited to stroke volume, heart rate, and cardiac output which again provides additional understanding of what is happening inside the fish. However, technology such as heart rate loggers are required to gather this information. Heart rate loggers are invasive and require the fish to be confined as the logger needs to be retrieved to download the information (Cooke et al. 2001). A quick and non-invasive technique to assess behavioural impairment of a fish is using reflex action mortality predictors (RAMP) (Davis 2007; Raby et al. 2015). Common scoring systems use a three-point scale which includes 1) ability to maintain equilibrium, 2) reaction to a tail grab (burst swimming), and 3) vestibular ocular response (i.e., eye tracking) before releasing a fish. This method can yield some information of the level of physiological stress since behaviour is linked to physiology. However, stress hormone levels cannot be determined (Raby et al. 2012).

Many of the measurements to assess the stress of angling are often done immediately after landing the fish (e.g., Donaldson et al. 2011) but miss the delayed physiological consequences of these events (Cooke 2013). Although initial mortality is not always observed during C&R events, the air exposure and injuries that occur while removing hooks from fish could cause issues that occur later in the lifespan of these fish that we do not observe (Cooke et al. 2001). Sublethal effects of C&R include decreased ability to acquire food and disruption of homeostasis (Campbell et al. 2010). Additional sublethal effect can include but are not limited to infections, disease, and reduction in growth which can also increase post-release predation (Dubois and Dubielzig 2004).

1.4 Reducing Injury and Mortality

Many strategies have been developed in an attempt to reduce injury and mortality, but none have been successful in reducing bleeding. Ways to decrease fish mortality include but are not limited to decreased air exposure, decreased handling times, quick unhooking, minimizing hooking injury, and unhooking strategies (Muoneke and Childress 1994; Bartholomew and Bohnsack 2005; Arlinghaus et al. 2007; Cooke and Schramm 2007). A comprehensive study that outlined many of the possible gear, intrinsic and environmental factors that lead to mortality demonstrated that many reports could conflict one another (Bartholomew and Bohnsack 2005). It is then expected that studies done today may also contradict one another.

A key factor that influences fish bleeding and mortality is the anatomical hooking locations (Lindsay et al. 2004; James et al. 2007; Moraga et al. 2015). Injuries in sensitive areas have a higher chance of leading to intense bleeding and mortality. Fish captured using live bait have increased likelihood of mortality for various species compared to artificial lures (Beukemaj 1970; Pauley and Thomas 1993; Masilan and Neethiselvan 2018). In some species, treble hooks increased mortality over single hooks, and in some species, barbless hooks had a lower mortality rate and overall decreased air exposure (Bartholomew and Bohnsack 2005). It is known that catch rates are influenced by the lure and hook type used but this also influences the severity of injuries and stress that can occur when a fish is caught and when the hooks are removed (Brownscombe et al. 2017).

When a hook penetrates a fish, there is always a possibility that they may bleed, particularly when they are injured in areas like the gullet and gills (Muoneke and Childress 1994; Bartholomew and Bohnsack 2005; Arlinghaus et al. 2007; Cooke and Schramm 2007). Although it is known that bleeding injuries occur during C&R events, currently there are no scientifically proven methods that reduce or stop said bleeding from occurring. To reduce the bleeding, some anglers have tried using unconventional tactics, but these alternative practices require further investigation before they can be supported by scientists, management, and policy makers.

1.5 Research Rationale and Objectives

The goal of my thesis was to identify strategies for mitigating injuries arising during recreational fishing events. To do so I explored two issues. Chapter 2 investigated if replacing treble hooks on lures with single hooks reduces injuries and fish handling times in Northern Pike (*Esox lucius*), Smallmouth Bass (*Micropterus dolomieu*) and Largemouth Bass (*Micropterus salmoides*). Lure type, hook type, number of hook points, unhooking time, use of hook removal gear, anatomical hooking location, hooking depth, deepest hook, reflex action mortality predictors (RAMP), and immediate mortality were measured to determine the efficacy and benefits of different fishing gear across these three species. Chapter 3 explored the practice of using carbonated beverages to reduce blood loss of gill injuries in angled Northern Pike. Bleeding cessation time, gill colour index as a proxy for blood loss, bleeding intensity values, survival and reflexes were quantified to evaluate the effects of using various carbonated beverages on Northern Pike. Collectively, these studies will lead to filling the gaps on how to potentially reduce fish mortality and injury. These findings will share knowledge with anglers, the scientific community, and policymakers on the best practices that should be implemented to encourage the survival of fish post-release.

Chapter 2. Influence of artificial lure hook type on hooking characteristics and injury of angled freshwater gamefish

2.1 Abstract

Catch-and-release is practiced in recreational fisheries under the premise that released fish will survive with negligible injury and stress. However, hooking injuries may prevent those outcomes from being realized. One way to potentially minimize injuries and maximize survival in angled fish is to replace treble hooks with single hooks on hard plastic lures, but the effectiveness of this tactic has yet to be tested. Our study investigated if replacing treble hooks with single hooks on hard plastic lures reduced injuries and handling times for angled Northern Pike (*Esox lucius*), Smallmouth Bass (*Micropterus dolomieu*) and Largemouth Bass (*Micropterus salmoides*). Furthermore, we compared fish handling time and injuries between fish that were captured with barbed and barbless hooks. Fish were angled using three types of conventional hard plastic lures (i.e., crankbaits, jerkbaits, and lipless crankbaits). Upon landing, total length of the fish, an array of hooking characteristics (i.e., number of hook points in the fish, anatomical hooking location(s)), and reflex impairment were recorded. Linear models indicated that using barbless J hooks on all lures yielded the shortest unhooking time for all species. For Smallmouth Bass caught on both crank and jerk baits, J hooks tended to result in more shallow hooking depth than treble hooks. Barbless treble hooks were more likely to be embedded in a sensitive location (e.g., foul hooked, gullet, gills, and/or eyes) compared to barbless J hooks in Smallmouth Bass. No other significant differences in hook types and anatomical locations were found for other species tested. Hook type and lure type did not influence reflex impairment or survival for any of the species. Using J hooks, especially barbless, on lures that traditionally have treble hooks should be considered when encouraging best angling practices for the freshwater gamefish studied here to expedite release although the extent to which this influences mortality remains unclear.

Key Words: recreational angling, catch-and-release, hooking, injury

2.2 Introduction

Recreational fishing is a popular activity around the globe. Although some fish are harvested, it is increasingly common that fish are released to comply with regulations or as a voluntary action linked to a conservation ethos (Arlinghaus et al. 2007). An assumption that underpins catch-and-release (C&R) as a conservation and management strategy is that mortality is low and that any injuries or sublethal disturbances are short lived (Wydoski 1977; Cooke and Schramm 2007). However, a growing body of research reveals that not all fish survive angling events (Muoneke and Childress 1994; Bartholomew and Bohnsack 2005; Arlinghaus et al. 2007). Mortality rates are highly variable and context dependent, (Bartholomew and Bohnsack 2005; Brownscombe et al. 2017) varying widely depending on environmental factors, angler behaviour, gear type used, and species-specific responses to stress (reviewed in Brownscombe et al. 2017).

Across C&R studies, a common factor has been identified as being the single largest determinant of fish survival - anatomical hooking location, whereby fish hooked in vital areas (e.g., the gullet and/or gills) tend to experience higher mortality and bleeding compared to fish hooked in the jaw (e.g., Pelzman 1978; Taylor and White 1992; reviewed in Bartholomew and Bohnsack 2005). Hooking location can be influenced by a variety of factors including lure/bait type, gear type, and angler experience (Muoneke and Childress 1994). For example, organic baits tend to result in deeper hooking locations than artificial baits while smaller baits tend to result in deeper hooking locations than larger baits (Arlinghaus et al. 2008; Fobert et al. 2009). Novice anglers are also more likely to deeply hook fish in comparison to more experienced anglers (Dunmall et al. 2001).

Hook type and hook number have also been shown to influence physical damage in recreationally angled fish (Muoneke and Childress 1994; Brownscombe et al. 2017). For instance, circle hooks tend to yield shallower hooking locations compared to J hooks (Cooke and Suski 2004). Many studies on salmonids have found that using single hooks on lures results in decreased mortality compared to the use of treble hooks (Hunsaker et al. 1970; Matlock et al. 1993; Nuhfer and Alexander 1992; Warner 1979). Similar findings have been found with Northern Pike, where using a single hook on lures instead of a treble hook tended to result in less mortality (Burkholder 1992). A recent study has suggested that using lures with fewer hooks and/or single hooks may help to reduce unhooking time and minimize air exposure in Largemouth Bass (Clarke et al. 2020). Single hooks have been shown to cause less injury and lower mortality in comparison to treble hooks in some contexts. However, there has been very little work done evaluating the effects of using a single hook on hard plastic fishing lures commonly used by anglers when targeting freshwater gamefish (Cooke and Suski 2005). Hook type and number can also influence handling time, which is another factor to consider when assessing impacts on recreationally angled fish (Brownscombe et al. 2017).

Traditionally, most artificial hard body lures use treble hooks, but this tradition has been slowly changing in the angling community. There are discussions in online forums as well as increasing number of fishing media stories about the merits of replacing treble hooks on hard bodied lures with single J style hooks (e.g., Landesfeind 2018; Waters 2019). Northern Pike, Smallmouth Bass, and Largemouth Bass are species that are traditionally caught with treble hooks when using hard plastic lures. As such, investigating the impacts of using single J hooks could provide insight on whether there is merit in replacing treble hooks on lures. The use of barbless hooks has also become a common practice (sometimes voluntary or mandated) in some jurisdictions as some studies suggest barbless hooks reduce injury during catch and release angling events (Meka 2004; Bartholomew and Bohnsack 2005).

The primary objective of this study was to investigate if replacing treble hooks on lures with single hooks reduces injuries and fish handling times. To do so, we focused on hard plastic lures (i.e., crankbaits, jerkbaits, and lipless crankbaits) which are commonly used to target freshwater gamefish, and for which barbed treble hooks are the default hook type at time of purchase. This study compared treble hooks (barbed and barbless) as well as J hooks (barbed and barbless) on the different lure types. Three species were included in the study (Northern Pike, Smallmouth Bass, and Largemouth Bass) representing some of the most popular freshwater gamefish in North America. Given interspecific variation in anatomy and hook performance we did not quantitatively compare outcomes among different species.

2.3 Methods

2.3.1 Animal Welfare

All experiments were conducted in accordance with regulations and guidelines set by the Canadian Council on Animal Care (Carleton University protocol AUP #110558). Fish were collected under Scientific Collection Permit #08577 from the Ontario Ministry of Natural Resources and Forestry.

2.3.2 Study Site and Fish Capture

Angling was conducted on Lake Opinicon (44.5590° N, 76.3280° W), Constance Lake (45.4090° N, 75.9797° W), Mississippi Lake (45.0321° N, 76.2029° W), Big Rideau Lake (44.7706° N, 76.2152° W), and the Rideau River (45.3151° N, 75.6971° W) from May to August in both 2019 and 2020. These bodies of water were chosen because they support popular sport fisheries where Largemouth Bass (n = 246), Smallmouth Bass (n = 103), and Northern Pike (n = 220) are targeted with lures. Water temperature varied from 10.5 - 29°C over the course of the study. All lakes are in eastern Ontario and have similar fish communities and characteristics (i.e., they support both cool water and warmwater fish communities).

2.3.3 Fishing Gear

To catch fish, anglers spent time casting and trolling from a boat between 05:00 to 22:00. Fish were captured by anglers of varying skill levels using crankbaits, jerkbaits, and lipless crankbaits. Crankbaits are hard plastic diving lures with a lip that were equipped with two hooks and the average size used was 6.5 ± 0.7cm. The most-used crankbaits were Strike King KVD Squarebill, Rapala DT (Dives-To) Series and Pro Model 6XD Crankbait. Lipless crankbaits are also hard plastic diving lures but do not have a lip and vibrate in the water. They were equipped with two hooks and the average size used was 7.0 ± 0.9 cm and were mostly Rapala Rippin' Raps and Cotton Cordell Super Spot. Jerkbaits are slender shallow lures that are retrieved with a jerking motion done by the angler. They were equipped with two hooks and the average size used was 10 cm, Rapala X-Raps. The study used treble hooks that came on the lures (barbed and barbless) as well as J hooks (7237 - Light Inline Single VMC). Single J hooks were either size 1/0 or 2/0 depending on the size of the lures (smaller lures had smaller hooks, and vice versa). To convert barbed hooks to barbless, the barb on each hook was pinched using pliers, not completely removed. Lures and hooks combinations were used in a randomized rotation by all anglers. Fish were caught on medium to medium-heavy spinning and baitcasting (2 – 2.1 m) rods with gear matched to the size of the lure and the target species paired with braided line (minimum 9 kg braid). Although anglers were of different skill levels, these lures are all fished actively and simply require holding a rod (trolling) or reeling in. This is unlike working soft plastic lures or live bait where bites may not be evident for the angler and where angler expertise can thus influence outcomes for fish (see Gutowsky et al. 2017; Clarke et al. 2020).

2.3.4 Hook Removal

Fish were landed as quickly as possible (always within 1 min) and netted when they were near the side of the boat. The fish were immediately transferred to a padded water-filled trough where hook removal was conducted while the fish was submerged in order record all variables accurately. Hook removal time was conducted by an experienced angler (at least 2 years of angling experience) and was defined as the length of time between the angler touching hook to when the hook was removed from the fish. The anatomical location (upper lip, lower lip, corner, etc.), number of embedded hooks, and depth of each hook point was recorded. Hooking depth was converted into a proportion in relation to total length to account for size differences among fish (Cooke et al. 2001; Gutowsky et al. 2017). When fish had more than one embedded hook point, the average of the hooking depth (in proportion to total length) was used for analysis. The type of lure and hook(s) that fish were caught on was also recorded. Hook removal was classified as "self", where the hook came out of the fish without any help from an angler, "hand", where anglers used their hands to remove hook, and "tool", where the angler used pliers or haemostats to remove hooks. Before release, total length of the fish was also measured to the nearest mm.

2.3.5 Reflexes and Survival

We recorded three key reflex action mortality predictors (RAMP) to evaluate if the fish was impaired following our treatments (Davis 2007; Raby et al. 2015): 1) ability to maintain equilibrium, 2) reaction to a tail grab (burst swimming), and 3) vestibular ocular response (i.e. eye tracking). The reflexes were scored as present or absent. Overall, six Northern Pike out of 569 fish were euthanized due to low RAMP score and inability to recovery and subsequently swim away. All other fish were released immediately following the evaluation.

2.3.6 Data Analysis

R Version 1.1.447 (R Core Team 2019) was used to conduct statistical analyses. Number of hooks, unhooking time, sensitive location, mean hook depth, depth of deepest hook, hook removal method, and reflexes/survival were compared among treatments using linear regression models. Significant effects were assessed using Type 2 sum of squares (Barbur et al. 1994). Post-hoc analysis was done when ANOVA results were significantly different using likelihood ratios (LR) or false discovery rate (FDR) (Benjamini and Hochberg 1995). Statistical significance was accepted at $\alpha = 0.05$ and, unless otherwise noted, all values were presented as means ± SEM.

2.4 Results

A total of 569 fish were captured and included in our study, including 220 Northern Pike, 103 Smallmouth Bass, and 246 Largemouth Bass (Table 2.1), from across the five different study lakes (Lake Opinicon n= 380, Constance Lake n = 53, Mississippi Lake n = 67, the Rideau River n = 12, Big Rideau Lake n = 60).

2.4.1 Number of Hook Points

The number of hook points in fish varied from 1 to 5 (of a maximum of 6 hook points on lures with two treble hooks). The number of hook points in fish did not vary significantly by hook type or lure type for any of the species (Table 2.2).

2.4.2 Unhooking Time

Unhooking time ranged from 0 - 305 seconds (mean = 10 ± 29 seconds). There was no difference in hook removal times among lures (Northern Pike, $F_2 = 2.74$, $p = 0.067$; Smallmouth Bass, $F_1 = 0.92$, $p = 0.34$ and Largemouth Bass, $F_2 = 2.69$, $p = 0.07$). Hook type had a significant effect on the unhooking time for all three species (Northern Pike, $F_3 = 9.30$, $p < 0.001$; Smallmouth Bass, $F_3 = 5.46$, $p = 0.002$ and Largemouth Bass, $F_3 = 11.32$, $p < 0.001$) (Figure 2.2). Across species, barbed treble hooks took the longest to remove while barbless single hooks were the quickest to remove (Table 2.3 and Table 2.4). For Northern Pike caught with crankbaits, lipless crankbaits, and jerkbaits, single barbless hooks had the fastest unhooking time and barbed treble had the slowest. For Smallmouth Bass caught with crankbaits, and jerkbaits, single barbless hooks had the fastest unhooking time and treble barbed hooks had the slowest. For Largemouth Bass caught with crankbaits, lipless crankbaits and jerkbaits, single barbless hooks had the fastest unhooking time and treble barbed had the slowest.

2.4.3 Use of Hook Removal Gear

There was a significant association between hook type and hook removal method for Northern Pike, Smallmouth Bass, and Largemouth Bass, (Northern Pike, $LR_2 = 43.32$, $p < 0.001$; Smallmouth Bass, $LR_2 = 19.05$, $p < 0.001$ and Largemouth Bass, $LR_2 = 62.79$, $p < 0.001$), although no post-hoc differences were observed. Differences in hook type did not influence the use of different hook removal gear, although pliers were used more frequently than bare hands to remove barbed treble hooks from Northern Pike (Z Ratio = -6.464, $p < 0.001$).

2.4.4 Anatomical Hooking Location

Hooking location was not influenced by lure types for any species. For Smallmouth Bass, there was an overall significant difference in the distribution of hook placement among the different hook types ($LR_3 = 10.3$, chi-squared = 0.016), but there were not any differences observed using a post-hoc test.

2.4.5 Hooking Depth

The average hook depth (measured from snout) ranged from 1 - 235 mm (mean 36 ± 26 mm, median = 31 mm). Once hooking depths were corrected to body length (as per Cooke et al. 2001), there were no significant differences in average relative hooking depth between lure or hook type in Northern Pike. Average hooking depth was significantly influenced by hook type in Smallmouth Bass and Largemouth Bass (LR_3 = 12.76, p = 0.005). For Smallmouth Bass, crankbait and jerkbait single barbless hooks were more shallow than treble barbed and barbless lures. Overall, there was a significant difference of average hooking depth in hook type in Largemouth Bass (LR_3 = 12.57, p = 0.006). However, there were no pairwise differences of average hook depth between hook types observed.

2.4.6 Deepest Hook

The deepest hook distance ranged from 1 - 235 mm (mean 39 ± 28 mm). There were no significant differences of deepest hook depth between lure or hook type in Northern Pike. Hook type had a significant effect on the deepest hooking location in Smallmouth Bass (LR_3 = 7.1, p < 0.001). The deepest hooks were barbless treble hooks followed respectively by barbed treble, barbless single, and barbed single, with barbed single being the most shallow hook (Figure 2.3). Hook type had an overall significant effect on the deepest hooking depth in Largemouth Bass (F_3 = 3.29, p = 0.021). There were no significant differences found in the post-hoc test.

2.4.7 Reflexes and Immediate Mortality

There was no significant effect of lure type on RAMP score detected for any species. Overall, there was a significant effect of hook type detected on RAMP score

($F_3 = 4.001$, $p = 0.008$) for Northern Pike, where treble barbed hooks were more likely to have a negative impact on reflexes followed by treble barbless then J barbed hooks. Only six Northern Pike were euthanized due to low RAMP score (two fish caught with jerkbaits that had barbed treble hooks, two fish caught with lipless crankbaits that had barbless treble hooks and one fish for both jerkbait single barbless and lipless crankbait treble barbed). In all instances, substantial bleeding was observed, and fish failed to swim away.

2.5 Discussion

Overall findings from our study demonstrate that replacing treble hooks with single hooks on hard plastic lures reduces deep hooking and unhooking time for Northern Pike, Smallmouth Bass, and Largemouth Bass. Across species, we found limited evidence or added benefit of using barbless hooks; the use of single hooks over treble hooks derived the greatest benefit. With barbed treble hooks being the most common and commercially available hook type on freshwater hard bodied plastic lures, and anecdotal evidence from social media showing that some anglers are replacing their treble hooks with single J hooks, the results of our study validate how changing hook types can minimize hooking injury and dehooking times.

Terminal gear selection influences the number of hook points that can penetrate a fish (Muoneke and Childress 1994). A treble hook is composed of three single hooks attached to a common shaft, which increases the potential of more hook points piercing a fish during angling events compared to a single hook. Decreasing the number of hooks on lures is thought to reduce injuries (Muoneke and Childress 1994). However, we did not find any significant differences in the number of hook points that pierced fish between treble and single hooks, or between lure types and hook types across all species observed in this study. This extended to no differences in bleeding or reflex impairment (i.e., equilibrium, burst swimming and/or vestibular ocular response). Given that individual fish will attack a lure in different ways given context, it is not entirely surprising that the number of hooks in a fish was similar across lure types and hook types. Aside from a study by Gutowsky et al. (2017), we know very little about how fish interact with baits and how this varies among individuals.

We found that using single barbless hooks on hard plastic baits expedited unhooking time for Northern Pike, Smallmouth Bass, and Largemouth Bass (Figure 2.1). In comparison to other studies (e.g., Arlinghaus et al. 2008; Clarke et al. 2020), we did not find any significant relationship between lure types and unhooking time for any of the species. Unhooking time is an important indicator of handling time and air exposure that fish experience during angling events (Cook et al. 2015). Although Northern Pike, Smallmouth Bass, and Largemouth Bass are somewhat resilient to air exposure, effects on behaviour and physiology have been documented (e.g., Cooke et al. 2002; Thompson et al. 2008; White et al. 2008; Arlinghaus et al. 2009) and may impact long-term fitness (Davis 2002; Coggins et al. 2007). In general, it is highly recommended that air exposure is minimized to decrease physiological (Pankhurst and Dedualk 1994; Cook et al. 2015; Gagne et al. 2017) and behavioural disturbances (Thorstad et al. 2004; Klefoth et al. 2008; Arlinghaus et al. 2009). Since Smallmouth

Bass and Largemouth Bass teeth cause minimal damage to anglers, the majority of the hooks were removed by hand. Northern Pike have multiple rows of sharp teeth which resulted in pliers being used more often to avoid angler injury. Barbed treble hooks had the longest unhooking time in Northern Pike, and the use of pliers to remove the hooks was more common. The large number of possible penetrating barbed hook points on the lures and the number of sharp teeth in Northern Pike increased the difficulty of removing hooks, compared to hook removal of Smallmouth Bass and Largemouth Bass. Although it is thought that removal of hooks with pliers is more efficient (Clarke et al. 2020), our study showed that using pliers was associated with significantly longer unhooking times compared to removing hooks by hand. This is confounded by the fact that easy-to-remove hooks did not require pliers. It is clear that tool intervention was required to remove hooks when in difficult (deep) anatomical locations, accounting for longer unhooking times.

Lure type did not have a significant impact on the hooking location in any of the species, which is in contrast from previous studies (e.g., Myers and Poarch 2000; Arlinghaus et al. 2008; Clarke et al. 2020). In other species like Chinook Salmon, anatomical hooking location was shown to be a key factor in predicting fish survival (Linsay et al. 2004). Salmon that were hooked in the gills, gullet, eyes, and tongue had higher mortality rates that fish hooked in less critical locations (Lindsay et al. 2004). Hooking in vital areas, such as the gills and gullet, can cause increased bleeding and reduced survival of fish captured by recreational angling (Muoneke and Childress 1994; Lyle et al. 2007; Arlinghaus et al. 2008; Clarke et al. 2020). For our study, hooking in sensitive locations was only influenced by hook type in Smallmouth Bass, which could be related to how this species attacks the bait. Largemouth Bass and Northern Pike are able to open their mouths more than Smallmouth Bass due to anatomical differences.

Hooking depth was significantly related to hook type in Smallmouth Bass and Largemouth Bass. Previously, it was found that treble hooks are deeply swallowed less often compared to single hooks (Muoneke and Childress 1994). However, our findings for Smallmouth Bass and Largemouth Bass contradict this. There was a significant increase in average hooking depth in Smallmouth Bass when treble hooks (barbed and barbless) were used vs barbless single hooks. Yet, hooking depth does not necessarily translate into hooks in the gullet or gills and in the case of hard plastic baits is often in the context of external (foul) hooking. For Largemouth Bass, there was a significant increase in average hooking depth when barbless treble hooks were used compared to barbed treble hooks as well as barbed treble hooks vs barbed single hooks. Similarly, the deepest hooking location in Smallmouth Bass was significantly different between hook types. Hooking depth ranged from shallow to deep as follows; single barbless hooks, single barbed hooks, barbless treble hooks, and treble barbed hooks (Figure 2.3). Treble hooks cause more damage once they are embedded into fish (Muoneke and Childress 1994) including Largemouth Bass (Clarke et al. 2020). Therefore, this contradictory finding supports the use of single hooks to minimize internal hooking damage and minimize average hooking depth in Smallmouth Bass and Largemouth Bass. Hooking depth is a key factor in determining injury intensities (Arlinghaus et al. 2007). When hooks are embedded deep in fish, they are harder to remove resulting in higher likelihood of injury, air exposure, and mortality (Arlinghaus et al. 2007; Bartholomew and Bohnsack 2005; Cooke and Suski 2005; Cooke et al. 2012). Therefore, to minimize negative outcomes associated with deep hooking events, using single hooks on hard plastic lures should be considered when targeting black bass.

Overall immediate mortality was minimal, likely due to the lack of major hooking injuries. When instances of mortality did occur, they were likely due to fish being hooked in sensitive locations and/or long unhooking time. Hook removal is usually accompanied with air exposure, however all hook removals in our study were done while fish were submerged. Although we standardized this in our study, removing hooks while fish are submerged in water is unlikely to occur when fish are landed by regular anglers. RAMP was assessed before fish were released and there were few cases where fish experienced any loss of equilibrium or any other reflex impairment. Fish were not monitored post-release, thus long-term effects are unknown. Wound severity, size of injury, or amount of bleeding were not taken into account in this study. However, previous studies have found that wound severity and bleeding were often greater when single hooks were used versus treble hooks (Muoneke 1992b; Nuhfer and Alexander, 1992). Yet, treble hooks may cause less mortality than single hooks because they are more difficult to swallow (Klein 1965; Muoneke 1992).

Fish were caught across two years in multiple seasons therefore water temperature was not consistent (10.5 - 29 °C), and the added effects of water temperature were not considered in this study. The effects of water temperature have been well studied, demonstrating increased stress and mortality in fish at higher water temperatures (Cooke and Suski 2005; Arlinghaus et al. 2007; Hühn and Arlinghaus 2011). However, with so little mortality observed here it is not possible to assess such relationships. Our study did not account for fight time which could have impacted injuries, and RAMP score. Also, for future studies, other lure types should be considered (i.e., top water lures, spybaits etc.), as well as other hook type combinations (i.e., circle hooks, octopus hooks, etc.). Additionally, although fishing with barbless hooks can decrease handling time (Meka 2004), it can also negatively influence catch rate (Alós et al. 2008). As such, future studies should consider investigating hooking to capture ratio for each hook type (barbed vs barbless) to see if a specific hook type increases success rates of capture.

2.6 Conclusion

Our research provides evidence that hook type on hard plastic lures used to capture Northern Pike, Smallmouth Bass, and Largemouth Bass is important for determining some welfare outcomes. Lure and hook type influenced the unhooking time, hooking location, average hook depth, and deepest hook in most cases. Specifically, barbed treble hooks typically took longer to remove compared to single barbless hooks. Angler education programs, fishing guides, and fishing media should promote scientifically tested species-specific best practices to potentially reduce population-level effects. This study provides a direct comparison of various lure and hook combinations and builds scientific knowledge on the benefits that come with replacing treble hooks with single hooks (also observed in Clarke et al. 2020), expediting unhooking time, and minimizing air exposure. Substituting treble hooks for single J hooks on hard plastic lures decreases unhooking time which can reduce injuries in Northern Pike, Smallmouth Bass, and Largemouth Bass. The ability to easily remove hooks can decrease air exposure (assuming most anglers do not remove hooks in water as we did here) creating better welfare outcomes for angled fish. Hook removal tools such as pliers and haemostats are beneficial to use when hooks are in difficult to remove locations and to promote angler safety, particularly for fish with sharp dentition such as Northern Pike. In situations where fish are hooked deeply and hook removal may cause intense bleeding, anglers should cut the hook to avoid mortality (Cooke and Danylchuk 2020). In conclusion, single barbless hooks on lures reduce hooking time compared to treble barbed hooks in Northern Pike, Smallmouth Bass, and Largemouth Bass. Anglers should consider their use when targeting these species. Future studies should investigate the long-term survival post-release for each possible combination of lure and hook type as well as investigate other factors that may promote long-term survival of fish post C&R events.

Figure Captions

A)

B)

Figure 2.1 Image of two jerkbaits used in the study with A) barbless treble hooks and B) barbed single J hooks.

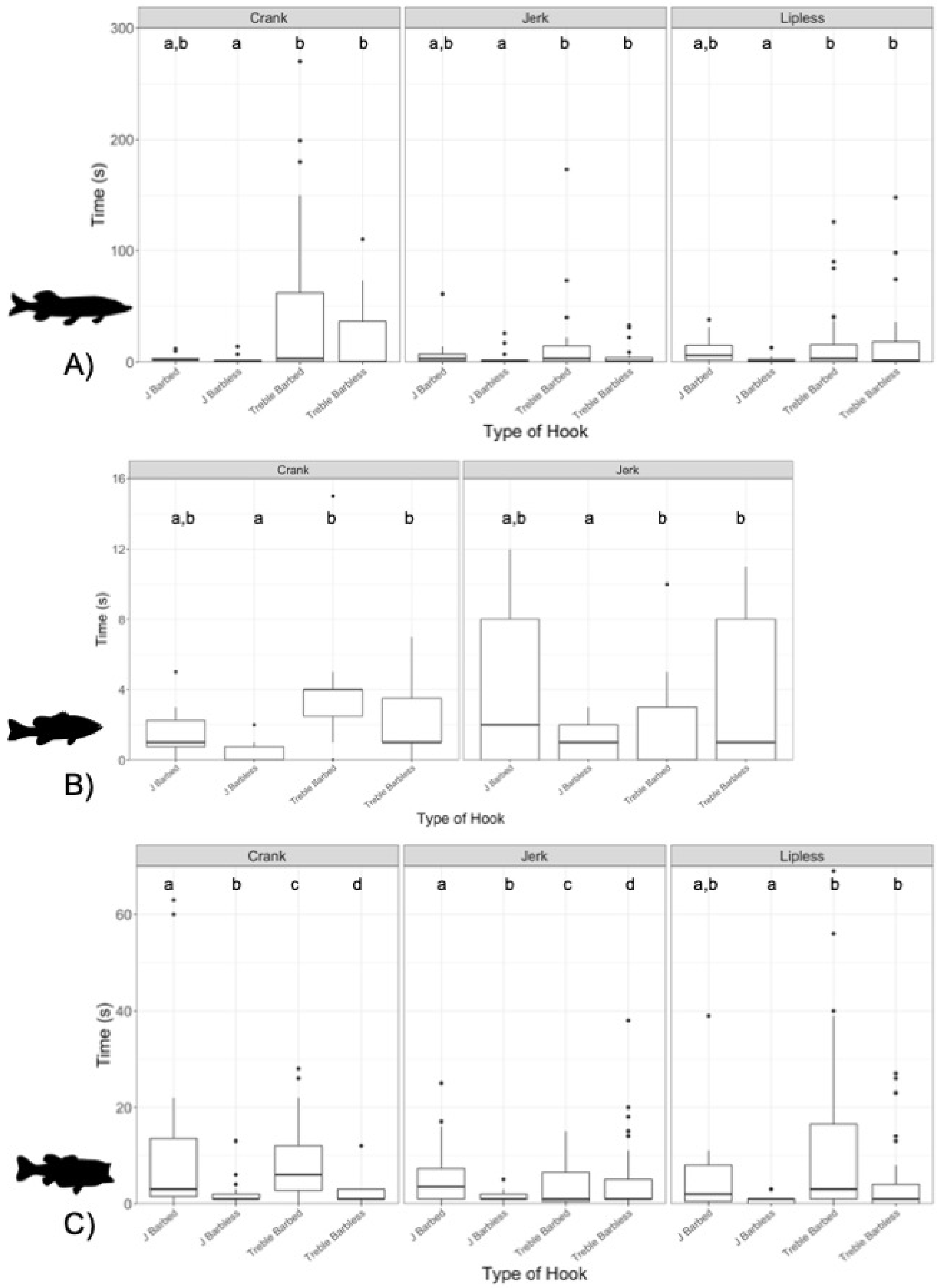

Figure 2.2 Time to remove various hook types (J Barbed, J Barbless, Treble Barbed and Treble Barbless) from A) Northern Pike (Crank Bait n = 65 , Jerk Bait n = 72, and Lipless Crank Bait n = 83), B) Smallmouth Bass (Crank Bait n = 45, and Jerk Bait n = 58) and C) Largemouth Bass (Crank Bait n = 76, Jerk Bait n = 90, and Lipless Crank Bait n = 80). One outlier from Smallmouth Bass was removed (Crank Bait Treble Barbed) and two points from Largemouth Bass were removed (Crank Bait Treble Barbed and J Barbed).

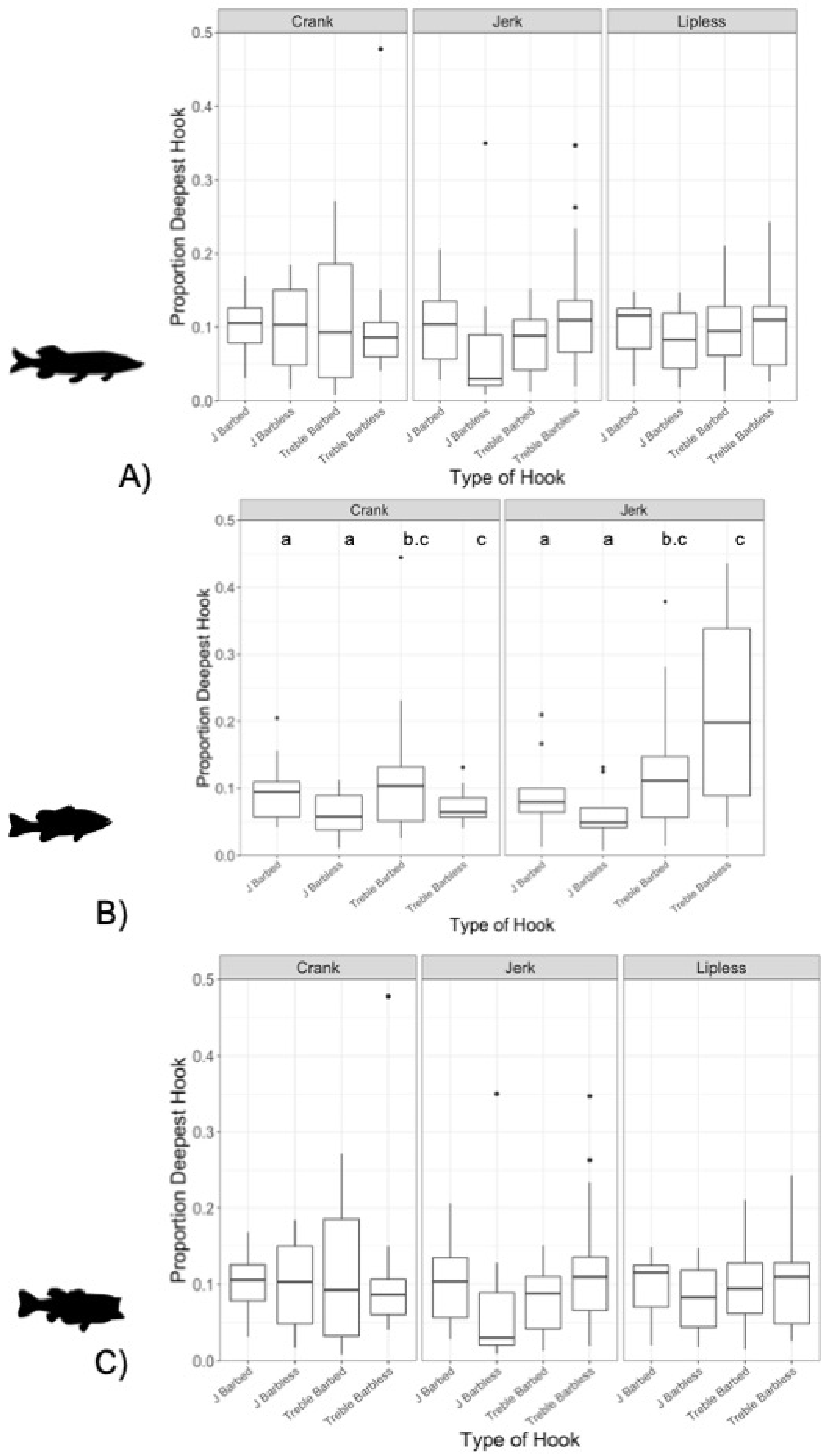

Figure 2.3 The deepest hook of various hook types (J Barbed, J Barbless, Treble Barbed and Treble Barbless) from A) Northern Pike (Crank Bait n = 65 , Jerk Bait n = 72, and Lipless Crank Bait n = 83), B) Smallmouth Bass (Crank Bait n = 45, and Jerk Bait n = 58) and C) Largemouth Bass (Crank Bait n = 76, Jerk Bait n = 90, and Lipless Crank Bait n = 80) in proportion to total length of fish.

Tables

Table 2.1 Size distribution and total number of fish caught for every combinator of lure and hook per species.

Species	Lure	Hook Type	Total (n)	Min TL (mm)	Max TL (mm)	Avg TL (mm)	SE +/- (mm)
Northern Pike	Crank Bait	J Barbless	11	400	800	626	132
		J Barbed	14	440	885	614	134
		Treble Barbed	29	465	647	559	53
		Treble Barbless	11	438	840	608	134
	Jerk Bait	J Barbless	14	445	855	565	120
		J Barbed	13	460	746	561	91
		Treble Barbed	23	257	785	532	122
		Treble Barbless	22	403	690	511	71
	Lipless Crank Bait	J Barbless	11	535	875	691	109
		J Barbed	13	409	779	552	112
		Treble Barbed	27	345	640	501	63
		Treble Barbless	32	320	735	515	86
Smallmouth Bass	Crank Bait	J Barbless	10	178	402	303	114
		J Barbed	12	169	389	275	64
		Treble Barbed	13	170	390	287	79
		Treble Barbless	10	175	444	289	92
	Jerk Bait	J Barbless	13	140	335	274	53
		J Barbed	19	250	430	335	55
		Treble Barbed	13	185	395	315	67
		Treble Barbless	13	195	420	292	68
Largemouth Bass	Crank Bait	J Barbless	17	189	446	317	68
		J Barbed	12	210	385	332	46
		Treble Barbed	33	215	380	299	45
		Treble Barbless	14	175	430	299	76

	Jerk Bait	J Barbless	11	100	500	345	112
		J Barbed	20	265	425	333	48
		Treble Barbed	11	224	365	303	47
		Treble Barbless	48	229	583	328	57
	Lipless Crank Bait	J Barbless	12	220	527	338	91
		J Barbed	11	218	411	316	54
		Treble Barbed	24	160	486	310	64
		Treble Barbless	33	235	425	330	41

Table 2.2 Anova outputs for number of hooks as predictor with lure type and hook type as responses for Northern Pike (n = 220), Smallmouth Bass (n = 103) and Largemouth Bass (n = 246). No significant differences were found therefore no posthoc analysis were done.

Species	Predictor	LR	DF	Chi-Squared
Northern Pike	Lure Type	0.858	2	0.651
	Hook Type	3.517	3	0.319
Smallmouth Bass	Lure Type	0.135	2	0.713
	Hook Type	4.67	3	0.198
Largemouth Bass	Lure Type	2.708	2	0.258
	Hook Type	4.222	3	0.239

Table 2.3 Anova outputs for unhooking time as predictor with lure type and hook type as responses for Northern Pike (n = 220), Smallmouth Bass (n = 103), and Largemouth Bass (n = 246). Significant differences are highlighted by bold italic font.

Species	Predictor	Sum Squares	DF	F value	p-value
Northern Pike	Lure Type	19.33	2	2.737	0.067
	Hook Type	***98.57***	***3***	***9.306***	***<0.001***
Smallmouth Bass	Lure Type	2.201	1	0.917	0.341
	Hook Type	***39.331***	***3***	***5.462***	***0.002***
Largemouth Bass	Lure Type	13.87	2	2.695	0.07
	Hook Type	***87.38***	***3***	***11.322***	***<0.001***

Table 2.4 Posthoc analysis outputs for unhooking time as predictor with lure type and hook type as responses for Northern Pike using FDR (n = 220). Significant differences are highlighted by bold italic font.

Species	Contrast	Estimate	SE	Z ratio	p value
Northern Pike	Crank J Barbed - Crank J Barbless	-0.194	0.106	1.837	0.12
	Crank J Barbed - Crank Treble Barbed	0.086	0.04	2.132	0.081
	Crank J Barbed - Crank Treble Barbless	0.059	0.042	0.042	0.205
	Crank J Barbless - Crank Treble Barbed	***0.28***	***0.099***	***2.835***	***0.038***
	Crank J Barbless - Crank Treble Barbless	***0.254***	***0.1***	***2.545***	***0.045***
	Crank Treble Barbed - Crank Treble Barbless	-0.026	0.019	1.413	0.205
	Jerk J Barbed - Jerk J Barbless	-0.195	0.106	1.837	0.12
	Jerk J Barbed - Jerk Treble Barbed	0.086	0.04	2.132	0.081
	Jerk J Barbed - Jerk Treble Barbless	0.059	0.042	1.399	0.205
	Jerk J Barbless - Jerk Treble Barbed	***0.28***	***0.1***	***2.835***	***0.03***
	Jerk J Barbless - Jerk Treble Barbless	***0.254***	***0.1***	***2.545***	***0.045***
	Jerk Treble Barbed - Jerk Treble Barbless	-0.026	0.018	1.413	0.205
	Lipless J Barbed - Lipless J Barbless	-0.195	0.106	1.837	0.12
	Lipless J Barbed - Lipless Treble Barbed	0.086	0.04	2.132	0.081
	Lipless J Barbed - Lipless Treble Barbless	0.059	0.042	1.399	0.205
	Lipless J Barbless - Lipless Treble Barbed	***0.28***	***0.099***	***2.835***	***0.038***
	Lipless J Barbless - Lipless Treble Barbless	***0.254***	***0.1***	***2.545***	***0.045***
	Lipless Treble Barbed - Lipless Treble Barbless	-0.026	0.018	-1.413	0.205
Smallmouth Bass	Crank J Barbed - Crank J Barbless	-0.64	0.307	-2.084	0.121
	Crank J Barbed - Crank Treble Barbed	0.139	0.088	1.575	0.195
	Crank J Barbed - Crank Treble Barbless	-0.021	0.123	-0.171	0.864
	Crank J Barbless - Crank Treble Barbed	0.779	0.3	2.595	0.088
	Crank J Barbless - Crank Treble Barbless	0.619	0.312	1.982	0.121
	Crank Treble Barbed - Crank Treble Barbless	-0.16	0.104	-1.533	0.195
	Jerk J Barbed - Jerk J Barbless	-0.64	0.307	-2.084	0.121
	Jerk J Barbed - Jerk Treble Barbed	0.139	0.088	1.575	0.195
	Jerk J Barbed - Jerk Treble Barbless	-0.021	0.123	-0.171	0.864
	Jerk J Barbless - Jerk Treble Barbed	0.779	0.3	2.595	0.088
	Jerk J Barbless - Jerk Treble Barbless	0.619	0.312	1.982	0.121
	Jerk Treble Barbed - Jerk Treble Barbless	-0.16	0.104	-1.533	0.195
Largemouth Bass	***Crank J Barbed - Crank J Barbless***	***-0.485***	***0.146***	***-3.322***	***0.004***
	Crank J Barbed - Crank Treble Barbed	-0.005	0.027	-0.185	0.853
	Crank J Barbed - Crank Treble Barbless	***-0.117***	***0.044***	***-2.683***	***0.018***
	Crank J Barbless - Crank Treble Barbed	***0.48***	***0.15***	***3.301***	***0.004***

	Crank J Barbless - Crank Treble Barbless	***0.368***	***0.15***	***2.472***	***0.024***
	Crank Treble Barbed - Crank Treble Barbless	***-0.112***	***0.042***	***2.652***	***0.018***
	Jerk J Barbed - Jerk J Barbless	***-0.485***	***0.146***	***-3.322***	***0.004***
	Jerk J Barbed - Jerk Treble Barbed	-0.005	0.027	-0.185	0.853
	Jerk J Barbed - Jerk Treble Barbless	***-0.117***	***0.044***	***-2.683***	***0.018***
	Jerk J Barbless - Jerk Treble Barbed	***0.48***	***0.146***	***3.301***	***0.004***
	Jerk J Barbless - Jerk Treble Barbless	***0.368***	***0.149***	***2.472***	***0.025***
	Jerk Treble Barbed - Jerk Treble Barbless	***-0.112***	***0.042***	***-2.652***	***0.018***
	Lipless J Barbed - Lipless J Barbless	-0.195	0.105	1.837	0.118
	Lipless J Barbed - Lipless Treble Barbed	0.086	0.04	2.132	0.081
	Lipless J Barbed - Lipless Treble Barbless	0.059	0.042	1.399	0.205
	Lipless J Barbless - Lipless Treble Barbed	***0.28***	***0.099***	***2.835***	***0.038***
	Lipless J Barbless - Lipless Treble Barbless	***0.254***	***0.1***	***2.545***	***0.045***
	Lipless Treble Barbed - Lipless Treble Barbless	-0.026	0.018	-1.413	0.205

Chapter 3. Do carbonated beverages reduce bleeding from gill injuries in angled Northern Pike?

3.1 Abstract

The premise of catch-and-release in recreational angling is that most fish have high post-release survival. Therefore, it is common for anglers, management agencies, and other 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 either Mountain Dew, Coca Cola, or carbonated lake water. The duration and intensity of bleeding, as well as overall blood loss (using gill colour as a proxy) was observed while the fish were held in a lake water bath. As a control, we had 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 ^{o}C and 24-27 ^{o}C) to determine if the effects of pouring carbonated beverages over injured gills is seasonally dependent. When compared to the control, we found that the duration and intensity of bleeding increased regardless of the type of carbonated beverages 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 for fish that have had their gills injured during recreational angling based on the context studied here. Our study reinforced the need to

scientifically test angler anecdotes and theories when it comes to best practices for catch-and-release fishing.

Key words: gill injury**,** blood loss, fishing, mitigation, catch-and-release

3.2 Introduction

Recreational angling is a common practice around the globe. Although some fish are harvested, a greater percentage of them are released (Cooke & Cowx, 2004). Catch-and-release (C&R) occurs when recreational anglers comply with local harvest regulations or when it is adopted voluntarily based on their conservation ethic (Arlinghaus et al. 2007). Regardless of the reason, the general premise with C&R is that most released fish will survive angling-released 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). Based on this, there has been considerable effort to develop techniques to reduce hooking injuries in critical areas, including bleeding that can occur where the hook penetrates the fish (reviewed in Brownscombe et al. 2017).

Using carbonated beverages, such as cola and citrus beverages, poured over 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. There is even a Facebook page, “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 carbon dioxide (CO_2) in the beverages causes vasoconstriction of the blood vessels to slow or stop bleeding (Pyzer 2015, 2019), or that phosphoric acid in carbonated 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 bleeding in injured fish.

In teleost fishes, it is well known that CO_2 has many effects on fish homeostasis. As a metabolic by-product of aerobic respiration, the accumulation of CO_2 in the blood (i.e. hypercarbia) can produce a state of respiratory acidosis (reviewed in Brauner and Baker 2009)). On the branchial epithelium, the expression of CO_2 receptors has been thought to occur in teleosts (reviewed in Gilmour and Milsom 2009) and exposures to high environmental P_{CO2} have been shown to induce bradycardia, heightened ventilatory rates, and hypertension in fishes (Sundin et al. 2000; Gilmour et al. 2005; Tuong et al. 2018; reviewed in Gilmour 2001). Together, these effects could exacerbate the effects of gill injury following angling if the animal's gills were doused in 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). As such, it might be 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. Together, these lines of physiological responses indicate that the addition of soft drinks to fish gills may cause harm to the animal and contrast the purported benefits of these substances.

Gills are a multifunctional organ 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 P_{CO2} 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). If this was the cause, this would cause a decrease in pH and 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 (Perry and Gilmour 2002). In addition, chemoreceptors that detect changes in CO_2 are located in the gill, 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, or slowing of heart rate, may transiently reduce bleeding (Perry and Desforges 2006). Also, caffeine is an non-specific adenosine receptor antagonist that may alter chemoreceptor signalling (Coe et al. 2017). The exact effects of a combination of these substances on gill function is unclear but it is possible the individual effects may all occur when these substances are applied together.

Regardless of the mechanism, the technique of pouring carbonated beverages over bleeding hook injuries of fish continues to be promoted, but debates as to the efficacy of its uses have also ensued (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 whether carbonated beverages should be considered a best practice for C&R. We aimed to investigate if they reduce or stop bleeding from the gills of injured fish. For this study, we angled Northern Pike (*Esox 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 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 use of chilled beverages influenced outcomes relative to beverages at ambient temperatures.

3.3 Methods

3.3.1 Animal Welfare

All experiments were conducted in accordance to regulations and guidelines set by the Canadian Council on Animal Care (Carleton University protocol AUP #110558).

Northern Pike Fish were collected under Scientific Collection Permit #08577 from the Ontario Ministry of Natural Resources and Forestry.

3.3.2 Fish Capture

Northern Pike were selected for study owing to their relevance in addressing the question of interest and their popularity as a target recreational angling species (Paukert et al. 2001). The study was conducted on wild Northern Pike from Lake Opinicon, Ontario, Canada (44.5590° N, 76.3280° W), and all fish were capture 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 minute, 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 hooking site or the gills upon capture (<10% of fish) were not used in the study and were immediately released. Following hook removal, the total length (TL, mm) of the fish was recorded.

3.3.3 Experimental Injury and Post-Injury Monitoring

Gill injuries were simulated by using end-cutting pliers to remove a section of the gill that had a length of 0.9 cm from the right middle gill arch Figure 3.1. A 0.9 cm section reflects a typical wound size based on observations of naturally hooked fish 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 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 treatments (e.g., cola) were being applied. When pouring carbonated beverages over the gills, a standardized volume of 150 mL was used. Following the procedure, fish were transferred to a white bottomed cooler (52 cm x 26.5 cm) containing nonaerated lake water (~25 liters) for observations. We also included a reference or baseline group of fish that did not have any gill filaments clipped nor liquid poured over the gills before being transferred to the cooler.

Experiments were conducted at two different time periods. *Experiment 1* was conducted in May 2019 when water temperature ranged between 11-18 °C, whereas *Experiment 2* took place in August 2019 when water temperature ranged from 24-27 °C. *Experiment 1* had five treatment groups: 1) baseline, where the gill was not injured, and no carbonated beverages were used; 2) post injury, fish were held in lake water (pH = 6.71) without any other treatment; 3) post injury, carbonated lake water (pH = 4.37) poured over the gills; 4) post injury, Mountain Dew™ (pH = 3.27) poured over the gills; and 5) post injury, Coca-Cola™ (pH = 2.56) poured over the gills. During *Experiment 1,* beverage temperature ranged from 11-18 °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), to mimic an angler either not using or using a cooler, respectively, to hold beverages.

Following treatment fish were individually held in a cooler and visually monitored for 20 min to quantify: 1) time to bleeding cessation; 2) gill colour, 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 colour was assessed against a 20-point colour gradient, with bright red (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, to nearly white (1) (Booth 1978) by one person throughout the entire experiment. Gill colour values were recorded 10 min and 20 min post-injury, as well as immediately before the gill injury occurred, serving as a reference. Relative bleeding intensity (BIN) was based on the following scale: 0, no bleeding; 1, minor bleeding, not obvious; 2, obviously bleeding, easily observed; and 3, intense bleeding, pulsatile blood flow. For all treatment groups other than the baseline group, we recorded bleeding intensity immediately before and after liquid was poured directly onto the wound while the fish was held in a water-filled trough. Additional bleeding intensity values were recorded at 3-min and 5-min post-injury. After 20 min in the cooler, the vigour and condition of the fish were recorded using reflex action mortality predictors (RAMP), and fish that were not moribund were released. Fish that were moribund were euthanized by cerebral percussion (3 fish in total; see below).

3.3.4 Survival and Reflexes

The presence or absence of basic reflexes can be used to predict post-release mortality of fishes (Davis 2007; Raby et al. 2015). Our RAMP scoring system evaluated: 1) ability to maintain equilibrium; 2) reaction to grasping the tail (burst swimming); and

3) vestibular-ocular response (i.e. eye tracking) to determine whether a fish was impaired post-treatment and should be euthanized or released.

3.3.5 Data Analysis

R Version 1.1.447, R Studio (R Core Team 2019) was used to conduct all statistical analyses. For both *Experiment 1* and *2*, bleeding time was compared among treatments using linear regression models and significant effects were assessed using Type 1 sum of squares. For bleeding intensity (BIN; ordinal scale from 0 to 3) and gill colour (ordinal scale from 1 to 20), ordinal logistic regression was applied to each time point at which BIN or gill colour 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 Information Criterion (AIC). When best fit models included interactions, ordinal logistic regression models were fit within each sampling time period to assess differences among treatments. Statistical significance was accepted at α = 0.05 and, unless otherwise noted, all values are presented as means ± SEM.

3.4 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, n = 38) 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).

3.4.1 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 3.2; $F_3 = 0.83$, $p = 0.48$). For *Experiment 2*, time to bleeding cessation ranged from 0 to 193 s (mean 87 ± 40 s) and also not different among treatments (Figure 3.2; $F_2 = 2.47$, $p = 0.10$).

3.4.2 Gill Colour Index

For *Experiment 1*, the best fitting model for gill colour index included a significant interaction between treatment and time (Table 3.1). Gill colour index did not differ among treatment groups prior to gill injury. Post injury, the baseline treatment (no injury) group exhibited significantly darker colour (higher score; Figure 3.3) 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 3.2). In *Experiment 2* there was also a significant interaction between treatment and time (Table 3.1). The baseline treatment (no injury) had significantly higher colour score (darker colour) than all other groups both 10 min ($t_{31} = 1.78$, $p < 0.001$) and 20 min ($t_{31} = 3.62$, $p < 0.001$) post-injury (Figure 3.4). There were no significant differences were detected among the control group and use of chilled Mountain Dew ($t < -0.206$, $p > 0.05$). Fish had a significantly lighter gill colour at 10 minutes when ambient temperature Mountain Dew was used in comparison to the control group ($t_{31} = -2.309$, $p = 0.021$; Table 3.2).

3.4.3 Bleeding Intensity Values

For *Experiment 1,* bleeding intensity was not different among treatments (BIN; Figure 3.5 A) as the best fitting model to the data included time as a fixed effect and individual as a random effect to account for repeated measures (Table 3.3). Similarly, treatment was not a significant contributing factor to variation in BIN in *Experiments 2* as the best fitting model to the data included time and individuals as independent predictors (Table 3.3).

3.4.4 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 chilled Mountain Dew exhibiting significantly higher impairment than the baseline group ($t_{46} = -3.43$, $p = 0.001$). Only three fish were euthanized owing to low RAMP score (2 fish in the carbonated lake water group and 1 in the Coca Cola™ group during *Experiment 1*).

3.5 Discussion

The main claim of those in the recreational angling community promoting the use of carbonated beverages is that this practice decreases the duration of bleeding related to hooking injury, particularly of the gills (Pyzer 2019). However, our study found no differences in the time to cessation of bleeding, gill colour (which was used as an index of blood loss), or bleeding intensity among three carbonated beverages poured over bleeding gills, or between the carbonated beverages and a control group. Differences were only detected between the baseline group where no simulated hooking injury occurred, and all other groups in which injury and bleeding happened. Overall, our study does not provide evidence that pouring carbonated soft drinks on gill injuries of Northern Pike is beneficial. Both carbonated water and carbonated beverages appear to result in similar bleeding scores, gill colouration, and impairment scores.

The presumed benefit of pouring CO_2 rich drinks on gills is that the combination of hypoxia- and CO_2-induced bradycardia may cause a positive effect on fish gills that are bleeding. Many anglers lift fish out of the water once landed, exposing them to air, 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 >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 mucous production, and alterations in the structural morphology of the gills in teleost fish (Meyer et al. 2009). Although many of these studies use more chronic exposures (i.e. > 24 h) and a different study species than used in our study, acute exposure may still have negative effects on fish (Meyer et al. 2009), particularly on ion and acid-base regulation (Wright and Wood 2009). Interestingly, Northern Pike are relatively tolerant of environmental acidification and have reported to survive in water with a pH of 4.2 to 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 on what changes the carbonated beverages may have (i.e., gill structure histology, ionoregulatory flux, and blood physiology changes in pH/PCO_2/HCO^-_3, etc.). Given that we limited our observations for 20 min during our study, we did not assess more chronic physiological and behavioral effects, as well as post-release 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 acutely injured during capture for this study, the longer-term consequences of pouring chemicals associated with carbonated sodas remains unknown. Future work involving telemetry or net pens would be useful for understanding longer-term consequences of this carbonated beverage technique. Lastly, our study used a simulated and standardized size of gill injury to 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, efforts should be taken to prevent injuries that result in severe bleeding. Possible solutions could include using hook styles that may prevent deep hooking/gill damage, 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 potential perception of the curtailment of bleeding anglers witness, especially if the combination of air exposure and CO_2 causes an immediate and severe bradycardia. We found no significant benefit or disbenefit with pouring carbonated beverages over the gills of Northern Pike, but it is possible that there are longer term impacts. Similarly, 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, Muskellunge), using other beverages, or applying the beverages in other ways (e.g., holding them in air for longer after applying 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 best practices being employed benefit fish (Brownscombe et al. 2017).

Figure Captions

Figure 3.1 Image of the standardized 0.9 cm by 0.9 cm section of gill filaments removed from Northern Pike (*Esox lucius*) to simulate hooking injury.

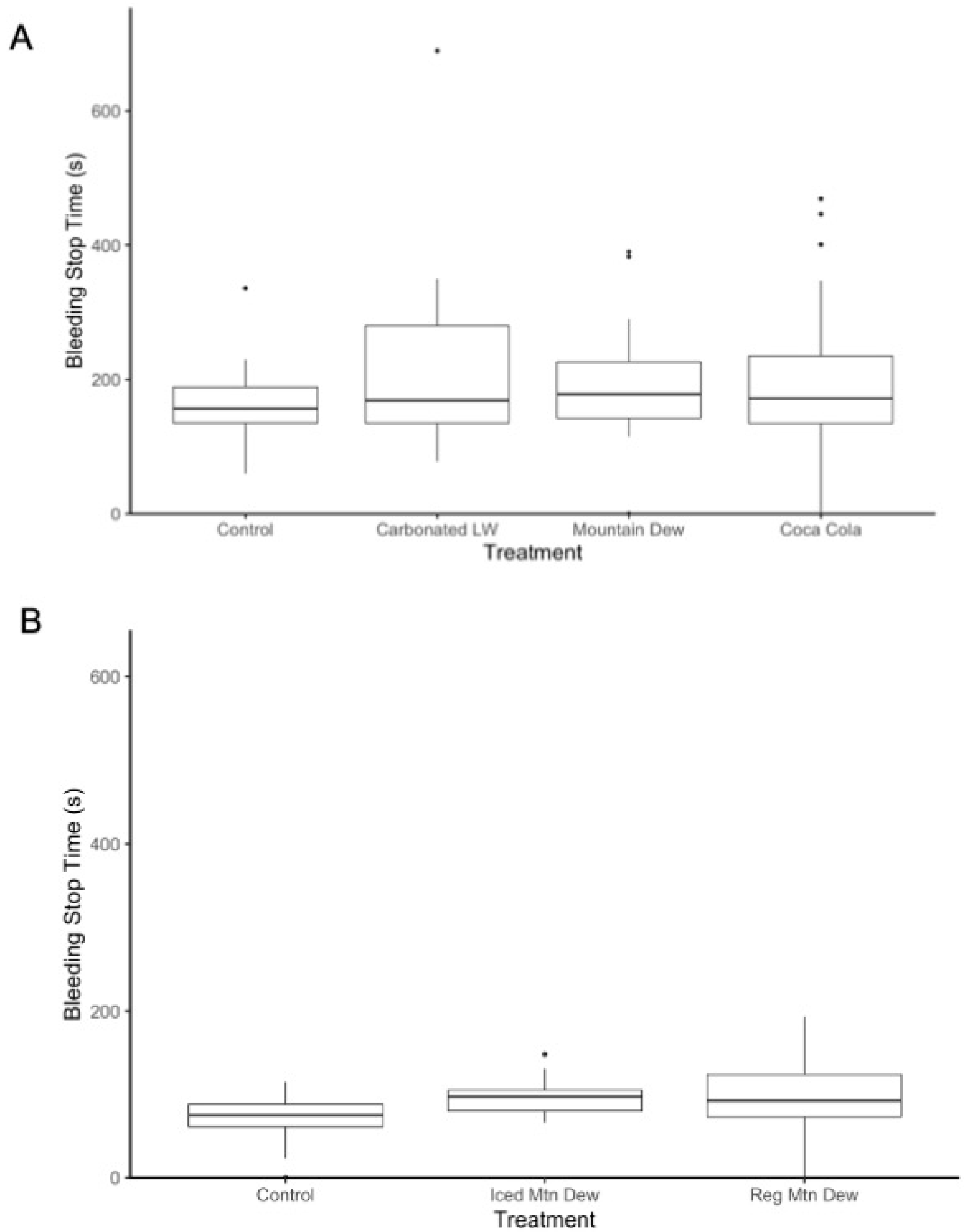

Figure 3.2 Bleeding cessation time following gill injury in Northern Pike in Experiment 1 (A) and Experiment 2 (B). In A, control is compared against carbonated lake water (Carbonated LW), Mountain Dew™, and Coca Cola™. In B) control is compared against Mountain Dew™ at 4-8 °C (chilled Mountain Dew™) and Mountain Dew™ at ambient (24-27 °C (Reg Mountain Dew™)).

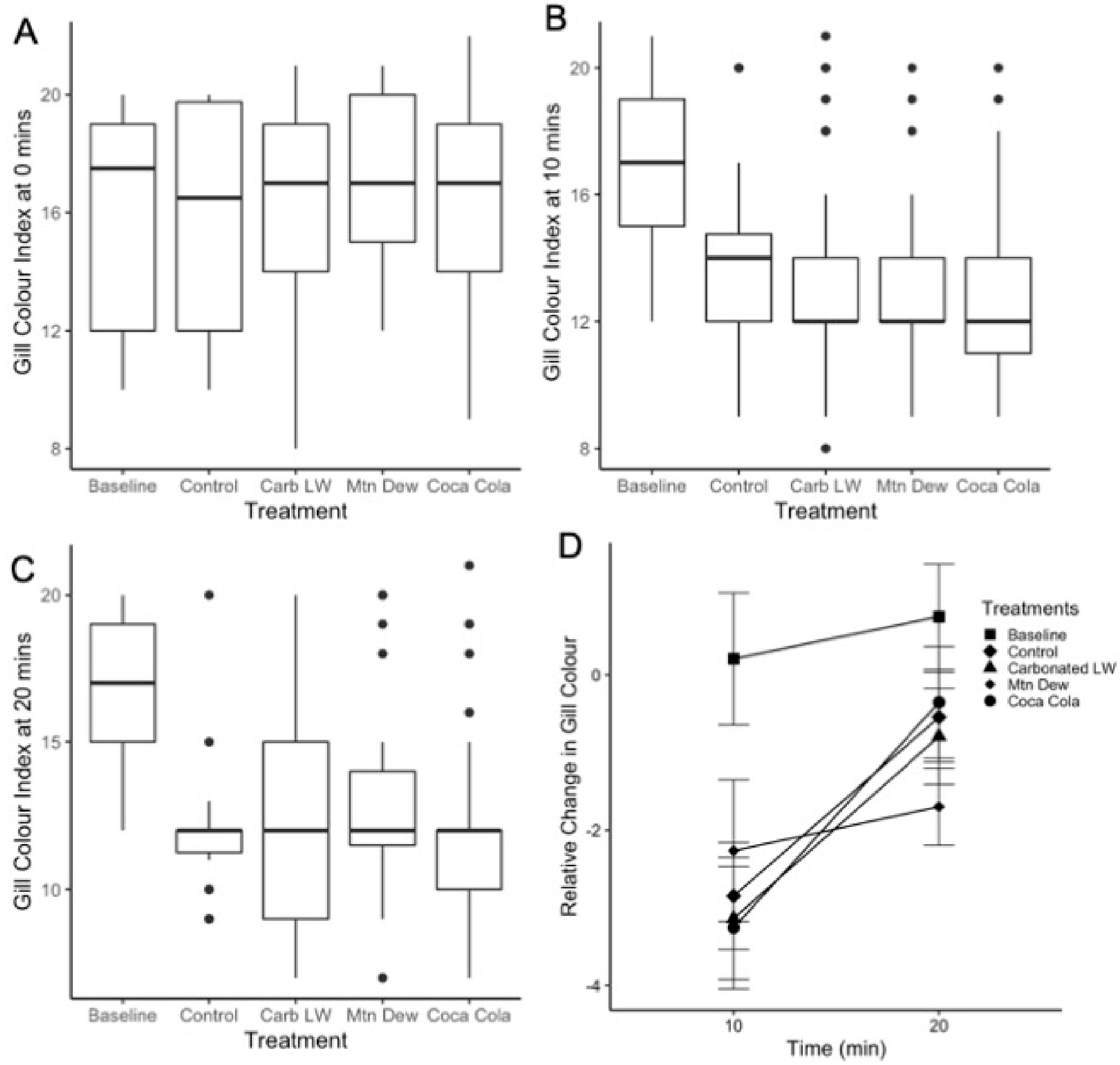

Figure 3.3 Gill Colour Index for Experiment 1 for 0 minutes (A), 10 minutes (B), 20 minutes (C) and the relative change from 0 to 10 minutes and 10 and 20 minutes (D). In all graphs control is compared against carbonated lake water (Carbonated LW), Mountain Dew™, and Coca Cola™.

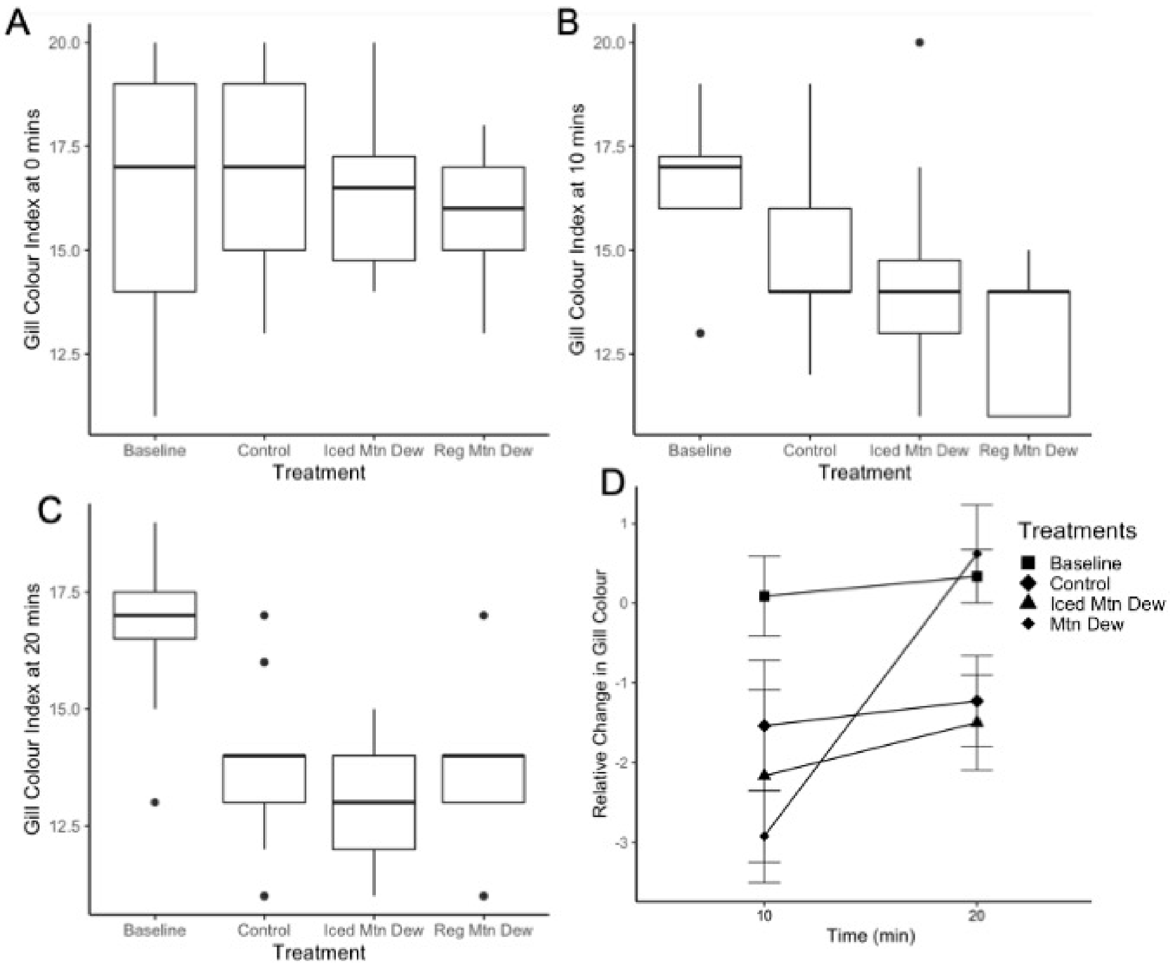

Figure 3.4 Gill Colour Index for Experiment 2 for 0 minutes (A), 10 minutes (B), 20 minutes (C) and the relative change from 0 to 10 minutes and 10 and 20 minutes (D). In all graphs control is compared against Mountain Dew™ at 4-8 °C (chilled Mountain Dew™) and Mountain Dew™ at ambient temperature, 24-27 °C (Reg Mountain Dew™).

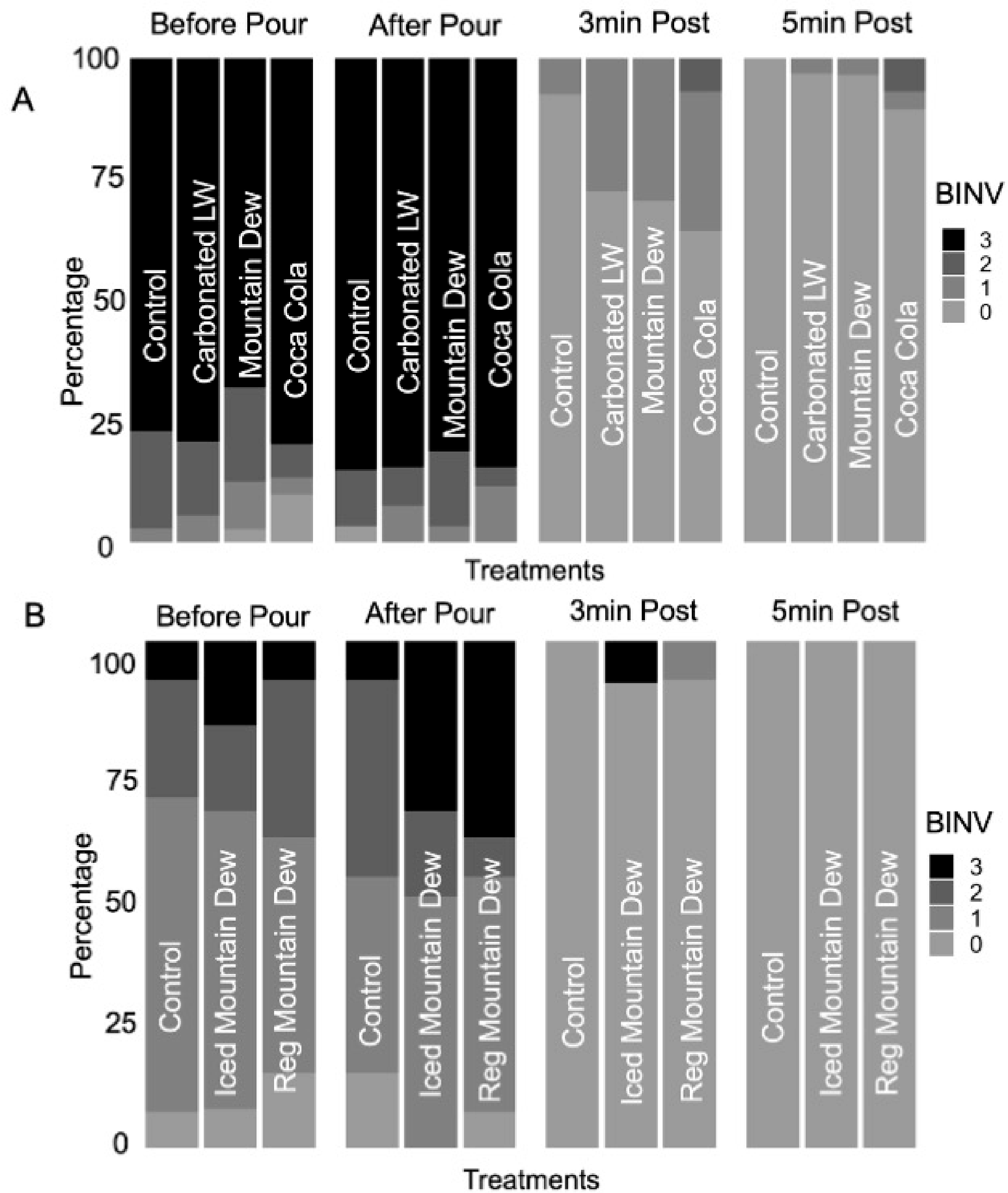

Figure 3.5 Bleeding intensity values following gill injury in Northern Pike in Experiment 1 (A) and Experiment 2 (B). Figures show the bleeding intensity values before the pour of any substance, after the cut (Before Pour), after the pour of a substance (After), after 3 minutes (3min Post) and 5 minutes after the substance was poured over the gills (5min Post). In A, control is compared against carbonated lake water (Carbonated LW), Mountain Dew, and Coca Cola. In B) control is compared against Mountain Dew™ at 4-8 °C (chilled Mountain Dew™) and Mountain Dew™ at ambient (24-27 °C (Reg Mountain Dew™)).

Tables

Table 3.1 Model selection outputs for ordinal logistic regression models. Full models included gill color as the response, the interaction between treatment and time as fixed effects, and individual as a random effect. Backward model selection was used to determine final model structure. Significant differences in model fit are highlighted by bold italic font.

Experiment 1 (n = 118), *Experiment 2* (n = 38)

Experiment	Model Specification	AIC	Residual Deviation	Loglikelihood	P value	DF
1	***Treatment*Time***	***2014.924***	***1954.924***	***25.575***	***<0.001***	***8***
	Treatment	2086.274	2046.274	-29.198	1.000	2
	Time	***2053.076***	***2017.075***	***59.600***	***<0.001***	***2***
2	***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***

Table 3.2 Ordinal logistic regression model outputs for gill colour index with treatment as a predictor using control values as reference group for comparisons at individual time periods, 0, 10 and 20 minutes. Significant differences are highlighted by bold italic font

Experiment 1 (n=118, df = 439)

Time	Comparisons	Value	Standard Error	T value	P-value
0 Minutes	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 Minutes	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 Minutes	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)

Time	Comparisons	Value	Standard Error	T value	P-value
0 Minutes	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
	Chilled Mountain Dew™	-0.8734	0.7416	-1.1777	0.2389

10 Minutes	***Regular Mountain Dew™***	***-1.6813***	***0.7281***	***-2.3093***	***0.0209***
	Baseline	***1.3084***	***0.7342***	***1.7821***	***0.0747***
20 Minutes	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***

Table 3.3 Model selection outputs for ordinal logistic regression models. Full models included bleeding intensity as the response, the interaction between treatment and time as fixed effects, and individual as a random effect. Backward model selection was used to determine final model structure. Significant differences in model fit are highlighted by bold italic font.

Experiment 1 (n = 118), *Experiment 2* (n = 38)

Experiment	Model Specification	AIC	Residual Deviation	Loglikelihood	P value	DF
1	Treatment*Time	531.4823	495.4823	9.473	0.394	9
	Treatment	1030.8998	1018.8998	0.059	0.996	3
	Time	***520.6760***	***508.6760***	***510.223***	***<0.001***	***1***
2	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***

Chapter 4.General Discussion

My thesis focused on identifying strategies to mitigate injuries to fish that are caught by recreational anglers. I investigated possible solutions for preventing hooking injuries in freshwater gamefish (Northern Pike, Smallmouth Bass, and Largemouth Bass) caught during C&R events and a possible solution to mitigate blood loss in gill injuries in Northern Pike. C&R angling occurs worldwide and can be used as a fisheries management technique to promote sustainability. However, long-term survival of fish may be compromised if substantial injuries occur during these events. In Chapter 2, Northern Pike, Smallmouth Bass, and Largemouth Bass were targeted with hard plastic lures with different hook characteristics to investigate the influence of hook type on injuries in angled freshwater gamefish. Using three lure types (jerkbaits, crankbaits, and lipless crankbaits) with four different hook types (treble barbed, treble barbless, single J barbed, and single J barbless) the influence of artificial lure hook type on hooking characteristics and injury of angled freshwater gamefish was investigated. In Chapter 3, the tactic of anglers using carbonated beverages (carbonated lake water, Mountain Dew™, and Coca Cola™) to mitigate blood loss (using bleeding intensity, gill colour as a proxy of blood loss, and bleeding time) on gill injuries was compared to allowing Northern Pike to recover without human intervention.

My findings suggest using single hooks over treble hooks to decrease unhooking time which inherently would decrease air exposure and increase survival post-release. Anglers should consider switching the treble hooks on their hard plastic lures to encourage post-release survival and less handling when removing hooks from fish. We also found that for Smallmouth Bass, hook type significantly influenced the probability of hooking in a sensitive location (gills, gullet, eyes). In Smallmouth Bass and Largemouth Bass, we found that switching from treble to single hooks encourages shallower hooking locations which could also lead to a lower frequency of hooks ending up in sensitive locations like the gullet. For Smallmouth Bass and Largemouth Bass, switching to single hooks will significantly encourage shorter unhooking times and shallower hooking locations which will, in turn, increase post-release survival and should be considered by anglers when C&R angling. This practice is also encouraged for anglers targeting Northern Pike as it will decrease unhooking times and the likelihood of having to use pliers to remove hooks in deep difficult to remove locations.

The decreased unhooking time, more shallow hooking location, average hook depth, and deepest hook support the practice of replacing treble hooks on hard plastic lures to single J hooks when targeting freshwater gamefish. If this practice is implemented within the angling industry, hooking injuries in angled fish could decrease and long-term survival could increase. This study is the first to compare hook types used on hard plastic lures on angled gamefish. The findings contribute to the growing body of literature with suggested best practices for anglers and policy makers to take into consideration during C&R fishing. Studies similar to this one can be used to create policies and management strategies underpinned by science-based best practice protocols.

We did not find that using carbonated beverages was beneficial on gill injuries on Northern Pike and recommend that the angler keep the beverages for themselves and return the fish to the water. Returning the fish to water also decreases air exposure which is beneficial for fish survival post-C&R events. Even though C&R anglers want to promote survival and decrease injuries, sometimes it is best to keep the fish in the water and let it recover without human intervention. However, since our study species was limited to Northern Pike, these findings may not apply universally to other species.

Our findings suggest returning the fish to the water and reserving carbonated beverages for angler consumption is the best practice. There are many best practices that can be used while angling to improve the outcomes for fish being released, but the use of carbonated beverages should not be considered one of them. Other best practices that have been scientifically proven to have benefits should be used instead, such as minimizing handling time and air exposure (Cooke et al. 2001; 2002; Meka and McCormick 2005; Arlinghaus et al. 2008; Cook et al. 2015).

Overall, this thesis aims to encourage anglers to consider switching from treble hooks to single hooks on hard plastic lures and to simply return the fish to the water as soon as possible to encourage the fish to survive long term and to potentially be caught again. The take-home message to anglers, fisheries management, and the industry is to consider the science of C&R fishing while engaging in these activities and implementing management strategies. We all want to be able to continue recreational fishing but to conserve fish populations, we must do our part in encouraging long-term survival following C&R events. Consider minimizing handling time and exposure by replacing treble hooks with single hooks on hard plastic lures and when injuries do occur, let the fish recover in the water without human interference.

Based on the results from Chapter 2 further investigation should establish the long-term behavioural and physiological effects of C&R events on freshwater fish while taking into account hook type. Despite some studies being done on the short-term effects of C&R events (Cooke et al. 2002; Bartholomew and Bohnsack 2005; Klefoth et al. 2008; Arlinghaus et al. 2009) and on small populations (Sass et al. 2018), future studies should consider the lures and hook types used and their long-term effects on other gamefish species. Fish should be able to swim freely, as holding them could alter their physiological responses and removes indirect mortality (i.e., predation), and could build on the study done by Sass et al. (2018) which focuses on Largemouth Bass and should additionally consider various populations of Northern Pike and Smallmouth Bass. As suggested by Pollock and Pine (2005), telemetry and tag-return could be used to help determine relative survival estimates. Physiological evaluations should include stress hormones such as cortisol, in addition to osmolality, chloride, glucose, and hemoglobin (Gustaveson et al. 1991). This research would provide anglers, policy makers and management alike a full picture of the effect of hook type on fish populations and what could be done during C&R to promote long-term survival and decrease injuries.

Although we did not find any significant benefits of using carbonated beverages on bleeding pike gills, the potential physiological effects should be investigated. The effects of CO_2 on fish have been well documented (Sundin et al. 2000; Gilmour et al. 2001; Gilmour et al. 2005; reviewed in Brauner and Baker 2009; reviewed in Gilmour and Milsom 2009; Tuong et al. 2018). However, carbonated beverages are made up of more than just CO_2, therefore the combination of effects of caffeine, sugar, and low pH on fish gills should be evaluated. The physiological effects could accumulate and cause detrimental damage to sensitive gill filaments and physiological imbalances within the fish. This research could be done in a laboratory setting to enable constant monitoring of the behavioural and physiological changes over time. Isolating caffeine, sugar, and low pH solutions could also be evaluated in order to pinpoint which effects each chemical has on the fish gills. Stress hormones such as cortisol should be considered in addition to the effects on individual proteins within the gills and their functions. This research could give a full picture as to all the effects of using carbonated beverages on gill injuries and enable management and policy makers to regulate their use during recreational fishing events. Having more evidence of the effects of using these beverages may also discourage more anglers from using this tactic.

There will undoubtedly be more research on C&R events in the future that will equip anglers and scientists alike with potential tools to encourage the long-term success of freshwater fish. Other tactics used by anglers to minimize bleeding in injured fish such as API Melafix™ (used to treat injured aquarium fish but also used by anglers) and G-Juice Livewell treatment should be tested. The chemical make-up of API

Melafix™ and G-Juice should be identified and should be tested in a controlled setting to investigate the behavioural and physiological impacts that these compounds have on fish. Many anglers on online forums swear by these products but the effects of them on fish, short- or long-term, remain unknown. The substances used on fish also end up in the body of water that they live in and could also have detrimental effects on their habitats, surrounding ecosystems, and environments and therefore should be investigated and regulated.

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Appendix

Appendix A. Additional Figures

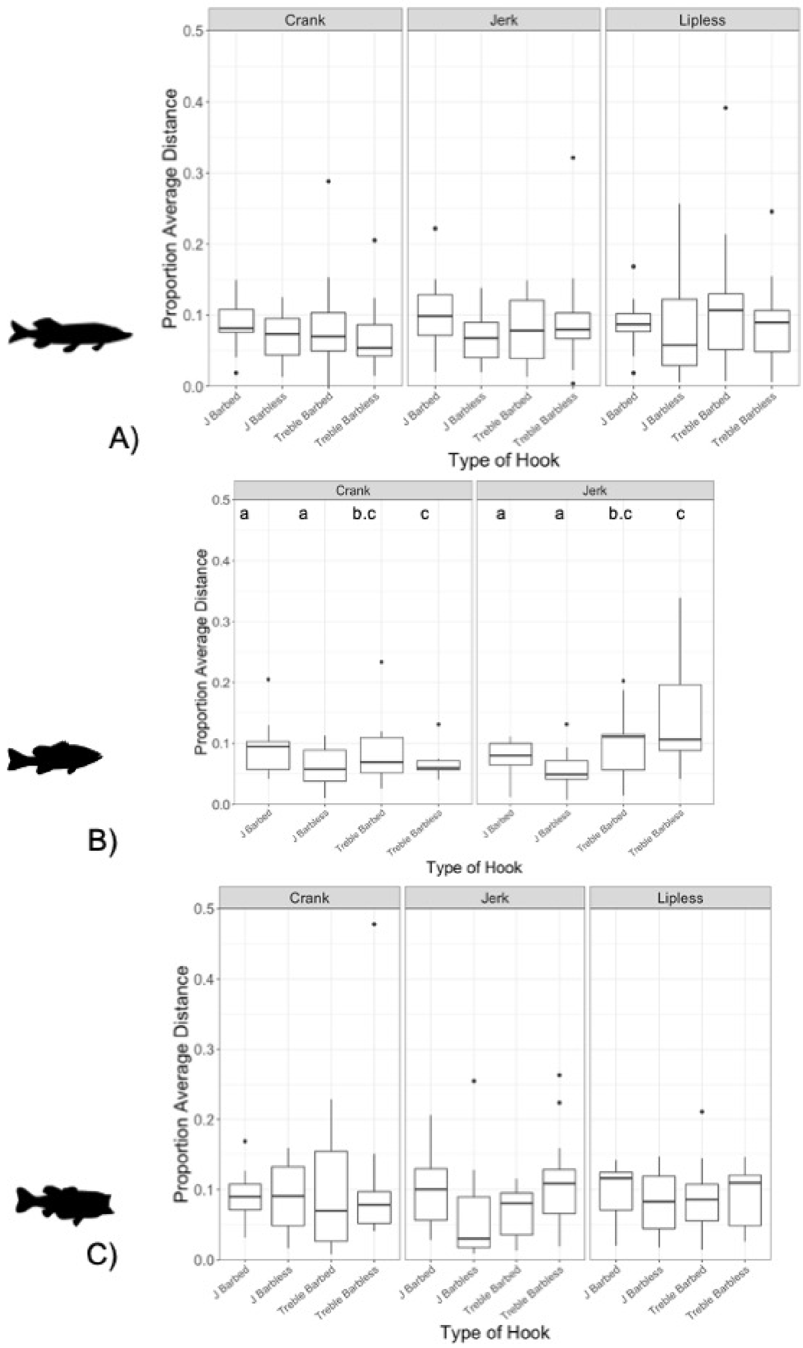

Figure 2.4 The average distance of various hook types (J Barbed, J Barbless, Treble Barbed and Treble Barbless) from A) Northern Pike (Crank Bait n = 65 , Jerk Bait n = 72, and Lipless Crank Bait n = 83), B) Smallmouth Bass (Crank Bait n = 45, and Jerk Bait n = 58) and C) Largemouth Bass (Crank Bait n = 76, Jerk Bait n = 90, and Lipless Crank Bait n = 80) in proportion to total length of fish.

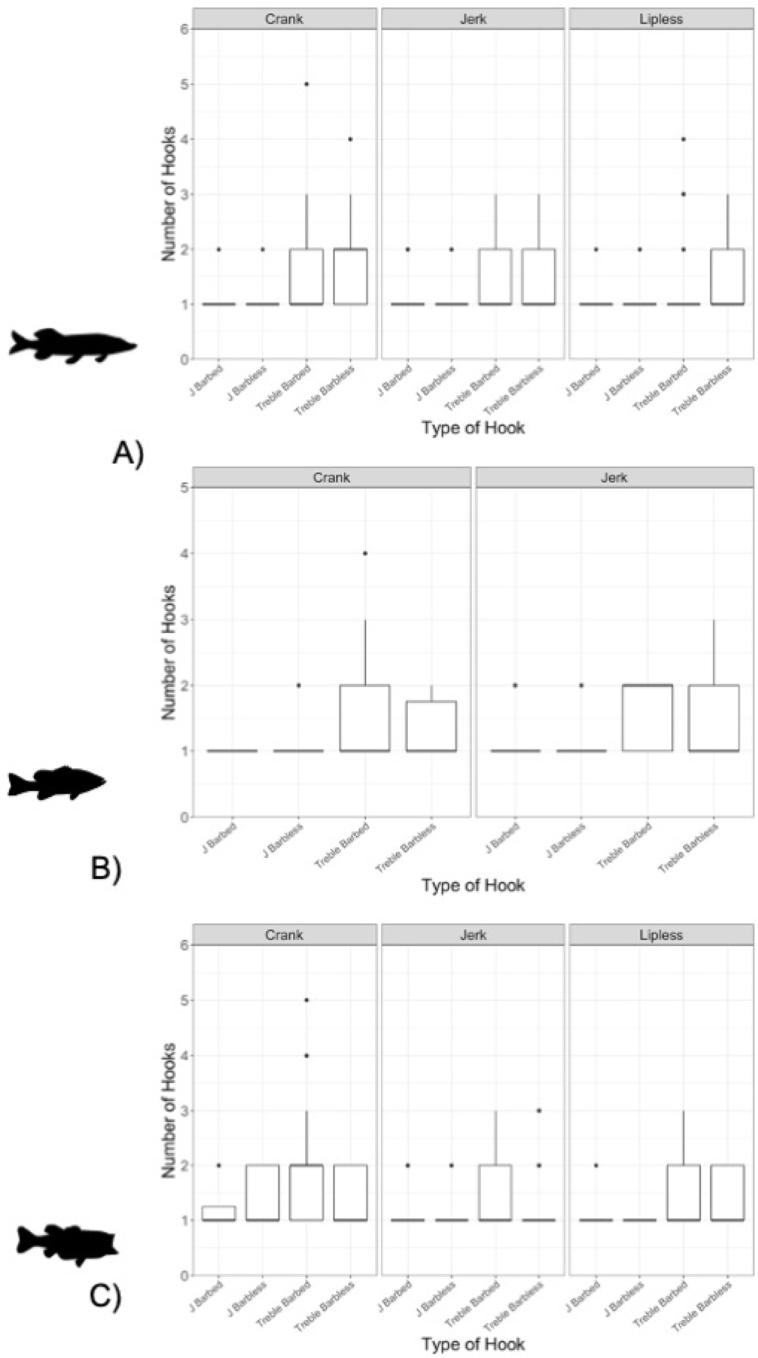

Figure 2.5 Number of hooks in A) Northern Pike (Crank Bait n = 65 , Jerk Bait n = 72, and Lipless Crank Bait n = 83), B) Smallmouth Bass (Crank Bait n = 45, and Jerk Bait n = 58) and C) Largemouth Bass (Crank Bait n = 76, Jerk Bait n = 90, and Lipless Crank Bait n = 80) with various hook types (J Barbed, J Barbless, Treble Barbed and Treble Barbless).

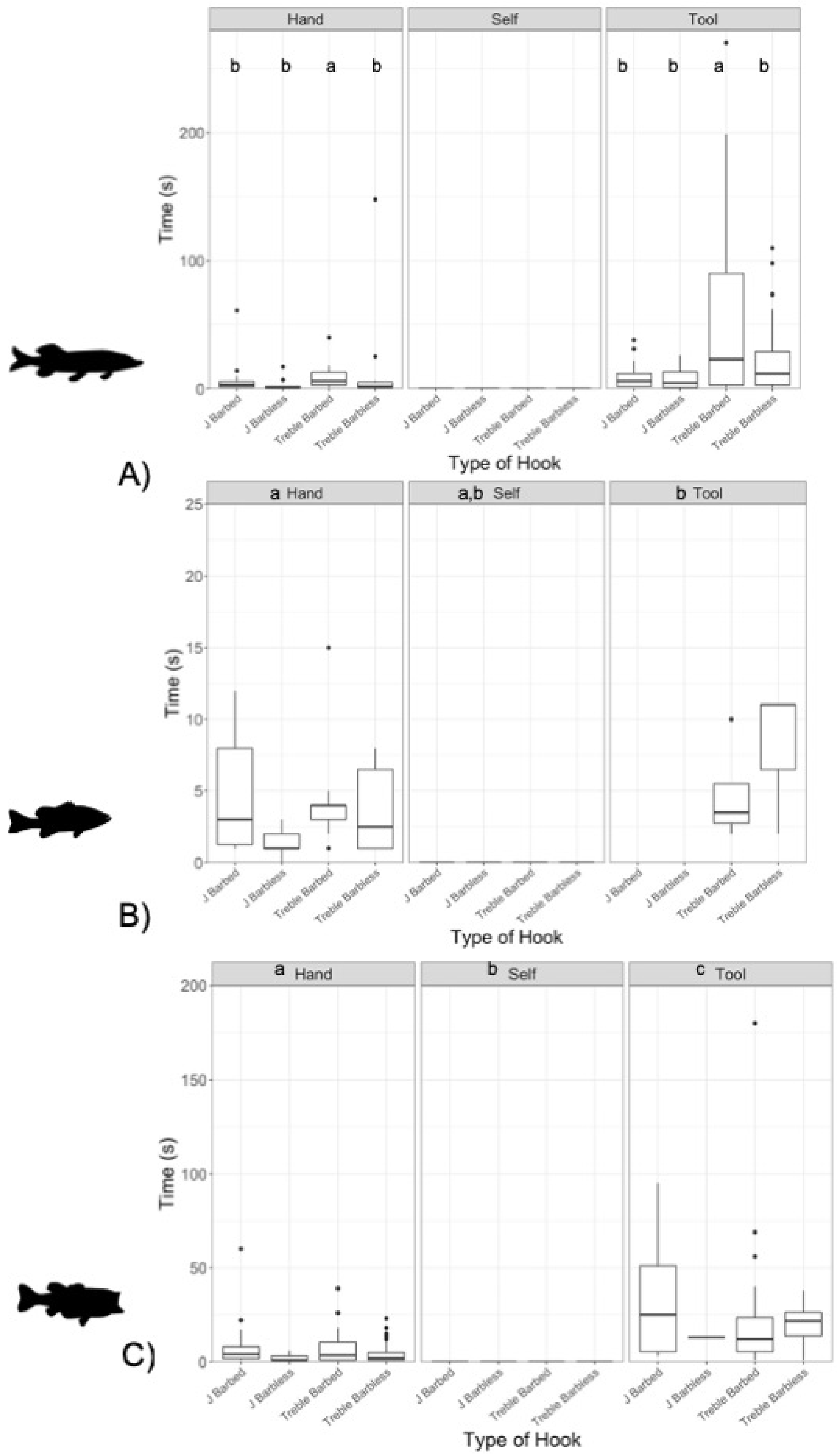

Figure 2.6 Time to remove various hook types (J Barbed, J Barbless, Treble Barbed and Treble Barbless) from A) Northern Pike (Crank Bait n = 65 , Jerk Bait n = 72, and Lipless Crank Bait n = 83), B) Smallmouth Bass (Crank Bait n = 45, and Jerk Bait n = 58) and C) Largemouth Bass (Crank Bait n = 76, Jerk Bait n = 90, and Lipless Crank Bait n = 80) using various removal techniques.

Appendix B. Additional Statistical Outputs

Table 2.5 Anova outputs for unhooking time as predictor with hook type and removal method as responses for Northern Pike (n = 220), Smallmouth Bass (n = 103), and Largemouth Bass (n = 246). Significant differences are highlighted by bold italic font.

Species	Predictor	LR Chisq	Df	Pr(>Chisq)
Northern Pike	***Hook Type***	***14.45***	***1***	***<0.001***
	Removal Method	***43.32***	***2***	***<0.001***
Smallmouth Bass	*Hook Type*	0.903	1	0.342
	Removal Method	***19.047***	***2***	***<0.001***
Largemouth Bass	Hook Type	0.59	1	0.442
	Removal Method	***62.791***	***2***	***<0.001***

Table 2.6 Posthoc analysis outputs for unhooking time as predictor with hook type and removal method as responses for Northern Pike (n = 220) Smallmouth Bass (n = 103), and Largemouth Bass (n = 246) using FDR. Significant differences are highlighted by bold italic font.

Species	Contrast	Estimate	SE	Z Ratio	p value
Northern Pike	***Hand - Self***	***14.2***	***6.11***	***2.320***	***0.02***
	Hand – Tool	***-19.9***	***5.97***	***3.329***	***0.001***
	Self - Tool	***-34.1***	***5.27***	***-6.464***	***<0.001***
	J Barbed - J Barbless	-3.68	7.79	-0.472	0.637
	J Barbed - Treble Barbed	***-23.33***	***6.77***	***-3.445***	***0.003***
	J Barbed - Treble Barbless	-8.93	6.97	-1.282	0.3
	J Barbless - Treble Barbed	***-19.65***	***6.72***	***-2.925***	***0.01***
	J Barbless - Treble Barbless	-5.26	6.96	-0.755	0.54
	Treble Barbed - Treble Barbless	***14.40***	***5.61***	***2.566***	***0.021***
Smallmouth Bass	Hand - Self	3.67	2.41	1.525	0.127
	Hand – Tool	***-16.15***	***4.38***	***-3.682***	***<0.001***
	Self - Tool	***-19.82***	***4.58***	***-4.328***	***<0.001***
Largemouth Bass	***Hand - Self***	***4.82***	***2.30***	***2.101***	***0.036***
	Hand – Tool	***-18.31***	***2.69***	***-6.815***	***<0.001***
	Self - Tool	***-23.14***	***3.18***	***-7.285***	***<0.001***

Table 2.7 Posthoc analysis outputs for unhooking time as predictor with hook type and removal method combined as responses for Northern Pike (n = 220), Smallmouth bass (n = 103), and Largemouth Bass (n = 246) using FDR. Significant differences are highlighted by bold italic font.

Species	Tool	Hook Contrast	Estimate	SE	Z Ratio	p value
Northern Pike	Hand	J Barbed - J Barbless	-3.675	7.792	-0.472	0.667
		J Barbed - Treble Barbed	***-23.326***	***6.771***	***-3.445***	***0.002***
		J Barbed - Treble Barbless	-8.931	6.965	-1.282	0.264
		J Barbless - Treble Barbed	***-19.651***	***6.719***	***-2.925***	***0.007***
		J Barbless - Treble Barbless	-5.255	6.961	-0.755	0.521
		Treble Barbed - Treble Barbless	***14.396***	***5.609***	***2.566***	***0.019***
	Self	J Barbed - J Barbless	-3.675	7.792	-0.472	0.667
		J Barbed - Treble Barbed	***-23.326***	***6.771***	***-3.445***	***0.002***
		J Barbed - Treble Barbless	-8.931	6.965	-1.282	0.264
		J Barbless - Treble Barbed	***-19.651***	***6.719***	***-2.925***	***0.007***
		J Barbless - Treble Barbless	-5.255	6.961	-0.755	0.521
		Treble Barbed - Treble Barbless	***14.396***	***5.609***	***2.566***	***0.019***
	Tool	J Barbed - J Barbless	-3.675	7.792	-0.472	0.667
		J Barbed - Treble Barbed	***-23.326***	***6.771***	***-3.445***	***0.002***
		J Barbed - Treble Barbless	-8.931	6.965	-1.282	0.264
		J Barbless - Treble Barbed	***-19.651***	***6.719***	***-2.925***	***0.007***
		J Barbless - Treble Barbless	-5.255	6.961	-0.755	0.521
		Treble Barbed - Treble Barbless	***14.396***	***5.609***	***2.566***	***0.019***
Smallmouth Bass	Hand	J Barbed - J Barbless	2.063	3.073	0.671	0.592
		J Barbed - Treble Barbed	-1.040	3.071	-0.339	0.808
		J Barbed - Treble Barbless	2.416	3.104	0.778	0.554
		J Barbless - Treble Barbed	-3.102	3.285	-0.944	0.478
		J Barbless - Treble Barbless	0.354	3.334	0.106	0.930
		Treble Barbed - Treble Barbless	3.456	3.191	1.083	0.418
	Self	J Barbed - J Barbless	2.063	3.073	0.671	0.592
		J Barbed - Treble Barbed	-1.040	3.071	-0.339	0.808
		J Barbed - Treble Barbless	2.416	3.104	0.778	0.554
		J Barbless - Treble Barbed	-3.102	3.285	-0.944	0.478
		J Barbless - Treble Barbless	0.354	3.334	0.106	0.930
		Treble Barbed - Treble Barbless	3.456	3.191	1.083	0.418
	Tool	J Barbed - J Barbless	2.063	3.073	0.671	0.592
		J Barbed - Treble Barbed	-1.040	3.071	-0.339	0.808

		J Barbed - Treble Barbless	2.416	3.104	0.778	0.554
		J Barbless - Treble Barbed	-3.102	3.285	-0.944	0.478
		J Barbless - Treble Barbless	0.354	3.334	0.106	0.930
		Treble Barbed - Treble Barbless	3.456	3.191	1.083	0.418
Largemouth Bass	Hand	J Barbed - J Barbless	6.03	3.16	1.91	0.75
		J Barbed - Treble Barbed	1.87	2.82	0.66	1.00
		J Barbed - Treble Barbless	4.36	2.64	1.65	0.89
		J Barbless - Treble Barbed	-4.16	2.97	-1.40	0.96
		J Barbless - Treble Barbless	-1.67	2.70	-0.62	1.00
		Treble Barbed - Treble Barbless	2.49	2.37	1.05	1.00
	Self	J Barbed - J Barbless	6.03	3.16	1.91	0.75
		J Barbed - Treble Barbed	1.87	2.82	0.66	1.00
		J Barbed - Treble Barbless	4.36	2.64	1.65	0.89
		J Barbless - Treble Barbed	-4.16	2.97	-1.40	0.96
		J Barbless - Treble Barbless	-1.67	2.70	-0.62	1.00
		Treble Barbed - Treble Barbless	2.49	2.37	1.05	1.00
	Tool	J Barbed - J Barbless	6.03	3.16	1.91	0.75
		J Barbed - Treble Barbed	1.87	2.82	0.66	1.00
		J Barbed - Treble Barbless	4.36	2.64	1.65	0.89
		J Barbless - Treble Barbed	-4.16	2.97	-1.40	0.96
		J Barbless - Treble Barbless	-1.67	2.70	-0.62	1.00
		Treble Barbed - Treble Barbless	2.49	2.37	1.05	1.00

Table 2.8 Anova outputs for sensitive location (foul, gullet, gills, eyes) as predictor with lure type and hook type as responses for Northern Pike (n = 220), Smallmouth Bass (n = 103), and Largemouth Bass (n = 246). Significant differences are highlighted by bold italic font.

Species	Predictor	LR	DF	Chi-Squared
Northern Pike	Lure Type	1.475	2	0.478
	Hook Type	2.823	3	0.42
Smallmouth Bass	Lure Type	<0.001	1	0.992
	Hook Type	***10.297***	***3***	***0.016***
Largemouth Bass	Lure Type	3.437	2	0.179
	Hook Type	1.606	3	0.659

Table 2.9 Posthoc analysis outputs for sensitive location (foul, gullet, gills, eyes) as predictor with lure type and hook type as responses for Smallmouth Bass using LR (n = 103). Significant differences are highlighted by bold italic font.

Contrast	Estimate	SE	Z ratio	p value
Crank J Barbed - Crank J Barbless	0.021	0.015	1.363	0.255
Crank J Barbed - Crank Treble Barbed	-0.014	0.015	-0.998	0.388
Crank J Barbed - Crank Treble Barbless	-0.035	0.015	-2.309	0.078
Crank J Barbless - Crank Treble Barbed	-0.035	0.016	2.239	0.078
Crank J Barbless - Crank Treble Barbless	***-0.056***	***0.016***	***-3.429***	***0.006***
Crank Treble Barbed - Crank Treble Barbless	-0.02	0.016	-1.290	0.263
Jerk J Barbed - Jerk J Barbless	0.021	0.015	1.363	0.255
Jerk J Barbed - Jerk Treble Barbed	-0.015	0.015	-0.998	0.388
Jerk J Barbed - Jerk Treble Barbless	-0.035	0.015	-2.309	0.078
Jerk J Barbless - Jerk Treble Barbed	-0.035	0.016	-2.239	0.078
Jerk J Barbless - Jerk Treble Barbless	***-0.056***	***0.016***	***-3.429***	***0.006***
Jerk Treble Barbed - Jerk Treble Barbless	-0.02	0.016	-1.290	0.263

Table 2.10 Anova outputs for average hooking depth as a proportion of total length as predictor with lure type and hook type as responses for Northern Pike (n = 220), Smallmouth Bass (n = 103), and Largemouth Bass (n = 246). No significant differences were found therefore no posthoc analysis were done.

Species	Predictor	LR	DF	p value
Northern Pike	Lure Type	0.08	2	0.961
	Hook Type	4.705	3	0.195
Smallmouth Bass	Lure Type	3.253	1	0.071
	Hook Type	**12.763**	***3***	**0.005**
Largemouth Bass	Lure Type	0.682	2	0.711
	Hook Type	**12.565**	**3**	**0.006**

Table 2.11 Posthoc outputs for average hooking depth as a proportion of total length as predictor with lure type and hook type as responses for Smallmouth Bass (n = 103) and Largemouth Bass (n = 246) using FDR. Significant differences are highlighted by bold italic font.

Species	Contrast	Estimate	SE	Z ratio	p value
Smallmouth Bass	Crank J Barbed - Crank J Barbless	0.021	0.015	1.363	0.255
	Crank J Barbed - Crank Treble Barbed	-0.015	0.015	-0.998	0.387
	Crank J Barbed - Crank Treble Barbless	**-0.035**	**0.015**	**-2.31**	**0.078**
	Crank J Barbless - Crank Treble Barbed	**-0.035**	**0.016**	**-2.239**	**0.078**
	Crank J Barbless - Crank Treble Barbless	**-0.056**	**0.016**	**-3.43**	**0.006**
	Crank Treble Barbed - Crank Treble Barbless	-0.020	0.016	-1.29	0.263

	Jerk J Barbed - Jerk J Barbless	0.021	0.015	1.363	0.255
	Jerk J Barbed - Jerk Treble Barbed	-0.015	0.015	-0.998	0.387
	Jerk J Barbed - Jerk Treble Barbless	**-0.035**	**0.015**	**-2.31**	**0.078**
	Jerk J Barbless - Jerk Treble Barbed	**-0.035**	**0.016**	**-2.239**	**0.078**
	Jerk J Barbless - Jerk Treble Barbless	**-0.056**	**0.016**	**-3.43**	**0.006**
	Jerk Treble Barbed - Jerk Treble Barbless	-0.02	0.016	-1.29	0.263
Largemouth Bass	Crank J Barbed - Crank J Barbless	0.14	0.068	2.039	0.124
	Crank J Barbed - Crank Treble Barbed	0.155	0.061	2.512	0.099
	Crank J Barbed - Crank Treble Barbless	<0.001	0.057	-0.008	0.994
	Crank J Barbless - Crank Treble Barbed	0.016	0.061	0.250	0.912
	Crank J Barbless - Crank Treble Barbless	-0.14	0.059	-2.343	0.115
	Crank Treble Barbed - Crank Treble Barbless	-0.156	0.052	-2.983	0.063
	Jerk J Barbed - Jerk J Barbless	0.14	0.068	2.039	0.124
	Jerk J Barbed - Jerk Treble Barbed	0.155	0.061	2.512	0.099
	Jerk J Barbed - Jerk Treble Barbless	<0.001	0.057	-0.008	0.994
	Jerk J Barbless - Jerk Treble Barbed	0.016	0.061	0.250	0.912
	Jerk J Barbless - Jerk Treble Barbless	-0.14	0.059	-2.343	0.115
	Jerk Treble Barbed - Jerk Treble Barbless	-0.156	0.052	-2.983	0.063
	Lipless J Barbed - Lipless J Barbless	0.14	0.068	2.039	0.124
	Lipless J Barbed - Lipless Treble Barbed	0.155	0.061	2.512	0.099
	Lipless J Barbed - Lipless Treble Barbless	<0.001	0.057	-0.008	0.994
	Lipless J Barbless - Lipless Treble Barbed	0.016	0.061	0.250	0.912
	Lipless J Barbless - Lipless Treble Barbless	-0.14	0.059	-2.343	0.115
	Lipless Treble Barbed - Lipless Treble Barbless	-0.156	0.052	-2.983	0.063

Table 2.12 Anova outputs for deepest hooking depth as a proportion of total length as predictor with lure type and hook type as responses for Northern Pike (n = 220), Smallmouth Bass (n = 103) and Largemouth Bass (n = 246). Significant differences are highlighted by bold italic font.

Species	Predictor	Sum Squares	DF	F value	p-value
Northern Pike	Lure Type	0.006	2	0.833	0.436
	Hook Type	0.014	3	1.391	0.246
Smallmouth Bass	Lure Type	1.888	1	3.918	0.051
	Hook Type	***10.263***	***3***	***7.102***	***<0.001***
Largemouth Bass	Lure Type	0.0734	2	0.35	0.705
	Hook Type	***1.041***	***3***	***3.288***	***0.021***

Table 2.13 Posthoc outputs for deepest hooking depth as a proportion of total length as predictor with lure type and hook type as responses for Smallmouth Bass (n = 103) and Largemouth Bass using FDR (n = 246). Significant differences are highlighted by bold italic font.

Species	Contrast	Estimate	SE	Z ratio	p value
Smallmouth Bass	Crank J Barbed - Crank J Barbless	-5.259	2.66	-1.976	0.08
	Crank J Barbed - Crank Treble Barbed	3.1	1.68	1.849	0.083
	Crank J Barbed - Crank Treble Barbless	***4.01***	***1.62***	***2.476***	***0.034***
	Crank J Barbless - Crank Treble Barbed	***8.36***	***2.53***	***3.298***	***0.005***
	Crank J Barbless - Crank Treble Barbless	***9.27***	***2.50***	***3.706***	***0.002***
	Crank Treble Barbed - Crank Treble Barbless	0.91	1.41	0.647	0.537
	Jerk J Barbed - Jerk J Barbless	-5.259	2.66	-1.976	0.08
	Jerk J Barbed - Jerk Treble Barbed	3.1	1.68	1.849	0.083
	Jerk J Barbed - Jerk Treble Barbless	***4.01***	***1.62***	***2.476***	***0.034***
	Jerk J Barbless - Jerk Treble Barbed	***8.36***	***2.53***	***3.298***	***0.005***
	Jerk J Barbless - Jerk Treble Barbless	***9.27***	***2.50***	***3.706***	***0.002***
	Jerk Treble Barbed - Jerk Treble Barbless	0.91	1.41	0.647	0.537
Largemouth Bass	Crank J Barbed - Crank J Barbless	0.141	0.072	1.964	0.198
	Crank J Barbed - Crank Treble Barbed	0.122	0.065	1.880	0.198
	Crank J Barbed - Crank Treble Barbless	-0.013	0.06	-0.224	0.876
	Crank J Barbless - Crank Treble Barbed	-0.019	0.065	-0.299	0.876
	Crank J Barbless - Crank Treble Barbless	-0.155	0.063	-2.464	0.151
	Crank Treble Barbed - Crank Treble Barbless	-0.135	0.055	-2.471	0.151
	Jerk J Barbed - Jerk J Barbless	0.141	0.072	1.964	0.198
	Jerk J Barbed - Jerk Treble Barbed	0.122	0.065	1.880	0.198
	Jerk J Barbed - Jerk Treble Barbless	-0.013	0.06	-0.224	0.876
	Jerk J Barbless - Jerk Treble Barbed	-0.019	0.065	-0.299	0.876
	Jerk J Barbless - Jerk Treble Barbless	-0.155	0.063	-2.464	0.151
	Jerk Treble Barbed - Jerk Treble Barbless	-0.135	0.055	-2.471	0.151
	Lipless J Barbed - Lipless J Barbless	0.141	0.072	1.964	0.198
	Lipless J Barbed - Lipless Treble Barbed	0.122	0.065	1.880	0.198
	Lipless J Barbed - Lipless Treble Barbless	-0.013	0.06	-0.224	0.876
	Lipless J Barbless - Lipless Treble Barbed	-0.019	0.065	-0.299	0.876
	Lipless J Barbless - Lipless Treble Barbless	-0.155	0.063	-2.464	0.151
	Lipless Treble Barbed - Lipless Treble Barbless	-0.135	0.055	-2.471	0.151