

**Sub-lethal consequences of net entrapment on freshwater turtles encountered as
bycatch in commercial fisheries**

By

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B.Sc., Lakehead University, 2011

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Master of Science
in
Biology

Carleton University
Ottawa, Ontario

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Dedication

For my family – Mom, Dad and Rachel – thanks for providing me with endless care packages and more importantly encouragement and support along the way.

Abstract

Freshwater bycatch is poorly documented despite the potential impact to aquatic biodiversity in a time of many other threats. As such, it is increasingly important to quantify individual consequences associated with bycatch, especially in long-lived species, where increases in adult mortality are especially likely to cause population declines. Here, I investigated the sub-lethal effects of fisheries interactions on freshwater turtles in a small-scale commercial fyke-net fishery in eastern Ontario. I experimentally exposed three frequently captured freshwater turtle species to entrapment stressors and revealed that there was evidence of physiological disturbances and behavioural impairments. Subsequently, I used fine-scale accelerometry to assess the effects of entrapment on locomotory behaviour and revealed that simulated entrapment resulted in locomotory impairment in two species over 6 hours after release. Collectively, this work will provide better insight into the sub-lethal effects associated with freshwater turtle bycatch in entrapment gear.

Acknowledgments

This thesis could not have been completed without the help and encouragement from many people. First and foremost, greatest thanks to my supervisors, Steve and Gabe, for providing me with constant support, instantaneous feedback and many opportunities throughout the past 2 years. I would also like to thank various members of the Cooke and Blouin-Demers lab. I am appreciative to all of you and thankful to call many of you good friends. My time in Ottawa, both socially and academically, would have not been the same without you. Also, my adoptive Ottawa family, the Massouds, provided me with countless family dinners, furniture and always made me feel welcome in their home – I cannot thank you enough.

For everyone that helped me in the field – Sarah Larocque, Keith Stamplecoskie and Andrew Weatherhead – thank you! Data collection would not have been possible without you! And to Nick – I couldn't have asked for a better Masters co-partner. Thanks for being a great colleague and more importantly friend over the last two years!

I would like to acknowledge the staff at Queen's University Biological Station for the use of their facilities. In addition, we would like to thank the Ontario Ministry of Natural Resources (OMNR) and Canadian Wildlife Federation (CWF) for funding. All work was conducted with Scientific Collection Permits obtained from the OMNR and Animal Care from the Canadian Council of Animal Care administered by Carleton University and Queen's University.

Co-Authorship

Chapter 2: Physiological disturbances and behavioural impairment associated with the incidental capture of freshwater turtles in a commercial fyke-net fishery. L.J.

Stoot, N.A. Cairns, G. Blouin-Demers and S.J. Cooke

While this study is my own, the research was undertaken as part of a collaborative effort and each co-author played a valuable role in its completion. The project was conceived by Stoot, Cairns, Blouin-Demers and Cooke. Fieldwork was completed by Stoot and Cairns. All computer and data analysis was conducted by Stoot and data was interpreted by all authors. All writing was conducted by Stoot. All co-authors provided comments and feedback on the manuscript. This manuscript has been accepted by *Endangered Species Research*.

Chapter 3: Biologgers reveal post-release behavioural impairments of freshwater turtles following fisheries interactions. L.J. Stoot, N.A. Cairns, J.D. Thiem, J.W.

Brownscombe, A.J. Danylchuk, G. Blouin-Demers and S.J. Cooke

While this study is my own, the research was undertaken as part of a collaborative effort and each co-author played a valuable role in its completion. The project was conceived by Stoot, Cairns, Blouin-Demers and Cooke. Fieldwork was completed by Stoot and Cairns, while accelerometers were provided by Danylchuk and Cooke. All computer and data analysis was conducted by Stoot. Data were interpreted by Stoot, Cairns, Brownscombe, and Thiem. All writing was conducted by Stoot. All co-authors

provided comments and feedback on the manuscript. This manuscript is in preparation for *Biological Conservation*.

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Chapter 1: General Introduction

Commercial fisheries in marine and freshwater ecosystems exploit mostly fish and crustaceans for human benefit. Commercial fisheries collectively account for approximately 92 million metric tonnes of global landings (FAO 2009). Although the majority of catches consist of targeted species, an estimated 7.3 metric tonnes caught in marine fisheries (or 8 %) are non-targeted organisms (Kelleher 2005). The capture of non-targeted species is known as bycatch and can include non-targeted fish, birds, mammals, and reptiles (Crowder & Murawski 1998; Hall *et al.* 2000). Bycatch occurs in long-line, trawl, and gill net fisheries. Recently, greater efforts have been deployed to quantify and prevent bycatch, although most efforts have been directed towards marine ecosystems with relatively little research devoted to freshwater (Allen *et al.* 2005; Raby *et al.* 2011). Lack of research in freshwater fisheries is concerning due to high extinction rates suffered by freshwater species along with the abundance of at risk bycatch species in freshwater (Riccardi & Rasmussen 1999; Dudgeon *et al.* 2006).

Bycatch can result in a variety of consequences and effects. Immediate mortality, such as in-net or on-hook mortality, has been reported in several frequently caught bycatch species including a variety of fish as well as marine mammals, sea birds, sharks, and marine turtles (e.g., Julian & Beeson 1998; Lewison *et al.* 2004; Zeeberg *et al.* 2006; Moore *et al.* 2008; Alfaro-Shigueto *et al.* 2011; Finkbeiner *et al.* 2011). Captured individuals are exposed to a wide range of injury and stressors from interacting with fishing gear (Davis 2002; Skomal 2007). Injuries may include abrasion and tissue damage from hooks and netting material (e.g., Aguilar *et al.* 1995; Lewison *et al.* 2004). Stressors can include confinement, exhaustion (from struggling), and for air breathing

species, inability to access oxygen (e.g., Hoopes *et al.* 2000; Snoddy *et al.* 2008). These stressors can cause immediate effects which can impair behaviour such as foraging ability or predator avoidance.

Although immediate mortality is a major concern, the presence of sub-lethal effects and post-release mortality can also have important population consequences. Furthermore, even if animals survive bycatch, their long-term condition (e.g., growth), health, and fitness may be altered. For that reason, the Species at Risk Act in Canada (2002, c.29, s.32) defines that it is illegal to kill, harm, harass, capture, or take any endangered or threatened species. Clearly, harm and harass fall within the category of sub-lethal effects emphasizing the need to not just document mortality, but to also understand what activities contribute to harm or harassment. Physiological and behavioural endpoints provide objective and ecologically relevant measures of harm and harassment.

Bycatch of marine mega-fauna, such as turtles, is especially concerning as they are long-lived and have delayed sexual maturity. Consequently, even slight increases in adult mortality, from sources such as bycatch, have the potential to cause population declines (Congdon *et al.* 1993; 1994). Freshwater turtles have similar life history traits, making them equally susceptible to population declines even with low levels of adult mortality. In eastern Ontario, a small-scale fyke net fishery exists on inland freshwater lakes and rivers. This fishery is a typical small-scale fishery employing approximately 50 fishers that use relatively small fishing vessels and collect fish mainly for local consumption. Target species include sunfish (*Lepomis* spp.) primarily although bullhead

(*Ameiurus* spp.), perch (*Perca flavescens*), black crappie (*Pomoxis nigromaculatus*), and rock bass (*Ambloplites rupestris*) are also harvested (Burns 2007; Larocque *et al.* 2012a). Fyke-nets are typically set in tandem with a lead line connecting each mouth (Fig. 1-1). Although fishing regulations state that the season is open from ice off in the early spring until ice-on in the fall, most fishers prefer to work in early spring or fall. Quantity of nets varies depending on area and license issued to each fisher, but some individuals are allowed to deploy up to 80 fyke-nets at once (Burns 2007). Habitats of freshwater turtles and commercial fish often overlap, thus increasing the possibility of turtle entrapment. Previous studies have confirmed that entrapment in fishing nets as a threat to freshwater turtles (Barko *et al.* 2004; Carrière *et al.* 2007; McClellan & Read 2009; Echwikhi *et al.*, 2011; Larocque *et al.* 2012ab). Depending on the season, typical setting of commercial fyke-nets does not require regular monitoring or provision of air spaces (Burns 2007), and as a result turtles that are caught often drown (Larocque *et al.* 2012b). Even low levels of anthropogenic mortality are problematic for freshwater turtles given their life-history characteristics and the fact that many populations are in decline from a variety of threats including boat strikes, vehicle collisions, and habitat loss/alteration (Gibbons *et al.* 2000). Currently, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) lists seven of eight turtle species present in Ontario as species at risk. Of these at-risk species, three are most likely to interact with commercial fishing gear: the eastern musk turtle (*Sternotherus odoratus*) designated as threatened, and the northern map turtle (*Graptemys geographica*) and common snapping turtle (*Chelydra serpentina*) both recognized as special concern. Painted turtles (*Chrysemys picta*) are also likely to interact with commercial fishing gear.

Research Objectives

The overall objective of my thesis was to provide insight into the sub-lethal effects of fisheries interactions on freshwater turtles. The eastern Ontario commercial fyke-net fishery was used as a study system given concern among natural resource managers regarding the effects of bycatch on turtle populations. In Chapter 2, I investigated the acute physiological consequences associated with incidental capture of freshwater turtles in fishing nets using an experimental approach. In addition, a secondary objective of Chapter 2 was to test for differences in responses to entrapment between sexes using painted turtles (*Chrysemys picta*) as a model. In Chapter 3, I investigated the sub-lethal effects associated with turtle bycatch using novel tri-axial accelerometers to monitor movement behaviour following release. Understanding both the immediate and post-release sub-lethal consequences related to turtle bycatch is important in determining potential post-release mortality or otherwise contribute to changes in turtle condition, health or fitness that may have significant population consequences.

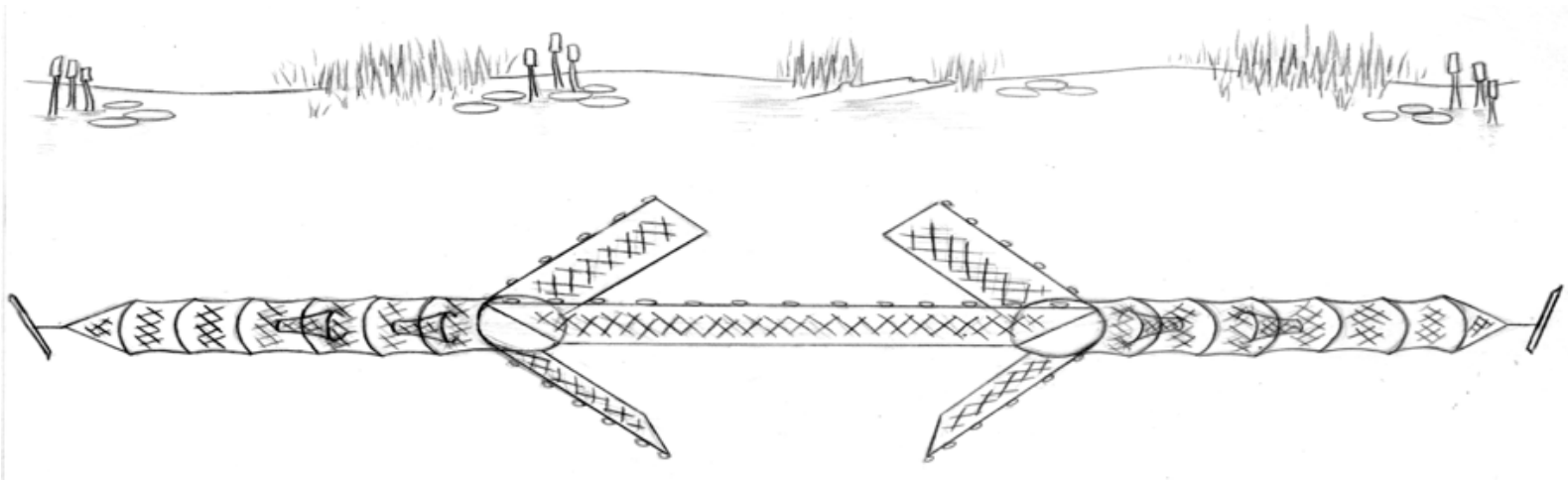
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Figure 1-1. Typical setting of a commercial fyke-net in eastern Ontario. Drawing reproduced from Larocque et al. 2012b.

Chapter 2: Physiological disturbances and behavioural impairment associated with incidental capture of freshwater turtles in a commercial fyke-net fishery

Abstract

Turtles are caught as bycatch in commercial fisheries in both inland and marine waters. Turtle mortality associated with bycatch is concerning as life-history characteristics of turtles, including high juvenile mortality and delayed sexual maturity, make them particularly susceptible to population declines following small increases in adult mortality. In eastern Ontario, freshwater turtles are encountered as bycatch in an inland commercial fyke-net fishery. Although some temperate turtle species can tolerate prolonged submergence, their ability to withstand submergence decreases as water temperatures increase such that turtles may experience severe physiological disturbances and mortality following prolonged forced submergence. The purpose of my study was to evaluate the sublethal physiological consequences and related behavioural impairments associated with fyke-net capture for three species of freshwater turtles (eastern musk turtle; *Sternotherus odoratus*, northern map turtle; *Graptemys geographica*, and painted turtle; *Chrysemys picta*). Individuals that were entrapped for 3 hours at elevated water temperatures (23-29°C) displayed considerably higher blood lactate and lower blood pH compared to free-living individuals. This trend was consistent across species and sexes. Both map and painted turtles displayed low responsiveness to behavioural assessments following entrapment, but musk turtles did not exhibit behavioural impairment from entrapment, despite having the largest increase in blood lactate. Sub-lethal responses can be used to identify potential harm or fitness impacts even in the absence of immediate mortality, which is important for protected species.

Introduction

Bycatch, the capture of non-targeted species, frequently occurs in commercial fisheries (Crowder & Murawski 1998; Hall *et al.* 2000; Hall & Mainprize 2005). Bycatch of marine species, especially turtles, has attracted considerable scientific and media attention (Lewison *et al.* 2004; Wallace *et al.* 2010). A variety of marine turtle bycatch reduction strategies have been developed and tested (UN FAO 2009) such as the turtle excluder device, which has prevented the accidental capture of turtles in the shrimp trawl fishery (Seidel & McVea 1982). Despite being less well documented (Raby *et al.* 2011), bycatch of freshwater turtles also occurs in a variety of inland fisheries (Barko *et al.* 2004; Carrière *et al.* 2009; McClellan *et al.* 2009; Larocque *et al.* 2012a). Turtles typically exhibit high juvenile mortality and delayed sexual maturity (Congdon *et al.* 1993). Thus slight increases in adult mortality, from sources such as bycatch, have the potential to cause population declines (Congdon *et al.* 1993; Gibbons *et al.* 2000). This is particularly concerning given the at-risk status of many marine and freshwater turtles (Burke *et al.* 2000).

Interactions with fishing gear can cause stress and injuries that alter the health, condition, behaviour, and potentially the survival of animals, something well studied in fish (e.g., Chopin & Arimoto 1995; Davis 2002). These interactions have been comparatively poorly studied (aside from immediate mortality) in other taxa such as mammals, birds, and reptiles, including marine turtles. Air breathing organisms are at particular risk of bycatch-related stress and mortality as a result of entrapment and entanglement in fishing gear that impedes their ability to reach the water surface and respire. The capacity to withstand the effects of submergence will dictate the likelihood

of mortality for a given species following capture in commercial fishing nets. Many freshwater turtle species can spend extended periods submerged. Some species, such as painted turtles (*Chrysemys picta*), are able to remain submerged with little to no oxygen for extensive periods during hibernation (Belkin 1963; Gatten 1981; Milton & Prentice 2007). In addition to reduced metabolic rate during submergence, painted turtles rely on anaerobic metabolism to further reduce oxygen requirements (Jackson 2000a). The mechanisms and ability to withstand low oxygen for prolonged periods is species-specific (Reese *et al.* 2001). For instance, some species have a better ability to sequester CO₂: painted turtles appear to be one of the most proficient species that is able to tolerate extensive periods in true anoxic conditions by using carbonate from their shells to buffer blood acidosis (Ultsch 1989; Ultsch & Jackson 1995; Jackson 2000a). Another mechanism frequently used by some turtles to acquire oxygen is the use of extrapulmonary gas exchange (e.g., eastern musk turtle; Ultsch *et al.* 1984; Ultsch & Cochran 1994). Individuals use their highly vascularized skin lining the cloaca and buccopharynx cavity to obtain oxygen from water (Feder & Burggren 1985; Ultsch 1988; Stone *et al.* 1992). Collectively, these mechanisms all contribute to a turtle's ability to withstand submergence associated with incidental capture.

For freshwater turtles, prolonged entrapment within nets is most likely stressful, particularly when water temperature is elevated (Fratto *et al.* 2008; Larocque 2012b). Elevated water temperatures and ensuing increased metabolic rates reduce the length of time a turtle can cope with submergence (Musacchia 1959; Herbert & Jackson 1985; Ultsch 1989). In addition, as water temperatures increase, dissolved water oxygen levels decrease, thus limiting the available oxygen for uptake. This can result in rapid lactate

accumulation even for species capable of extrapulmonary gas exchange (Frankel *et al.* 1966; Dejours 1994). Finally, entrapment exposes turtles to a variety of additional stressors, in particular the exertion associated with trying to escape from nets and interactions with other species, both target and bycatch, in a confined area.

The objective of our study was to assess the sub-lethal consequences of fyke-net entrapment on three species of freshwater turtles. In particular, we evaluated blood lactate and pH, as well as behavioural responsiveness following experimental entrapment. We also evaluated whether male and female painted turtles differed in their physiological and behavioural responses to entrapment. We focused on freshwater turtles that are incidentally captured in a small-scale commercial fyke-net fishery in eastern Ontario (Carrière *et al.* 2009; Larocque *et al.* 2012ab). Upon capture, fishers are required to discard turtles (whether alive or dead) along with other bycatch (e.g., gamefish) as soon as possible after landing. Commercial fishing in that region occurs on inland lakes and rivers, with sunfish (*Lepomis* spp.) being the primary targeted species, in addition to bullhead (*Ameiurus* spp.), yellow perch (*Perca flavescens*), black crappie (*Pomoxis nigromaculatus*), and rock bass (*Ambloplites rupestris*; Burns 2007; Larocque *et al.* 2012a). The habitats of freshwater turtles and targeted commercial fish overlap, thus increasing the possibility of turtle entrapment (Carrière *et al.* 2009; McClellan & Read 2009; Larocque *et al.* 2012a). Presently, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) lists seven of the eight turtle species present in Ontario as species at-risk. Of these seven at-risk species, two are most likely to interact with commercial fishing gear and hence were the focus of our study: the eastern musk turtle (*Sternotherus odoratus*) and the northern map turtle (*Graptemys geographica*) are both

recognized as Special Concern. In addition, the non-threatened painted turtle (*Chrysemys picta*) was used as a model as it is the most commonly captured species in this fishery.

Materials and methods

Study area

We conducted the study on Lake Opinicon (44° 34' N, 76° 19' W) approximately 100 km southwest of Ottawa, Ontario, Canada. Experiments were conducted between 30 June and 28 July 2011 when water temperatures ranged from 23 to 29°C. Free-living control turtles were captured between 14 June and 17 September 2011 when water temperatures ranged from 19 to 28°C and between 29 May and 3 June 2012 when water temperatures ranged from 20 to 25°C. For all experiments dissolved oxygen was at or near saturation (e.g., 6-8 mg·l⁻¹).

Fyke-nets

We used fyke-nets similar to the ones used in the local commercial fishery to collect turtles following the methods outlined in Larocque *et al.* (2012a). Nets were fished in pairs and were attached by leads (see Fig. 1 in Larocque *et al.* 2012a). Each net contained seven steel hoops, which were 0.5 m apart and measured 0.9 m in diameter, and two throats were located in the second and fourth hoop. Two wings and a lead were attached vertically to the mouth of each net. Wings measured 4.6 m long by 0.9 m high while each lead measured 10.7 m long by 0.9 m high. All gear was fabricated with 2.54 cm square, 5.08 cm stretch, nylon mesh.

Experimental procedure

For this experiment, we used 73 turtles of 3 different species for both treatment and control (see Table 1). We used painted turtles to test for sex-specific differences despite an approximately equal sex-capture ratio of 1:1 to minimize any potential sub-lethal effects on females. Indeed, that was a condition of our Scientific Collection Permit and Species-at-Risk Permit from the Ontario Ministry of Natural Resources. We used two groups to determine the physiological effects and behavioural impairments associated with entrapment in fyke-nets: a treatment group in which turtles were submerged in a fyke-net for 3 hrs, and a control group of free-living turtles. This submergence time was chosen to provide sufficient time for blood physiology to respond to capture stress while avoiding immediate mortality (Larocque *et al.* 2012b). It should be noted that our treatment length of 3 hrs is not representative of typical entrapment length within the commercial fishery in Ontario, as fishers are required to check their nets every 2 to 7 days, depending on the season. Our estimates of physiological disturbance are therefore very conservative.

Control turtles were “free-living” individuals which were caught via dip-net or snorkelling and sampled immediately (within 3 minutes of capture) to obtain baseline blood physiology values and behaviour tests. Turtles were transported back to the laboratory for morphometric measurements. Turtles were held outdoors in ~700 L fibreglass tanks at ambient temperatures until they could be returned to capture location. Tanks were supplied with lake water using a flow through system and turtles were not fed, but were provided with basking platforms and exposed to ambient sunlight.

Treatment individuals were collected initially by fyke-nets equipped with floats, which were checked daily and captured turtles were transported back to the lab to obtain morphometric measurements. Turtles were held in outdoor ~700 L fibreglass tanks at ambient temperature for a minimum of 48 h before experimental trials to eliminate any potential effects of initial capture stress. After a minimum of 48 hours, individuals were subjected to simulated entrapment for 3 hours using a fyke-net that was modified to prevent escape or entry. The net was completely submerged in the lake in approximately 1.5 m of water which ranged from 23 to 29°C over the course of this experiment.

Blood physiology

All blood samples were taken within 3 minutes of removal from net or of capture. Approximately 0.5 ml of blood was taken from the caudal vasculature on the dorsal portion of the tail using a 1 ml Tuberculin slip tip with a sodium-heparinized (10 000 USP units ml⁻¹, Sandoz, Québec, Canada) coated syringe with a 25 gauge, 38 mm needle for painted and map turtles (Becton Dickinson and Company, Franklin Lakes, New Jersey). Smaller 28.5 gauge needles and syringes were used for musk turtles (Becton Dickinson and Company, Franklin Lakes, New Jersey). Blood lactate was measured immediately after collection using a Lactate ProTM meter (Arkray Inc., Japan) that was previously validated with teleost fish (Brown *et al.* 2008). Remaining blood was transported back to the field laboratory in the syringe on ice in a cooler. Blood pH was measured using a field physiology meter within 2 hours of collection with a 3-point calibrated minilab IQ128 Elite pH meter (IQ Scientific Instruments Inc., California) at ambient temperature.

Behavioural assessments

Both experimental and control groups were tested for behavioural responses. Involuntary response to a variety of stimuli such as touch, gravity, and sound can be helpful in predicting mortality. These assessments to predict mortality have been used on a variety of vertebrates (Davis 2007; 2010), including freshwater turtles (LeDain *et al.* in press). We used six behavioural tests, similar to the ones employed by LeDain *et al.* (in press) that included escape ability, righting ability (both on land and in water), response to startles (audible/pressure and visual), and tactile stimuli to the head, limbs and tail (Table 2-2). The ability of turtles to complete these tests can serve as indicators of their condition. We combined tactile stimuli into a single response, as there was no variation between the reactions to head and limb/tail stimuli. If the individual responded to the stimulus, the test was scored as present; lack of reaction indicated an absent response. Each test was scored as present (1) or absent (0) and converted into a Behaviour Impairment Index (BII) which is an overall score of impairment for each individual based on the number of tests performed where $BII = 1 - (\text{sum of individual test scores} / \text{total possible score of 6})$. The BII ranges from 0, which indicates that the individual was not impaired, to a maximum score of 1, which indicates that the turtle was completely impaired.

Statistical analyses

We compared blood lactate and pH levels of control and treatment groups between male turtles of three species. Blood lactate and pH residuals did not violate the assumptions of normality and homogeneity of variance, therefore we used a two-way

ANOVA to test the effect of species and group on blood lactate and pH. To assess whether control and experimental groups differed within a species, we used follow-up two sample t-tests that assumed unequal variance. We also evaluated physiological differences between males and females in painted turtles from control and treatment groups. Both blood lactate and pH residuals did not violate the assumptions of normality and homogeneity of variance, therefore we used a two-way ANOVA to test the effect of species and group on BII. Behavioural data were also analyzed using a two-way ANOVA since BII score residuals did not violate the assumptions of normality and homogeneity of variance. We again used follow-up two-sample t-tests that assumed unequal variance to assess whether control and experimental groups differed within each species. All statistical tests were performed using JMP (Version 9.0.1, SAS Institute). Significance was accepted at $\alpha < 0.05$.

Results

Of the 73 individuals sampled, all were able to complete the trial and no turtles died. Our test of the physiological effects of entrapment in males of three species revealed a significant interaction between species and experimental group for blood lactate (Partial $R^2 = 0.02$, $F_{(2,48)} = 14.19$, $p < 0.001$) and marginally significant for blood pH (Partial $R^2 = 0.01$, $F_{(2,48)} = 3.18$, $p = 0.051$). These interactions indicate that the effect of treatment on blood lactate and pH differs by species. Post-hoc two sample t-tests revealed that blood lactate was significantly higher in treatment than in control groups in all three species: map turtles ($t_{(17)} = 18.82$, $p < 0.001$), musk turtles ($t_{(9)} = 20.81$, $p < 0.001$), and painted turtles ($t_{(11)} = 16.39$, $p < 0.001$; Fig. 2-1). Accordingly, blood pH was also lower in treatment groups than in control groups in all three species: map turtles ($t_{(13)}$

= 9.684, $p < 0.001$), musk turtles ($t_{(16)} = 17.313$, $p < 0.001$), and painted turtles ($t_{(8)} = 10.82$, $p < 0.001$; Fig. 2-1). Significant interactions resulted from musk and painted turtles having marked increases in blood lactate and decreases in pH between control and treatment groups, while map turtles exhibited less dramatic differences (Fig. 2-1).

We investigated behavioural impairment associated with entrapment in males of three species. There was a significant interaction between species and experimental group for the behavioural scores (Partial $R^2 = 0.16$, $F_{(2,48)} = 14.07$, $p < 0.001$), indicating that the three species were not impaired to the same extent. Post-hoc two sample t-tests revealed that the BII scores were significantly different between control and treatment groups for map turtles ($t_{(9)} = 6.8206$, $p < 0.001$), and painted turtles ($t_{(12)} = 4.8179$, $p < 0.001$), but there was no significant difference in musk turtles ($t_{(9)} = 1$, $p = 0.3434$; Fig. 2-2).

When we investigated sex differences in physiological responses to entrapment in painted turtles, there were no significant sex by group interactions for blood lactate (Partial $R^2 < 0.001$, $F_{(1,31)} = 0.05$, $p = 0.83$) or blood pH (Partial $R^2 = 0.01$, $F_{(1,31)} = 3.76$, $p = 0.062$). In addition, there was no significant difference between males and females in blood lactate (Partial $R^2 < 0.001$, $F_{(1,31)} = 0.09$, $p = 0.77$) or blood pH (Partial $R^2 < 0.001$, $F_{(1,31)} = 0.07$, $p = 0.79$, Fig. 2-1). There was a significant difference between painted turtle control and submerged groups when combining both sexes in both blood lactate (Partial $R^2 = 0.94$, $F_{(1,31)} = 809.95$, $p < 0.001$) and blood pH (Partial $R^2 = 0.91$, $F_{(1,31)} = 336.37$, $p < 0.001$; Fig. 2-1).

BII scores between male and female painted turtles also lacked a sex by group interaction (Partial $R^2 < 0.001$, $F_{(1,31)} = 0.014$, $p = 0.906$). In addition, there was no

significant difference between males and females (Partial $R^2 < 0.001$, $F_{(1,31)} = 0.027$, $p = 0.871$; Fig. 2-2). There was a significant difference between painted turtle control and submerged group behaviour scores (Partial $R^2 = 0.57$, $F_{(1,31)} = 43.18$, $p < 0.0001$; Fig. 2-2).

Discussion

Our main objective was to determine the sub-lethal consequences of fyke-net entrapment on three species of freshwater turtles. It was evident that simulated incidental capture causes significant changes to blood physiology compared to free-living individuals in all three species. Although the literature lacks baseline blood physiology values for musk and map turtles at temperatures similar to our study, all three species displayed control values similar to baseline blood lactate ($\sim 1.5 \text{ mmol l}^{-1}$) and pH (~ 7.8) found in painted turtles at similar temperatures to our study ($\sim 22^\circ\text{C}$; Keiver *et al.* 1992ab; Warren & Jackson 2004). Blood lactate and pH trends associated with submergence were similar to trends found by Larocque *et al.* (2012b) where research focused on painted turtles submerged for 4 hours. Clearly, treatment turtles in our experiment experienced stress related to prolonged forced submergence. Submergence of painted turtles for 12 hours in small cages that prevented extensive movements also resulted in similarly higher blood lactate and lower pH values to those we found (Fig. 2-1; LeDain *et al.* in press). Entrapment in nets has the potential to cause exhaustion more rapidly in turtles as a result of the ability to move within the net and the active attempts to escape (Larocque *et al.* 2012b). All three species displayed a decline of 0.7 or greater in blood pH between control and treatment groups (Fig. 2-1). Decline in blood pH of approximately one unit can be lethal for the freshwater turtles assessed in this study (Ultsch *et al.* 1984). In

addition, lactate values of entrapped turtles were similar to those found in hibernating painted turtles after 100-150 days (approximately 20-25 mmol l⁻¹ Reese *et al.* 2004) at 3°C despite entrapment for only 3 hrs. Additionally, increased rate and intensity of movement in the net as a result of warmer temperatures, would result in a higher rate of oxygen consumption, therefore increasing acidosis (Robin *et al.* 1964; Jackson & Silverblatt 1974; Herbert & Jackson 1985). These findings agree with previous research that suggests that water temperature, regardless of the oxygen content, can limit duration of survival (Ultsch 1989). Similar studies have assessed the physiological effects of various types of fishing gear, such as trawl nets (Stabenau *et al.* 1991; Harms *et al.* 2003) and gillnets (Snoddy *et al.* 2009) on blood physiology of marine turtles and have shown similar effects of entrapment on blood physiology as our study.

Fyke-net entrapment and associated submergence led to species-specific physiological responses, pointing to the potential danger of using a single model species as a surrogate for the various species potentially captured as bycatch (Fig. 2-1). Painted turtles exhibited a physiological response typical of a non-bimodally breathing turtle which relies on extreme anoxia tolerance. Denial of oxygen stimulates a physiological switch to anaerobic metabolism, which is characterized by the accumulation of blood lactate and decline in blood pH (Jackson 2000b). Despite having the ability to tolerate true anoxia in cold water, when submerged in elevated normoxic conditions (such as in this study), painted turtles were unable to acquire sufficient oxygen through secondary gas exchange mechanisms to remain aerobic (Ultsch & Jackson 1982; Jackson *et al.* 2000; Reese *et al.* 2001) as indicated in our study by elevated blood lactate and reduced blood pH. Therefore, painted turtles must metabolize energy anaerobically resulting in

blood acidosis (Ultsch & Jackson 1982; Ultsch *et al.* 1999; Jackson *et al.* 2000; Reese *et al.* 2001). Another mechanism of tolerating submergence is the use of gas exchange via extra pulmonary oxygen uptake or bimodal respiration, which is typified by musk and map turtles (Ultsch *et al.* 1984; Reese *et al.* 2001). Both species are known to be generally intolerant of prolonged submergence in anoxic conditions, but can survive extended periods of submergence in normoxic conditions during hibernation by remaining aerobic while accumulating relatively little blood lactate (Reese *et al.* 2001; Reese *et al.* 2003). Despite their tolerance of submergence in normoxic conditions, lactate accumulation in both species over 3 hours was relatively high in our study (Fig. 2-1A). We observed turtles swimming within the net and actively searching for exits. Increased activity, exacerbated by the elevated temperature could be also contributing to the observed increase in blood lactate and decline in pH (Dejours 1994). Gatten (1984) compared free-diving and forcibly submerged loggerhead musk turtles (*Sternotherus minor*) and found that involuntary submergence led to altered physiology and behaviour, including the accumulation of lactate. Stress associated with forced submergence may to some extent inhibit turtles from buffering acidosis, therefore altering associated blood physiology.

Furthermore, behavioural tests support our contention that the responses to simulated capture are species-specific. BII scores of submerged individuals were significantly lower than those of control in painted and map turtles (Fig. 2-2). This indicates that physiological stress associated with submergence is manifested in behavioural impairments, such as righting ability (Fig. 2-3). Interestingly, map turtles had the smallest percent increase in lactate and decrease in pH, despite being the most

behaviourally impaired species. This is not unexpected as map turtles are known for their poor ability to tolerate blood acidosis (Reese *et al.* 2001). These results point to a species-specific sensitivity and some incongruence between blood physiology and behavioural indicators, again outlining the potential danger of relying on data from a single species to make management recommendations. Surprisingly, musk turtles did not have significantly different BII scores between control and treatment groups despite having significantly different blood physiology values. Out of the six reflex tests, the behaviour was present for all individuals from control and treatment groups for five of the tests (Fig. 2-3). Their ability to obtain oxygen from normoxic water (Ultsch *et al.* 1984) could account for their behavioural responsiveness, although we would expect to see corresponding physiological results. While accumulation of lactate occurs rather quickly, studies have shown that it takes several hours for lactate to be metabolized (Bennett 1978; Seymour 1982). Quick accumulation and potential lag time in metabolizing lactate could account for incongruence between blood physiology and behavioural responses. Increased activity as a result of elevated temperature could result in increased lactate levels and decreased pH, but our behavioural results do not support this idea. Compared to fish, where there are several examples of incongruence between physiological and reflex (including some behaviours) metrics associated with fisheries interactions (Davis *et al.* 2001; Thompson *et al.* 2008), there are no published examples that have evaluated both types of endpoints in freshwater turtles to determine whether our observations are unique.

Sex appeared to have no influence on physiological or behavioural impairment following submergence in painted turtles as similar responses were seen in males and

females (Fig. 2-1 and 2-2). Significant differences were seen in blood lactate and pH as well as BII scores between both control and submerged turtles in both sexes. Some studies investigating normal blood physiology profiles for various turtle species (e.g., *Chelonia mydas*, *Podocnemis expansa*) have determined that males and females have similar physiological profiles at rest (Bolten & Bjorndal 1992; Oliveira-Júnior *et al.* 2009). Alternatively, some studies suggest significant differences in hematology between sexes, including the New Guinea snapping turtles (*Elseya novaeguineae*; Anderson *et al.* 1997), bog turtles (*Clemmys muhlenbergii*; Brenner *et al.* 2002), and Mediterranean pond turtle (*Mauremys leprosa*; Hidalgo-Vila *et al.* 2007). Because turtles are ectotherms, “normal” values can vary as a result of many factors such as activity, temperature, and seasonality. Understanding potential sex-specific fitness consequences is important in slow-maturing, long-lived species where females are more ecologically important than males. Other species should be investigated to determine if this pattern is consistent (including across sexually dimorphic or imperiled species) as the use of surrogates is clearly not ideal.

Although freshwater turtles are particularly adept at tolerating submergence, I found that incidental capture causes significant species-specific changes in physiology and can lead to behavioural impairments. These changes in blood physiology were seen despite a submergence period much shorter than typically occurs in the commercial fishery in eastern Ontario. Despite lack of immediate mortality, some species exhibited behavioural impairments, which would compromise their activity and potentially result in post-release mortality, such as drowning. Additional non-lethal consequences associated with submergence are currently unknown, and should be the focus of future research. Our

findings point to intrinsic differences in blood physiology and behaviour among species and reflect the need for management decisions to account for inter-specific variation. Painted turtles have previously been used as surrogates for at-risk species in the eastern Ontario fyke-net fishery (Larocque *et al.* 2012b). Our study highlights the limitations of using a surrogate species to gauge the physiological response to incidental capture. The use of a conservative approach with safe and pre-determined endpoints allowed us to determine the effects of incidental capture on two additional at-risk turtle species. Although mortality is an ecologically relevant endpoint for studying the effects of bycatch in an experimental context, it is not ethically appropriate when studying at-risk animals (Putman 1995; Minter & Collins 2005). Sub-lethal consequences, such as physiological disturbance and behavioural impairments are becoming recognized as useful and relevant tools in conservation science (Wikelski & Cooke 2006; Cooke *et al.* in press a). In particular, physiological (e.g., Davis 2002; Cooke *et al.* in press b) and reflex (e.g., Davis 2010) endpoints serve as objective indicators of animal welfare in fisheries (Diggles *et al.* 2011; Cooke *et al.* in press b) and can be used to inform conservation actions (Wikelski & Cooke 2006; Seebacher & Franklin 2012) given their utility in defining cause and effect relationships and elucidating mechanisms of mortality (Cooke & O'Connor 2010; Cooke *et al.* In press a). By determining how species respond to forced submergence associated with incidental capture, we can assist managers in the development of successful and sustainable bycatch reduction strategies. Given that most endangered species legislations extend beyond mortality to include sublethal disturbances (e.g., “harm” and “harassment” are forbidden by the Canadian Species at Risk Act), efforts to document physiological and behavioural impairments could provide an

objective means of evaluating consequences of different types of fisheries activities while also informing welfare (Diggles *et al.* 2011).

Tables

Table 2-1 – Sample sizes, carapace length, and mass of three species of turtles used to assess the sub-lethal consequences of entrapment in commercial fishing nets in Lake Opinicon, Ontario, Canada.

Species	Sex	Total <i>N</i> (Control, Treatment)	Mean carapace length \pm SE (mm)	Mean mass \pm SE (g)
Eastern musk turtle (<i>Sternotherus odoratus</i>)	Male	18 (8,10)	107 \pm 4	186 \pm 16
Northern map turtle (<i>Graptemys geographica</i>)	Male	20 (10,10)	115 \pm 6	178 \pm 13
Painted turtle (<i>Chrysemys picta</i>)	Male	16 (6,10)	139 \pm 2	330 \pm 12
	Female	19 (9,10)	154 \pm 3	481 \pm 20

Table 2-2 – Description of the six behavioural tests, adapted from LeDain *et al.* (in press), that were performed on three species of turtles used to assess the sub-lethal consequences of entrapment in commercial fishing nets in Lake Opinicon, Ontario, Canada.

Behavioural Test	Description
Escape	Turtles were held posteriorly and completely submerged for 10 seconds – if any attempt to escape (moving limbs, moving neck/head), the behaviour was present.
Righting (Land)	Turtles were placed on their carapace, on land, and left for 10 seconds - if successfully able to right or any attempts (limb or head movement) were made, the behaviour was present.
Righting (Water)	Turtles were placed on their carapace, while being held underwater in a tub - if successfully able to right or any attempts were made (limb or head movement), the behaviour was present.
Audible startle	Turtles were placed in a 14 gallon plastic tub, and were not held. The sides of the tub were gently tapped – if any signs of startle were made (retract head, abrupt change in direction), the behaviour was present.
Visual startle	Turtles were placed in a 14 gallon plastic tub, and were not held. We waved a hand within 10 cm of their face - if any attempt to retract their head, the behaviour was present.
Tactile stimuli	Turtles were held posteriorly and we gently pinched their tail, a limb and their head - any attempt to retract their limb/head/tail was scored as present.

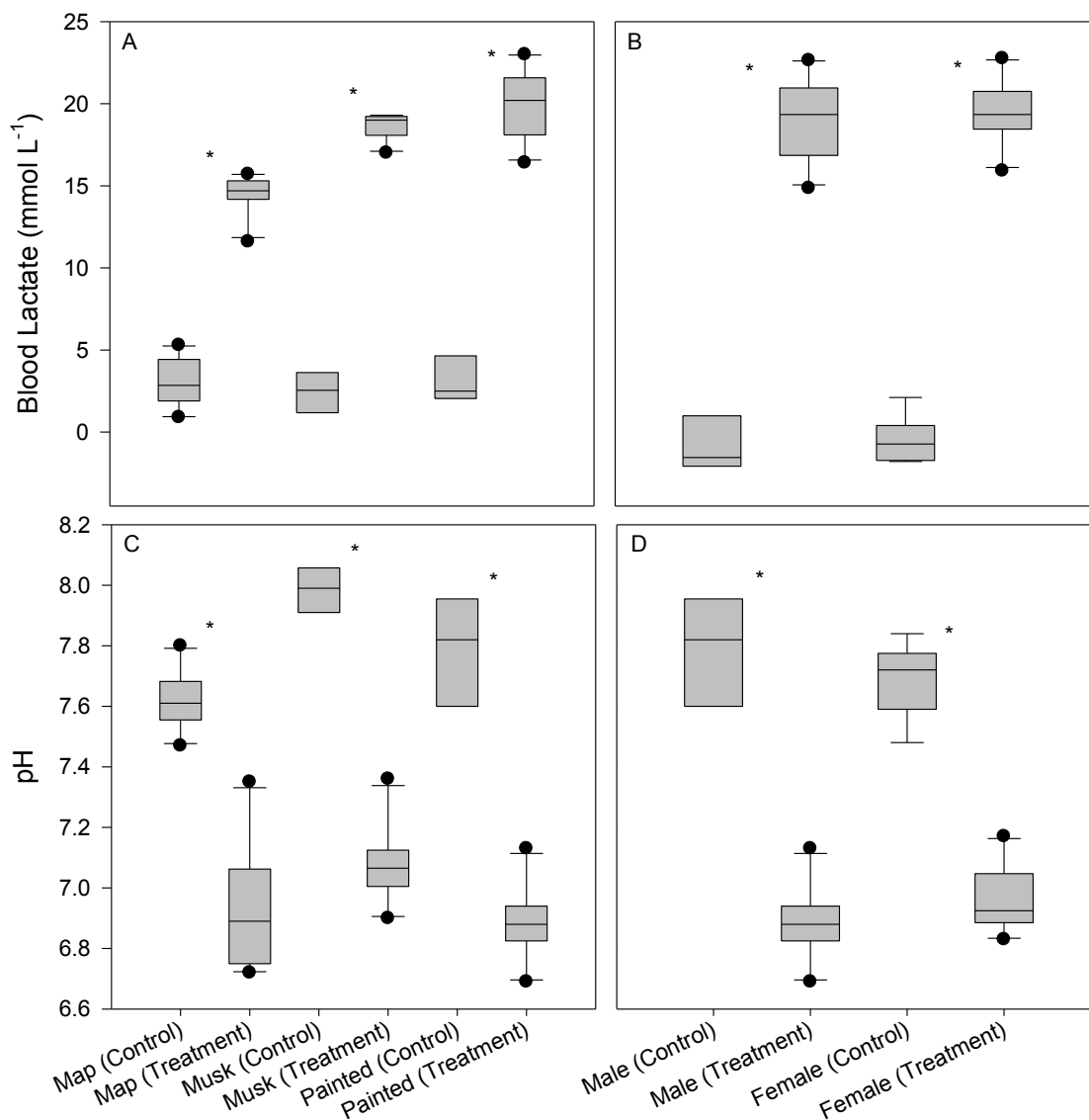


Figure 2-1 – Blood lactate (top) compared between species (A) and sexes (B) and pH (bottom) compared between species (C) and sexes (D) of control and submerged individuals. Median is reported along with the 25th and 75th percentiles. Bars represent the 10th and 90th percentiles and any outliers (black dots) are reported. Significant difference ($p < 0.05$) between control and submerged individuals are represented by asterisks (*).

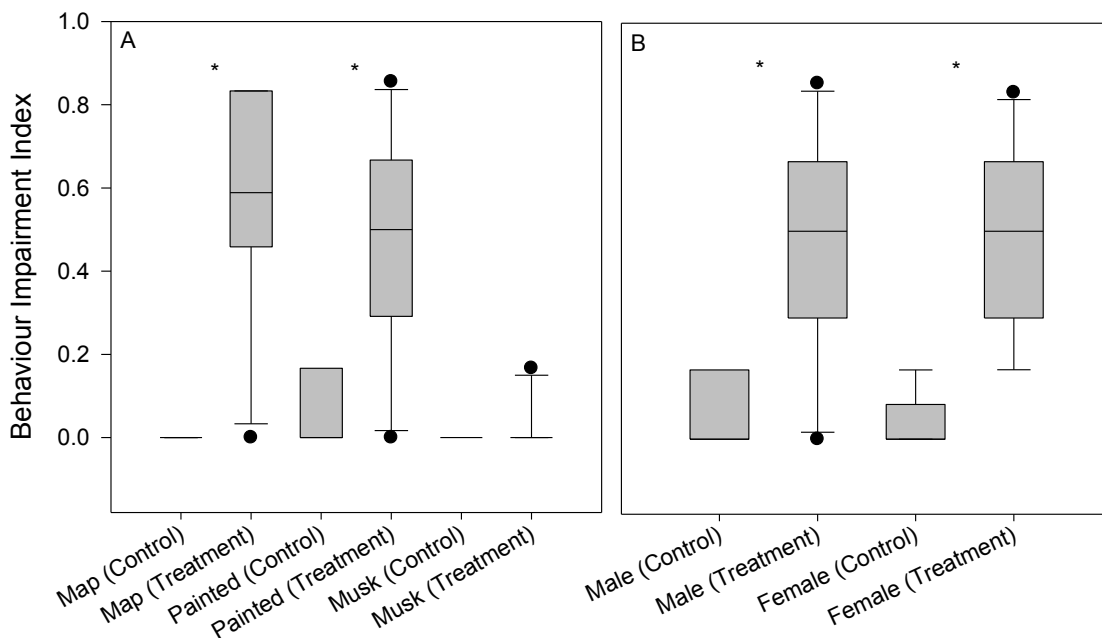


Figure 2-2 – Behaviour Impairment Index (BII) scores after six behavioural tests of control and submerged individuals compared between species (A) and sexes of painted turtles (B). Scores range from 0, which suggests that the individual was not impaired, to a maximum score of 1, which indicates that the turtle was completely impaired. Median is reported along with the 25th and 75th percentiles. Bars represent the 10th and 90th percentiles and any outliers (black dots) are reported. Significant difference ($p < 0.05$) between control and submerged individuals are represented by asterisks (*).

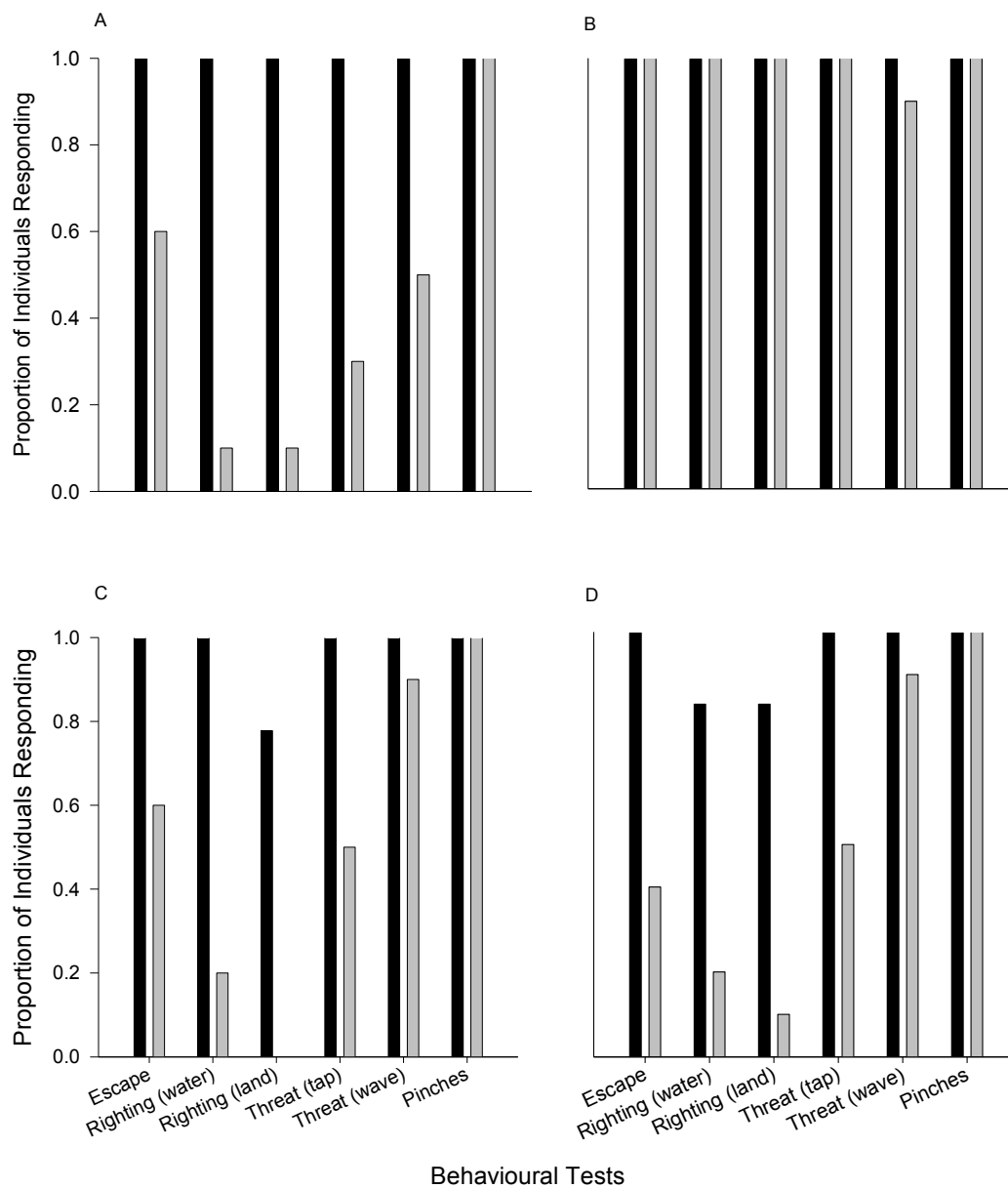


Figure 2-3 – Proportion of individuals that responded with a positive response for each behavioural test for each species; northern map turtle (*Graptemys geographica*; A), common musk turtle (*Sternotherus odoratus*; B), female painted turtle (*Chrysemys picta*; C) and male painted turtle (D). Black bars represent control individuals and submerged individuals in grey.

Chapter 3: Biologgers reveal post-release behavioural impairments of freshwater turtles following fisheries interactions

Abstract

Bycatch, the incidental capture of non-target organisms, occurs in both marine and freshwater commercial fisheries. Although immediate bycatch mortality frequently occurs and is a major concern, detrimental sub-lethal effects and potential post-release mortality are also of concern. Turtle populations are especially vulnerable to slight increases in adult mortality from sources such as bycatch. In eastern Ontario, turtles are frequently captured as bycatch in a small-scale freshwater commercial fyke-net fishery and, currently, the fate of discarded turtles is unknown. The purpose of our study was to determine the effect of fyke-net capture on fate and post-release behaviour in two species of freshwater turtles (eastern musk turtle; *Sternotherus odoratus*, and painted turtle; *Chrysemys picta*). We used tri-axial accelerometers to study fine-scale movement behaviour of entrapped (exposed to forced submergence for 4 hrs at water temperatures of 23-29°C) and control turtles upon release. We used overall dynamic body acceleration (ODBA) as a measure of movement and assessed differences between control and entrapped turtles over three time intervals up to 48 hours after release. Post-release mortality was not detected in any individuals exposed to simulated capture. Individuals of both species that were exposed to simulated entrapment displayed significantly lower locomotory levels over the first 6 hours. However, that difference disappeared after 48 hours, suggesting the ability to recover, despite decreasing locomotory levels. The use of tri-axial accelerometers in freshwater turtles is novel and allowed us to quantify uninterrupted, fine-scale movement behaviour. Quantifying the post-release and sub-

lethal effects of entrapment is important in estimating the population effects associated with bycatch, which is especially important for species at-risk.

Introduction

Bycatch, the incidental capture of non-target organisms, occurs in most commercial fisheries (Crowder & Murawski 1998; Hall *et al.* 2000; Hall & Mainprize, 2005). Fish bycatch accounted for approximately 28% of total landings in the United States in 2002 (Harrington *et al.* 2005). Although mortality frequently occurs, not all individuals caught as bycatch die immediately (Davis 2002). Post-release mortality and detrimental sub-lethal effects have been reported in a variety of frequently caught bycatch species including marine mammals, sea birds, sharks, and marine turtles (Julian & Beeson 1998; Lewison *et al.* 2004; Moore *et al.* 2008; Finkbeiner *et al.* 2011). Detrimental sub-lethal effects and post-release mortality can have important negative population consequences that are not always immediately evident and remain largely undocumented (Alverson *et al.* 1994; Chopin & Arimoto 1995; Davis 2002; Lewison *et al.* 2004). Unknown additional mortality rates are especially concerning for species that have slow maturation and long generation times, and that are at-risk (Crowder *et al.* 1998; Hall *et al.* 2000). Injuries and negative physiological effects sustained as a result of bycatch, such as net entanglement (e.g., Frick *et al.* 2010), hooking injuries (e.g., Aguilar *et al.* 1995), and stress associated with prolonged submergence in air breathing organisms (e.g., Lutcavage & Lutz 1991; Harms *et al.* 2003; Lewison *et al.* 2005; Snoddy *et al.* 2009) can be non-lethal, but could still impair behaviour. Behavioural impairments, such as reduced mobility and diminished foraging ability, can increase the risk of mortality (Olla *et al.* 1997; Schreck *et al.* 1997; Davis 2002). Furthermore, sub-lethal effects of entrapment

have the potential to affect the health and fitness of the individual (Davis 2002; Skomal 2007). Various studies have focused on how sub-lethal effects associated with capture manifest themselves behaviourally in several frequently caught species, mostly fish (e.g. sablefish; Davis & Parker 2011). In addition, studies on fish have indicated that there are interspecific differences in the effects of sub-lethal stressors (Ryer *et al.* 2004). Identifying how behaviour is impaired following capture, especially fine-scale movement behaviour, is crucial to estimate the overall negative population consequences of bycatch, particularly in at-risk species.

The use of animal-attached sensors or biologgers is becoming increasingly widespread to study fine-scale, continuous animal behaviour and movement in nature (Cooke *et al.* 2004; Wilson *et al.* 2008; Cooke 2008; Rutz & Hays 2009; Bograd *et al.* 2010). Biologgers encompass a wide range of devices that can measure various parameters, such as temperature, location, acceleration, and pressure (Rutz & Hays 2009). Biologgers have been used to assess post-release behaviour and associated delayed mortality in marine turtles following interaction with fishing gear (e.g., Chaloupka *et al.* 2004; Swimmer *et al.* 2006; McClellan & Read 2009; Snoddy & Williard 2010). Surprisingly, little information exists on the post-release behaviour of freshwater turtles following interaction with fishing gear, despite freshwater turtles being frequently encountered as bycatch in many inland fisheries (Barko *et al.* 2004; McClellan *et al.* 2009; Raby *et al.* 2011; Larocque *et al.* 2012a). Unlike marine turtles that are most frequently captured in long line and trawl fisheries, freshwater turtles are mostly encountered in trap net fisheries, such as hoop and fyke-nets (Barko *et al.* 2004; McClellan *et al.* 2009; Larocque *et al.* 2012a). Despite the ability of freshwater turtles to

withstand extended periods of submergence, prolonged entrapment in fishing nets can result in drowning (Larocque *et al.* 2012a). In addition, there are acute physiological and behavioural impairments associated with entrapment in fyke-nets in several freshwater turtles (Ledain *et al.* In press; Stoot *et al.* In press). Turtles are particularly susceptible to population declines following even slight increases in adult mortality, such as bycatch mortality, because of their naturally high juvenile mortality and delayed sexual maturity (Congdon *et al.* 1993; 1994; Gibbons *et al.* 2000).

The purpose of our study was to determine the post-release movement behaviour and fate of freshwater turtles caught as bycatch in commercial fyke-nets. As study species, we chose two representative, but ecologically different, freshwater turtles: the painted turtle (*Chrysemys picta*) and the musk turtle (*Sternotherus odoratus*). We exposed individuals of both species to simulated capture stressors and measured their fine-scale movement behaviour with tri-axial accelerometers. Tri-axial accelerometers measure acceleration in three axes (x, y and z) to estimate animal orientation and both static and dynamic movements within natural habitats (Yoda *et al.* 1999; Wilson *et al.* 2008; Shepard *et al.* 2008). Both of our study species are frequently caught as bycatch in a small-scale commercial fyke-net fishery on inland lakes and rivers in eastern Ontario (Carrière *et al.* 2009; McClellan & Read, 2009; Larocque *et al.* 2012ab). This small-scale commercial fishery targets sunfish (*Lepomis* spp.) although bullhead (*Ameiurus* spp.), yellow perch (*Perca flavescens*), black crappie (*Pomoxis nigromaculatus*), and rock bass (*Ambloplites rupestris*) are also harvested (Burns 2007; Larocque *et al.* 2012a). Upon landing, all bycatch species are required to be discarded immediately, whether dead or alive.

Previous studies have assessed the post-release mortality of commonly encountered fish

bycatch in this fishery (e.g., northern pike; Colotelo *et al.* 2013), but the fate of turtles encountered as bycatch is currently unknown.

Materials and Methods

Study area and turtle collection

We conducted the study on Lake Opinicon (44° 34' N, 76° 19' W) approximately 100 km southwest of Ottawa, Ontario, Canada. Experiments were conducted between 14 May and 19 June 2012 when lake water temperatures ranged from 18 to 26°C. For all experiments, dissolved oxygen was at or near saturation (i.e., 6-8 mg·l⁻¹). All turtles were initially collected using fyke-nets, which were set throughout the lake in 2-3 m of water and left for approximately 24 hours with floats to provide air pockets. We used fyke-nets similar to the ones used in the local commercial fishery. Nets were fished in pairs and were attached by leads (Fig. 1 in Larocque *et al.* 2012a). Each net contained seven steel hoops, which were 0.5 m apart and measured 0.9 m in diameter, and two throats were located in the second and fourth hoop. Two wings and a lead were attached vertically to the mouth of each net. Wings measured 4.6 m long by 0.9 m high while each lead measured 10.7 m long by 0.9 m high. All gear was fabricated with 2.54 cm square, 5.08 cm stretch, nylon mesh. Upon retrieval from the nets, all turtles were returned to the field laboratory where we measured mass and carapace length, and determined the sex based on external characteristics. Individuals were held outdoors in ~700 L fiberglass tanks at ambient temperatures for approximately 48 hours to let any potential capture stress wane. Tanks were supplied with lake water using a flow through system and turtles were not fed, but were provided with basking platforms and exposed to ambient sunlight.

Experimental procedure

We used 32 male turtles of two species for both treatment and control (Table 3-1). We used a treatment group in which turtles were submerged for 4 hours in a closed fyke-net set in 1.5 m of water (23 to 29°C), and a control group in which turtles were placed singly into a tank with access to oxygen and a basking platform exposed to ambient sunlight for 4 hours. Four hours of submergence was chosen to provide sufficient time for impairment to occur while avoiding immediate mortality (Larocque *et al.* 2012b). It should be noted that our submergence length is not representative of typical entrapment length in the Ontario commercial fishery, which can be up to 2-7 days, depending on the season. We used a matched pair design in which, for each individual undergoing the entrapment treatment, we selected a control individual that was matched for species, sex, size, capture day, and capture location. Both individuals from a given pair were released simultaneously at the same location (their capture location).

After initial capture, attachment points were created for the accelerometers by drilling two small holes between the 10th and 12th marginal scutes on the left side. Pairs of control and treatment individuals were then left to recover for a minimum of 48 hours prior to experiments, after which they underwent their respective treatments. After 4 hours in their respective treatments, individuals were removed and tested for basic behavioural responses to assess impairment. Following behavioural assessment, tri-axial accelerometer loggers (model CEFAS G6, 18 g in air, 10 Hz recording frequency, 1 Hz for temperature and depth; CEFAS Technology Limited, Suffolk, United Kingdom) were attached. Tri-axial accelerometers measure both dynamic and static acceleration in units

of gravity (g). To allow tag retrieval, unique-frequency radio-telemetry transmitters (Model BD-2, 3.2 g in air, 20 cm trailing whip antenna, Holohill Systems Inc., Carp, ON, Canada) were also attached to the accelerometers and the entire package weighed approximately 24 g in air and ~12 g in water (approximately 5% of body mass). Tags were attached externally to the posterior left side of the carapace using 13.6 kg stainless steel trolling line (Figure 1). After logger and transmitter attachment, which lasted 3 minutes on average, both control and treatment turtles were immediately transported to their capture location in large, plastic containers with lids and were released simultaneously. Accelerometers were set to record for 48 hours and then shut off. Forty-eight hours post-release, turtles were located using a hand-held radio-tracking receiver (Biotracker; Lotek Engineering, Inc.; Newmarket, ON, Canada) and 3-element yagi antenna (AF Antronics, Urbana, IL). Turtles were then recaptured and both tags recovered.

Behavioural assessments

The use of reflex tests to assess involuntary responses to stimuli, such as touch and gravity have been shown to be useful predictors of mortality in a variety of vertebrates, most notably fish (Davis 2007; 2010). Although not a measure of true reflexes, a variety of behavioural tests have been used to assess impairment related to submergence in freshwater turtles (Ledain *et al.* In press; Stoot *et al.* In press). We used six behavioural tests, identical to the ones used in Stoot *et al.* (In press), which assessed escape ability, righting ability (on both land and water), response to startles (audible/pressure and visual) and tactile stimuli to the head, limbs and tail (See Table

2.2). If the individual responded to the stimulus, the test was scored as present (1); lack of reaction showed that the response was absent (0). These scores were converted into a Behaviour Impairment Index (BII), which is an overall score of impairment for each individual based on the number of tests performed. The BII ranges from 0, which indicated that the individual was not impaired, to a maximum score of 1, which indicates that the turtle was completely impaired and is calculated where $BII = 1 - (\text{sum of individual test scores} / \text{total possible score of 6})$.

Data processing and analysis

Accelerometers were set to record total acceleration (g) at 10 Hz in three (x, y and z) axes. Total acceleration was calculated as the sum of static and dynamic acceleration. Tags were calibrated before deployment by rotating the device through known angles to real g (9.8 m s^{-2} ; Gleiss *et al.* 2010). Static and dynamic acceleration were separated by weighted smoothing at an interval of 3 sec based on the methods of Shepard *et al.* (2008) using Igor Pro 6.0 software (WaveMetrics Inc., Lake Oswego, OR). Median ODBA (overall dynamic body acceleration) values were calculated for both species for each time interval of interest.

To ensure that tag burden did not affect experimental groups differently, we ran a one-way t-test to compare the combined mass of tags between control and treatment individuals. To assess the effects of entrapment on movement, we calculated median ODBA values over three time periods post-release for both species: 10 minute intervals for the first hour, 1 hour intervals for the first 6 hours, and 6 hour intervals for the entire 48 hours. Since we sampled the same individuals repeatedly, we used a repeated

measures two-way ANOVA to test for the effect of time and experimental group on ODBA. We tested for sphericity using Mauchly's test, and if it was violated, we corrected degrees of freedom using Greenhouse-Geisser estimates of sphericity. We conducted follow-up one-way ANOVAs looking at the effect of treatment when there was indication of an interaction or of a main effect. Since our sample size was modest, we conducted follow-up one-way ANOVAs when $p < 0.150$. To test for differences in behavioural impairment scores, we used a one-way ANOVA, as residuals did not violate the assumptions of normality and homogeneity of variance, to compare experimental groups within species. All statistical tests were performed using JMP (Version 9.0.1, SAS Institute) and significance was accepted at $\alpha < 0.05$.

Results

Of the 16 pairs of turtles that underwent treatment, all but one painted turtle (from the control treatment) survived for 48 hours. Therefore, we used 7 pairs of painted turtles and 8 pairs of musk turtles to document the effect of entrapment on movement (Table 3-1). The mass of turtles was similar for control and treatment individuals for both musk ($t_{(14)} = 0.121, p = 0.547$) and painted turtles ($t_{(12)} = 0.326, p = 0.625$), which indicates that tag burden was equal in both groups for both species.

We examined ODBA as a proxy for locomotory activity every 10 minutes for the first hour post-release and found no significant interaction between time and treatment in painted turtles ($F_{(1.99, 23.93)} = 0.668, p = 0.522$) or in musk turtles ($F_{(5, 10)} = 1.986, p = 0.167$). There was no significant difference in movement over time in painted turtles ($F_{(1.99, 23.93)} = 2.426, p = 0.110$) or in musk turtles ($F_{(5, 10)} = 1.283, p = 0.344$), although there was some evidence that painted turtles experienced some decrease in movement

over time (Fig. 3-2A & 3-2B). In addition, we did not find significance differences in ODBA between experimental groups in painted turtles ($F_{(1, 12)} = 3.454, p = 0.088$) or in musk turtles ($F_{(1,14)} = 2.837, p = 0.114$), although the difference approached statistical significance in both cases. Therefore, we conducted follow-up one-way ANOVAs that revealed that experimental groups had significantly lower ODBA in musk turtles during 0-10, 10-20 and 30-40 minutes after release, as well as during 40-50 minutes after release in painted turtles. This suggests that submerged individuals had impaired movements during these time intervals (Table 3-2).

We examined ODBA as a proxy for locomotory activity each hour for the first 6 hours post-release and found no significant interaction between time and treatment in painted turtles ($F_{(2,25, 26,94)} = 1.142, p = 0.339$) or in musk turtles ($F_{(2,32, 32,48)} = 0.515, p = 0.629$). There was a significant difference in movement over time in painted turtles ($F_{(2,25, 26,94)} = 4.325, p = 0.020$) that tended to have decreased movement over time, but not in musk turtles ($F_{(2,32, 32,48)} = 2.576, p = 0.084$) although again the difference approached significance with musk turtles tending to show decreased movement over time (Fig. 3-2C & 3-2D). We did find significant differences in ODBA between experimental groups in painted turtles ($F_{(1, 12)} = 5.912, p = 0.032$) and in musk turtles ($F_{(1,14)} = 4.588, p = 0.050$) where entrapped individuals had lower levels of locomotory activity compared to controls.

We examined ODBA as a proxy for locomotory activity every 6 hours for 48 hours post-release and found no significant interaction between time and treatment in painted turtles ($F_{(2,61, 31,33)} = 0.102, p = 0.943$) or in musk turtles ($F_{(7, 8)} = 0.427, p = 0.861$). There was no difference over time in painted turtles ($F_{(2,61, 31,33)} = 2.859, p = 0.059$) or in

musk turtles ($F_{(7, 8)} = 2.273, p = 0.136$), but again the differences approached significance with both species tending to move more with time (Fig. 3-2E & 3-2F). We found no significant difference in ODBA between experimental groups in painted turtles ($F_{(1, 12)} = 0.097, p = 0.761$) or in musk turtles ($F_{(1, 14)} = 2.823, p = 0.115$) over this longer time interval.

We also investigated the immediate impairment associated with entrapment by using a behavioural impairment index (BII). There was a significant difference in BII scores between control and submerged groups for painted turtles (partial $R^2 = 0.56, F_{(1,12)} = 15.38, p = 0.002$). Experimentally submerged painted turtles had significantly lower BII scores, which suggests impairment after 4 hours of simulated fyke-net entrapment. No significant difference was found between control and submerged musk turtles (partial $R^2 = 0.13, F_{(1,14)} = 2.00, p = 0.179$; Fig. 3-3 & 3-4), suggesting that submerged musk turtles were not behaviourally impaired.

Discussion

Our main objective was to determine the post-release consequences of fyke-net entrapment on two species of freshwater turtles commonly encountered as bycatch. We only found delayed mortality in one individual, a control painted turtle. Mortality occurred shortly after release and the cause of mortality is unknown as the individual was not exposed to the submerged treatment and had access to oxygen throughout its trial. Although there are previous reports of external electronic tags of painted turtles becoming fouled by algae or macrophytes (Boarman *et al.* 1998), there was no fouling of the biogger on the dead turtle upon retrieval. We used tri-axial accelerometers to measure fine scale movement, which enabled us to investigate potential impairment over

three time periods post-release: initial (1 hour post-release at 10 minute intervals), short-term (6 hours post-release at 1 hour time intervals), and medium-term (48 hours post-release at 6 hour time intervals). We did find evidence suggesting that individuals from both species exposed to simulated net entrapment had impaired locomotory activity relative to control animals, at least in the first 6 hours after release. Studies looking at post-release mortality rates in marine turtles are relatively common (Chaloupka *et al.* 2004; Swimmer *et al.* 2006; Snoddy & Willard 2010). To our knowledge, however, there are no previous studies looking at post-release mortality and locomotory impairments associated with entrapment in freshwater turtles, despite its importance in understanding the fate of turtles encountered as bycatch.

Previous studies have assessed the physiological response to fyke-net entrapment in painted and musk turtles and revealed that both species experience significant physiological impairment after 3 hours of simulated entrapment (Stoot *et al.* In press). Furthermore, despite similar physiological impairment, not all species were behaviourally impaired following entrapment; musk turtles did not have significant behavioural impairment whereas painted turtles were significantly impaired (Stoot *et al.* In press), as was the case in the present study. Although painted turtles are tolerant of true anoxic conditions in cold water, submergence in elevated normoxic conditions makes them incapable of sequestering sufficient oxygen through secondary gas exchange mechanisms to remain aerobic (Ultsch & Jackson 1982; Jackson *et al.* 2000; Reese *et al.* 2001), thus requiring them to metabolize energy anaerobically, which leads to blood acidosis (Ultsch & Jackson 1982; Ultsch *et al.* 1999; Jackson *et al.* 2000; Reese *et al.* 2001). Unlike painted turtles, musk turtles are intolerant of submergence in anoxic conditions, but use

an alternative strategy to tolerate submergence in normoxic conditions called bimodal respiration, which is the gas exchange via extrapulmonary oxygen uptake (Ultsch *et al.* 1984; Reese *et al.* 2001).

Overall, our research suggests that the movements of both painted and musk turtles are impaired (i.e., decreased locomotory activity levels) to some extent within the first 6 hours after release from simulated incidental capture. Therefore, simulated entrapment in fyke-nets for 4 hours can cause impairments, which affect locomotory ability. This suggests that turtles released alive from commercial fishing nets may have reduced abilities to flee when faced with predators, or rapidly approaching boats (Galois & Ouellet, 2007; Bulté *et al.* 2010).

Painted turtles had higher overall median ODBA values through all three time periods compared to musk turtles, which is most likely a result of their superior locomotory ability. Painted turtles are known to be free-swimming while musk turtles tend to be more bottom crawlers (Ernst *et al.* 1994). Tag weight could also have contributed to interspecific differences in locomotion because painted turtles are heavier than musk turtles and, therefore, the tags represented a smaller burden proportionally. Nonetheless, the tag mass in water was ~12 g which constitutes, on average, only ~4% of the mass of painted turtles and ~6% of the mass of musk turtles used in this study. General rules for vertebrates suggest tag mass should not exceed 3% of body mass (Putman 1995). Tags were positioned well back on the carapace, consistent with best practices for turtle tagging (Boarman *et al.* 1998; Balazs 1999).

Behavioural tests indicated that the two species respond differently to simulated capture. These behavioural tests have previously been used in freshwater turtles to assess behavioural impairment associated with entrapment (Ledain *et al.* In press; Stoot *et al.* In press). As indicated, BII scores of submerged individuals were significantly lower than those of control in painted turtles, but musk turtles did not have significantly different BII scores between experimental groups suggesting that they were not impaired. Musk turtles had similar proportions of submerged individuals responding to each test as the control individuals. From these results, we would not have expected to detect impaired locomotory behaviour in musk turtles, which highlights the usefulness of measuring fine-scale movement behaviour to detect subtle impairments.

We also wanted to quantify the duration of potential impairment in both musk and painted turtles. Despite seeing impairment effects over 6 hours after release, we did not find significant locomotory differences between control and submerged groups over 48 hours after release. In addition, activity levels seem to stabilize and converge over time between experimental groups in both species. This suggests that individuals have recovered and are no longer experiencing locomotory impairment past 6 hours. Within the eastern Ontario fyke-net fishery, turtles can typically remain entrapped in nets for up to 48 hours at elevated water temperatures (similar to this study) and up to 7 days at cooler temperatures (~10-12°C). We would expect to see more obvious locomotory impairments in both species when exposing individuals to longer submergence periods. In fact, these prolonged submergence times result in high mortality rates (Larocque *et al.* 2012a).

Our study shows the importance of evaluating post-release fate on various time scales. Assessing fine-scale locomotory activity over the short-term revealed impairment patterns that were not present over the longer-term. Impaired locomotion can increase the risk of mortality and predation (Davis 2002). In addition, it is important to identify post-release effects as harm and harassment of species at-risk violates the Species at Risk Act in Canada (2002, c.29, s.32). By identifying species-specific responses to entrapment, we can determine how species respond to incidental capture and use this information to inform management. Electronic tagging techniques such as those used here hold much promise for the study of post-release bycatch behaviour and fate (Cooke 2008; Donaldson *et al.* 2008). The obvious next step is to quantify in more detail the increased risk of mortality that results from locomotory impairments within the first 6 hours following release from nets in freshwater turtles.

Tables

Table 3-1 - Sample sizes, mean pair carapace length, and mean pair mass of two species of turtles used to assess the post-release of entrapment in commercial fishing nets in Lake Opinicon, Ontario, Canada.

Species	Sex	Number of pairs	Mean pair carapace length \pm SE (mm)	Mean pair mass \pm SE (g)
Eastern musk turtle (<i>Sternotherus odoratus</i>)	Male	8	116 \pm 1	218 \pm 4
Painted turtle (<i>Chrysemys picta</i>)	Male	7	137 \pm 3	309 \pm 17

Table 3-2 - Follow-up one-way ANOVA results between experimental groups looking at 10 minute intervals over the initial first hour after release. Significant results are denoted with an asterisk (*).

Species	Time period (minutes)	Partial R ²	F ratio	Degrees of freedom	p-value
Musk	0-10	0.277	5.370	1,14	0.036*
Musk	10-20	0.299	5.978	1,14	0.028*
Musk	20-30	0.002	0.278	1,14	0.607
Musk	30-40	0.292	5.789	1,14	0.031*
Musk	40-50	0.023	0.327	1,14	0.577
Musk	50-60	0.089	1.366	1,14	0.262
Painted	0-10	0.034	0.425	1,12	0.527
Painted	10-20	0.000	0.010	1,12	0.922
Painted	20-30	0.208	3.154	1,12	0.101
Painted	30-40	0.164	2.348	1,12	0.151
Painted	40-50	0.324	5.765	1,12	0.034*
Painted	50-60	0.198	2.961	1,12	0.111

Table 3-3 – Follow-up one-way ANOVA results between experimental groups looking at 1 hour intervals over the initial 6 hours after release. Significant results are denoted with an asterisk (*).

Species	Time period (hrs)	Partial R²	F ratio	Degrees of freedom	p-value
Musk	0-1	0.143	2.34	1,14	0.148
Musk	1-2	0.132	2.126	1,14	0.167
Musk	2-3	0.218	3.895	1,14	0.069
Musk	3-4	0.094	1.458	1,14	0.247
Musk	4-5	0.084	1.286	1,14	0.276
Musk	5-6	0.180	3.082	1,14	0.101
Painted	0-1	0.019	2.882	1,12	0.115
Painted	1-2	0.025	0.303	1,12	0.592
Painted	2-3	0.004	0.049	1,12	0.829
Painted	3-4	0.063	0.806	1,12	0.3871
Painted	4-5	0.140	1.947	1,12	0.1882
Painted	5-6	0.026	0.319	1,12	0.5833

Table 3-4 – Follow-up one-way ANOVA results between experimental groups looking at 6 hour intervals over 48 hours after release. Significant results are denoted with an asterisk (*).

Species	Time period (hrs)	Partial R²	F- ratio	Degrees of freedom	p-value
Musk	0-6	2.133	3.796	1,14	0.072
Musk	6-12	0.177	3.016	1,14	0.105
Musk	12-18	0.069	1.038	1,14	0.326
Musk	18-24	0.958	1.484	1,14	0.243
Musk	24-30	0.041	0.605	1,14	0.450
Musk	30-36	0.000	0.005	1,14	0.944
Musk	36-42	0.076	1.156	1,14	0.301
Musk	42-48	0.037	0.531	1,14	0.478
Painted	0-6	0.113	1.523	1,12	0.241
Painted	6-12	0.035	0.436	1,12	0.522
Painted	12-18	-7.000	0.000	1,12	1.000
Painted	18-24	0.152	2.148	1,12	0.169
Painted	24-30	0.003	0.040	1,12	0.846
Painted	30-36	0.003	0.319	1,12	0.583
Painted	36-42	0.001	0.013	1,12	0.910
Painted	42-48	0.008	0.095	1,12	0.763

Figures

Figure 3-1 – Placement of accelerometer device on left side of a tagged painted along with a radio transmitter. Arrows show direction of x, y and z-axis.

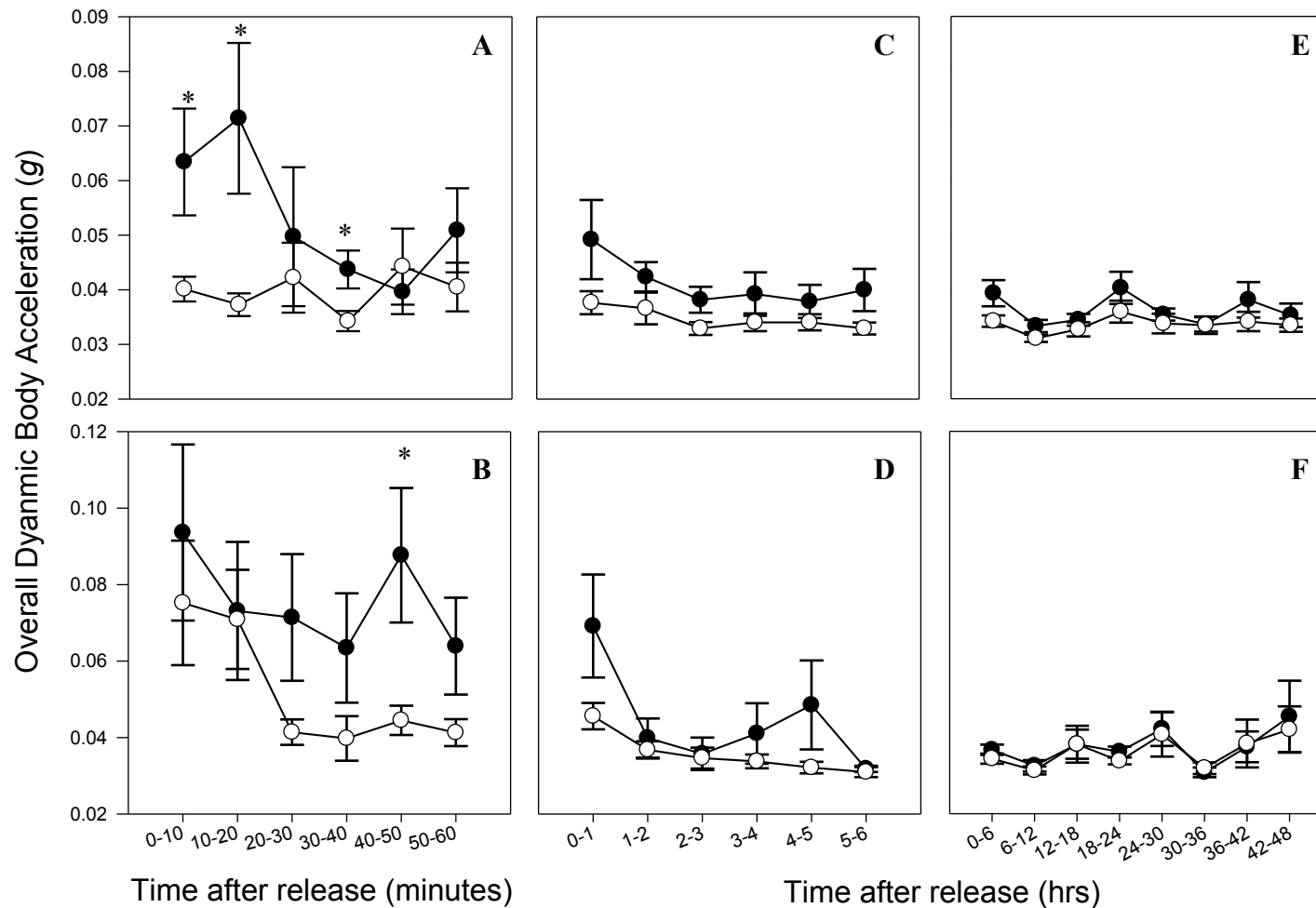


Figure 3-2 –Least squares means of ODBA (y-axis in g) over three time periods looking at eastern musk turtles (*Sternotherus odoratus*; top) and painted turtles (*Chrysemys picta*; bottom) over three time periods; 1st hour post-release (A & B), 6 hours post-release (C & D) and 48 hours post-release (E & F). Control individuals (black circles) are compared to submerged individuals (white circles) with error bars representing standard error. Significant follow-up one-way ANOVAs are denoted with an asterisk (*). Note: y-axis differs between species.

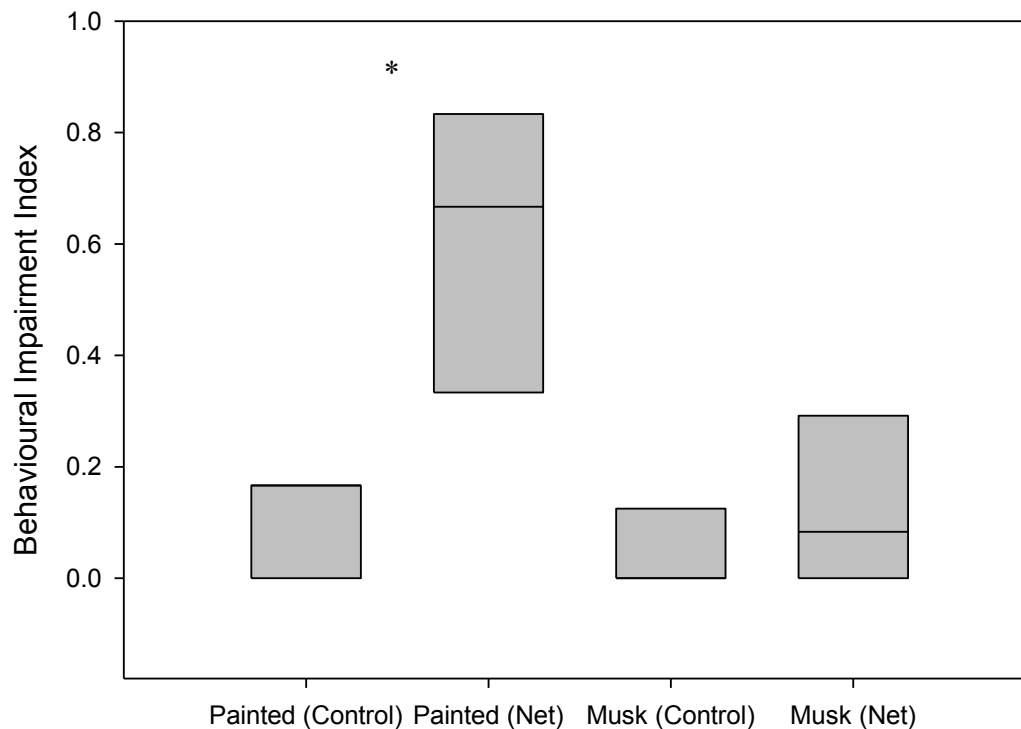


Figure 3-3 – Behaviour Impairment Index (BII) scores after six behavioural tests of control and net submerged individuals compared between species. Scores range from 0, which suggests that the individual was not impaired, to a maximum score of 1, which indicates that the turtle was completely impaired. Median is reported along with the 25th and 75th percentiles. Significant difference ($p < 0.05$) between control and submerged individuals are represented by asterisks (*).

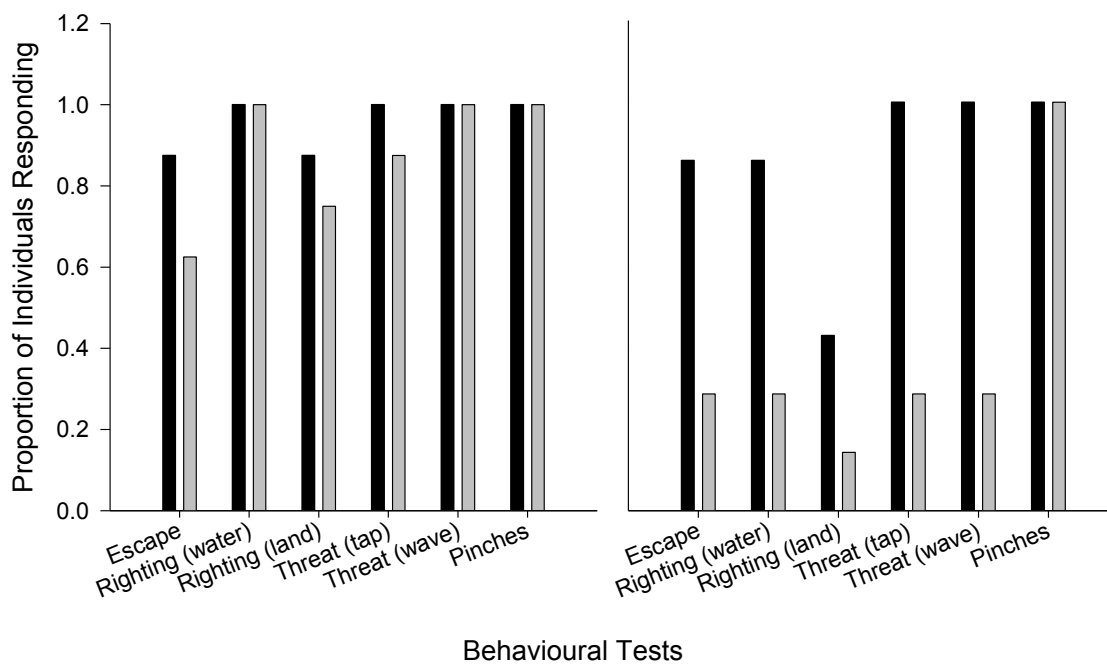


Figure 3-4 - Proportion of individuals that responded with a positive response for each behavioural test for each species; eastern musk turtles (*Sternotherus odoratus*; left) and painted turtle (*Chrysemys picta*; right). Black bars represent control individuals and submerged individuals in grey.

Chapter 4: General Discussion

The purpose of this thesis was to assess sub-lethal consequences associated with freshwater turtle bycatch in a small-scale commercial fishery. Immediate effects were investigated in Chapter 2 by assessing impairment related to entrapment using physiological parameters, such as blood lactate and pH as well as behavioural and reflex indicators. This investigation revealed the presence of species-specific responses to submergence and the potential for incongruence between physiological and behavioural responses. In Chapter 3, I evaluated the post-release locomotory consequences and potential delayed mortality related to entrapment.

Findings and Implications

To date, very few studies have investigated freshwater turtle bycatch (e.g., Barko *et al.* 2004; Lowry *et al.* 2005; Fratto *et al.* 2008; Larocque *et al.* 2012abc). Although immediate mortality is a clear concern, the presence of sublethal effects and associated post-release mortality remain largely undocumented and unknown (Davis 2002). Sub-lethal effects and associated mortality can have important population consequences, especially in long-lived, slow-growing species such as turtles (Congdon *et al.* 1993; 1994). I revealed that entrapment of three species of freshwater turtles (map, painted and musk) caused physiological impairments compared to control groups. Behaviour was also impaired following submergence in both painted and map turtles, although musk turtles were not impaired after exposure to simulated entrapment conditions. These results suggest that despite the presence of physiological impairment following entrapment, these effects are not always manifested behaviourally, suggesting a disconnect between

physiology and behaviour. Furthermore, I did not see any differences between males and females when exposed to simulated entrapment, which implies that both sexes respond similarly. I have also demonstrated the limitations of using surrogate species in a diverse turtle community.

In Chapter 3, I used fine-scale tri-axial accelerometers to assess the presence of locomotory impairment post-release in freshwater turtles exposed to simulated entrapment conditions. Until now, the post-release effects and associated fate of released freshwater turtles were unknown. I used overall dynamic body acceleration (ODBA) as an estimate of locomotory activity over three time periods: initial (1 hour post-release at 10 minute intervals), short-term (6 hours post-release at 1 hour time intervals) and medium-term (48 hours post-release at 6 hour time intervals) using painted turtles (*Chrysemys picta*) and eastern musk turtles (*Sternotherus odoratus*). Although I did not find post-release mortality in any individual exposed to simulated capture, submergence time (4 hours) was very conservative as entrapment times at similar temperatures can range up to 48 hours and or even longer (e.g., up to 7 days in Ontario) at cooler temperatures during real fishing. Furthermore, individuals of both species that were exposed to simulated entrapment displayed lower levels of locomotory activity over the first 6 hours following release. However, that difference disappeared after 48 hours, suggesting the ability to recover.

In conclusion, my results from Chapters 2 and 3 suggest that turtles encountered as bycatch display sub-lethal effects immediately after capture and several hours post-release. Managers will need to acknowledge and incorporate the presence of these sub-

lethal effects into management plans. Capture in fyke-nets results in harm and harassment of these at-risk freshwater turtles, which is in direct violation of the Canadian Species at-Risk Act (2002, c.29, s.32). Furthermore, documenting the presence of species-specific responses and tolerance is useful when strategizing the use of various bycatch reduction programs (Hall *et al.* 2000). Knowing how species respond to submergence and associated tolerances can be useful when implementing bycatch reduction devices, as 100% exclusion rate is rarely possible. This thesis will give managers better information on how the sub-lethal effects associated with incidental capture are affecting freshwater turtles both immediately and post-release.

Future Directions

This thesis contributed important information towards quantifying the sub-lethal effects associated with freshwater turtle bycatch. This is the first step towards identifying the individual consequences associated with entrapment, excluding individuals that succumb. Further steps would be to evaluate the sub-lethal effects on all turtle species encountered in this fishery. Although I was able to study the presence of post-release mortality in musk and painted turtles, map turtles are particularly poor at tolerating submergence (Reese *et al.* 2001). In addition, further research should investigate the effect of longer entrapment periods, more typical of the actual fishery. My entrapment durations (3 to 4 hours) were conservative as I wanted to expose animals to reasonable stressors without causing mortality. Conducting these studies earlier in the season at cooler temperatures would allow longer, more realistic submergence periods to be used.

In terms of assessing long-term effects associated with entrapment, important next steps should evaluate how sub-lethal effects associated with entrapment influence the fitness of individuals. Furthermore, using this information, we could determine how sub-lethal effects influence populations and if these effects can result in population declines. Using this information to assess the conservation status of these populations and the effects of incidental capture on freshwater turtles can help managers make the necessary decisions and changes.

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