The consequences of retained lures on free swimming fish: physiological, behavioural and fitness perspectives

by

Christopher Ernest Pullen
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Dedication

This thesis is dedicated to my wife Dr. Kristen Hayes. Without her unfailing support and dedication this undertaking quite simply would not have happened. Kristen, you encouraged me throughout this journey in full knowledge of the challenges it would bring. You carried the load when this project and my work took me away for weeks on end. You sat with me for hours working through the complexities of the analysis and empowered me with your intellect and insight as we grappled with aspects I found daunting. You reminded me that even the data that "doesn't work" is important and adds to the story we are trying to tell. This research is as much yours as it is mine. I can't thank you enough DB.

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Abstract

The objective of this thesis was to determine what happens to free swimming northern pike (*Esox lucius*) following breakoff from an angling event. In a laboratory setting, retained lures did not affect metabolic rate, blood physiology or locomotor activity of pike. Gill ventilation rate was elevated in deeply hooked fish suggesting that lures in obstructive locations may challenge recovery from exercise. However, elevated cortisol levels in these fish suggest that confinement produces prolonged stress that may affect physiological responses and behaviour. Quantification of lure shedding rates in free swimming fish determined that barbless hooks were shed faster than barbed hooks. Hooking location did not influence shedding rate and fish were able to rid themselves of all lure types. Retained lures reduced the activity of pike that were released suggesting that lures do affect normal activities. These findings will assist fisheries managers in developing enhanced conservation and angling management policies.

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Acronyms

ANOVA: Analysis of variance

AST: Aspartate Transaminase

Bl: Blade Length (lures/spoons)

BPM: Beats per Minute

CR: Catch and Release

FL: Fork length

GIS: Geographic Information System

GPS: Global Positioning System

hr: Hour

mmol/l: millimol per litre

QUBS: Queen’s University Biological Field Station

SE: Standard error

SL: Standard length

SMR: Standard Metabolic Rate

TL: Total length

Chapter 1 General Introduction

"Fishing is a... discipline in the equality of men - for all men are equal before fish". ~Herbert Hoover

Few things frustrate an angler more than getting their catch to the side of the boat, only to have it thrash and be gone. The sense of disappointment is deepened when the angler realises that their prized lure or fly has gone with the fleeing fish. While many anglers focus on the cost to them (e.g. the cost to replace the lost lure or the time to retie one), perhaps it is only the biologist who wonders what the cost is to the fish? In this project, I will provide answers to this important question using the popular northern pike (*Esox lucius*) as an experimental system.

Humans cannot live without water. As a result, we have centered much of our development and culture in its proximity (Gleick, 1996). The water systems upon which humans have relied have always teemed with life and over time humans have developed progressively more ingenious ways to exploit the fish and aquatic organisms living therein. Humankind took to the water to harvest its resources, catching large volumes to sell or to feed their families. Our approaches are wide-ranging: from single fishing poles, to small boats with cast nets, to large industrial fleets seining the water to pull in vast qualities of fish in a single haul (Pitcher & Hollingworth, 2002). Along the way, the "first man who [snuck] away to the creek when the tribe did not really need fish" (Haig-Brown, 1946) discovered that a day spent fishing was a fine way to spend the day. Images of anglers fishing for pleasure have been found in Egypt as far back as 3000 years ago (Pitcher & Hollingworth, 2002). In the centuries that followed, opinions on the merit

of angling as a pastime have metamorphosed from the vocation of the marginalized peasant (Arlinghaus *et al.*, 2007; Pitcher & Hollingworth, 2002) to the noble pursuit of high society written about by Izaak Walton in his 1653 book *The Complete Angler* (Walton, 1653) (i.e. the evolution of fly fishing and fishing lodges in Scotland) to where it resides now as a broadly accepted leisure activity that supports a multi-billion dollar global industry (Arlinghaus & Cooke, 2008; Cowx, 2002; Parkkila *et al.*, 2010; Policansky, 2002).

Not until recently, in the latter half of the 20th century, has attention been focused on understanding the reasons for declining global fish stocks in inland (Post *et al.*, 2002) and marine (Myers & Worm, 2003) systems. The impact on fish populations of over-exploitation by commercial fisheries has been well documented (Musick *et al.*, 2000; Worm *et al.*, 2009) and there is growing evidence that recreational fishing also contributes to declines in fishery resources, particularly in coastal environments (Coleman *et al.*, 2004; McPhee *et al.*, 2002) and inland waters (S. J. Cooke & Cowx, 2004; S. J. Cooke & Cowx, 2006; Post *et al.*, 2002). Considering the socioeconomic benefits of recreational angling (Arlinghaus & Cooke, 2008), understanding how to minimize its negative impacts on fish stocks and aquatic ecosystems is essential for sustaining recreational fisheries.

Catch and Release Management

As previously suggested, anglers have shifted most of their fishing from necessity to pleasurable pastime including those who wander rivers and streams to pursue trout with fly rod, sophisticated high-tech tournament anglers, and urban anglers who try their

luck at landing a pan fish or large carp in urban waters. At first consideration we might assume the impact of recreational angling to be small when compared to the size and composition of the global commercial fishery. However, estimates for Canada alone suggest that, on an annual basis, a significant number of fish are caught through recreational fishing. A recent Department of Fisheries and Ocean's Angler survey (DFO, 2012) found that 193 million fish were caught by recreational anglers, more than half the number coming from Ontario alone. Of that total, 63 million fish were kept indicating that 130 million fish were returned to the water. These are not small numbers and the return rate illustrates that many of the angling population, either by regulation or personal motivation, are practicing catch-and-release (C&R) angling. C&R angling has been endorsed as a reasonable conservation measure by which anglers can pursue fish while alleviating the pressure on fish stocks (Arlinghaus *et al.*, 2007). The primary motivation behind the practice is the premise that a fish that has been angled and released will return to support the population (S. J. Cooke & Schramm, 2007) and will be available to be caught again (Quinn, 1996). As C&R angling has been promoted, the philosophy and science behind this type of angling has become broadly accepted by recreational anglers and encouraged by fisheries managers (Bartholomew & Bohnsack, 2005; S. J. Cooke & Suski, 2005; Quinn, 1996; Reeves & Bruesewitz, 2007). Increasingly, C&R angling has become standard practice where anglers voluntarily return their catch as part of the fishing experience, or fish in predominantly total C&R managed fisheries (Arlinghaus *et al.*, 2007; Cowx, 2002). Specialist anglers in particular (Landsman *et al.*, 2011b) focus almost exclusively on C&R.

Estimates suggest that 60% of fish captured recreationally are released (S. J. Cooke & Cowx, 2004). A review of marine angling in coastal United States fisheries suggests that releases increased from 34.2% 1981 to 58.7% in 1999 while total overall landing decreased by 28% (Bartholomew & Bohnsack, 2005). In cases such as specialized trout (e.g. steelhead *Oncorhynchus mykiss*), predator (e.g. muskellunge *Esox masquinongy*) and saltwater fisheries (e.g. bonefish *Albula spp*) near 100% return is anticipated (Arlinghaus *et al.*, 2007). Whether C&R is voluntary or is a by-product of harvest regulations, this practice has become an important component of most contemporary recreational fisheries as a measure to ensure that catchable fish will be available from year to year (Arlinghaus *et al.*, 2007; Bartholomew & Bohnsack, 2005).

Concurrent with the rise in C&R angling is an increase in targeted research on the impact of this practice on fish and fish populations. A fundamental assumption about C&R is that once released, the fish will survive to be captured again and/or to reproduce. However, growing evidence suggests that C&R may result in increased fish mortality, which in turn can have a negative consequence for the population (Pine *et al.*, 2008). Populations may also be impacted even if C&R is not lethal for fish; the impact of a C&R event may cause sublethal effects (physiological and/or behavioural) that negatively affect the overall fitness of the fish (Arlinghaus *et al.*, 2007; Donaldson *et al.*, 2008).

Based upon the existing body of information, Cooke and Suski (2005) proposed five variables about which generalizations can be made with relative certainty to predict the effects of C&R angling on fish survival and fitness: 1) angling duration, 2) air exposure 3) water temperature, 4) tackle type and 5) angling during the fish reproductive

period. Much of the current research has focused on how these variables affect fish response and recovery after a C&R angling event.

Physiological Response to Catch and Release

Wild fish are exposed to both natural (e.g. escape from a predator) and anthropogenic (e.g. catch and release angling) stressors. These stressors produce physiological changes, the "stress response" (Wendelaar Bonga, 1997) that can affect both short term and long term behavior and survival of the fish. The stress response is mediated by the release of cortisol, a corticosteroid hormone that normally regulates energy metabolism and water/ mineral balance. Initially, this response frees energy resources that allow the fish to cope with the present challenge and to recover from the stressor in the short term. However, if the stressor is severe or sustained, this physiological response can have a detrimental effect on the fish's physical well-being.

A number of laboratory studies have reviewed indicators that can be reliably used to evaluate the stress response in fish. Release of cortisol causes a rise in metabolic rate associated with an increase in plasma glucose concentration, an increase in the flux of water and ions, an increase in respiratory rate and oxygen consumption, an increase in hematocrit, and a decrease in liver carbohydrate reserves (Barton, 2002; Mommsen *et al.*, 1999; Wendelaar Bonga, 1997).

The physiological stress responses after angling and physiological recovery after release have been well documented using both laboratory and field-physiology techniques (Bartholomew & Bohnsack, 2005; S. J. Cooke *et al.*, 2013; C. D. Suski *et al.*,

2007). In most species, stressors associated with C&R angling (such as exhaustive exercise, injury and air exposure) produce short term physiological disturbances that recover within 8-12 hours (Kieffer, 2000). However, these disturbances have sublethal effects that impact fish behavior, fitness and mortality (S. J. Cooke *et al.*, 2002; Donaldson *et al.*, 2008).

Implications of Break off

One question that has perplexed anglers but has received little formal attention from the research community is: after a fish snaps the line and disappears to the depth of the lake to hide, the hook still embedded in its mouth, what has the encounter cost the fish?

Although a number of studies have shown that a fish that has been released often quickly returns to its baseline physiological and behavioural state (Arlinghaus *et al.*, 2007; Kieffer, 2000), fewer studies have described the accrued sub-lethal impacts to fish that are released or that break away from the line with gear (e.g. hooks or lures) still embedded in their mouth, a process described as "break off"(Arlinghaus *et al.*, 2008a; Henry *et al.*, 2009). Break off typically occurs as a result of fish behavior after hooking or during the line retrieve. Either the line breaks after contact with submerged logs or rock, or it becomes entangled in submerged vegetation as the fish tries to rid itself of the hook. Alternatively, the fish could be lost as a result of gear failure; either the knots used to attach line to leader fail, the line and tackle has become worn and abraded, or the angler mishandles the reel drag, snapping the line. In the case of fish with sharp dentition

(e.g., northern pike, barracuda (*Sphyraena barracuda)*), lines can be cut after coming in contact with the teeth of the fish.

The short and long term behavioural and physiological implications to the fish after break off with artificial lures are largely unknown and are often discussed only in the context of C&R fishing as the similarities (i.e. the fish breaks off or is brought to the boat and released). In the break off scenario, some variables affecting stress reactions would occur (e.g. exhaustive exercise) while other elements would be minimized or eliminated (e.g. effects of handling and air exposure) (Bartholomew & Bohnsack, 2005; S. J. Cooke & Suski, 2005; C. D. Suski *et al.*, 2007) as the fish is typically not removed from the water. Most of the studies to date that evaluate the effects of hook retention on fish have focused on single hook ingestion (deep hooking) typical of fresh or live bait tactics (Fobert *et al.*, 2009; Margenau, 2007; Tsuboi *et al.*, 2006). A few studies have evaluated impacts of artificial baits (Henry *et al.*, 2009) on fish behavior and physiology. Only two studies by Arlinghaus *et al.* (2008a) and Klefoth (2008) evaluate the behavioural response of pike after release with artificial lures to simulate break off. In these studies telemetry was used to track fish post-release and to determine short and long term movement and habitat use. The results of the studies show that lure retention causes some short term behavioural changes (e.g. lack of movement), but that typical behavioural patterns resume as quickly as 24 hours after the fish are released. While these studies provide some preliminary insights into the effects of retained lures on free swimming fish, the long term implications of swimming free with a retained hook on the well-being of the fish and its ultimate survival are unclear. Indeed, the length of lure retention, the physiological response of the fish, and the subsequent sub lethal effects of

the lure are still unknown. Clearly, retaining a lure in the mouth has the potential to impact feeding and respiration which could have serious fitness consequences. Evaluation of both behavioural and physiological indicators such as cortisol, glucose and ion concentrations as well as oxygen consumption (indicators of stress), lactate (indicator of exhaustive exercise), and hematocrit (indicators of stress and/or blood loss) over the duration that the lure is retained could provide an insight into how a fish adapts to and/or is affected by break off.

Implications for Fisheries Management

Little information exists on the rate of lure loss rate in recreational or commercial fisheries, but we do have data on numbers of recreational anglers. In Canada in 2010, DFO estimated 3.3 million licensed recreational anglers participated in the fishery. Canadian numbers are dwarfed by an estimated 33.1 million anglers in the US in 2011 (USFWS, 2012). Assuming that half the anglers in North America lose at least one lure or hook during an angling season, 18.2 million fish could be swimming free with a retained lure. Losing a lure is not specific to catch and release angling. Subsistence fishermen as well as anglers focused in specialized C&R fisheries such as muskellunge (Landsman *et al.*, 2011b) can lose lures following break off from an angling event.

Effective management of fish stock depends upon an accurate estimate of fish mortality and this estimate is not only affected by an accurate estimate of fish that survive release after angling, but also that survive break off. Pollock and Pine (2007) pointed out that while measuring changes in survival after release is one of the more relevant pieces of information for fisheries managers, history has suggested that accurate measure

of mortality has been difficult to obtain. A challenge to studies that attempt to accurately estimate angling-related survival and mortality has been that additional stress may be imposed by the study design (e.g. tagging or penning) which may influence the outcome (S. J. Cooke *et al.*, 2013; S. J. Cooke & Schramm, 2007). Recent studies have attempted to address these deficiencies by working *in situ* using both direct observations and telemetry (S. J. Cooke *et al.*, 2002; A. J. Danylchuk *et al.*, 2007; S. E. Danylchuk *et al.*, 2007) the results of which can be used to improve management decisions. Studying the impacts of break off and associated injury, stress and survival of the fish is critical for estimating mortality, determining harvest limits, or managing fishing gear allowances.

Northern Pike Biology

Northern pike (*Esox lucius*) have a circumpolar distribution across the northern hemisphere (Scot & Crossman, 1973), with a range that has expanded into other northern areas as a result of introductions (Casselman & Lewis, 1996; Harvey, 2009). In North America pike are found in 45% of the total freshwater area (Carlander *et al.*, 1978) and in Canada they are distributed across the country with the exception of southern British Columbia and the Maritimes (Harvey, 2009). In the ecosystems where they are present, pike are a keystone piscivore and can influence species composition, abundance and distribution of many species (including other pike, through cannibalism and competition) in a fish community (Craig, 2008; Scot & Crossman, 1973). Northern pike spawn in spring shortly after ice-out when water temperatures reach 8–12°C. Males and females congregate in marginal areas and flooded areas and spawn over vegetation in spring (Casselman & Lewis, 1996). Young pike stay in spawning areas feeding on emerging

benthic invertebrates and other young- of- the- year fish moving to deeper water as spring floods recede and the spawning areas dry up. Pike spawn earlier than muskellunge, and in situations when rapid temperature warming results in overlapping spawning periods, hybridization between the two species can occur, resulting in the tiger musky.

Northern pike are visual predators and are crepuscular feeders and prefer aquatic macrophytes as cove for their ambush predation style. Vegetation provides a refuge from predation for the young, and cover to conceal feeding fish of all sizes (Inskip, 1982).

Pike are of interest in the recreational angling context as they are a sought after game fish, particularly in the trophy class where larger mature fish are targeted (Harvey, 2009). Further, their role as a keystone predator defines the trophic structure of the systems they inhabit. Near shore habitat alteration and loss, coupled with eutrophication have decreased pike abundance as their spawning, rearing and foraging habitat have been lost. Using commercial harvest as an indicator, data from the1990's suggests that pike populations have declined through the Great Lakes (Casselman & Lewis, 1996). Pike are also frequently by catch of anglers targeting bass and other near shore species. Given the lighter tackle used for those species and the aggressive detention of pike, pike are more likely to break off gear. For these reasons, pike are of particular interest in this study: the impacts of free swimming pike with retained gear are not well understood and the possible management implications are not clearly identified or refined.

Research Objectives and Predictions

This thesis includes three separate studies that evaluate a) the physiological consequences (i.e. the stress response) of lure retention in a laboratory setting, b) the behavioural effects of lure retention in a laboratory setting and c) an in situ evaluation of fish survival related to lure retention. The overall objective of this research is to determine how swimming free with a lure after break off affects fish health, behaviour and survival.

For all three studies, northern pike was used as a model species. Pike were chosen because they are a common game fish that readily bites artificial lures. The mouth morphology and dentition make them highly susceptible to line breakage, particularly when they are not an angler's target species and metal leaders were not used (i.e. angling for bass and walleye) (Arlinghaus *et al.*, 2007). In addition, their flight response behaviour after hooking increases the probability of lure loss as they typically head for deep cover resulting in entanglement and line abrasion. Additionally, pike are a surrogate species for muskellunge, a highly desirable game fish species that are not as abundant and are afforded protection in some jurisdictions. The findings from this study will be relevant to many toothy predators in both freshwater and marine environments (e.g. barracuda, tiger fish (*Hydrocynus vittatus),* etc.).

Rationale and Expectations for Chapter 2

The physiological effects of C&R angling have been described in many species including northern pike (Arlinghaus *et al.*, 2009). While northern pike are more susceptible to break off during angling and subsequent lure retention, there is little information about the physiological effects of sustained stress related to a retained lure in

pike (Arlinghaus *et al.*, 2007). This study will evaluate the physiological effects of prolonged exposure to a retained lure in three different hooking locations in a laboratory setting to simulate a break off situation. In general, it is expected that lure retention will increase and or prolong the stress response as measured by stress response indicators (i.e. oxygen consumption/ metabolic rate and blood levels of glucose, lactate, cortisol, hematocrit, AST and ion balance). It is also anticipated that deep hooking will create a greater and more prolonged stress response than shallow hooking.

Rationale and Expectations for Chapter 3

The behavioural effects on pike of swimming free with a retained lure are unclear. A few studies evaluated pike mobility following release with a retained lure in the mouth; these studies found short term behavior impairments (i.e. less mobility than controls) and a rapid return to normal behavior (Arlinghaus *et al.*, 2008a; Klefoth *et al.*, 2008). This study will evaluate and quantify the behavioural effects of prolonged exposure to a retained lure in three different hooking locations after break off in the laboratory setting. Specifically, behavioural responses of fish to a retained lure and the length of time necessary for fish to expel lures in aquaria will be assessed. Fish swimming capacity will be characterized to identify the potential fitness impairments of retaining lures. In general, it is anticipated that lure retention will produce behavioural impairments until the lure is expelled. Deep hooking is expected to produce the most prolonged impairments as deep-set hooks are likely to take the longest times for the fish to expel.

Rationale and Expectations for Chapter 4

Although some research has evaluated the behavioural consequences of fish free swimming with a lure post break off (Arlinghaus *et al.*, 2008a; Klefoth *et al.*, 2008), these studies were limited because, once the fish was released, the researchers did not know if and for how long the lure was retained and were unable to ascertain definitively if the observed behaviour was impacted by prolonged exposure to the lure. This study assesses the behavior of free swimming pike released with a retained lure in the mouth in three different hooking locations. The novel aspect of this study is the inclusion of radio transmitters in the lure to determine behavioural changes and the duration for which lures remain with the fish based on hooking location. By tracking the lure, this study also provides a more accurate estimate of survival by using post-release monitoring tools that most closely represent normal angling activity, and by reducing pre-treatment handling (e.g. tagging the fish). In general it is expected that lure retention will cause behavioural impairments that are related to hooking location. Further, it is expected that deep hooking will result in longer lure retention, greater behavioural impacts and lower fish survival.

Chapter 2: Consequences of oral lure retention on the physiology of adult northern pike (*Esox Lucius L.*)

Introduction

All wild fish are exposed to a variety of stressors, both natural (e.g. escape from a predator) or anthropogenic (e.g. escape from an angler). These stressors produce physiological changes, via stress response (Wendelaar Bonga, 1997) that can affect both short and long term survival of the fish. In fish, a stressor activates the sympathetic nervous system (and the release of catecholamines) which then stimulates the release of corticosteroid hormones (i.e. cortisol). The release of cortisol, which normally regulates energy metabolism and water/ mineral balance, frees energy resources (i.e. glucose) that allow the fish to cope with and recover from the stressor in the short term (Barton, 2002). Thus the stress response is an important physiological adaptation that is crucial for fish to survive an acute challenge. However, if the stressor is severe or sustained, this physiological response can have a negative effect on the fish's well-being, behavior and ultimate survival as metabolic energy is redirected from growth and reproduction in an effort to maintain homeostasis (Barton, 2002). Specifically, cortisol release causes secondary responses such as increases in ion and water flux, metabolic rate, gluconeogenesis (increasing plasma glucose levels), respiratory rate, oxygen consumption and hematocrit and a decrease in liver carbohydrate reserves (Mommsen *et al.*, 1999; Wendelaar Bonga, 1997). Sustained release of cortisol caused by chronic stressors results in tertiary responses such as inhibition of growth, reduction of appetite suppression of the immune system and a negative effect on reproduction (reviewed in (Wendelaar Bonga, 1997).

Catch and release angling is associated with stressors such as exhaustion, injury, capture and air exposure that induce a physiological stress response in the fish (C. D. Suski *et al.*, 2007). Although the fish may recover from the initial challenge, prolonged stress responses may have sublethal effects on fish behavior, fitness and survival (Arlinghaus *et al.*, 2007; S. J. Cooke & Schramm, 2007; Donaldson *et al.*, 2008). The impacts of C&R stressors have been studied in laboratory settings (Arlinghaus *et al.*, 2007; Arlinghaus *et al.*, 2009; S. J. Cooke & Suski, 2005). For example, exhaustive exercise (i.e. simulating a C&R angling event) in northern pike causes increases in plasma glucose, plasma and muscle lactate, changes in plasma ionic status (increased potassium and sodium along with decreased chloride), decreases in energy resources (ATP and PCr) and decreases in blood pH (with associated decreased plasma HCO_3^- and increased PCO_2) (Arlinghaus *et al.*, 2009; Schwalme & Mackay, 1985a; Schwalme & Mackay, 1985b).

Understanding recovery from exhaustive exercise and the stress response is important as the rate at which an individual fish is able to restore homeostasis after angling will directly impact its ability to survive following release. In laboratory settings the rate of recovery depends on the species of fish and on its life history and physiological requirements (Arlinghaus *et al.*, 2009; C. D. Suski *et al.*, 2007), the recovery environment (Milligan, 1996; C. Suski *et al.*, 2006), the duration and intensity of the stressor (Schreer *et al.*, 2005) and the physiological variable being measured (Donaldson *et al.*, 2010). Although most of the physiological disturbances that occur during C&R angling typically take about 8-12 hours to fully resolve in the majority of fish species (Kieffer, 2000), studies on recovery rates in pike have varied (Arlinghaus *et al.*, 2009; Schwalme & Mackay, 1985a; Schwalme & Mackay, 1985b; Soivio & Oikari, 1976). Even after

recovery, it is likely that a sub lethal C&R event modulates how a fish is able to respond to subsequent stressors (McConnachie *et al.*, 2012).

What are the consequences of a C&R event in which the lure is retained? The physiological impact of free swimming with retained gear is not well understood. Short term lure retention (24 hours) in angled smallmouth bass (*Micropterus dolomieu*) produces elevations in blood glucose levels but little change in lactate concentrations and hematocrit (Henry *et al.*, 2009). However, mortality and physiological disturbances in bluegill (*Lepomis macrochirus)* are lower for fish in which a hook was left in place compared to those fish in which the hook was removed (upon capture) 10 days after the initial angling event (Fobert *et al.*, 2009).

Information about the physiological effects of C&R angling and lure retention in pike is critical. Northern pike are an integral part of recreational angling; they are a sought after game fish, particularly in the trophy class where larger mature fish are targeted and are also frequently by-catch of anglers targeting bass and other near shore. Given the sharp dentition of pike, their flight response after hooking and lighter tackle that is used to target other game fish species, pike are more likely break off gear (Arlinghaus *et al.*, 2008a). However, the physiological effects of lure retention and the long term implications of swimming free with a retained hook as the fish engages in life functions (e.g. foraging and spawning) are unclear. To that end, the objective of this study was to quantify the physiological consequences of prolonged exposure to a retained lure (simulated break off) in a laboratory setting using northern pike as a model. Specifically, we used a combination of blood-based physiological metrics and metabolic rate measurements to provide a more comprehensive overview of the physiological impacts of lure retention in this species.

Materials and Methods

This study was undertaken at the Queens University Biological Station (QUBS) in May 2008. Northern Pike were collected from Lake Opinicon (Figure 2-1) which has an abundant pike population. Pike were collected by conventional hook-and-line angling from a variety of locations throughout the lake and on a given day, a number of locations were sampled to ensure that fish were not collected from the same area. Angling involved casting and trolling using conventional angling gear with a target of collecting eight fish per day. Barbless hooks were used to minimize injury and to increase ease of hook removal (Alos *et al.*, 2008). Upon capture fish were immediately brought to the boat and netted with an effort to keep angling time shorter than 60 seconds. Fish that were angled for longer periods of time or to exhaustion were not included in the study. Following collection and hook removal, fish were visually assessed. Fish in good condition (i.e. no visible signs of excessive injury or bleeding) were retained and transported to the QUBS wet lab facility in an onboard live well which was regularly refreshed with lake water. To date, the most accurate method to establish baseline physiological parameters is to measure values in fish captured and sampled quickly (wild controls) before physiological changes due to angling can occur (S. J. Cooke *et al.*, 2013). For this reason, some fish were captured, sampled for blood immediately and then released back into the lake.

At the QUBS wet lab, pike were held in three, 1200 litre (152 cm diameter) shaded flow-through holding tanks for 24 hours to allow the fish to return to a baseline resting state following methods similar to Suski *et al.* (2007). Fish were distributed among three tanks to minimize density effects (average 5 fish per tank). After a 24 hour holding time, the fish were carefully netted from their tank and randomly allocated to a control group or one of six different treatment

groups to represent realistic angling break off scenarios: (1) a small spoon (5 cm blade length (bl)) placed in the jaw, 2) a small spoon hooked deeply into the tissue at the base of the tongue (3) a small spoon hooked in both the upper and lower jaw (4) a large spoon (12 cm bl) placed in the jaw, (5) a large spoon hooked deeply into the tissue at the base of the tongue and (6) a large spoon hooked in both the upper and lower jaw (Figure 2-2). To apply the hook treatment, fish were held ventral side down in a foam-padded v-shaped sampling trough filled with fresh lake water. Hooks were placed into position using pliers and pushed through the tissues with a direct uni-direction application of force to simulate hooking that would occur during an angling event. Control fish were handled in an identical manner to treatment fish but did not receive hooks.

Metabolic Rate

To quantify the effect of hooking treatment on metabolic rate, a 12 hour static respirometry assessment was completed using 38 northern pike (mean total length 491 ± 41 mm) with (treatment groups) and without (control group) retained lures (Table 2-1). Standard metabolic rate (SMR) was determined using computerized, intermittent-flow respirometry (LoligSystems, Hobro, Denmark) (Steffensen, 1989). During each experimental cycle (12 hour overnight tests) four hooking treatments in four fish were assessed.

To calibrate the equipment prior to the assessment, each fish netted from the 24 hour holding tank was placed in a water-filled displacement tube from which water was expelled into a calibrated flask to determine the volume of each fish. After the hook treatment was applied, fish were transferred to a glass chamber (746 mm length x 140 mm wide) outfitted with fibre optic oxygen probes. The tubes were split between two tanks (152 cm X 61 cm X 61 cm) filled to a depth of approximately 24 cm with lake water (from Lake Opinicon) at ambient

temperatures. Water was circulated between the two tanks to ensure all fish were subjected to the same water mix and two large air stones from the laboratory's central air system oxygenated each tank. The water was exchanged between tests to prevent build-up of wastes.

Each glass chamber (holding one fish) was connected to two aquarium pumps; one pump recirculated water through the chamber, and the other flushed ambient, oxygenated water into the chamber. The total volume per set up, including the glass chamber, two pumps, and all associated tubing and pumps was 11.48 L. Oxygen consumption in each individual chamber was quantified within 15-minute cycles that consisted of an 8-minute measurement phase, a 4 minute flush period to replace water in each chamber, and a 3-minute wait period following each flushing prior to commencing measurements. Water from the chambers was continually circulated over the fiber optic oxygen probes to ensure adequate mixing during each measurement cycle. The change in oxygen concentration (α) for each chamber was calculated as slope (ΔO2saturation/Δ*t*), and oxygen consumption rate (MO2, mg O2 kg-1 h-1) for each fish was calculated by:

$$MO_2 = \alpha Vresp\ \beta M_b^{-1}$$

where Vresp is the volume of each glass chamber minus the volume of the fish (L), β is oxygen solubility (adjusted daily for both temperature and barometric pressure), and M_b is the fish mass (kg) measured before placing in the respirometer chamber. All calculated dissolved oxygen values were corrected for background oxygen consumptions generated for each specific fish and chamber prior to commencing experiments. Regular calibration of the fiber optic oxygen probes occurred with oxygen-free water and fully saturated water through the experiments. Data were recorded with AutoResp software Version 1.4 (Schurmann & Steffensen, 1997; Steffensen,

1989). SMR values were calculated as the average of six lowest values recorded between 2000 and 0600 as very minimal human disturbance occurred in the laboratory during these hours (Gingerich *et al.*, 2010; Schurmann & Steffensen, 1997). Respirometry trials were completed between May 31st, and June 10th, 2008.

Exercise and Recovery

To quantify the effects of lure retention on exercise and recovery, we used a conventional chasing protocol to induce physiological disturbances (i.e. to simulate a C&R event). Pike (n=85; mean total length 505 ± 58 mm) were removed from the 24 hour holding tanks and then exercised for 60 seconds using tail pinches (C. D. Suski *et al.*, 2007) in a circular (92cm in diameter) tank half full of lake water. Following exercise, pike in treatment groups were netted, transferred to a foam-padded v-shaped trough filled with fresh lake water and a lure treatment was applied. Six groups of treatment fish were hooked using the protocol described above (Table 2).

Each fish was then transferred to one of ten isolation boxes. Each 79 cm x 15 cm x 15 cm (L x L x H) box was constructed from black, 6 mm acrylic sheet with a total volume of 16.5 litres. The boxes were placed on racks over two 152 cm x 61 cm x 61 cm fiberglass tanks continually supplied with lake water. Oxygenated lake water was pumped to a header pipe and then directed to each chamber through a hole in the removable lid into a small overflow chamber where the flow was dissipated and then overflowed into the main raceway where the fish were held. Fish were oriented into the flow with water passing over them to the drain. The drain end consisted of a false back which created a weir over which the water flowed into the small overflow chamber and through a drain hole set in the end. The overflow served to maintain

water level and further block ambient light that may have entered through the drain hole. To eliminate the risk of the fish dislodging the lids, each lid was inset and held in place with webbing straps secured with plastic cam lock buckles. Flow into the chambers was set to average 0.87 l/min for a turnover rate of 14.36 litres per hour.

One hour post exercise, fish were removed from the isolation box and quickly transferred to the sampling trough filled with fresh lake water, sampled for blood, and then returned to the box as quickly as possible to avoid air exposure. Following 24 hours, fish were again removed from the isolation chamber, sampled and then transferred to a lake water filled cooler in which they were transported to the lake and released. The few fish that showed loss of equilibrium or other behavioural impairments were held until they resumed normal activity and then released.

Four different control groups of fish were also tested including: C1: fish were angled, sampled for blood and immediately released (wild controls); C2: fish were exercised with no lure treatment, transferred to isolation chambers and sampled at 1 hour and 24 hours post exercise; C3: fish were exercised, a lure applied and then removed, sampled for blood immediately and then transferred to isolation chambers (i.e. a simulated C&R event). This group of fish were sampled again at 1 hour and 24 hours post exercise; C4: fish were transferred to isolation chambers (no exercise) and sampled after 24 hours (Table 2).

Blood Sampling

Following the exercise protocol described above, fish were non-lethally sampled for blood. For this procedure, pike were held supine in a padded v-shaped trough filled with fresh lake water. A blood sample was drawn from each fish using caudal puncture with a 3.8 cm, 21-

gauge needle and a 2 ml heparinized vacutainer (lithium heparin, Becton-Dickson, NJ, USA). A portion of whole blood was used to quantify hematocrit (the percentage of red blood cells within the total volume of blood) using microhematocrit capillary tubes centrifuged for 5 minutes (using a CritSpin-Micro-Hematocrit Centrifuge). The remaining whole blood was stored in ice slurry for up to 1 hour until it could be processed.

Blood was centrifuged at 10,000 g for 5 min (Clay Adams Compact II Centrifuge) and lactate and glucose concentrations were quantified on-site from plasma using hand-held lactate (Lactate Pro LT-1710 portable lactate analyser; Arkray Inc., Kyoto, Japan) and glucose (ACCU-CHEK glucose meter; Roche Diagnostics, Basel, Switzerland) meters. The devices have previously been calibrated for use on fish (S. J. Cooke *et al.*, 2008). Plasma was then placed in a dewar and shipped to Carleton University where it was held in a -80°C ultracold freezer until analysis. Plasma ion assays (sodium, potassium, chloride) were completed using a Roche-Hitachi 917 analyzer (Basal, Switzerland) and based upon the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). To ensure that the integrity of the analysis was maintained, laboratory personnel followed the Veterinary Laboratory Association Quality Assurance Program, New York State Department of Health, College of American Pathologists, and the Canadian Food Inspection Agency External Proficiency Panel guidelines.

Plasma cortisol was determined in a single assay using a commercial kit (ImmunoChem Cortisol ^{125}I RIA kit; MP Biomedicals, Orangeburg, NY) and a Cobra Auto-Gammer (Hewlett-Packard Inc., Palo Alto, CA) following the methods outlined in Gamperl et al. (1994). Intra-assay variability (% CV) was 10.45 %. (Gamperl *et al.*, 1994))

Statistical Analysis

A Levene's test was used to determine homogeneity of variances across all treatment groups. A one way analysis of variance (ANOVA) was then used to quantify differences in metabolic rate and blood physiology between control and treatment groups. A two way ANOVA was used to quantify the interaction between treatment (lure retention) and sampling time (1 hour and 24 hours) after exercise for all physiological variables (continuous variable). Where ANOVA determined statistical significance, a Tukey honestly significant difference (HSD) test (homogenous variances) or Dunnett's test (heterogenous variance) was used to determine statistical significance between means. All analyses were conducted using JMP v10 (SAS Institute, Cary, NC). Data are expressed as mean ± standard deviation and significance was evaluated $\alpha < 0.05$.

Results

Four fish died during the course of our study. One T1 fish died at 1 hour post exercise during blood sampling and one T2 fish was found dead in the isolation chamber at the one hour sampling time. Two fish were found dead at 24 hours, a T4 and a C3 fish; in the case of these fish it was determined that the flow of water had become interrupted asphyxiating the fish. Over the course of the study, 14 fish were able to expel their lures in the isolation chambers. Of these, 3 lures were expelled at 1 hour and 11 lures expelled at 24 hours. Across treatments, four T1 fish, four T3 fish, four T4 fish and three T6 fish expelled lures. No T2 or T5 fish expelled hooks. Fish that died or expelled the lure during the study were not analysed and were replaced.

Effect of lure retention on metabolic rate

The metabolic rate for control fish did not differ from that of any treatment fish ($F=1.0061$, $p=0.4184$). The mean metabolic rate for control fish was 126 ± 21 $mgO_2\ kg^{-1}\ h^{-1}$ ($n=13$). Mean metabolic rates for treatment fish were 128 ± 16 $mgO_2\ kg^{-1}\ h^{-1}$ (T1; $n=5$), 122 ± 25 $mgO_2\ kg^{-1}\ h^{-1}$ (T3; $n=7$) and 117 ± 19 $mgO_2\ kg^{-1}\ h^{-1}$ (T6; $n=4$). (Figure 2-3)

Baseline physiological values

To assess the physiological effect of holding pike in our laboratory, blood physiology of confined control pike was compared to that of wild controls. Fish that were held for 24 hours and then exercised (C3) had significantly higher levels of plasma lactate, glucose, cortisol and hematocrit than wild controls (C1) but no significant difference in plasma levels of sodium, potassium, chloride or AST (Table 3).

Fish that were held in a holding tank for 24 hours and then in isolation for an additional 24 hours without being exercised (C4) had glucose, cortisol and AST concentrations that were significantly higher than those of wild controls. Fish in this group also had significantly lower concentrations of plasma chloride than wild controls. Plasma levels of lactate, hematocrit, sodium and potassium in C4 fish were not different from those found in the wild controls (Table 3).

Because treatment fish (i.e. fish with a retained lure) experienced conditions similar to those in the C3 control group, baseline levels of physiological indicators from these fish were used to compare responses in treatment fish.

Effect of lure retention on physiological responses to exhaustive exercise (simulated C&R event)

One minute of exhaustive exercise produced physiological disturbances in all fish that were exercised (Table 2-4; Figure 2-3).

Lactate

The effects of metabolic exhaustion after exercise were demonstrated by significant increases in plasma lactate levels at one hour post exercise relative to control ($F=3.80$; $p=0.001$) in all groups of fish. Twenty-four hours after exercise, lactate concentrations in all groups had dropped to levels significantly below control values ($F=8.22$; $p<0.0001$) and that were not different from lactate concentrations measured in C4 fish ($F=1.47$; $p=0.188$). There were no significant differences in the lactate response to exercise between any of the groups (treatment or control) at either one hour or 24 hours post exercise ($F=0.748$; $p=0.632$) indicating that lure retention had no impact on this parameter (Table 2-4; Figure 2-4).

Glucose

Exhaustive exercise produced significant increases in plasma glucose relative to control one hour post exercise for fish in groups T2, T4 and T5 ($F=2.76$; $p=0.011$). Although there was a trend for glucose to increase in other groups of fish (C2, C3, T1, T3 and T6), these increases were not statistically significant. By 24 hours post exercise, plasma glucose concentrations in all groups had returned to and were not significantly different from control values or from levels measured in C4 fish ($F=1.488$; $p=0.169$). No significant differences in glucose levels were identified between any of the groups (either control or treatment fish) at either one hour or 24

hours post exercise (F=0.331; p=0.939) suggesting that lure retention had little effect on glucose concentrations. (Table 2-4: Figure 2-4)

Cortisol

Exhaustive exercise produced no significant changes in plasma cortisol relative to control at one hour post exercise (F=1.13; p=0.354). After 24 hours, cortisol concentrations were not significantly different from levels measured at one hour post exercise (0.409; p=0.895) and were also not different from control values or from those measured in C4 fish (F=0.549; p=0.834). No significant differences in cortisol levels were identified between any of the groups (either control or treatment fish) at either one hour or 24 hours post exercise (F=0.409; p=0.895) (Table 2-4; Figure 2-5).

Hematocrit

Although ANOVA indicated that there were significant changes in plasma hematocrit at one hour post exercise (F=2.36; p=0.027), the Tukey follow up test (a more conservative test) was not significant for any pair of means indicating that there were no significant changes in hematocrit at this time. By 24 hours post exercise, hematocrit was significantly decreased (F=4.96; $p<0.0001$) from control in all groups except T6 and these values were not different from those measured in C4 fish. No significant differences in hematocrit were identified between any of the groups (either control or treatment fish) at either one hour or 24 hours post exercise (F=0.342; p=0.933) suggesting that lure retention had little influence on this parameter (Table 2-4 and Figure 2-5).

Aspartate Transaminase (AST)

Exhaustive exercise produced no significant changes in plasma AST relative to control at one hour post exercise (F=0.724; p=0.670). After 24 hours, AST levels were not significantly different from those measured at one hour post exercise (F=0.549; p=0.0.796) and were also not different from control values or from those measured in C4 fish (F=0.956; p=0.484). No significant differences in AST levels were identified between any of the groups (either control or treatment fish) at either one hour or 24 hours post exercise (F=0.549; p=0.0.796) (Table 2-4).

Ions (Sodium, Potassium and Chloride)

Exhaustive exercise produced variable changes in plasma ion concentrations. Exercise produced no significant changes in plasma sodium measured one hour post exercise (F=1.82; p=0.090). Twenty-four hours post exercise, plasma sodium was not significantly changed from concentrations measured at one hour (F=0.618; p=0.739) and was not different from levels measured in C4 fish. Significant decreases in sodium from control were detected in groups C2, T1, T2 and T5 (F=3.46; p=0.001) at this time.

Exercise produced no significant changes in plasma potassium measured at either one hour post exercise (F=0.747; p=0.650) or 24 hour post exercise (F=1.42; p=0.196) relative to control or to C4 control fish.

Exercise tended to decrease plasma chloride with significant decreases occurring one hour post exercise in all groups except T3 and T4 (F=3.44; p=0.002). At 24 hours post exercise, plasma chloride was significantly lower than control in C2, T1, T2 and T5 groups (F=3.16;

p=0.003) but was not significantly different in any groups from measurement one hour post exercise and in C4 fish.

No significant differences in sodium (F=0.619; p=0.739), potassium (F=1.77; p=0.099) or chloride (F=0.397; p=0.902) were found between any groups at either 1 hour or 24 hours post exercise indicating that lure retention has little effect on ion changes following exercise (Table 2-4).

Discussion

The results from our study were unexpected and interesting. In our laboratory setting, retention of a lure did not appear to affect the physiological ability of pike to respond to, and recover from, a simulated catch and release event. More specifically, we did not see any differences in the responses of any of the blood-based physiological variables to exhaustive exercise between any of the control or lure treatment groups. However, it is important to note that these results were obtained from pike in a laboratory setting and that physiological responses may have been impacted by the effect of confinement in this particular species. In contrast with our results, lure retention has caused changes in some blood-based parameters in other fish species. For example, nesting smallmouth bass with a retained lure show elevated plasma glucose concentrations relative to controls, but not changes in plasma lactate and hematocrit (Henry *et al.*, 2009). It is possible that pike are a hardy species and that they recover more easily from multiple stressors (i.e. exhaustive exercise and lure retention) than other species. Some support for this idea is provided by a study by Arlinghaus et al (2009) in which air exposure had little effect on the physiological response to exercise in pike and that pike recovered from the exercise rapidly.

Remarkably, metabolic rate was not elevated in fish that had a retained lure relative to appropriate controls. We had anticipated that fish with a retained lure would have had either difficulty with respiration such that it would be reduced or possibly would attempt to rid themselves of the lure via physical activity (despite being confined) and experience elevated metabolic demands. It is still possible that lure retention may affect respiration when fish are recovering from an oxygen debt after actively swimming or after burst exercise (pike are sit and wait predators), an effect that our static respirometry study may not have identified. Metabolic rates for pike have varied across studies (Armstrong & Hawkins, 2008). The metabolic rate of pike in our study falls within the range reported in previous studies (reviewed in (Armstrong & Hawkins, 2008). Differences in measurements may be related to the amount of time the fish were allowed to acclimatize, different sizes of pike sampled and water temperature all of which can affect resting metabolic rate (Armstrong & Hawkins, 2008). Our results indicate that pike are able to respire normally even with a retained lure in the mouth.

The physiological response to angling-related stress is thought to be similar to that elicited by exhaustive exercise in the laboratory setting and has been well documented in many species of fish including northern pike (Arlinghaus *et al.*, 2009; Schwalme & Mackay, 1985a; Schwalme & Mackay, 1985b). This intense exercise results in anaerobic respiration and the production of lactate along with stimulation of the sympathetic nervous system and the subsequent release of catecholamines and cortisol (Barton, 2002). Cortisol in turn, generates a cascade of physiological changes designed to resort homeostasis including the release of glucose, release of red blood cells, increased cardiac output and recruitment of gill lamellae to increase oxygen uptake. In our study, exhaustive exercise produced physiological disturbances including increases in plasma lactate, plasma glucose and decreases in plasma sodium and chloride. These

changes are in general in agreement with the existing literature on stress responses in fish (Wendelaar Bonga, 1997) and specifically with those in pike (Arlinghaus *et al.*, 2009; Schwalme & Mackay, 1985a; Schwalme & Mackay, 1985b; Soivio & Oikari, 1976).

Our study was not designed to track recovery of physiological indicators; however, while plasma lactate and glucose did recover to baseline values within 24 hours following exercise, plasma sodium and chloride levels had not recovered by this sampling period. We did not see the rapid recovery of lactate that was previously reported by Arlinghaus *et al.* (2009) in pike in which full recovery of muscle lactate occurred within 1 hour post exercise. In our study, plasma lactate concentrations were still elevated above control values at 1 hour post exercise in agreement with another study (Schwalme & Mackay, 1985b) in which plasma lactate and glucose recovered more slowly. Because pike are able to remove lactate without conversion to glucose (Schwalme & Mackay, 1985b), the elevations in glucose may be attributed to the stress response and mobilization of energy stores rather than as a result of lactate clearance. Together, our results indicate that the retention of a lure in the mouth of northern pike does not result in any significant physiological impairment to the magnitude of exercise, or the timeline for recovery.

The measurement of hematocrit can be used as an indicator of blood loss and overall condition for a fish as well as an indicator of stress as a result of erythrotic swelling, fluid shifts or splenic contractions (Barton, 2002). In our study, hematocrit levels in fish that were confined were higher than those in wild controls likely indicating a stress response to confinement. Although hematocrit levels decreased over 24 hours, they did not fall below those of wild controls; therefore, the decreases in hematocrit are likely due to a recovery from stress and not

due to deterioration of the fish. Further, AST levels did not increase over the study period indicating that little tissue damage occurred in these fish.

Cortisol levels in our study were significantly higher in pike that were contained in a holding tank than in those that were sampled immediately after angling (wild controls) indicating that confinement produced significant stress that persisted even after a "rest" period of 24 hours and, in one group of control fish, after an additional 24 hour period in an isolation chamber. In addition, plasma cortisol levels did not increase in response to exhaustive exercise as expected and remained elevated (relative to field controls) throughout the study period. Although many species of fish adapt to laboratory conditions, pike may experience significant stress related to confinement. The failure to see a cortisol response could indicate that cortisol release had reached a maximum and further increases were not physiologically possible even in response to additional stress. Indeed, Edeline et al (2010) showed that doubling pike density (increased social stress) in large (5 metre diameter) ponds caused a neuroendocrine stress response although no significant increases in plasma cortisol. Interestingly, cortisol values recorded by the authors of this study were higher than the levels that we measured in wild controls indicating that even in large ponds, confinement likely produces some stress in this species (Edeline *et al.*, 2010).

The high concentrations of plasma cortisol and the lack of a cortisol response to exercise complicate the conclusion that lure retention has no effect on the ability of pike to recover following the angling event and break off. It may be possible that in wild fish, recovery from an angling event and break off are hindered by a lure retained in the mouth but that in the laboratory, we are unable to detect this effect due to the unnaturally high stress created by confinement in this species. We were able to see a change in some physiological variables in

response to stress (i.e. disturbances in lactate, glucose and ions) indicating that these fish were able to respond physiologically to the exercise challenge. However, it is possible that this response was attenuated because cortisol release was at a maximal level and that the effect of any additional stressor (i.e. lure in the mouth) to this response was masked. It has been observed that the stress response in some fish is altered by artificial elevation of cortisol and these fish are less able to meet challenges of additional physiological stressors (McConnachie *et al.*, 2012).

Our results provide an important observation about laboratory studies using wild northern pike, particular larger adults of the species. Confinement appears to create significant stress in pike that persists even after a "rest" period of 24 hours and isolation for an additional 24 hours. This reaction makes it difficult to obtain appropriate baseline physiological controls in the laboratory setting. Certainly, wild fish that are quickly captured and sampled before changes in physiological indicators occur provide the most accurate "resting" control values and can be used to reveal stress due to confinement (S. J. Cooke *et al.*, 2013). Further, the physiological response to this stress complicates our ability to interpret effects of additional stressors in this setting. For example, Arlinghaus et al (2009) reported that air exposure does not impact the physiological recovery to exercise in pike; as pike in that study had plasma glucose concentrations similar to those obtained in our confined fish, high stress levels may have masked the additional effect of air exposure in these fish.

In conclusion, we have demonstrated that lure retention has little effect on either resting metabolic rate or the physiological stress response to exhaustive exercise in the laboratory setting. However, our results also suggest that northern pike are highly sensitive to captivity which may affect the ability to test stress responses in a laboratory setting in this species. Future

studies should be designed to minimize the effects of confinement. In addition, in order to interpret results, plasma cortisol should be measured and referenced to values obtained by quick capture and sampling of wild fish. Finally, although we failed to document consistent negative consequences associated with lure retention in a laboratory context, we caution that there may still be physiological and/or behavioural consequences outside of the laboratory environment (i.e. in the field) where fish must engage in locomotory activity to forage and avoid predators.

Tables

Table 2-1: Description of treatment groups used to assess the effect on metabolic rate of retaining a small or large lure in three different hooking locations.

Group	Treatment
T1	Small Spoon/Shallow Hooking
T2	Small Spoon/ Deep Hooking
T3	Small Spoon/ Hooking in Upper & Lower Jaw
T6	Large Spoon/ Hooking in Upper & Lower Jaw
Control	No Hook

Table 2-2: Description of treatment groups to assess the effect of retaining a small or large lure in three different hooking locations on blood physiology in response to exhaustive exercise. Following angling or exhaustive exercise, fish were sampled immediately (0 hour) and/or at one hour post exercise and again at 24 hours post exercise. [a]Blood samples measured in the C3 group immediately after exercise (0 hr) served as the baseline for effect of lure retention

Group	Exercise	Treatment	Sample time		
			0 hr	1 hr	24 hr
T1 (n=8)	60 sec	Small spoon/ shallow hooking	-	√	√
T2 (n=9)	60 sec	Small spoon/ deep hooking	-	√	√
T3 (n=8)	60 sec	Small spoon/ hooking in upper & lower jaw	-	√	√
T4 (n=8)	60 sec	Large spoon / shallow hooking	-	√	√
T5 (n=8)	60 sec	Large spoon/ deep hooking	-	√	√
T6 (n=9)	60 sec	Large spoon/ hooking in upper & lower jaw	-	√	√
C1 (n=9)	angling	Wild controls angled from lake	√	-	-
C2 (n=10)	60 sec	No hooking	-	√	√
C3 (n=7)	60 sec	Hooking and then hook removed (simulated C& R)	√[a]	√	√
C4 (n=9)	-	No hooking	-	-	√

Table 2-3: Comparison of blood physiology between fish sampled immediately after angling (C1; n=9) and confined control fish, C3 (sampled at time 0; n=6) and C4 (n=9) to assess baseline physiological parameters. Values are presented as mean ± SD. Levels with dissimilar letters are significantly different (P<0.05).

Variables	C1	C3 (sampled at time 0)	C4	F ratio	Prob>F
Lactate (mmol l^{-1})	0.8 ± 0.3[a]	6.9 ±2.9[b]	1.7 ± 1.1[a]	27.4	**<0.0001**
Glucose (mmol l^{-1})	3.1 ± 1.2 [a]	6.8 ± 0.9 [b]	6.5 ± 0.9 [b]	33.3	**<0.0001**
Cortisol (ng ml^{-1})	1.4 ± 1.5 [a]	589 ± 239 [b]	435 ± 295 [b]	14.9	**<0.0001**
Hematocrit (% PVC)	18 ± 2.8 [a]	24 ± 3.3 [b]	18 ± 4.6 [a]	5.14	**0.015**
Sodium (mmol l^{-1})	141 ± 4.3 [ab]	149 ± 4.4 [a]	136 ± 8.6 [b]	6.98	**0.005**
Potassium (mmol l^{-1})	3.9 ± 2.1	4.0 ± 0.6	4.9 ± 3.4	0.490	0.620
Chloride (mmol l^{-1})	124 ± 3.3 [a]	126 ± 4.2 [a]	117 ± 5.7 [b]	6.97	**0.005**
AST (IU L^{-1})	750 ± 261 [a]	1725 ± 804 [ab]	2867 ± 2240 [b]	4.83	**0.019**

Table 2-4: Evaluation of the effects of exercise on eight physiological parameters for control pike and pike with a retained lure. Values are presented as mean ± SD. Within each physiological parameter, levels with dissimilar letters are significantly different P<0.05).

Time	Group	Lactate (mmol l^{-1})	Glucose (mmol l^{-1})	Cortisol (ng ml^{-1})	Hematocrit (%PVC)	AST (IU L^{-1})	Na+ (mmol l^{-1})	K+ (mmol l^{-1})	Cl- (mmol l^{-1})
0 hour control	**C3**	**6.9 ± 2.9^{a}**	**6.8 ± 0.9^{a}**	**589 ± 239^{a}**	**24 ± 3.3^{a}**	**1725 ± 804^{a}**	**149 ± 4.4^{a}**	**4.0 ± 0.6^{a}**	**126 ± 4.2^{a}**
1 hour	C2	**11 ± 2.1^{b}**	9.8 ± 2.0^{a}	356 ± 184^{a}	19 ± 3.5^{a}	1526 ± 670^{a}	137 ± 6.6^{a}	4.7 ± 2.8^{a}	**110 ± 9.5^{b}**
	C3	**12 ± 0.7^{b}**	12 ± 2.4^{a}	475 ± 166^{a}	18 ± 1.6^{a}	1489 ± 798^{a}	144 ± 12^{a}	4.7 ± 1.7^{a}	**112± 6.7^{b}**
	T1	**9.7 ± 3.0^{b}**	9.6 ± 2.5^{a}	408 ± 308^{a}	19 ± 2.7^{a}	1836 ± 942^{a}	135 ± 11^{a}	3.8 ± 1.7^{a}	**106 ± 7.7^{b}**
	T2	**11 ± 1.3^{b}**	**12 ± 3.6^{b}**	493 ± 269^{a}	19 ± 2.8^{a}	1275 ± 587^{a}	137 ± 7.6^{a}	5.1 ± 3.4^{a}	**109 ± 8.0^{b}**
	T3	**11 ± 0.9^{b}**	10 ± 2.3^{a}	400 ± 139^{a}	21 ± 1.3^{a}	2038 ± 1424^{a}	140 ± 6.7^{a}	5.6 ± 3.7^{a}	113 ± 2.8^{a}
	T4	**10 ± 1.7^{b}**	**12 ± 3.1^{b}**	447 ± 343^{a}	19 ± 6.7^{a}	1527 ± 579^{a}	141 ± 9.1^{a}	6.2 ± 4.0^{a}	113 ± 8.8^{a}
	T5	**11 ± 1.9^{b}**	**13 ± 4.4^{b}**	568 ± 264^{a}	22 ± 3.5^{a}	1251 ± 602^{a}	139 ± 11^{a}	5.8 ± 1.7^{a}	**110 ± 9.9^{b}**
	T6	**11 ± 0.9^{b}**	11 ± 2.8^{a}	306 ± 190^{a}	23 ± 4.6^{a}	1664 ± 954^{a}	144 ± 8.9^{a}	4.2 ± 1.2^{a}	**112 ± 4.5^{b}**
24 hour	C2	2.6 ± 2.8^{c}	6.0 ± 2.0^{a}	451 ± 355^{a}	**14 ± 3.1^{b}**	2941 ± 2843^{a}	**132 ± 8.3^{b}**	3.7 ± 1.2^{a}	**110 ± 8.0^{bc}**
	C3	1.9 ± 1.7^{c}	5.9 ± 1.4^{a}	435 ± 329^{a}	**13 ± 2.0^{b}**	3230 ± 1839^{a}	134 ± 2.2^{a}	2.5 ± 0.6^{a}	114 ± 3.5^{abc}
	C4	1.7 ± 1.1^{c}	6.5 ± 0.9^{a}	435 ± 295^{a}	**18 ± 4.6^{b}**	2867 ± 2240^{a}	136 ± 8.6^{a}	4.9 ± 3.4^{a}	**117 ± 5.7^{bc}**
	T1	1.5 ± 0.6^{c}	5.4 ± 2.1^{a}	565 ± 439^{a}	**15 ± 1.9^{b}**	3977 ± 3302^{a}	**127 ± 8.3^{b}**	5.2 ± 2.7^{a}	**102 ± 16^{bc}**
	T2	1.6 ± 0.8^{c}	6.8 ± 2.2^{a}	444 ± 576^{a}	**16 ± 5.6^{b}**	2211 ± 805^{a}	**128 ± 14^{b}**	3.7 ± 2.1^{a}	**109 ± 13^{bc}**
	T3	0.8 ± 0. 5^{c}	5.8 ± 0.7^{a}	447 ± 423^{a}	**15 ± 3.8^{b}**	3636 ± 2872^{a}	139 ± 3.9^{a}	3.4 ± 1.8^{a}	119 ± 5.6^{ac}
	T4	0.6 ± 0.4^{c}	6.8 ± 1.5^{a}	440 ± 231^{a}	**14 ± 4.1^{b}**	3382 ± 1862^{a}	135 ± 14^{a}	2.9 ± 1.5^{a}	113 ± 13^{ac}
	T5	1.6 ± 0.7^{c}	7.8 ± 2.4^{a}	420 ± 261^{a}	**16 ± 3.6^{b}**	2968 ± 2292^{a}	**128 ± 11^{b}**	3.4 ± 1.0^{a}	**109 ± 12^{bc}**
	T6	1.5 ± 1.3^{c}	5.6 ± 1.8^{a}	233 ± 161^{a}	**18 ± 2.4^{b}**	5162 ± 5042^{a}	133 ± 5.8^{a}	5.1 ± 3.2^{a}	114 ± 4.8^{abc}

Figures

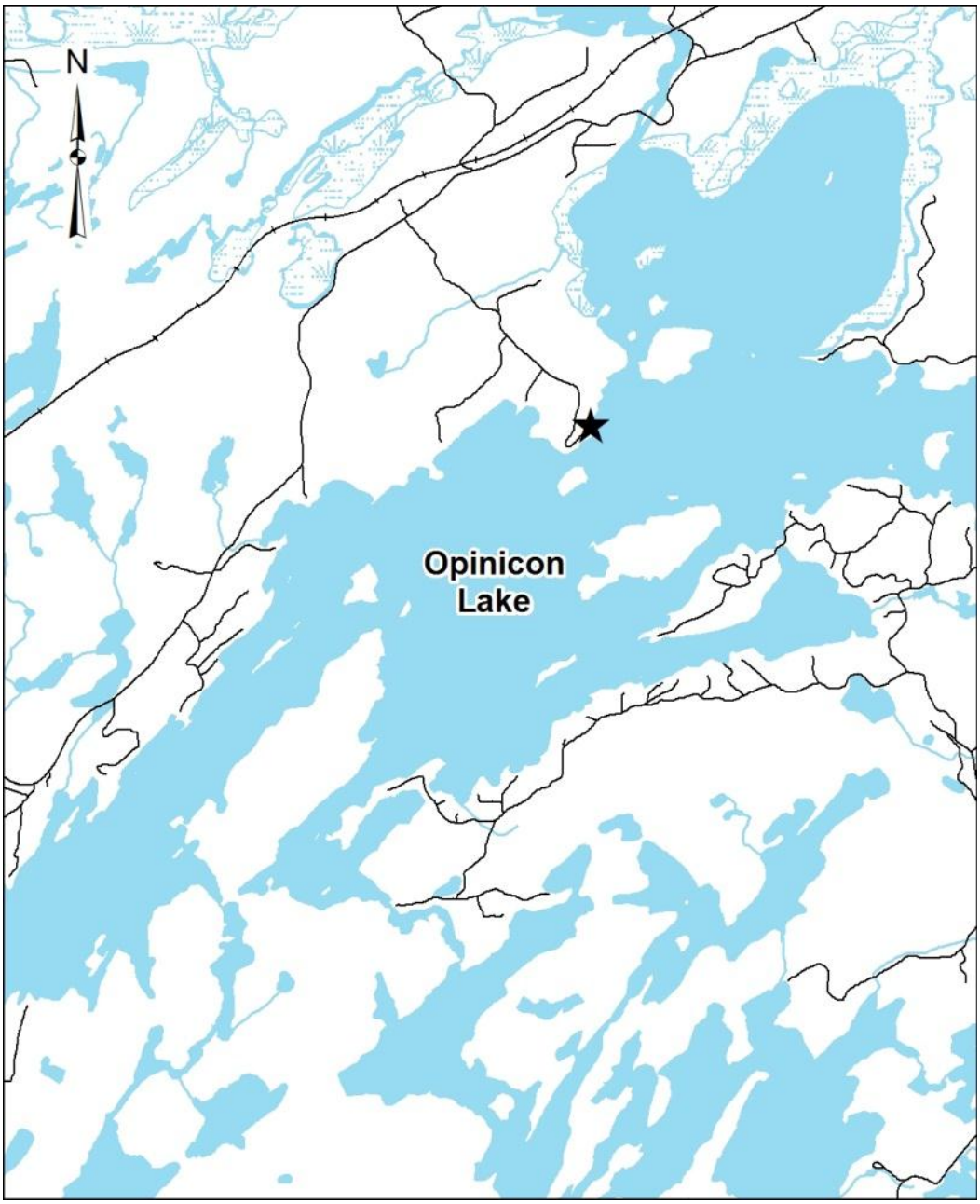


Figure 2-1: Map of Lake Opinicon, Ontario (UTM 18T 394923 4935549). The star denotes the location of the Queens University Biological Station (QUBS) and the location of the common release site for all fish used in the telemetry study.

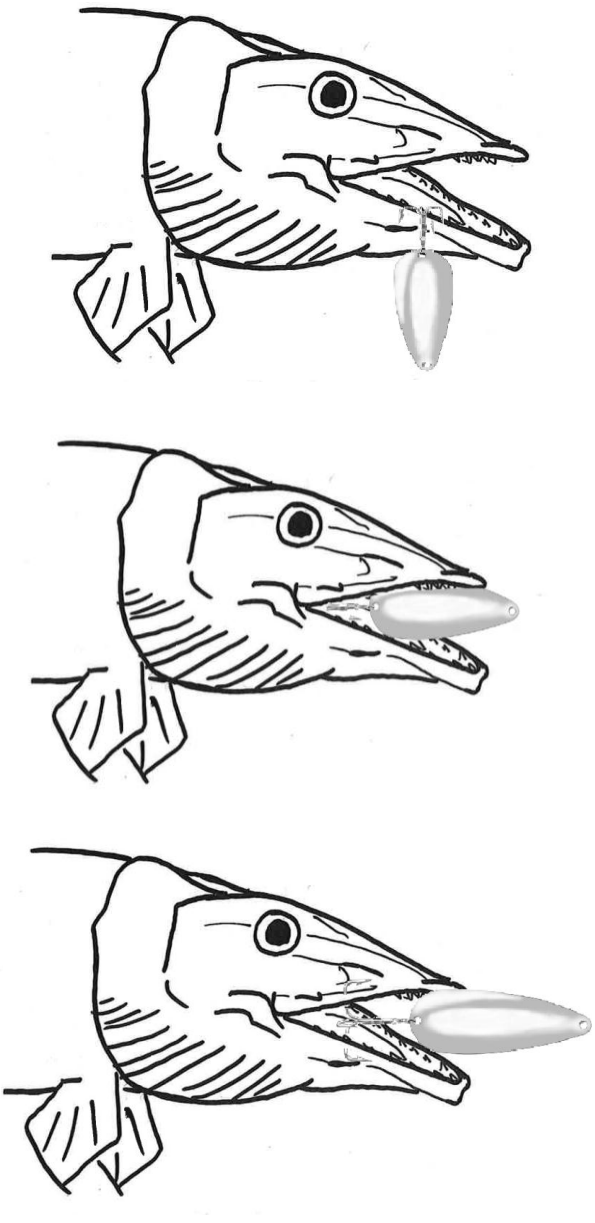


Figure 2-2: Hooking locations for lure treatments. (A) Shallow hook through lower jaw. (B) Deep hook through soft tissue at the base of the tongue, (C) Upper and lower jaw hooking, one hook each through the tissue of the upper and lower jaw. Both small (5 cm blade length) and large (12 cm blade length) were used.

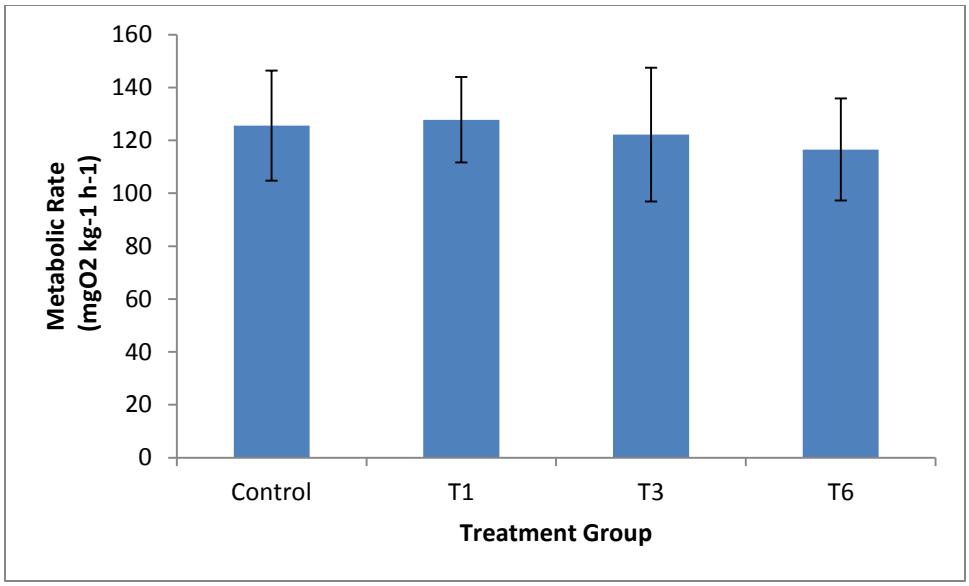


Figure 2-3: Mean metabolic rate of control pike (control; n=13) and pike that received a hooking treatment (T1, n=5; T3, n=7; T6, n=4). The metabolic rate for control fish did not differ from that of any treatment group.

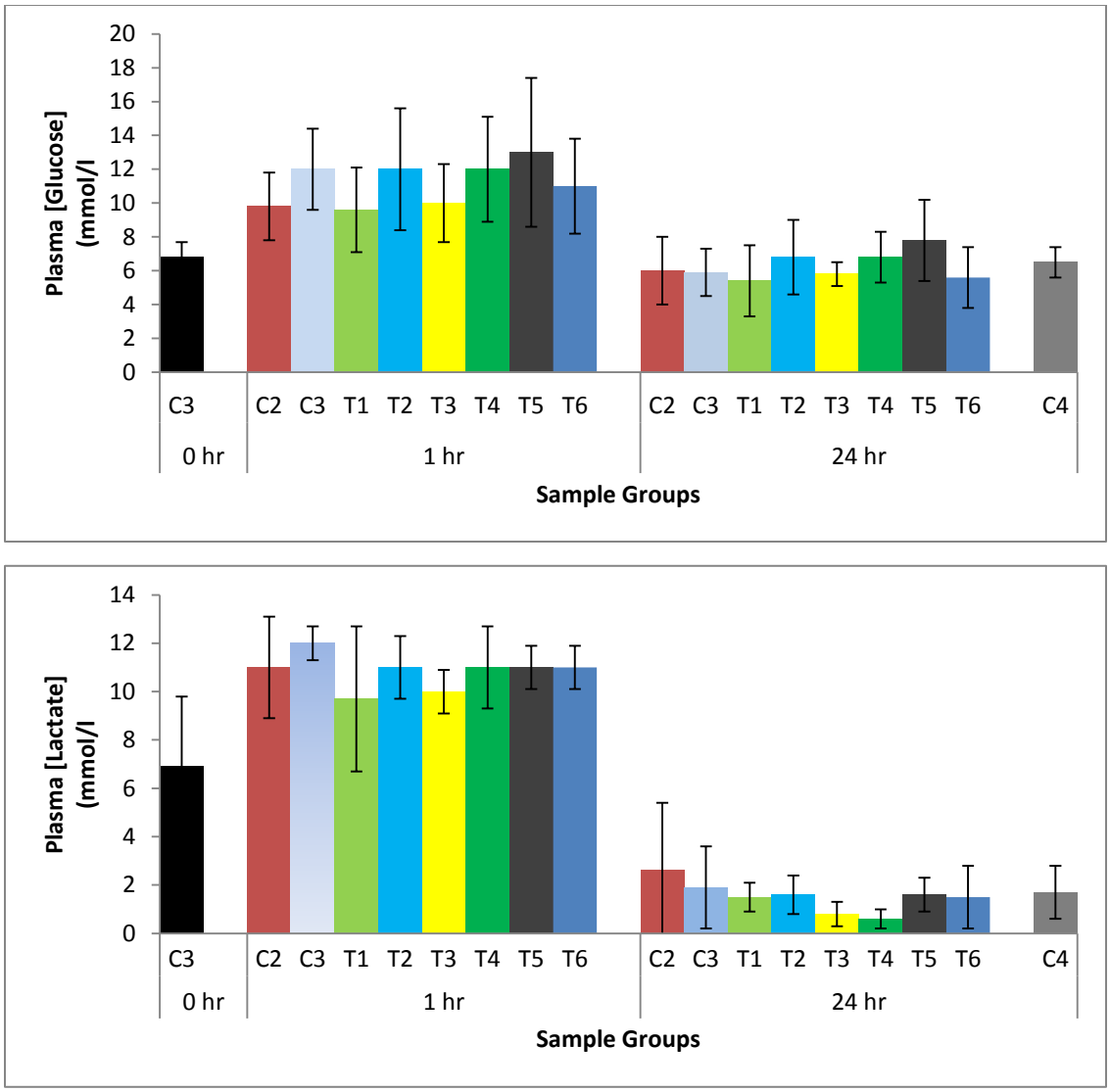


Figure 2-4: Concentrations of plasma glucose and lactate for baseline control fish (C3, time 0 hr) and treatment and control fish sampled at 1 hour and 24 hours after exhaustive exercise. Responses of these parameters to exercise between any of the groups (treatment or control) at either one hour or 24 hours post exercise were not significantly different.

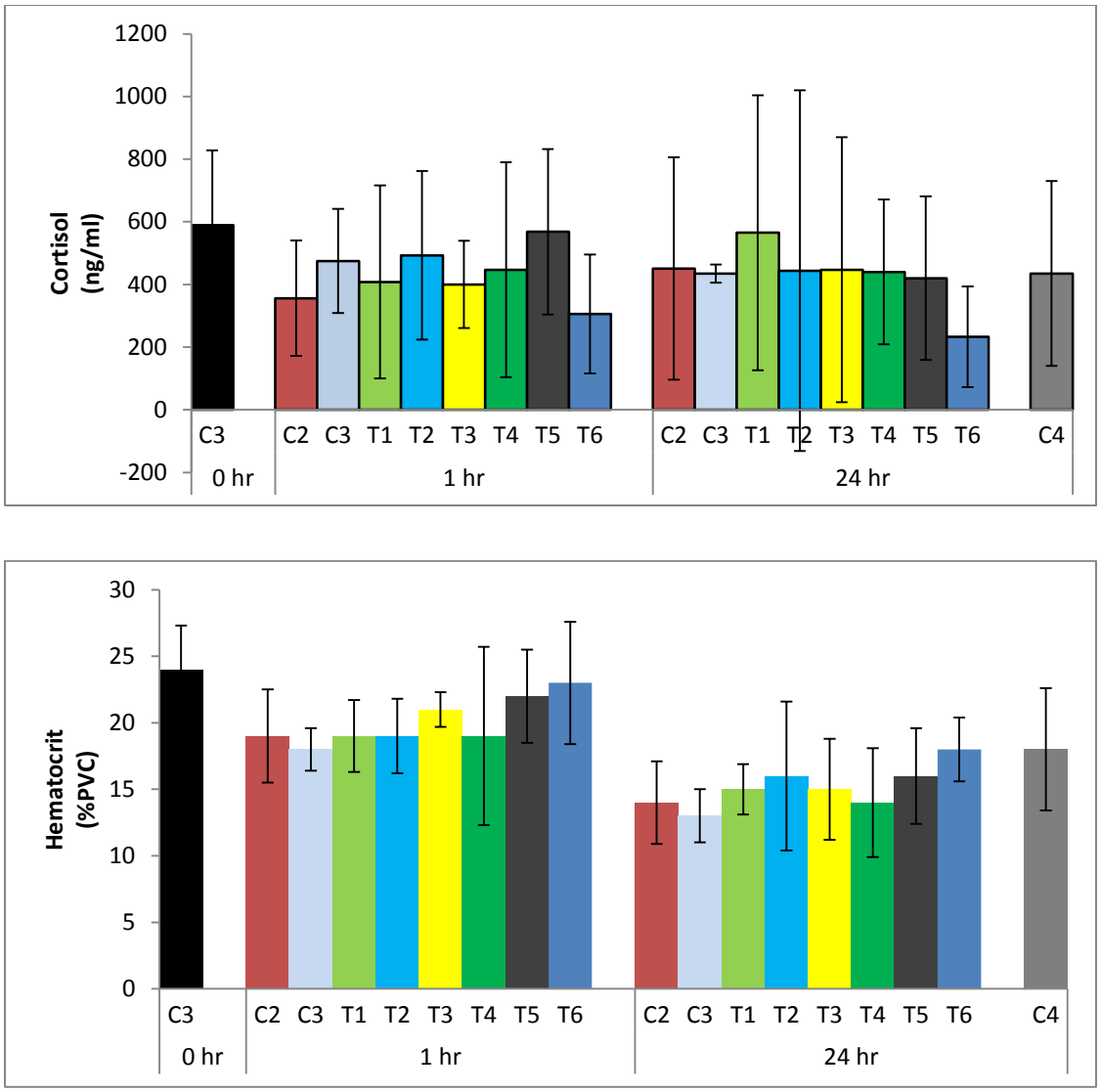


Figure 2-5: Concentrations of plasma cortisol and hematocrit for baseline control fish (C3, time 0 hr) and treatment and control fish sampled at 1 hour and 24 hours after exhaustive exercise. Responses of these parameters to exercise between any of the groups (treatment or control) at either one hour or 24 hours post exercise were not significantly different.

Chapter 3: Behavioural observations of pike held in observations tanks with retained lures

Introduction

Studies that examine the fate of fish caught and released by recreational anglers are increasingly common (summarized in (Arlinghaus *et al.*, 2007). One aspect of recreational fisheries interactions that has received less attention involves fish that escape prior to landing (Chopin & Arimoto, 1995). In some cases fish simply become unhooked; however, sometimes fish break the line and swim away with hooks still embedded in their tissue with the bait or lure in tow (termed break off). Field observations of northern pike released with retained lures suggest that pike move very little following initial break off (or release) from the angling event (Arlinghaus *et al.*, 2008a; Klefoth *et al.*, 2008); however little is known about what the fish are doing during this recovery phase. Specifically, in the case of retained gear, it is unknown whether they are they trying to rid themselves of the encumbrance or if they rest.

A number of factors likely interact to affect post release behaviour of fish following break off. Physiological reactions likely alter behavior of fish post release (S. J. Cooke *et al.*, 2002) but studies looking into the effects post break off are limited (Arlinghaus *et al.*, 2008a; Henry *et al.*, 2009; Klefoth *et al.*, 2008). Sub lethal effects quantified from C&R studies include a combination of injury, behavioural changes and physiological disruptions post release (S. J. Cooke *et al.*, 2002; C. D. Suski *et al.*, 2007). As described in Chapter 2, pike appear to have a longer recovery period than other fish

(Kieffer, 2000). For example, elevated glucose levels were observed in pike up to 96 hours post angling (Schwalme & Mackay, 1985b) which may likely contribute to their behavioural response following an angling event.

Based on observations from telemetry studies, the behavioural reactions of fish post release appear to vary across species. For example, Gurshin and Szedlmayer (2004) noted reduced movement in the shortnosed shark (*Rhizoprionodon terrenovae*) and reduced swimming speeds were observed in lemon shark (*Negaprion brevirostris*) (Sundstrom & Gruber, 2002). Conversely, hyperactivity was observed in largemouth bass (*Micropterus salmoides*) (S. J. Cooke *et al.*, 2000) following release. Dispersal studies looking at tournament-caught bass found that only 14% of largemouth bass returned to their original capture location (Wilde, 2003) while non-tournament caught fish showed a higher rate of return (Richardson-Heft *et al.*, 2000; Ridgway, 2002). These results indicate that compounding effects of tournament handing (e.g. prolonged air exposure, repeated handling, livewell conditions) slows the fish's recovery and return to baseline behaviour after release. Nguyen *et al*.(2009) found that tournament-caught bass that experience barotrauma and fizzing (deflating the swim bladder using a hypodermic needle) showed similar movement activity. However, their data did suggest that fizzed fish moved twice as far as non-fizzed fish (Nguyen *et al.*, 2009), providing another example of compounding effects that alter fish response from an angling event. The challenge with telemetry studies that look at post release behaviour is that tagging can confound the observations (Bettoli & Osborne, 1998; S. J. Cooke *et al.*, 2002). Klefoth (2008) addressed this limitation by allowing pike to recover for two weeks post tagging to

allow for a more complete recovery. Theoretically this time allowed the fish to return to a pre-angling state prior to being angled again for the study.

The objective of this study was to collect direct, fine-scale video observations of pike following simulated break off with a retained lure in the laboratory, specifically, direct observational data of pike interaction with a retained lure and quantification of their activity patterns for 24 hours. The data collected was correlated with the results of field observations noted in previous studies. A challenge was to minimize tank stress to the greatest extent possible; however the risks associated with observations in tanks were considered less invasive than tagging for short term observations. The use of video to observe pike in tanks is not new (Frith & Blake, 1991; Schriefer & Hale, 2004) and this technique is used extensively for the study of fish swimming dynamics (Chadwell *et al.*, 2012; Domenici *et al.*, 2004; New *et al.*, 2001) and response to toxicological effects (Bjerselius *et al.*, 2001; Hansen *et al.*, 1999).

Methods

Northern pike were collected from Lake Opinicon (Figure 2-1) following the protocol described in Chapter 2. In this study, two observation tanks were used, allowing for observations of two fish to be collected in each 24 hour period. Pike were held in 1200 litre (152 cm diameter) shaded flow through holding tanks for 24 hours to allow the fish to return to a baseline resting state following methods similar to Suski *et al.* (2007). Following the 24 hour hold time, the fish were randomly allocated to treatment and control groups.

Prior to being transferred to observation tanks, fish were exercised for 60 seconds using tail pinching, transferred to a padded trough with fresh lake water, and then hooked based on one of three treatment groups. For this experiment large spoons were used and either hooked shallow (T4=lower jaw), deep (T5= base of tongue), upper and lower jaw (T6) and control (no hook). After the hook was attached, fish were immediately transferred to 60 cm by 76 cm observation tanks with a 56 cm by 38 cm glass viewing window. The bottom of each tank was covered with a sheet of white plastic lattice (similar to the material used to screen commercial fluorescent light fixtures) to raise the bottom of the tank while maintaining good water circulation through the tanks. This was done to keep the fish in the viewing window even if they rested on the bottom of the tank. A wood frame was attached to the front of each observation tank that was covered in black plastic sheet. At the apex of the cone, approximately 60 cm from the viewing pane, a small observation hole was created where the lens of a video camera (Sony HDD 2000) was placed at a suitable focal length for collecting video footage while minimizing disturbance to the fish in the tank. The same video camera was used for both tanks and moved for each observation period. The top of each tank was covered with a screened cover to limit the possibility of pike jumping from the tank. Each tank was connected to the laboratory's lake water system and was continuously supplied with fresh lake water. In addition, air stones were added to the tank to ensure adequate oxygenation. Strict use of tank and laboratory isolation, the frequent turnover of tank water with fresh lake water, and supplemental aeration were expected to ameliorate some of the confounding influences of captivity on the pike.

Data were collected during five minute digital video observation periods at 5, 20, 35 and 50 minutes and then again at 6, 12 and 24 hours. The video was transferred to a computer and visually analysed every 10 seconds over the course of the five minute observation period. The behavioural data collected during the observation period and recorded on data sheets included the following: swimming within the tank water column, darting (sudden burst movements about the tank), jumping in an attempt to clear the water, rubbing against the tank, head shaking, resting and/or exploring (probing the water surface). Physical data collected during the observational period included: injury severity (e.g. lacerations), evidence of bleeding, change in lure location and opercular pumping (ventilation). The same independent observer analysed all video data.

Statistical Analysis

A two way analysis of variance (ANOVA) was used to compare behavioural scopes among treatment groups with treatment group and time as independent variables. When significant differences were found a post-hoc Tukey HSD test was used to determine where differences in means occurred. A two way ANOVA was also used to compare ventilation rates among treatment groups with treatment group and time scale as independent variables. For all analysis time comparisons were made at 1 (50 minute mark), 6, 12 and 24 hours. All analyses were conducted using JMP v10 (SAS Institute, Cary, NC). Data are expressed as mean ± standard deviation and significance was evaluated $\alpha < 0.05$.

Results

Thirty-nine pike (523 ± 71 mm, range 225-640 mm) were placed in four treatment groups (T4= 496 ± 63 mm; T5 = 499 ± 107 mm; T6 = 545 ± 45 mm; Control = 496 ± 63 mm). Four mortalities occurred during this component of the study. One T4 pike was dead at three hours, one T6 pike was dead at 6 hours, and one T6 pike was dead at 24hrs. The latter fish had started to lose equilibrium at 6 hrs. Fish that died were not included in the analysis. One control fish jumped out of the tank after 12 hours and not included in the analysis. Seven lures were shed during the observation period or 22% of the total (n=32) and the distribution was even across treatments (three T4, two T5 and two T6 fish).

No differences in behaviour were observed among the different treatment groups and controls (Table 3-1). Generally, fish remained in a resting position on the bottom of the tanks with little activity recorded. Fish made little or no effort to actively dislodge their lures. Momentary (<5 seconds) bursts of hyperactivity were observed; however this type of activity was infrequent. Occasionally, a fish would be observed either actively hovering or exploring the surface of the tank; however, while still noteworthy, none of these activities were sustained and did not affect the outcome of our analysis.

All treatments groups showed a significant decrease in opercular pumping rate (i.e. ventilation rate) over time (Figure 3-1). For all fish (both treatment and control), ventilation rate peaked between 20 and 50 minutes post hooking and declined steadily thereafter with significant differences noted between the first hour (50 min) of observation (59 ± 2 beats per minute (bpm)) and at 24 hours (47 ± 27 bpm). Among

treatment groups, a difference was noted for fish in the T5 group that showed significantly higher ($F=2.98$, $p=0.0025$) opercula pumping rates throughout the observation period (60.44 ± 1 bpm) than fish in other groups (Figure 3-2).

Discussion

In our laboratory setting, retention of a lure in the mouth does not appear to impact activity levels in pike in the short term (within 24 hours a hooking event). Specifically, we did not observe any differences in movement or behaviour between fish with and without a retained lure; all pike observed (both treatment and control) showed limited activity within the 24 hour study period. Our results do not agree with those of Arlinghaus *et al*. (2008a) who, in a field study, observed that pike with a retained lure had significantly lower movement in the first hour post release compared to fish with no lure. Although their observations were of pike in a natural setting, this study design did not allow for direct observation of the fish to quantify the fine scale behaviour that occurred within this time period. It is possible that the differences observed in our study are due to the impact of confinement on fish behaviour. Based on our physiology results from Chapter 2, confined pike have elevated levels of cortisol and glucose, indictors of a stress response. Stress related to confinement may have impacted our ability to see a behavioural reaction to the added stress of the lure. Interestingly, ventilation rates of pike did gradually decline over the 24 hour study period. This gradual decrease in opercular pumping could indicate a recovery from exhaustive exercise similar to the recovery in plasma lactate after exercise that we observed reported in Chapter 2.

Alternatively, the decreases in ventilation rate could also signal acclimatization of pike to the tank (Gibson & Mathis, 2006).

Contrary to our expectations, pike spent little time trying to disengage the retained lure. These observations are similar to those made by Henry et al (2009) of nesting male smallmouth bass released with retained lures following simulated break off. The authors of that study observed that while fish with buoyant type lures appeared to actively attempt to remove the lure, fish with neutrally buoyant lures, such as soft plastics and jig heads, were less active or disturbed by the presence of the lure. The lures used in our study were negatively buoyant spoons and also appeared to not elicit significant responses.

Increased opercular movement occurs in response to stress and has been used as an indicator of stress in fish (Gibson & Mathis, 2006). For example, exposing fish to external stimuli (Huuskonen & Karjalainen, 1997), application of electrical current to water (Jansen & Green, 1970) or application of chemical stimulants to water (Gibson & Mathis, 2006; James *et al.*, 2003; Thomas & Rice, 1975) will all increase ventilation rate. We used this indicator to determine if lure retention causes increased stress in fish as measured by increases in ventilation rate.

Although all fish experienced a similar ventilation response to exercise, ventilation rates in fish that were hooked deeply (T5 group) were significantly greater than those in other fish. Fish accomplish ventilations by a synchronous expansion and contraction of the buccal and opercular cavities (Moyle & Cech, 2004). The expansion and contraction creates a continual unidirectional flow which provides a supply of fresh

water over the gills. Fish increase gill ventilation to compensate for low dissolved oxygen levels in hypoxic water. Increasing the volume of water over the gills, either by increasing the volume pumped (ventilator stroke volume) or the rate at which it is pumped (ventilator frequency) (Moyle & Cech, 2004), is a mechanism to compensate for the low dissolved oxygen in the water column. The oxygen levels in the tanks were adequate given the oxygenation supplied through the air stones and frequent water turn over and since ventilation rate declined over time for all fish some other mechanism was affecting the T5 fish.

Interruptions in ventilator strokes (opercular pumping) occur when a fish creates brief reversals of flow that appear as coughs (Moyle & Cech, 2004) to the observer. Fish use this action to clear foreign matter and mucosa from the gills. Toxicology studies in brook trout (*Salvelinus fontinalis*) showed that cough frequency increased when fish were challenged with excessive concentrations of copper in the water and that cough frequency can be used as a sub lethal indicator of stress (Drummond *et al.*, 1973). While coughing was not quantified, the T5 treatment group with the hook deep in the throat may have been inhibited in their ability to "cough" and to clear the gill area on a regular basis. They may therefore have increased their ventilation rate to compensate for their inability to clear foreign matter and mucosa from the gills. For deeply hooked fish then, it could be hypothesized that their reduced activity patterns are not only a result of recovery from exercise and stress, but also in response to directing energy to other activities such as respiration.

The results of this laboratory behavioural study show that lure retention has no effect on movement or behaviour in the short term. Some caution should be used in interpreting these results as confinement stress may mask behavioural indicators of fish interacting with the lure. Hooking location did affect ventilation rate; specifically elevated ventilation was observed in deeply hooked pike compared to fish with less obtrusive hooking locations or fish with no lure. Future work could consider using tri-axial acoustic accelerometers to assess fine scale activity patterns of pike with retained lures in the 24 hours post release. This approach has been used by other researchers (Landsman, 2011; O'Toole *et al.*, 2010) to determine activity rates of muskellunge and great barracuda.

Tables

Table 3-1 Results of a two way ANOVA comparing behavioural variables for pike with treatment groups and time interval (1, 12, 24 hours; mean±S.E.) as independent variables. No significant differences were found among variables or interactions.

Variables	Treatment Group	Mean (Count)	ANOVA Output		
			Treatment	Time Interval	Interaction
Resting	Control	26±1.6	F=0.20, p=0.899	F=2.1, p=0.125	F=0.29, p=0.939
	T4	25±1.7			
	T5	25±1.8			
	T6	26±1.9			
Exploring	Control	0.63±0.35	F=0.25, p=0.862	F=1.2, p=0.318	F=0.31, p=0.929
	T4	4.4e-16±0.37			
	T5	0.22±0.39			
	T6	0.43±0.41			
Swimming	Control	1.1±0.51	F=0.68, p=0.560	F=0.47, p=0.624	F=0.55, p=0.773
	T4	1.5±0.53			
	T5	0.65±0.55			
	T6	0.92±0.59			
Hovering	Control	2.3±1.4	F=0.26, p=0.855	F=1.8, p=0.166	F=0.30, p=0.931
	T4	3.4±1.5			
	T5	3.6±1.5			
	T6	2.6±1.6			
Darting	Control	0.34±0.17	F=0.49, p=0.690	F=0.64, p=0.529	F=1.7, p=0.138
	T4	0.37±0.17			
	T5	0.31±0.18			
	T6	0.14±0.19			
Jumping	Control	0.71±0.37	F=0.19, p=0.905	F=1.5, p=0.237	F=0.27, p=0.951
	T4	0.22±0.39			
	T5	0.22±0.41			
	T6	0.61±0.44			
Rubbing	Control	0.05±0.05	F=0.37, p=0.775	F=1.2, p=0.322	F=0.22, p=0.968
	T4	0.11±0.05			
	T5	0.03±0.05			
	T6	0.07±0.06			
Head Shakes	Control	0.05±0.17	F=0.94, p=0.425	F=0.56, p=0.574	F=0.70, p=0.652
	T4	0.49±0.18			
	T5	0.34±0.18			
	T6	0.21±0.20			

Figures

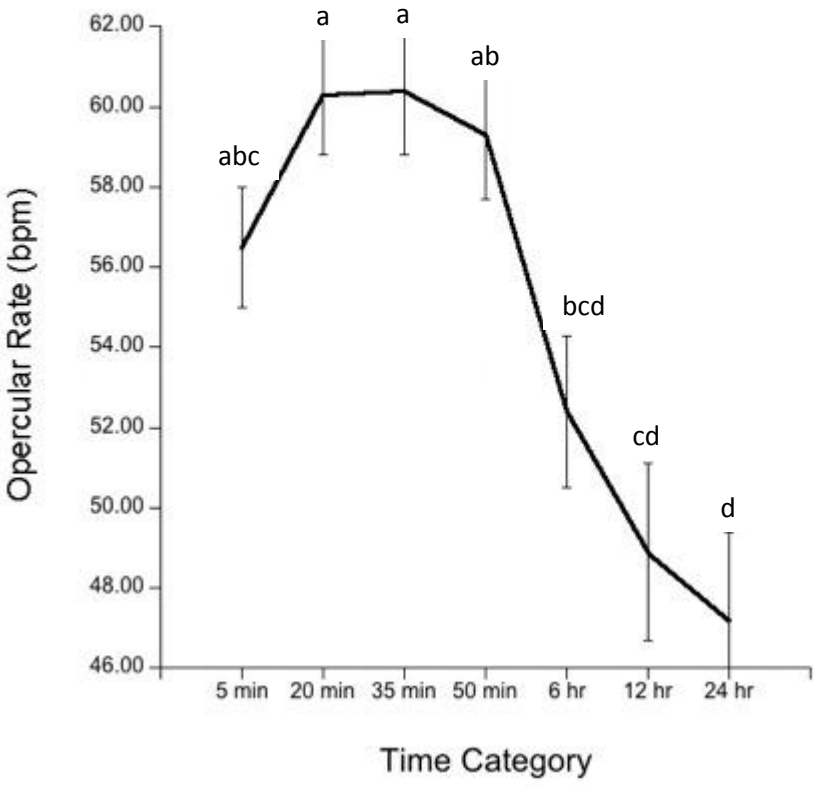


Figure 3-1 Comparison of mean ventilation rate over time for all treatment groups and controls. Ventilation rate peaked at 35 minutes and declined over the course of the observation period for all groups. Ventilation rate at 50 minutes was significantly higher than at 24 hours. Levels with dissimilar letters are significantly different.

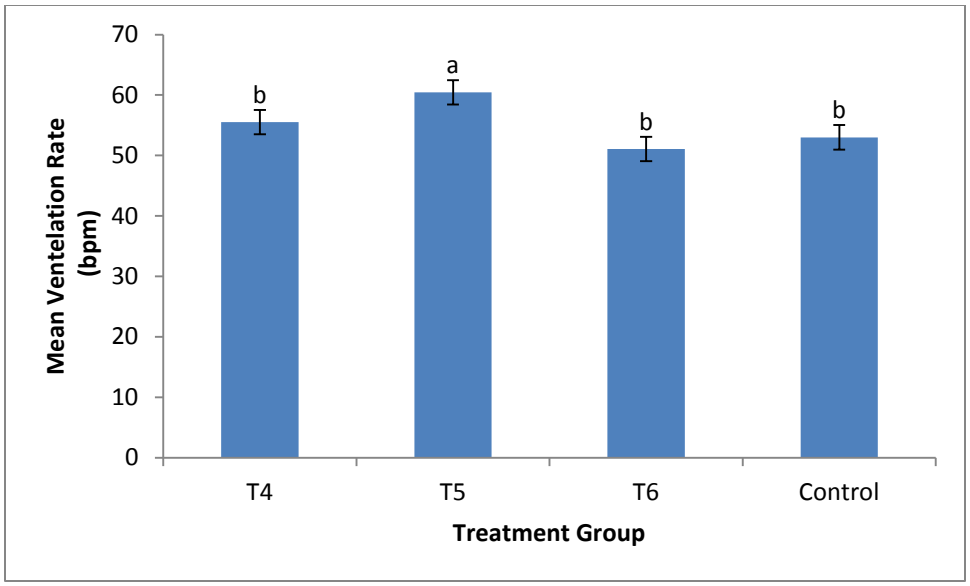


Figure 3-2 Comparison of average ventilation rates (calculated over 24 hours) between treatment groups and control fish. Levels with dissimilar letter are significantly different. Ventilation rates of T5 fish, with deep hooking in the esphogeal passage, were significantly higher than the other two treatment groups and the controls.

Chapter 4: Tracking lentic northern pike with retained lures: Assessment of lure retention times in relation to hooking location and hook type.

Introduction

Biotelemetry devices enable researchers to continually monitor animals from a distance (Lucas & Baras, 2000) alleviating many of the complications related to collecting direct observations (such as recording data while pursuing the animal). For fisheries research, using electronic telemetry tags creates enormous potential to gather *in situ* data from fish while they are not influenced by the presence of an observer or stressed by being held in observation facilities.

The use of biotelemetry as a research tool in understanding fish ecology and behaviour has steadily increased in the past three decades (Landsman *et al.*, 2011a). The availability of this technology creates the opportunity to gather real time field data on the fate of fish with a lure retained following break off. Telemetry allows us to locate (Lucas & Baras, 2000) and in some cases monitor the physiology and behaviour (S. Cooke *et al.*, 2004; Ropert-Coudert & Wilson, 2005) of free swimming fish. Options have also greatly improved with the introduction of simple passive integrated transponder (PIT) tags, more complex radio and acoustic telemetry systems and even remote satellite tags and archival biologging devices (S. Cooke *et al.*, 2004; Lucas & Baras, 2000; Ropert-Coudert & Wilson, 2005; Wilson *et al.*, 2006).

Limited work has been done to understand the effects of retained lures on the well-being and survival of fish. Some studies have assessed the impact of deep hooking on various fish species (Fobert *et al.*, 2009; Margenau, 2007; Tsuboi *et al.*, 2006) but typically these studies have used hooks without a lure. Fewer studies have assessed the behavioural consequences of swimming free with a lure retained in the mouth (but see (Arlinghaus *et al.*, 2008a; Henry *et al.*, 2009). A fish that has managed to break an angler's line and escape with a retained lure may suffer immediate mortality either from injury or predation (Arlinghaus *et al.*, 2007; S. Cooke & Philipp, 2004; S. E. Danylchuk *et al.*, 2007), or may starve as a result of impaired feeding ability (Tsuboi *et al.*, 2006). If the fish survives, it may still experience sublethal impairments such as a reduced ability to evade predators over the longer term (S. Cooke & Philipp, 2004; Schreer *et al.*, 2005; White *et al.*, 2008) or succumb to a variety of delayed physiological impairments. The various documented changes in behaviour caused by angling-related stressors (Gingerich *et al.*, 2007; Klefoth *et al.*, 2011; Stålhammar *et al.*, 2012; White *et al.*, 2008) may be indicative of an altered or impaired ability of a fish to sense and respond to its environment; this impairment could be attributed to the presence of a retained lure.

Radio telemetry in combination with an experimental approach designed to quantify behavioural reactions in fish (Donaldson *et al.*, 2008) is a powerful and sensitive tool which can be employed to assess direct and sublethal behavioural impacts of a retained lure (Olla *et al.*, 1997). Arlinghaus *et al*. (2008a) evaluated the impacts on behaviour of free swimming pike with retained lures using a combination of visual tracking for short term movement (using a float attached to the dorsal fin) and traditional radio tracking to follow fish for a number of weeks post release. The limitation of this

study was that the researchers did not know if and when the fish lost the lure and whether the retained lure was still influencing the observed behaviour. The novel aspect of the current study was to track the lure, rather than the fish in order to 1) determine the length of time the fish is affected by retained fishing gear and 2) increase the resolution of the behavioural observations and movement patterns and 3) relate the behavioural observations specifically to the duration that the lure is impacting the fish.

Methods

This study was completed between June 16th and July 11th, 2009. All fish were collected from Lake Opinicon in eastern Ontario (Figure 2-1). On a given day, northern pike were angled from a variety of locations throughout lake to ensure that treatment fish were not all collected from the same area. Angling involved casting and/or trolling until enough fish were captured to complete a round of releases, typically no more than five at a time. Once a fish was hooked it was immediately retrieved (< 60 seconds), netted with a rubberized, non-marring fish net and transferred to a 1200 litre onboard live well. The live well was covered and regularly flushed with fresh lake water to enhance stable conditions with holding periods less than 2 hours (Arlinghaus *et al.*, 2008a; Arlinghaus *et al.*, 2009; Klefoth *et al.*, 2008). Any fish that were injured (e.g. bleeding) were not used in the study. Barbless hooks were used to minimize injury and increase ease of hook removal. Upon return to the QUBS research station, fish were transferred from the on board live well to individual lake water filled coolers and left for one hour prior to treatment. Water was circulated regularly through the coolers to ensure water quality.

Every effort was made to reduce air exposure and maintain an optimal environment for the fish in the water.

Transmitter Lures

Medium size hollow crank bait type lures (90 mm TL x 30 mm H X 90 mm circumference at widest point were constructed from components ordered from a commercial lure components company (Luremaking.com). These materials were used because plastic crank baits can float and the transmitter could be placed inside the lure. Radio transmitters (Model PD-2, 3.8 g radio transmitters: Holohil Systems Ltd, Carp Ontario Canada, serial number 144041 to 144090) with pulses 0.34 to 0.37 and three month battery life were used. To assist buoyancy, a small amount of foam was placed in each lure to ensure that the lure would float in the event damage broke the water tight seal. As the lures would not be used for angling they were assembled with one treble hook at the posterior and the transmitter antenna exiting the body through the anterior end where the eye for line attachment would normally be. In this way, the bait and lure would lay alongside the body of the fish with the transmitter antenna trailing. The second treble hook was not attached. As the same components were used for all lures, the impact of differences in lure mass for our study was considered negligible.

Lures were divided into treatment groups and painted with high visibility fluorescent paint for each group (Figure 4-1). The colour not only facilitated the visual identification of the various lures for treatments, but assisted with lure location on the water surface by increasing visibility from a distance.

Lure Treatment

On each sampling day, captured fish were randomly assigned to treatment groups to ensure that not all fish from the same location and day received the same treatment. Prior to tagging, each fish was held ventral side down in a foam-padded v-shaped sampling trough filled with fresh lake water, measured for total length (mm), and macroscopically evaluated for injury. Lures with unique frequency radio-telemetry transmitters were externally affixed to the mouth of treatment fish in one of three orientations: shallow hooking through lower jaw, deep hooking at the base of the tongue or hooking through both the upper and lower jaw. Hooks were held with pliers and embedded with force similar to being hooked on a line, by way of a strong unidirectional pull. Sham tags made of rubber and a wire antenna was affixed with a dorsal backpack to the base of the dorsal fish and control fish received a transmitter in the same fashion following the methods of Cooke (2003). In brief, to affix sham tags and transmitters to the base of the dorsal fins, two 22-gauge hypodermic needles mounted on 3 ml syringes were pushed through the dorsal musculature ventral and posterior to the origin of the dorsal fin. Wires attached to the transmitter (20-gauge surgical stainless steel) were threaded through the needles and pulled out on the opposite side of the fish through a small (10 mm × 5 mm × 1 mm) backing plate made from rubber gasket material (Colotelo *et al.,* 2013). The wires were twisted until the transmitter was snug to the fish and trimmed to minimize potential for snagging on lake debris and macrophytes. No anaesthetic was used during the transmitter attachment.

Dispersal

Tagged pike were released sequentially from the shoreline adjacent to the Queen's University Biological Station at points at least five metres apart. The release site was located in a shaded area adjacent to the dock complex to ensure that the fish were not released into an area of active boat traffic, but close enough that the dock could be used for initial tracking and observation. Upon release, the behaviour of each fish was observed and the time it took the fish to move out of release site (<1 metre) was recorded. The fish was then tracked using a combination of visual observation and manual radio tracking (Thompson *et al.*, 2008) to determine their location within defined areas: 1 metre, 10 metres and 100 metres from the release point. The location of the fish on the first day of tracking (day following initial release) served as the final release location to determine if the fish had moved more than 100 metres from the release point within 24 hours of release. This data was collected to evaluate the short term behaviour of the fish following the first moments after break off. Arlinghaus *et al.* (2008a) and Klefoth *et al.* (2008) noted that fish tended to limit movement after initial release from an angling event and then ranged further afield.

Tracking

Radio tracking was completed manually from a research vessel using a hand-held radio receiver (R1000 Telemetry Receiver, Communications Specialists Inc., Orange, California, USA) and a three-element Yagi antenna. . Tracking occurred between 0800

and 2000 and every attempt was made to locate one position per fish per day. When a transmitter signal was located, the tracking boat would refine the search until the fish was positioned within 5 metres of the boat. At each location the habitat type was observed and the location was recorded with a handheld Garmin eTrex GPS. Habitat type was noted as it may be an important factor for how and when fish are able to expel lures or to determine if fish select a specific location to recover. Each day the tracking boat would survey the lake starting at the last known location of each fish and continue tracking until the fish was located.

Each fish was tracked for a minimum of two weeks. Tracking was terminated once the lure was located and recovered (lost by the fish), or the fish could not be located within the study area. In cases where a lure stopped moving it was assumed that the lure had been caught in submerged debris or sank; however the position of the signal was recorded for the remainder of the two week period. Where accessible, snorkelers were deployed to observe the fish or recover the lure. A reward was offered to anglers who caught the fish and returned the lure and provided an approximate location of the catch.

Data Analysis

Dispersal

Data was organized by time to leave 1 metre, 10 metre and 100 meter zones. Google Earth™ was used to plot the position of each fish the day following release (day one of tracking) and the distance measured from the common release point. Statistical analysis was performed using a one way ANOVA with hooking location as the predicting factor and the mean time to disperse 1metre, 10 metres and 100 meters from the release

point as the dependant variable. Additionally, the probability of each treatment group having moved 100 meters from the release site was determined using a univariate survival analysis with censoring.

Tracking

Data were organized by number of days in the water for each fish (independent of date of release). The daily position of each fish was plotted on a map of Lake Opinicon using GIS (ESRI ArcGIS version 10.1) and from these plots, the movement activity was calculated including total distance from the release site to lure loss or, at the end of the two week tracking period, daily distance moved. Continuous variables (e.g. distance moved and distance to release point) were contrasted between the treatment groups and the control groups using a one way ANOVA. A one-way ANOVA was also used to contrast the mean number of days to lure loss. Homogeneity of variances for the dependent variables within factors was tested by Levene's test (variance homogeneity). In case of deviations ($P < 0.05$), continuous data were log ($X + 1$)-transformed for statistical analysis, but data are presented untransformed. All statistical analyses were performed using JMP Version 10.0 (SAS Institute, Cary, NC) and the level of significance for all tests was set to $\alpha<0.05$.

Results

Fifty-four fish (total length 416 - 690 mm; mean 519 ± 64 mm) were released with lure transmitters and back pack transmitters (controls). Water temperature ranged from 20-25 °C over the course of the field program and the predominant wind direction

was out of the north east. Two fish died immediately upon release during the dispersal component of the program. These fish were considered to have succumbed to the handling stress of transport and tagging and were not used as part of the movement study.

Dispersal

The majority of fish dispersed the initial release area (< 1 meter) in under 10 seconds regardless of treatment. Only two fish, a T4 treatment fish and a control fish, remained in the immediate release site for over a minute (90 and 360 seconds respectively). In general, after entry into the water fish moved to the cover of macrophytes and then moved in an easterly direction into the basin in front of the research station which served as the release area. All fish left the 10 metre area within an hour of release and 78% of the fish had left the 100 metre zone within 24 hours including one fish to left the study area all together. Differences in dispersal rate from the release area among the five treatment groups were not found to be significant (1metre $F = 1.0463$, $p=0.394$; 10 metres $F= 1.3103$, $p = 0.281$; 100 metres $F=0.3611$, $p=0.835$).

Only one lure was shed during the initial release period (T4 fish) which was observed floating 125 minutes after release. Two additional lures (T1 and T4 fish) were found in the release area 24 hours following release (first day of tracking). The precise time of lure loss was not known, but lure loss was considered to have occurred on day two for analytical purposes (the day following release).

After 10 hours (the time that dispersal tracking typically ended because of night fall) 17 fish (3 T1 fish, one T2 fish, one control fish; four T3 fish and four T4 fish) had

not moved beyond 100 meters from the release site. Based on the univariate survival analysis the mean time for 50 % of fish to disperse beyond 100 metres was 233 ± 33 min (T1); 206 ± 20 min (T2); 217 ± 49 min (T3); 252 ± 41 min (T4) and 158 ± 18 min (control) (Figure 4-2). After 24 hours the geographical position of each fish was pinpointed and mean distance from the release site among the groups was compared. No significant difference ($f=0.2221$ $p = 0.925$) was noted among treatment groups and controls groups (Table 4-1).

Tracking

During the study period one fish was confirmed to have died and was located by snorkelers (T3 group). Fish that were not relocated for more than three consecutive tracking days were not included in the data set. Three control fish left the study area during the program and were never relocated. Release and tracking days were staggered throughout the 26 day period, for computational purposes the data set was standardized to 14 tracking days. Over the course of the program 28 lures (70%) were recovered floating on the lake, including one returned by anglers, and an additional 10 (25%) were suspected to have been dropped by fish, but not recovered.

GIS plots confirmed two observations in the field. First, plotted tracks revealed an unusual pattern: some fish that initially moved in one direction suddenly changed direction and followed the prevailing wind, ending up on the windward side of the lake. These lures were tracked to the same location for a number of days prior to being located and recovered. Based on these observations, lures found on the windward side of the lake following the movement pattern described above were considered to have been shed

prior to the movement across the lake. Tracking plots for these fish were truncated accordingly.

The second observation related to fish that appeared to cease daily movements and remained in the same location for the remainder of the study period. In some instances, snorkelers were unable to locate the fish or the lure because of depth, turbidity or dense macrophytes. Although it is possible that these fish died, analysis of the data and literature suggested that it was more likely that the lures had been shed and lost. This decision is based upon the following rational:

- Both control and treatment fish typically appeared to move each day (e.g. > 50 m) which is consistent with other studies on pike daily movement (Arlinghaus *et al.*, 2008a; Muscatello & Janz, 2009);
- None of the control fish died over the course of the two week tracking period based on their continual movement;
- No mortalities were observed in previous studies that tracked fish with retained lures in the same lake and conditions
- One fish was captured by anglers following the loss of the lure and identified by the transmitter code on the sham; however, the lure was still transmitting from the lake indicating that it was likely entangled in macrophytes or had been damaged and sank.

In cases where a fish did not move more than 50 metres for three or more consecutive days, (whether the lure was found or not) the first 50 metre point was selected to represent the location where the fish separated from the lure. A distance of fifty metres

was selected for the following reasons. Arlinghaus *et al.* (2008a) reports that their study team could track a fish to within two meters before a fish would move as a result of their presence. Given that handheld GPS units typically have an error of ± 5 metres, the location recorded could be substantially different from the observation point. Finally, the effect of lake depth and the direct distance of the boat from the fish add an additional 5 meters of variability in boat position to the fish. In total, a minimum movement factor of 2*5*5=50 meters is appropriate. By requiring more than three or more consecutive days of less than 50 metre movement, random events of reduced movement could be accounted for.

Days to lure loss were compared among the four treatment groups (controls not included). T1 fish retained the lure significantly longer than T4 fish ($f=3.6793$, $p = 0.0208$) (Figure 4-3). No difference was identified between T1, T2 and T3 fish nor T2, T3 and T4 fish. Control fish were not included in the comparison. For analysis of mean daily distance moved, data were log transformed. A significant difference between the mean daily movement of the control group and the treatment groups (Figure 4-4) was identified ($F=4.8746$, $p = 0.025$).

While not statistically significant, a visual analysis of the data against the mean of the treatment groups highlights that the mean daily movement of T4 and the control group was approximately 100 metres greater than the T2 group which had the lowest mean daily movement among the groups.

Discussion

The results of our study provide additional resolution on the fate of fish in the wild that break off during an angling event and swim away with a retained lure. Following break off, the short term impacts to the fish are similar to those encountered during a C&R event in that the fish is exposed to a period of brief exhaustive exercise and associated physiological changes (e.g. elevated glucose and lactate). The fish may experience injury from the hook, but ultimately returns to the lake to swim free. Unlike C&R, in a break off event, the fish may not experience air exposure, the exercise may be short term and there is little or no impact caused by handling the fish; however, the fish does end up with a hook embedded in its jaw or soft tissue. It has been speculated that the one that "got away" and more specifically got away with the lure will have shed the lure and recovered. Surely the fish would thrash to rid itself of the encumbrance and if mechanical agitation did not dislodge the lure, the hook would rust out in days. Little evidence has been presented to support these assertions, but none the less anglers optimistically assume that the fish would not spend the rest of its days with a lure hanging from its jaw.

Research to date on this subject has focused mainly on the consequences of deeply embedded single hooks using some variety of live or organic bait (DuBois & Kuklinski, 2004; Schill, 1996; Tsuboi *et al.*, 2006; Warner, 1979). Conclusions from these studies focused on the impacts of cutting the line as opposed to retrieving the hook from the fish. Arlinghaus et al. (2008a) investigated the consequences of lure retention in a recreational angling context using northern pike as a model. They evaluated the

behaviour and survival of fish released with retained lures in the short term after release and over a longer (weeks) post release period using radio transmitters. No mortality was observed after monitoring fish for a 3 week period and although there were some behavioural alterations in fish with retained lures relative to controls, these disturbances were short lived (Arlinghaus *et al.*, 2008a). Henry *et al.* (2009) also looked at the impacts of break off in nesting male smallmouth bass. They evaluated physiological changes and short term behaviour to assess the impacts on parental care and fitness of the fish. Different lure types caused short term impacts expressed as reduced nest care, less vigorous nest guarding and increased nest abandonment. This study was able to collect data based upon direct observation of the fish while the lure was still retained in the mouth and noted that the fish did engage in activities that could be interpreted as trying to actively dislodge the lure. In addition they noted variation in response related to the lure type. Fish appeared to more actively try to rid themselves of floating, buoyant lures and were less impacted by the presence of neutrally buoyant lures such as soft plastic worms and jigs (Henry *et al.*, 2009).

Fish in our study left the initial dispersal area quickly and most had moved beyond 10 metres within the first 10 minutes of release. This finding differs from observations by Arlinghaus *et al.* (2008a) who reported that pike showed very little initial movement and that fish with a lure moved less actively than fish without a lure. In addition, we saw no evidence to suggest that the lure affected the treatment fish to the extent observed by Arlinghaus *et al.* (2008a). However the lures that we used were of a floating type and, based on the observations of Henry *et al.* (2009), may have caused the pike to more actively work to dislodge the lure. This active response to our lures may

explain the differences between our observations and those of Arlinghaus *et al.* (2008a); perhaps fish increased their overall movement rates to those of control fish in an attempt to rid themselves of the lure. Arlinghaus *et al.* (2008a) used neutrally buoyant lures which may not have elicited an active response from the pike. Certainly, we have observed that confined pike with retained spoons (negatively buoyant lures) did not appear to actively interact with the lure (see Chapter 3 of this thesis).

Perhaps one of the most interesting outcomes of our study is related to the shedding rate of lures from free swimming fish post break off, the first time that this type of data has been collected in this manner. The majority of lures that we released on fish were shed within the observation period of each fish. As expected, barbless lures were shed in the least amount of time (3 ± 0.4 days). The barbless lure treatment (T4 group) consisted of one of the treble hooks embedded through the lower jaw. Interestingly, the T1 lure treatment, (the same treatment as the T4 group but using a barbed hook) took the longest time to shed (6 ± 1.1 days). One T1 fish retained its lure throughout the entire study period (14 days). This fish made movements in the range of 100 to 500 metres for five days post release and took up residence in an area west of the research station. Subsequent daily movements did not pass the 50 metre reduction test as over the remaining period the fish was located at points ranging from 20 to 75 metres from the release site. Even removing this fish from the data set or truncating it does not alter the mean days to shedding for the T1 group.

Why does the addition of a barb on the hook cause such a marked difference in shedding rate? In our study, the answer may relate to the effect of the lure body on the

hook. Barbless hooks affixed to a floating lure in the lower jaw may be dislodged more easily when facilitated by the resistance created by swimming movements of the fish and subsequent drag and pressure on the lure body. A barbed hook may have sufficient hold to limit expedited passive shedding and overcome any additional resistance created by the lure body. Stein *et al.* (2012) found no significant difference in hook retention rates for bone fish (*Albual vulpes*) hooked in the jaw with either barbed or barbless hooks without an associated lure. The time required for bonefish to shed either type of hook was similar to the time it took pike in our study to lose the barbed hook (T1 group). Perhaps, the reason that barbless hooks were shed at a faster rate in our study was the addition of the floating lure body which facilitated its removal. Alos *et al.* (2008) looked at the effect of using barbless hooks in a marine recreational fishery on catch per unit effort (CPUE). Barbless hooks resulted in less injury and because they were easier to remove resulted in lower CPUE. In our study, the combined effect of ease of removal of barbless hooks and resistance created by the floating lure body likely explains the faster shedding rates of this group.

Most of the work on barbed versus barbless hooks has focused on delayed mortality of fish that have been caught and then released. Burkholder (1992) reported mortality rates between 0 and 4.8% for pike caught with a variety of treble hooks and barbless lures. Studies evaluating the effect of bait type and size on injury to pike reported a 2.4% mortality rate following release (Arlinghaus *et al.*, 2008b). These rates are not that divergent from studies that reported delayed mortality rates for other fish (DuBois & Dubielzig, 2004; Reeves & Bruesewitz, 2007). Higher C&R mortality appears to be more related to hooking location (i.e. deep versus shallow hooking) or

angler behaviour (e.g. time it takes to remove the hook (Arlinghaus *et al.*, 2008b)) rather than from impacts of specific hook types. While it appears that mortality is not significantly attributed to hook type (barbed versus barbless) our work suggests that for fish that breaks off, a barbless hook does reduce the time that it retains the lure.

In our study, hooking location did not significantly impact the rate at which pike shed the lures; fish with a single hook in the lower jaw were able to lose their lures as quickly as fish that were hooked deeply and those with a hook through both upper and lower jaws. Although intuitively it seems that a single hook through the lower jaw would be easier to shed than lures embedded in other hooking locations, perhaps the rate at which the lures are shed are directly related to the amount of effort the fish allocates to dislodge the lure. For example, a single hook may be unobtrusive enough that the fish may not work as actively to remove it as compared to other treatments (e.g. deep hooking). In our tank behaviour study (Chapter 3), we observed increased opercular pumping in fish that were hooked deeply likely related to obstruction of the airway. Because of the impact on respiration, these fish may have worked more actively to expel the lure. Studies in other species have also shown that fish that are deep hooked are able to expel the hook in a matter of days (Fobert *et al.*, 2009) although expulsion rates may vary depending on hook size and style (Robert *et al.*, 2012). The effect of being hooked through the upper and lower jaw may have limited feeding or hampered the fish's ability to ambush prey again stimulating greater effort to dislodge the lure. In our study, the addition of the floating crank bait body to the hook may have facilitated a fish's ability to expel the deep hook.

Mean daily movement rates were not significantly different among treatment groups, but were significantly different between the treatment groups and the control groups with the control groups moving more each day. Our results differ from those observed by Arlinghaus *et al.* (2008a) where they noted that fish with a lure, after reduced movement in the first 24 hours post release, moved greater distances each day than the control fish. They attributed the control fish behaviour to faster recovery and resumption of normal activity (holding in marcrophytes/ambush predation) than the fish coping with the presence of a lure. The differences in movement patterns observed between the two studies may be related to the length of time the fish were tracked (our study tracked fish for a shorter length of time). It is also possible that results from the previous study would have been different if they were able to determine if or when treatment fish lost the lure.

The key outcome of our work on shedding rates of retained lures following a simulated breakoff event is data that quantifies retention times for lures in wild fish. Indeed, our study is the first to report this information for this species. Our results show that the behaviour of pike is impacted by lure retention expressed as decreased movement following release with a lure indicating some sublethal effects of lure retention. Surprisingly, hooking location of the retained lure did not impact this behavioural response nor did it impact the rate at which fish were able to expel the lure. However, barbless hooks were able to be shed at a faster rate than barbed hooks.

Caution must be used in the interpretation of our results given that not all lures were recovered from the lake and mortality cannot be definitely ruled out. One way to

address this limitation in future studies would be to simultaneously track the fish as well as the lure. This technique would help define more quantitatively the shedding point of the lure. Finally, tracking the fish for a longer period of time following shedding of the lure would help to quantify delayed mortality as a result of sub lethal effects. Recapture of the fish such as was done by Klefoth *et al*. (2011) would provide follow up physiological data to help further understand these sublethal impacts *in situ*.

Tables

Table 4-1 Results of a oneway ANOVA comparing mean distance traveled by fish with a retained lure and control group 24 hours post release from the common release site.

Treatment Group	N	Mean (m)	SD	F Ratio	p value
T1	11	217	175	0.2221	0.925
T2	9	213	109		
T3	13	269	246		
T4	8	223	160		
Control	9	210	120		

Figures

Figure 4-1: Lure treatments included one treble hook at the posterior end of the lure assembly. The transmitter was embedded inside the lure body with the antenna extending out the anterior of the lure: Each colour was assigned to a specific treatment group for ease of recognition once assigned to a fish as well as for locating at a distance on the surface of the lake. Each lure was labelled with transmitter information, research affiliation and contact number. A nominal reward was provided to individuals who returned found lures.

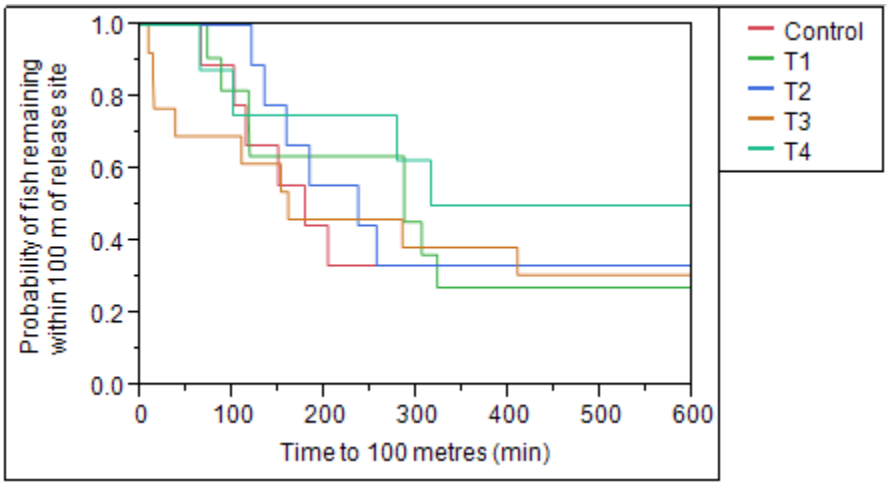


Figure 4-2: Probability of being within 100m of release site for fish with lure in lower jaw (T1 N = 11), deep hooked (T2 N = 9), hooked through the upper and lower jaw (T3 N = 12), lower jaw/barbless hook (T4 N= 8) and control, no hook (N = 9). There is a higher probability that fish in the control group leave the release area in the least amount of time (<250 min), whereas fish in the T3 group have a higher probability of prolonged residency.

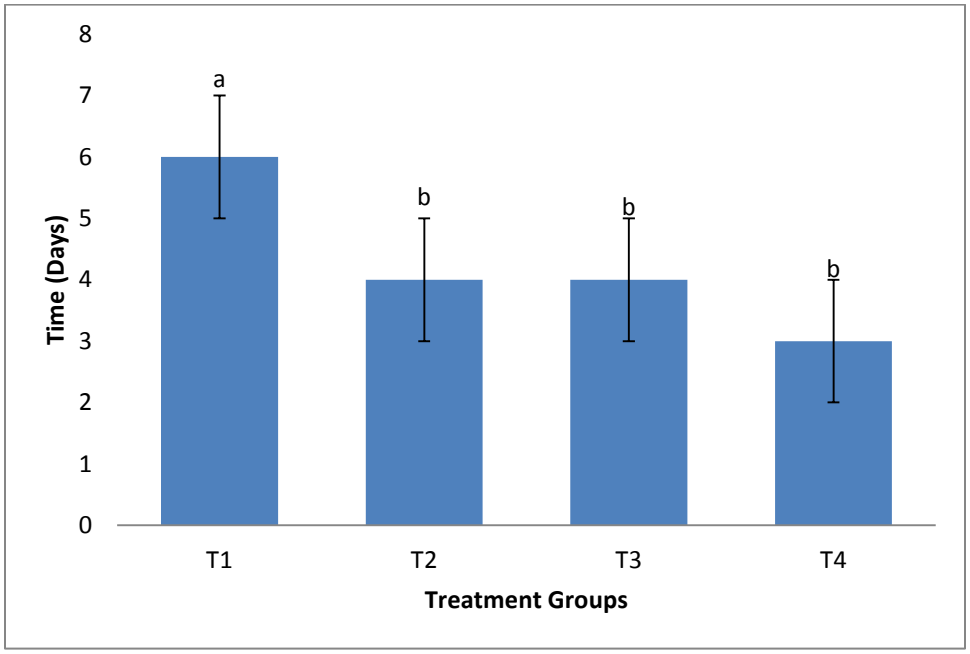


Figure 4-3: Results of Dunnetts test for significance among groups for days to lure loss for fish with lure in lower jaw (T1 N = 11), deep hooked (T2 N = 9), hooked through the upper and lower jaw (T3 N = 12), and lower jaw/barbless hook (T4 N= 9). Results show a significant difference between T1 and T4 treatment groups when T4 is used as the control. Levels with dissimilar letter are significantly different.

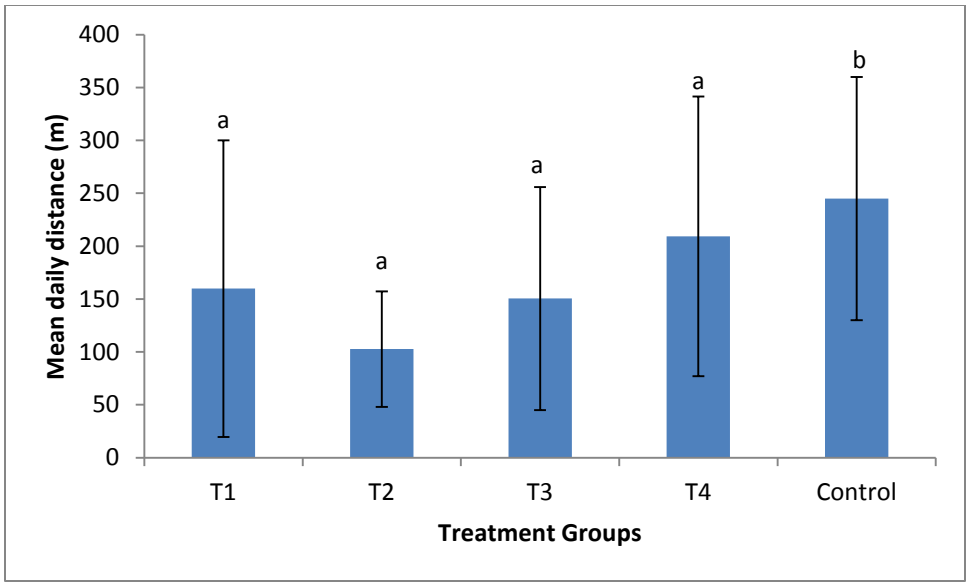


Figure 4.4 Variation in mean daily distance moved (Log transformed) among treatment groups for fish with lure lower jaw (T1 N = 11), deep hooked (T2 N = 9), hooked through the upper and lower jaw (T3 N = 11), lower jaw/barbless hook (T4 N= 9) and Control (N = 8). Results show a significant difference in mean distance per day between the control group and the treatment groups. There is no difference between the treatment groups. Levels with dissimilar letter are significantly different.

Chapter 5 General Discussion

In the wide ranging field of fisheries science the study of recreational angling represents a small portion of the body of work. The evaluation of impacts as a result of lure break off on free swimming fish is a much smaller portion still. However, with an estimated 36.4 million licensed recreational anglers in North America alone (DFO, 2012; USFWS, 2012) fishing each season, the number of fish swimming with a retained lure is substantial. While a boon for the tackle industry, the implications to the fish are not fully understood. Although most anglers optimistically assume that lures lost due to break off are eventually shed by the fish, the additional stress that the fish experiences may hamper its recovery from the angling event. These sub lethal impacts may cause long term impairment of fish fitness, growth or result in delayed mortality. High rates of lure loss occur in a number of specialized fisheries such as muskellunge fishing. The nature of musky fishing targets larger, mature fish (Landsman *et al.*, 2011b) and lost lures could have a detrimental effect on survival or reproductive potential. A higher incidence of break off could also occur in targeted bass angling where tackle selection increases the risk of break off by pike which share similar habitat as bass and are hooked incidentally. Marine species are not immune. While heavier gear is typically used, the dentition of many marine species (e.g. sharks, barracuda) and the sheer size of fish (e.g. marlin and sailfish) results in high rates of lure loss. Further, in fisheries where the desire is to land fish on the lightest tackle as possible (e.g. trout and bonefish fisheries) lure loss rates may be potentially higher than average. The extent to which lure loss occurs for individual fish species is largely anecdotal as data is limited and research to date has focused primarily on lead impacts to waterfowl (Radomski *et al.*, 2006). Accounting for lure loss

and the impact on fish should be an important consideration for estimating mortality, determining harvest limits, managing fishing gear allowances (barbless only) and informing angling practices (e.g. that using barbless gear is important for hook removal and shedding time) . As well, the welfare of fish should be considered and recommendations made for improving angling practices that minimize break off and increase the likelihood that the fish can successfully expel a retained lure in as short a time as possible.

In this thesis I used both physiological and behavioural techniques to study the effects of lure retention on free swimming fish in an effort to provide information about this little considered impact of angling.

Findings and Implications

In Chapter 2, I determined that in a laboratory setting, the ability of pike to recover from exhaustive exercise (i.e. a simulated C&R event) was not affected by either large or small retained lures or the hooking location of a retained lure. In addition, the presence of a lure in the mouth did not impair respiration or affect metabolic rate when the pike was at rest. This study also provided baseline physiological data about pike physiology in response to stress, information that is currently lacking in the literature. Most interestingly, my results have highlighted some experimental variables that must be considered when studying pike in a laboratory setting. Pike in this study were found to have very high cortisol levels and correspondingly high glucose levels compared to wild controls even after a 24 hour "rest" period to acclimatize to the environment. Although fish that were exercised did recover from metabolic exhaustion, the high levels of these

secondary stress indicators (i.e. cortisol and glucose) persisted throughout the study period. Even pike that were not exercised and simply rested in the holding tank (24 hours) and then in an isolation chamber (an additional 24 hours) showed physiological signs of stress. The significant effect of the high levels of cortisol in these fish must be considered when interpreting our results and those of other laboratory studies in this species. Overall, our results suggest that pike are quite robust in the short term when confronted with a stress challenge and appear to recover quickly even with the additional stress of a retained lure.

In Chapter 3, I investigated the behavioural impacts of lure retention in a laboratory setting and determined that lure retention does not affect the locomotor activity level of pike within the first 24 hours after break off. My results do not agree with results of a previous field study in which the activity of pike was changed by the presence of a retained lure (Arlinghaus *et al.*, 2008a). In the laboratory setting, the overall activity level of pike was low and fish spent the majority of time in a resting state, with occasional bursts of activity and within the study period 22% were able to shed their lures. Ventilation rate declined for all groups over the 24 hour observation period indicating that the fish were recovering from the initial exercise and perhaps becoming acclimatized to the tanks. Fish that were deeply hooked recovered as well, but had significantly higher ventilation rates throughout the observation period. This observation suggests that the deeply hooked lure does impact recovery and indicates that wild free-swimming fish with retained lures have additional challenges to recovery from the angling event. Finally, behavioural observations show that pike do not spend a significant amount of time reacting to the presence of the lure, but rather spend most time

in a resting state. Brief periods of hyperactivity occur in which they may be trying to rid themselves of the lure.

In Chapter 4 using telemetry to collect data from free-swimming pike, I determined that the presence of a retained lure does impact the movement of these fish *in situ*. Specifically, fish with lures moved less per day up to the time of lure loss compared to fish without lures. I also determined that most of the pike were able to shed the lure (95%) but that fish treated with barbless hooks were able to shed the lure in the shortest duration. Hooking location was not a determining factor in the ability of the fish to lose the lure or in the behavioural impact of the lure.

Summary and Future Research Directions

This research has demonstrated that retained lures do have an impact on northern pike. In the laboratory setting in the short-term, lure retention does not appear to have significant physiological or behavioural impacts although these results may be complicated by the effects of confinement in this species. In the field, pike released with a lure do show a period of reduced activity, eventually resuming normal movements within the lake. Pike appear to be able to rid themselves of retained lures following break off and there is a difference in retention time based on hook type but not hooking location. Overall my results coupled with the few other studies evaluating post break off impacts on fish have highlighted that short term mortality is low (at least for pike), fish seem to resume normal activities shortly after recovery and eventually lose the lure. In my view, the challenges that I confronted in this study present excellent opportunities for

future work to further refine our understanding of the sub lethal effects and fate of fish swimming with retained lures and fishing gear.

Holding wild pike in captivity

Pike are an important model research species as a result of their global abundance in north temperate regions, role as predator and popularity as a sport fish. Pike also serve as a model for muskellunge, a highly prized target of a specialized angling community, and can assist in our understanding the ecology and physiology of that species. Refinement in the study design for this species will assist all future research. Our study highlighted the sensitivity of adult wild pike to confinement. The use of wild fish is integral to understanding the nuances of stress and sub lethal impacts of angling which cannot be achieved with hatchery reared fish. Future research should focus on root causes of the confinement stress in pike and methods to reduce it. Further, techniques to understand the masking effect of an elevated stress response in pike (and other species) should be developed to identify the impacts of multiple stressors.

Behaviour observations

Recent work by Stalhmmar *et al.* (2012) has shown that work with adult pike is possible in tanks and natural behaviour (e.g. feeding) can be achieved. This work can be expanded to understand how retained lures impact specific aspects of pike behaviour such as burst swimming performance during ambush predation and pray capture. Indeed, physiological parameters could be coupled with these experiments to understand how

stress may impact these behaviours or to add to the understanding how foraging success affects growth and fitness.

In Situ Field Studies

Observing fish in their natural environment is unquestionably the best opportunity to understand how stressors impact fishes behaviour and survival. The research presented here could be further developed by testing the shedding rates and behavioural impacts of different lure types and hooking locations. One key question that remains to be explored relates to the fate of the fish after it sheds the lure; part of this answer may be accomplished by tracking both the retained lure and the fish simultaneously. This technique would more definitively address the question of mortality, both immediate and delayed, exact time of shedding and post shedding behavioural changes.

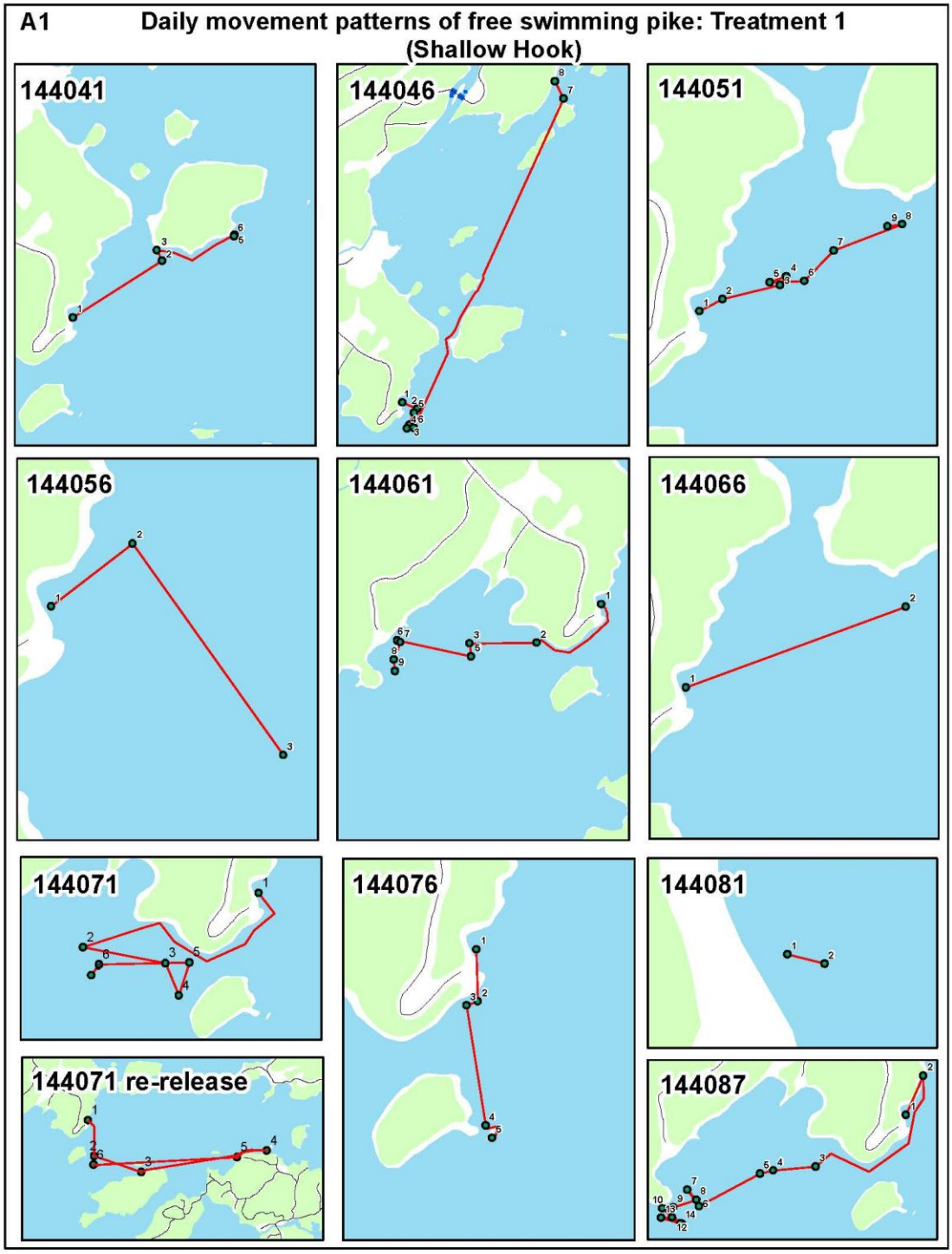
Overall Conclusions

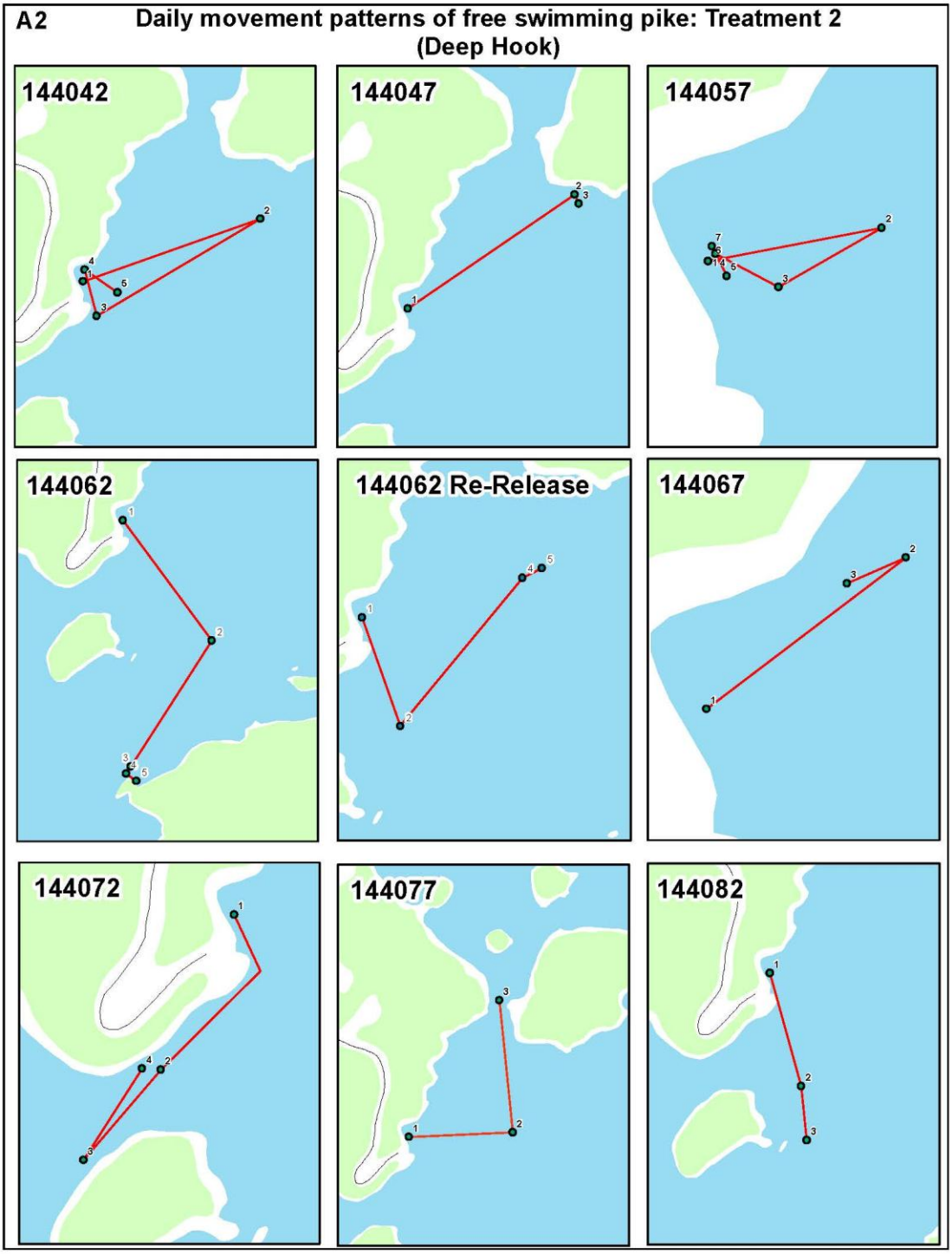
1. Retained lures do not appear to impact resting metabolic rate or blood physiology of pike in a laboratory setting. Pike in confinement, while able to recover from exhaustive exercise, experience prolonged elevated cortisol levels which could mask the effects of the retained lure.
2. Behavioural observations of pike indicate that lure retention has no impact on locomotor activity levels. Respiration rates (i.e. gill ventilation) were elevated in fish that were deep hooked suggesting that the presence of the lure in certain obstructive hooking locations challenges the fish and slows recovery from exercise.

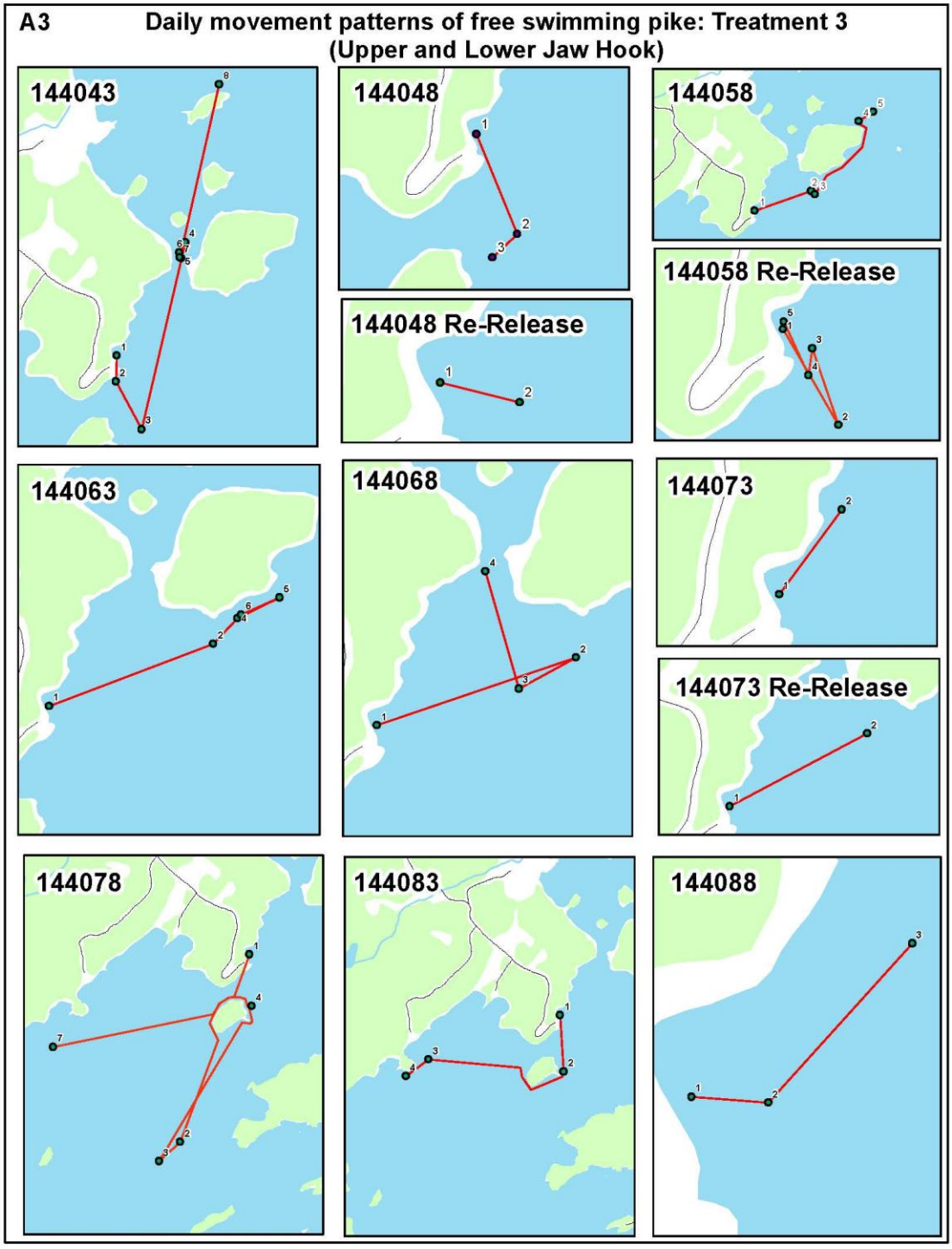
3. This study was the first to attempt to quantify shedding rates of retained lures in free swimming fish. The results show that barbless hooks are lost by pike faster than barbed hooks. Hooking location did not have a bearing on shedding rate and pike were able to rid themselves of shallow, deep and upper and lower jaw treatment in a similar number of days.
4. The presence of the retained lure reduces the activity of pike released into the lake suggesting that the lure does has an effect on the fish movement that could limit life history activities such as the ability to forage.

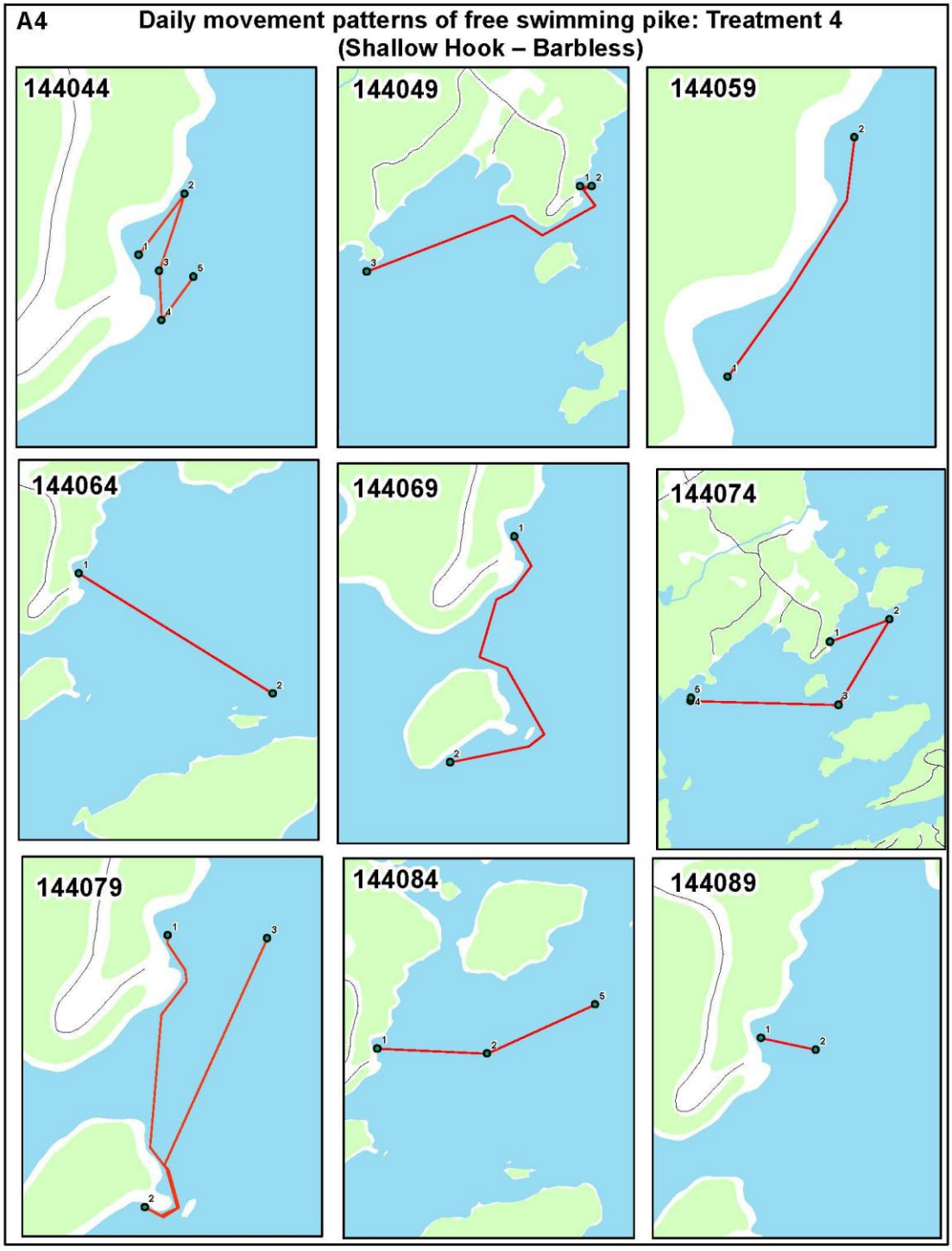
Appendices

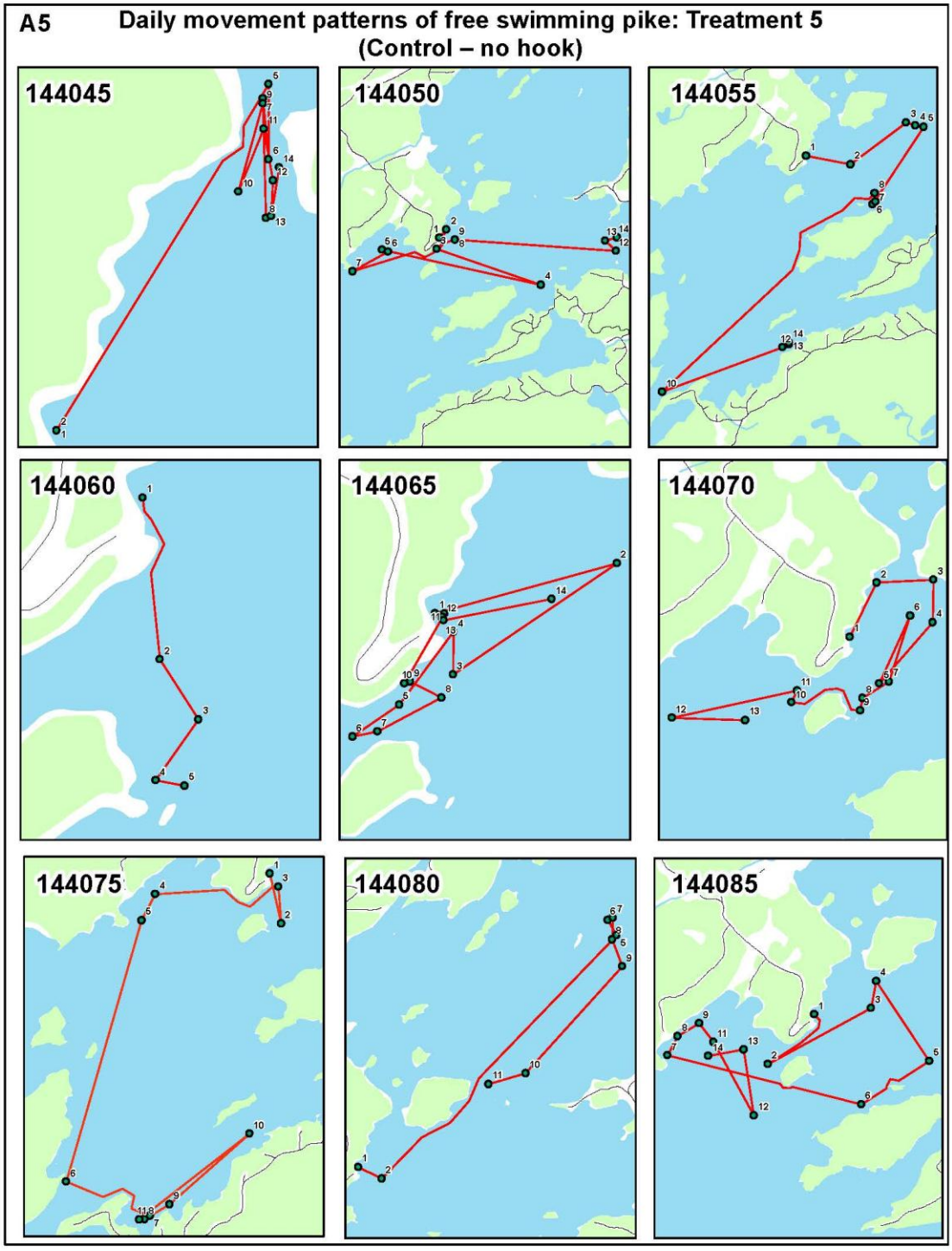
Summary Figures of Pike Movements: Treatment and Control Groups released into Lake Opinicon June/July 2009.


A1
Daily movement patterns of free swimming pike: Treatment 1
(Shallow Hook)
144041
144046
144051
144056
144061
144066
144071
144076
144081
144071 re-release
144087


A2
Daily movement patterns of free swimming pike: Treatment 2
(Deep Hook)
144042
144047
144057
144062
144062 Re-Release
144067
144072
144077
144082


A3
Daily movement patterns of free swimming pike: Treatment 3
(Upper and Lower Jaw Hook)
144043
144048
144058
144048 Re-Release
144058 Re-Release
144063
144068
144073
144073 Re-Release
144078
144083
144088


A4
Daily movement patterns of free swimming pike: Treatment 4
(Shallow Hook – Barbless)
144044
144049
144059
144064
144069
144074
144079
144084
144089


A5
Daily movement patterns of free swimming pike: Treatment 5
(Control – no hook)
144045
144050
144055
144060
144065
144070
144075
144080
144085

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