LIFE-SKILLS TRAINING FOR JUVENILE LAKE STURGEON (*ACIPENSER FULVESCENS*)

A Thesis Submitted to the College of

Graduate Studies and Research

in Partial Fulfillment of the Requirements

for the Degree of Master of Science

in the Department of Biology

University of Saskatchewan

Saskatoon

By

Janelle R. Sloychuk

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Abstract

Hatchery supplementation of declining fish populations is used for increasing year-class strength, particularly when fish are released with knowledge of local predators. The ability of young-of-the-year lake sturgeon (*Acipenser fulvescens*) to avoid predation, as well as their vulnerability to predation, remains undocumented. The objective of my thesis was to determine: 1) whether hatchery-reared, predator-naive juvenile sturgeon would respond to alarm cues from injured conspecific cues, a reliable indicator of predation risk in other fishes; and 2) if sturgeon would learn to identify unknown predators through a Pavlovian-like conditioning with conspecific alarm cues. Releaser-induced recognition learning is a variant of Pavlovian learning in which recognition of a previously neutral stimulus is acquired through the experience of pairing a behaviourally active releasing stimulus and a novel stimulus. Sturgeon were initially conditioned using a behaviourally active stimulus of sturgeon alarm cue, paired with a behaviourally neutral stimulus of novel northern pike (*Esox lucius)* odour, or were pseudo-conditioned with distilled water paired with pike odour. Following conditioning, sturgeon were tested for recognition of the predator odour 24 hours later. The first population of fish (Rainy River) showed a dramatic antipredator response to alarm cues from the skin of conspecifics, but failed to exhibit learning of a novel predator through conditioning with alarm cues obtained from the skin of conspecifics. However, when Rainy River fish were conditioned with alarm cues from the whole body of conspecifics, they showed strong learning of the predator. Conditioning Wolf River fish to recognize predators with whole body extract had no effect on response to predator odours. However, when the fish were conditioned multiple times there was evidence of predator learning. These results highlight potential opportunities and limitation to life-skill training of artificially reared sturgeon for future conservation initiatives.

Acknowledgments

Table of Contents

List of Figures

Chapter 1: Introduction

1.1. The lake sturgeon (*Acipenser fulvescens*): ecology, population status and conservation

1.1.1 Sturgeon ecology and life history

Lake sturgeon (*Acipenser fulvescens)* evolved between 200 and 300 million years ago during the lower Jurassic period and have since remained relatively similar morphologically, and likely behaviourally and ecologically (Sulak and Randall 2002). They belong to the order Acipensiformes, and the genera *Acipenser,* along with 15 other extant species of sturgeon. The life history of the lake sturgeon is characterized by high fecundity, late maturation and high early mortality in eggs and early life stages (Winemiller and Rose 1992). Lake sturgeon in the Wolf River (Wisconsin, USA) become sexually mature as late as 20–25 years in females, while males first spawn at age 12–15 (Bruch *et al* 2001). Age to maturity is later in northernmost populations with males maturing by age 19, and females maturing between 22–26 years old in the South Saskatchewan River (McLeod *et al* 1999). The delay in maturation in sturgeon is due to a disproportionate amount of energy allocated towards somatic growth during the juvenile stage (Beamish *et al* 1996), thus maximising the size at maturity and limiting mortality to the earliest life stages (Peterson *et al* 2007). Sturgeon spawn periodically, with females spawning every 3–5 years and males spawning every other year (Bruch *et al* 2001). Despite spawning periodicity, the longevity of lake sturgeon, with a life-span greater than 50 years (Birstein 1993), increases the likelihood that they will contribute to at least one spawning event that results in successful recruitment (Duong *et al* 2013).

Optimal spawning habitats for sturgeon are typically flowing streams and rivers with clean rock or cobble substrate to allow for proper egg adhesion as well as interstitial space for newly hatched larval sturgeon (Bruch and Binkowski 2002). Fecundity of sturgeon in the South Saskatchewan River has a reported range of 117,450 (25 years of age; 15.5 kg) to 607,400 eggs (51 years of age; 45.5 kg) (McLeod *et al* 1999). Sturgeon are aggregate spawners, and do not participate in parental care or nest preparation, thus larval lake sturgeon experience extremely high mortality (Duong *et al* 2011). While lake sturgeon populations are thought to be sustained by infrequent episodes of highly successful recruitment under environmentally favourable conditions (Bruch and Binkowski 2002), recruitment failure is a significant cause for the decline of many North American sturgeon species (Jager *et al* 2001). Low water flows affect the survival of embryos as they experience low oxygen concentrations, variable water temperatures, as well as desiccation. Juvenile sturgeon may be trapped in shallow pools and subject to temperature and oxygen stress (Ferguson and Duckworth 1997).

Life histories of most populations of sturgeon are poorly studied (Kynard *et al* 2010), and in particular, a knowledge gap exists for juvenile life stages (Secor *et al* 2002). Specifically, early life stage mortality, movement and distribution, and the effects of predation on recruitment of age-0 sturgeon remain unclear (Caroffino 2009). It is imperative to identify life history information for juvenile sturgeon as it is thought that juvenile fish measuring less than 600 mm have the most significant influence over population growth (Gross *et al* 2002; Barth *et al* 2009). One common cause of mortality of fish in early life stages is predation (Gadomski and Parsley 2005a), which may also be amplified in low water conditions (Ferguson and Duckworth 1997). Early life mortality can play a major role in shaping population dynamics of a species (Houde 1987), however, early life stages of sturgeon are generally very difficult to sample *in situ* (Secor

et al 2002), and little empirical information exists regarding natural sturgeon growth, mortality

and survival (Secor *et al* 2002). Research on the vulnerability of sturgeon larvae and young

juveniles to predation is lacking (Gadomski and Parsley 2005a) but it is generally accepted that

natural mortality of sturgeon is mostly limited to younger life stages (Peterson et al 2006).

1.1.2 Population status

Of the 27 species of paddlefish and sturgeons recognized worldwide, most are currently

viewed as either endangered, threatened or functionally extirpated (Birstein 1993). All sturgeon

species are now listed as endangered in part of their ranges, and most in all of their ranges

(Birstein *et al* 1997; Billard and Lecointre 2001). Lake sturgeon are widespread in Canada and

are found from Alberta to Quebec (Ferguson and Duckworth 1997). Historically, lake sturgeon

were harvested for Aboriginal use, and later by commercial fisheries for caviar, meat, and

isinglass (Harkness and Dymond 1961). High initial yields quickly and permanently declined,

which led to the closure of many North American sturgeon fisheries (Ferguson and Duckworth

1997).

In addition to overexploitation, habitat alteration is a key component in the decline of

sturgeon populations. The construction of hydroelectric dams contributed significantly to the loss

of habitat as well as population fragmentation. Dams were often constructed at prime sturgeon

spawning grounds, and water fluctuations due to hydroelectric generation may prevent optimal

flows required for successful recruitment (Ferguson and Duckworth 1997). Hard-bottom and

structured habitats are critical as spawning substrates (Parsley *et al* 1993), and successful

spawning events are dependent upon flow and temperature conditions (Jager *et al* 2001). Thus,

variable flow regimes have accounted for a lack of egg adhesion sites and physical cover for

larvae (reviewed by Coutant 2004; McAdams, Walters, Nistor 2005). Dredging, road

3

construction, and floodplain abstraction have also degraded critical sturgeon habitat as well as the water quality necessary for recruitment (Ferguson and Duckworth 1997). Sturgeon larvae and eggs are susceptible to suffocation by the additional sediment, and the benthic community, which is the primary food source of juvenile lake sturgeon, may be disrupted by habitat degradation due to industrial activities (Caroffino 2009; Kempinger 1996).

Similar to other North American populations, lake sturgeon in the North and South Saskatchewan Rivers have been subjected to population-threatening habitat alteration and overharvesting (North/South Consultants Inc. 2002). Commercial harvests during the early 1900s marketed as much as 56,000 kg/year in the early 1900s but the harvest quickly declined to 6000 kg/year by the 1930s, declining further to 1000 kg/year during the 1990s. Index fishing programs implemented in 1996 by the Saskatchewan River Management Board revealed a dangerously low population of 1300 fish, 8 kg and over that was still subject to overharvesting (North/South Consultants Inc. 2002). The construction of hydroelectric dams on the Saskatchewan and South Saskatchewan Rivers during the 1960s fragmented the sturgeon into several sub-populations (McLeod, *et al* 1999). A fishing moratorium on commercial and sport fishing harvests has been enforced in Saskatchewan since 1996 (Peterson *et al* 2007) and the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) has listed the Saskatchewan River populations as endangered (2006).

1.1.3 Conservation efforts and stocking programs

With sturgeon in danger of extirpation world-wide (Birstein *et al* 1997), harvesting bans on caviar and sturgeon meat fisheries, in addition to sport-fishing bans, have been imposed in many regions to protect sturgeon populations from extinction. However, these management actions have been futile in slowing the decline of these species. The life history attributes of

sturgeon present a particular challenge to conservation managers. Traits such as late age-at-maturity, infrequent periodic reproduction, and long life expectancy make population assessments challenging. Regional harvest management becomes difficult in highly migratory sturgeon populations, as certain populations may migrate as much as 500 km over their life cycle (DeVore *et al* 1999).

The global decline of sturgeon has caused the development of culture methods for conservation stocking (Doroshov 1985) as well as for the commercial sale of meat and caviar. Conservation stocking for lake sturgeon has been employed in some regions to reintroduce or rehabilitate sturgeon to create self-sustaining populations in their native ranges. Evaluations of hatchery-produced sturgeon indicate that they can survive to adulthood and contribute to fisheries and spawning populations, particularly in depressed populations (Secor *et al* 2000). However, there is no guarantee that hatchery programs will always initiate natural recovery. As wild brood stocks are used for hatchery programs, a problem of depressed natural populations means that the number of breeding females is limited. Thus, program managers need to be able to minimize inbreeding and artificial selection, a particular challenge with slow-maturing, long-lived species. Another challenge exists in the inadequate understanding of how artificial rearing environments affect the post-release behaviour of these fish (Secor *et al* 2002; Crossman *et al* 2011). Lake sturgeon culture has typically been performed using water sources that are different than the natal river or the river in which the sturgeon will be placed, which poses a problem as this species that is known to imprint and return to natal rivers (DeHaan *et al* 2006; Crossman *et al* 2011,2014). A greater understanding of habitat requirements, early life history traits as well as population biology, is essential in order for rehabilitation stocking to be an effective recovery strategy.

On account of their life history characteristics, lake sturgeon stocking programs are difficult to assess in the long-term, and only two published studies have evaluated the efficacy of stocking. A report on the St. Louis River, Lake Superior, developed from 1985 through 2007, determined that the majority of stocked sturgeon did not migrate to other areas of the lake from where they were stocked (Schram 2007). Additional work by Drauch and Rhodes (2007) evaluated the genetic composition of supplemented lake sturgeon populations, and emphasized the importance of multi-year stocking strategies to prevent the loss of genetic diversity from the source to introduced populations. Assessment of lake sturgeon stocking programs require a minimum of 15–20 years for comprehensive program results, thus many programs have not been adequately evaluated and rely on intermittent, short-term or anecdotal indicators of program success.

1.2. Antipredator defences

Predation is a major selective force that can affect the morphological and behavioural characteristics of animals (Lima and Dill 1990). While in this interaction a predator can bear the cost of losing a meal, the prey bears the disproportionate cost of losing its life (Dawkins and Krebs 1979). Thus, prey animals are under pressure to respond to the threat of predation. Prey may adapt by selecting safe habitats for reproduction and foraging, and may also develop social behaviour, such as shoaling, to avoid predation. They may also adapt through learning how to identify predators and how to assess the risk that a particular predator may pose.

Natural environments are dynamic, and the risk of predation is temporally and spatially variable throughout the lifetime of a prey animal. While prey can develop specific evolutionary phenotypes, a single defensive phenotype in prey may not always be an optimal strategy under variable threat levels (Dewitt 1998). Strategies and traits to improve survival can be quite costly

6

to an organism, thus phenotypic plasticity provides an adaptive strategy for responding to threats in an environment (Dewitt 1998). Morphological, life-history, and behavioural traits can all be influenced by the threat of predation (Brown *et al* 2013b).

Prey animals can determine risk by using a variety of visual, chemical and mechanical cues, but much of the work in aquatic systems has focussed on chemosensory information, specifically semiochemicals, detected by prey. There are three classes of semiochemicals: kairomones, disturbance cues, and damage-released alarm cues (Ferrari *et al* 2010a). Kairomones are cues emitted by one species that are detected by another, such as the scent of predators detected by prey. Kairomones are adaptively favourable to the receiver but not the sender, and enable prey to detect and avoid potential predators (Ferrari *et al* 2010a). Disturbance cues may be emitted in the form of urinary ammonia from disturbed or startled prey (Kiesecker *et al* 1999; Brown and Vavrek 2003). Finally, damage-released alarm cues are derived from epidermal club cells and are detectable by conspecifics when skin is damaged. Karl von Frisch discovered that a fright reaction can be elicited in European minnows (*Phoxinus phoxinus*) when they are exposed to cues from injured conspecifics (von Frisch 1941). These substances advertise a foraging predator and are a reliable indicator of risk. Damage-released alarm cues have been recognized in a wide range of fish species, including ostariophysans, salmonids, gobies, poeciliids, percids, cottids, cichlids and centrarchids (Chivers and Smith 1998; Brown 2003). Upon detection by conspecifics, damage-released chemical alarm cues elicit dramatic changes in morphology, life-history and behaviour (Ferrari *et al* 2010a). At the time I began my thesis work, it was not known if sturgeon possessed or responded to damage-released chemical alarm cues that warn conspecifics of danger.

1.2.1 Morphological adaptation

To cope with unknown threats, some prey species have defensive features such as spines, armour, or other physical adaptations that have acquired over evolutionary time. These morphological adaptations, while costly to the organism, protect prey in environments that contain a predictably high amount of risk of predation (Brown *et al* 2013b). Morphological changes, that provide defense against predation, can also be induced by predators in a number of aquatic prey (Kusch 1993; Lass and Spaak 2003; Ferrari *et al* 2010a). For example, crucian carp (*Carassius carassius*) may alter their depth-to-length ratio, growing deeper in response to cues from piscivorous northern pike (Brönmark & Miner 1992; Brönmark & Pettersson 1994; Nilsson et al. 1995). Increasing the depth-to-length ratio is an effective defence against gape limited predators. Shell morphologies of snails are likewise affected by predation cues. The morphological response can even be modulated by specific types of predators that threaten them, with snails growing narrow apertures in response to crayfish feeding cues, and wider apertures in response to predacious water bugs (Hoverman *et al* 2005).

Adult sturgeon are large armoured fish with five rows of large, bony scutes (2 lateral, 2 lateroventral and 1 dorsal), as well as a subopercle bone over the gills that may provide morphological defense against predation. This morphological defense in sturgeon likely evolved under Mesozoic Era conditions with predation pressure from large piscivores (Sulak and Randall 2002). Larval sturgeon lack protective scutes and participate in drifting behaviour that may make them more susceptible to predation, however, documentation of predation of larval fish is difficult due to small body size and rapid digestion (Kim and DeVries 2001; Caroffino *et al* 2010). Sturgeon develop scutes during the larval stage at approximately 25 mm total length

(Gadomski and Parsley 2005a). In young lake sturgeon, the scutes are very sharp and form prominent apical hooks, but gradually become smoother with age and are eventually nearly absorbed in adults (Scott and Crossman 1973). Unlike lake sturgeon, anadromous species of sturgeon may retain scutes that continue to grow throughout their life (Vescei 2011). Thus, the reabsorption of scutes by lake sturgeon may demonstrate a functional morphological trade-off. Scutes may bear a greater cost than benefit in the absence of large predators.

Another morphological defence in sturgeon is their cryptic colouration. Larvae develop dark pigmentation at 15 mm total length. The changes in colour morphology of lake sturgeon as they develop from early juvenile to adulthood is very pronounced (Peterson *et al* 2007), with black speckling and black "saddle marks" on fish <300 mm lightening and eventually disappearing with age. Juvenile lake sturgeon may depend on this cryptic colouration and morphological defense to avoid predation (Caroffino *et al* 2010),

Any consideration of behavioural defences of lake sturgeon needs to keep in mind that the fish also have morphological defences that function to reduce detection and capture. As sturgeon develop morphological defences, such as the growth of sharp bony scutes, they may rely less upon antipredator behaviour (i.e. compensation correlation between antipredator morphology and behaviour) (Wishingrad et al 2014a). Alternatively, behavioural and morphological traits can either jointly (i.e. complementation) or discretely (i.e. cospecialization) provide effective protection against predation. Since larval sturgeon do not possess effective morphological defenses of bony scutes or cryptic colouration, potential antipredator behaviour may be most obvious at this stage if a compensation relationship exists between morphology and behaviour. Investigation of the potential antipredator behaviour of juvenile sturgeon may provide further insight on the relationship between morphological and behavioural defense traits.

1.2.2 Behavioural adaptations

Compared to morphological adaptations and life-history switches, behavioural adaptations in response to experience are far more plastic (Brown *et al* 2013b) and may be a less costly method of avoiding predation. With advanced detection of predators, prey may perform adaptive antipredator responses such as avoiding risky areas, increasing vigilance, shoaling and shelter use, or decreasing activity (Lawrence and Smith 1989; Lima and Dill 1990; Sih 1995; Ferrari *et al* 2010a).

Newly hatched lake sturgeon are negatively phototactic, benthic, and actively seek cover within the interstitial spaces of rocky substrates (Harkness and Dymond 1961; Kempinger 1988; Peterson *et al* 2007). Lake sturgeon prolarvae have an undeveloped mouth and eyes and will remain in hiding for approximately 7 days (Czeskleba *et al* 1985; Hastings *et al* 2013). After this period, prolarvae will shed their anal plug at the onset of exogenous feeding, marking the transition to the larval stage. At about 17–18 mm in size, the larvae will disperse downstream by drifting during the night, often over several kilometres, before settling on the river bottom. Larval lake sturgeon were found to decrease their preferences of rock cover after 20 days post hatch and increase their use of open bottom habitats, and time spent in the water column. The larval stage lasts for several weeks, depending on water temperature. The transition from larval to juvenile sturgeon is marked by the complete formation of definitive fin structures, at around 40 mm total length (Kempinger 1988). At this point their activity level places them at greater risk and hence we may expect behavioural defences to increase at this point in time. As individuals grow their ability to escape should also increase and this change could influence their responses to predation threats.

1.2.3 Learned predator recognition

In order for prey to display appropriate antipredator responses to threats, they must first recognize predators as dangerous. While a few cues are innately categorized as risky, such as injured conspecific cues, most predator-specific cues need to be learned. If prey are instead able to learn or acquire a response to ecologically-relevant stimulus through experience, they might perform more accurate risk assessment and make decisions according to the level of perceived threat. Exposure to a novel stimulus in the presence of an aversive (risky) cue can facilitate an association of risk with the novel stimulus. This type of acquired recognition of predators is not only limited to direct experience, but can also occur by cultural transmission or social learning (Mathis *et al* 1996).

Traditional Pavlovian learning is defined by a set of procedures by which a neutral conditioned stimulus (CS) is paired with a biologically-significant unconditioned stimulus (US). The US elicits strong innate behavioural responses, and as a result of CS-US pairings, the CS becomes associated with the US. For Pavlovian learning, the unconditioned response and the conditioned response are the same response, however the unconditioned stimulus becomes the releasing stimulus of this behaviour as a result of conditioning. The conditioned response (CR) results from the pairing. In the case of chemically-mediated learning in aquatic prey animals, the US can be conspecific alarm cue that elicits an innate behavioural response, and the CS is a neutral stimulus, such as the odour of a novel potential predator (Siegel and Kreutzer 1997). Pavlovian conditioned responses result from the specific temporal combinations of the US and CS.

Releaser-induced recognition learning is a variant of Pavlovian conditioning (Suboski 1990). By exposing an animal to a behaviourally active (releasing US) stimulus and a behaviourally neutral (CS) stimulus, the pairing will result in a behavioural change based on the experience. Through the pairing of the stimuli, the previously neutral stimulus evokes appropriate behavioural responses. An important distinction of Milton Suboski's releaser-induced recognition-learning model (1990), is that pairings of stimuli are not limited to the predictive temporal relationships of Pavlovian conditioning. While timing of the presentation of stimulus is integral to traditional Pavlovian conditioning, the releaser-induced recognition learning model suggests that various stimulus relationships can activate the transfer-of-control mechanism. While in traditional Pavlovian learning the conditioned stimulus must be presented before the unconditioned stimulus, the presentation of damage-released alarm cue and novel predator odour do not have to occur simultaneously in order for a conditioned response to result. The alarm cue keeps the prey animal in a receptive state towards novel conditioned (Wisenden 2011), with chemical signals degrading as soon as 15 minutes depending on environmental conditions (i.e. Carbon dioxide concentration (Chivers *et al* 2014)), and persisting for as long as several hours (Wisenden *et al* 2009). Zebra fish (*Danio rerio*) have formed associations between the CS and the CR when alarm cue and pike odour were presented as long as five minutes apart (Korpi and Wisenden 2001). Another unique property of releaser-induced recognition learning is that a single conditioning event of the CS with the US can result in near-permanent associations between the CS and the CR (Suboski 1990; Wisenden 2011). Thus, the releaser-induced recognition model suggests that evolutionary learning processes have been opportunistic in allowing animals to recognize biologically important objects or events, with slow learners becoming efficiently removed from the gene pool over evolutionary time.

12

1.3. Life-skills training

Hatchery-reared fish are routinely stocked into natural waterways in an attempt to restore or maintain stable populations. One of the pitfalls of these stocking programs is the dramatic mortality of newly stocked individuals (Suboski and Templeton 1989; Brown and Smith 1997, 1998). Learned recognition of novel predators has been demonstrated to significantly increase an individual's survival during subsequent predator encounters (Mirza and Chivers 2000; Gazdewich and Chivers 2002). Thus, predator training has been employed in attempts to condition hatchery-reared fish (predator-naive) prior to release. Berejikian *et al* (1999) found that juvenile chinook salmon (*Oncorhynchus tshawytscha)* experienced increased survival rates upon training with alarm cues to recognize cutthroat trout *(Oncorhynchus clarkii)*. The fish that received the paired alarm substance may have been better able to recognize and respond to the presence of cutthroat trout, thereby reducing risk of capture. The benefit of this type of conditioning may be highest in the first few days following release, after which acquired predator recognition will likely occur in the post-release environment. The effects of aquaculture on salmonids have been studied extensively. However, inferring results from salmon to sturgeon may not be valid on account of the significantly different life histories between these families (Jager 2005). Thus, understanding the effects of aquaculture on sturgeon, and other fishes with unique life histories, requires further investigation.

1.4. Research objectives

My overall objective is to examine the antipredator behaviour of lake sturgeon. In this thesis, I present three data chapters examining how juvenile sturgeon respond to chemical alarm

cues and if they are able to form a learned recognition of a novel predator odour through conditioning with alarm cues. Specifically, I used northern pike (*Esox lucius)* and juvenile sturgeon from Rainy River and Wolf River to answer the following questions:

Do sturgeon exhibit antipredator behaviour to chemical alarm cues? If yes, what is the behaviour?

In Chapter 2 of my thesis, I observe antipredator behaviour in juvenile sturgeon from the Rainy River population to alarm cues prepared from the skin of conspecific skin. Since sturgeon may be opportunistic and cannibalistic feeders, I also isolate the response of juvenile lake sturgeon to conspecific alarm cue by exposing the fish to other novel cues, including food odours.

Can sturgeon exhibit learned predator recognition?

In Chapter 2, I also test whether juvenile sturgeon from Rainy River could learn to recognise a novel predator through the pairing of predator odours with alarm cues from the skin of injured conspecifics. Given the failure of the fish to learn, in Chapter 3, I tested whether juvenile sturgeon from both Rainy River and Wolf River populations could learn to recognise a novel predator through the pairing of whole body alarm cues and predator odour. Associative learning can occur after a single conditioning event (Ferrari *et al* 2010a), however increased opportunities to learn may result in increased information regarding the threat of predation. In Chapter 4, I investigate whether Wolf River sturgeon may be conditioned to recognize a novel predator when they are exposed to a series of conditioning events, rather than just a single event.

Anticipated significance

My research is aimed at exploring questions about behaviour of juvenile lake sturgeon in relation to predation. Little is known about the behaviour of juvenile sturgeon and their vulnerability to predation. A few studies have explored the potential for predation on juvenile white sturgeon (*Acipenser transmontanus)*; however, no studies to date have explored the potential for learned predator recognition in sturgeon species (Wishingrad *et al* 2014b). I use a number of novel paired cues in this experiment to determine typical antipredator behaviour of juvenile sturgeon, and whether or not sturgeon exhibit predator recognition through learning. Since predation may limit successful recruitment of threatened or endangered sturgeon populations, an understanding of juvenile sturgeon survival behaviour is essential for conservation programs.

Chapter 2: Antipredator behaviour and learned predator recognition by sturgeon

2.1. Introduction

Environmental cues can inform aquatic animals of the presence of food, predators or reproductive opportunities. Cues such as amino acids can indicate the presence of food, while sex pheromones may stimulate receptors in other individuals. These types of cues initiate behavioural responses in a mechanical, stimulus-response manner (Wisenden 2011). Predatory cues, however, differ from foraging and reproduction indicators in that risk varies greatly over ecological space and time, as well throughout the ontogenetic life stages of an individual. While foraging and reproduction are limited to a narrow range of predictable cues, the size, shape, odour, and sound of predators are highly variable for prey animals. A mistake made by prey in detecting and responding appropriately to antipredator cues is far more costly than an error in detecting and responding to foraging or reproduction cues. Damage-released alarm cues are a reliable indicator of predator attacks, and not surprisingly evoke strong and immediate antipredator responses in conspecifics. Antipredator responses to alarm cues have been identified in many aquatic species (Smith 1992; Chivers and Smith 1998; Ferrari *et al* 2010a), but until recently, had not been confirmed in Acipenseriformes (Wishingrad *et al* 2014a, b, c). Alarm cues are contained in distinctive club cells in the epidermis of Ostariophysan fish, and are only released by mechanical damage to the skin. Thus, established protocol for the preparation of an alarm cue stimulus is to remove skin fillets from donor fish to create a homogenate which is then filtered for use as an indicator of risk to conspecifics. When this alarm cue homogenate is presented in conjunction with a novel neutral stimulus, like the odour of a novel predator, conspecifics subsequently learn to associate the neutral stimulus with danger. This learned

association is called releaser-induced recognition learning, a variant of Pavlovian conditioning (Suboski 1990).

Prey that display antipredator behaviour in response to alarm cue gain significant survival benefits (Mathis and Smith, 1993). During conditioning, prey may perform various antipredator behaviours including changing activity, using shelter, or area avoidance. A typical response that is measured under laboratory conditions is a decrease in activity levels when comparing the period before versus after the addition of the stimulus. Many animals reduce activity to reduce their visibility by hunting predators (Lima and Dill 1990). In contrast, other animals may initiate a fleeing response to risk.

The objectives of this Chapter were two-fold. First, to document the responses of juvenile lake sturgeon to conspecific skin extract and a variety of other odours including food and novel fish odours. The second objective was to test whether sturgeon could learn to recognize the predator through the pairing of conspecific alarm cue and novel predator odour prepared using established protocol. When a fish detects a potential threat it has a variety of behavioural options. Fish could stop moving thereby increasing their crypsis and avoiding detection by the predator. In contrast, they could exhibit dashing behaviour and flee away from the predator. For fishes that typically live in rivers, fleeing into the water column could help them to escape benthic predators, such as crayfish and other bottom dwelling fish, and indeed Kynard et al (2010) reported on the swim-up behaviour of disturbed juvenile sturgeon. Since sturgeon are benthic foragers and may flee into the water column in response to risk, I hypothesized that sturgeon would spend more time in the bottom third of the tank in response to feeding cues than alarm cues. At the time I initiated my work, there was nothing known about behavioural responses of lake sturgeon to alarm cues, but both increases and decreases in activity are explainable in the

17

context of antipredator behaviour. The critical issue in interpretation of behavioural responses is whether we can differentiate feeding responses from responses to risk. Subsequent to me initiating my thesis work, Wishingrad et al. (2014 a, b, c) documented that juvenile sturgeon show dramatic fleeing responses to alarm cues. I hypothesized that the pairing of conspecific skin odour with a novel predator odour would result in learned predator recognition. This could be manifested through either a reduction in activity or a fleeing response, including movement into the water column.

2.2. Methodology

2.2.1 Test Species

Juvenile lake sturgeon used in this experiment were obtained as larval sturgeon from Sustainable Sturgeon in Emo, Ontario in May 2012. These sturgeon originated from brood fish from the Rainy River, and are referred to throughout the experiments as the Rainy River population. Sturgeon were kept in 70-L tanks as larvae and moved to a 650-L circular flow-through tank once they were free-swimming juveniles. Fish were fed adequate food depending on their size and stage (larval sturgeon: brine shrimp larvae; juvenile sturgeon: frozen blood worm). Larval sturgeon were fed every 4 hours to satiation in accordance with the white sturgeon standard operation procedures provided by the University of Saskatchewan Aquatic Toxicology Centre (Tompsett, *et al* 2010). The temperature of the holding tanks for juvenile sturgeon was maintained at 15 ± 1ºC while the temperature of the experimental tanks was held at 18±1 ºC. The photoperiod was held constant at 14h L:10h D hr in both holding tanks and experimental tanks. The experiments were carried out from October 15 to November 5, 2012, when the Rainy River sturgeon were between 140 and 161 days post hatch (mean ± SD Total Length 7.1 ± 0.7 cm).

Predator odour used for all experiments in this thesis was collected from piscivorous northern pike. Six northern pike were captured in June 2013 from Pike Lake, Saskatchewan, using seine nets. The pike were housed in a 6000-L flow-through circular tank at 12 ± 1ºC. Pike were fed *ad libitum* with live fathead minnows.

2.2.2 Stimulus preparation

Sturgeon skin extract

Skin-derived alarm cue was prepared following established protocols (Ferrari *et al* 2005). In sturgeon, skin-derived alarm cue was shown to be effective indicator of risk at a concentration of 25 cm^2 of skin $\cdot$ L^{-1} (Wishingrad *et al* 2014b*)*. The skin fillets were obtained from six juvenile sturgeon 6.4 ± 0.4 cm. In order to ensure that solutions were prepared with equal amounts of tissue, surface area of tissue used for sturgeon alarm cue preparation was converted into a weight measurement. Thus, approximately 3.2 g of tissue was diluted in 1 L of distilled water, homogenized using a Polytron homogenizer (Brinkman Instruments, Rexdale ON) and filtered through glass wool. Solutions were frozen in 50 mL aliquots at -20 ºC until required.

Pike odour

Five pike (mean $\pm$ SD total length $= 20.8 \pm 10.3$ cm) were fasted in their holding tank for 7 days to avoid the presence of diet cues (Brown *et al* 1995). Pike were then moved to individual aerated, unfiltered 30-L stimulus collection tanks. The pike remained in stimulus-collection tanks for 24 h, at which time they were transferred back to their original holding tank. Water from individual pike was combined and frozen in 60 mL aliquots at -20 ºC until required.

Carp odour

Common carp (*Cyprinus carpio)* odour was obtained from five laboratory-raised carp (mean $\pm$ SD total length $= 12.2 \pm 1.7$ cm). The carp, fed daily on a diet of floating pellets Top

Fin® Pond Fish Food, were fasted in their holding tank for 3 days prior to being moved to individual aerated, unfiltered 30-L stimulus collection tanks. The carp remained in stimulus-collection tanks for 24 h, at which they were transferred back to their original holding tank. Water from individual carp was combined and frozen in 60 mL aliquots at −20 ºC until required.

Bloodworm extract

Bloodworm (family Chironimidae) extract was prepared from frozen bloodworms (Hikari Bio-Pure Frozen Blood Worms) that had been thawed and drained. Bloodworms were then weighed and 100 mL of distilled water was added. The mixture was homogenized using a Polytron homogenizer (Brinkman Instruments, Rexdale ON) and filtered through glass wool. Distilled water was added until the solution was diluted to ~ 3.2 g · L^{-1}, a concentration comparable to that of skin-derived sturgeon skin extract. The solution was frozen in 50 mL aliquots at −20 ºC until required.

Minnow extract

Minnow extract was prepared from six fathead minnows (mean ± SD total length = 3.5 ± 0.3 cm). Minnows were killed by cervical dislocation (in accordance with the Canadian Council on Animal Care) and weighed. The whole-body minnows were placed in 100 mL of distilled water and homogenized using a Polytron homogenizer (Brinkman Instruments, Rexdale ON). The solution was filtered through glass wool to remove remaining tissue before diluting the extract to ~ 3.2 g · L^{-1}. Minnow extract was frozen in 50 mL aliquots at −20 ºC until required.

2.2.3 Experimental protocol

The experiment consisted of two phases: conditioning trials followed by recognition trials. During conditioning trials, sturgeon were exposed to 1 of 5 cues: (1) skin-derived sturgeon alarm cue paired with pike odour. This pairing is known to facilitate learned recognition of

predator odour in many fish species (Ferrari et al. 2010a for a review); (2) minnow alarm cue derived from whole body grind paired with pike odour. Using skin cues from distantly related heterospecific should not induce a learned response and hence is used as a negative control (Ferrari et al. 2010a for a review), (3) bloodworm extract paired with pike odour. This allowed us to compare the behavioural response to a risk cue (sturgeon alarm cues) to that from a feeding cue (bloodworm), (4) distilled water paired with pike odour, to control for the possibility of innate recognition of the pike odour. Finally, we added an extra group exposed to (5) distilled water paired with carp odour alone to ensure those fish do not show a fear response to novel, neutral odour like that of a non-predatory fish. Behavioural observations were undertaken and the responses to the 5 cues compared across groups.

During the testing trials, which occurred 24 h after the conditioning trials, the conditioned fish from the previous phase were exposed to pike odour alone, to assess whether or not they would display an antipredator response to this cue. Fish from group 5 (carp odour) were removed, as this group only served as a control for the conditioning response.

Conditioning trials

Groups of three sturgeon were placed in 37-L tanks, filled with dechlorinated tap water. The tanks had a 3×6 grid was drawn on the front of the tank for behavioural observations (Figure 2.1), while the sides and back of the tank were covered in black plastic sheets to block visual disturbances. The tanks contained a sand substrate and an air stone. A 2-m long piece of plastic tubing was attached near the air stone, and used to inject test stimuli into the tanks while the air stone aided in distribution of the stimuli. Prior to testing, sturgeon were acclimated for a 24-h period in their test tanks (water at 19.0ºC, 14:10 h light:dark cycle).

All trials were conducted between 1000 and 2200 h. Observations consisted of an 8-min pre-stimulus and an 8-min post-stimulus injection period. Prior to the pre-stimulus period, I withdrew and discarded 60 mL of water from the injection tubes (to remove any stagnant water) and then withdrew and retained an additional 60 mL of water for slowly flushing the stimulus into the tank after injection. Following the pre-stimulus period, I injected either 20 mL of pike odour paired with 20 mL of sturgeon extract, minnow extract, bloodworm extract or distilled water, or 20 mL of distilled water paired with 20 mL of carp odour. Upon addition of the stimulus to 37-L tanks, the final dilution was 0.09g of tissue $\cdot$ L^{-1}. Once the stimuli were fully injected, I began the post-stimulus observation period.

As a measure of antipredator response, I recorded the vertical use index of a focal fish (randomly chosen but consistent for the duration of the trial) every 15 sec (1: fish on the bottom row of the tank; 2: fish in the middle row of the tank; 3: fish in the top row of the tank). The number of line crosses (using the 3 x 6 grid pattern drawn on the front of the tank) was also recorded for one of the three sturgeon (randomly selected, the same fish was observed until the end of the conditioning period). All trials were performed blind and the order of stimulus injection was randomized.

Recognition trials

One hour after the end of the conditioning trials, the fish were moved to a similar 37-L tank filled with clean dechlorinated tap water. After 24 h, recognition trials were performed. Following the pre-stimulus period, I injected 20 mL of pike odour into the tank. Once the stimuli were fully injected, I began the post-stimulus observation period. All trials were performed blind and the order of testing was randomized. I tested 100 sturgeon (mean $\pm$ S.D total length. $= 6.4 \pm$ 0.4 cm) in total with $N = 20$ per treatment.
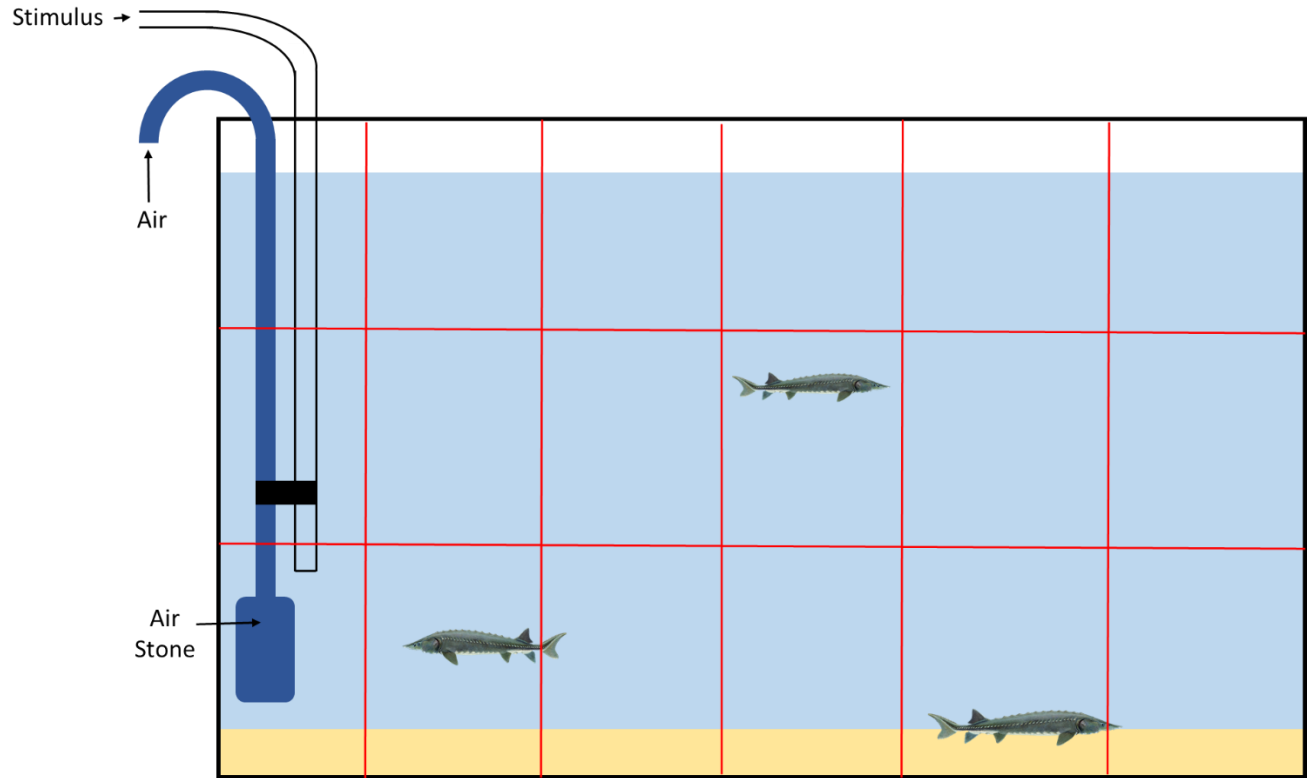
Figure 2.1: Schematic diagram (front view) of tanks used in all conditioning and recognition trials. Sturgeon are drawn to scale.

2.2.4 Statistical analysis

To test whether the fish differed in their behaviour prior to the injection of the stimulus, I ran a 1-way ANOVA on the pre-stimulus data for each of the two response variables (activity and vertical use), for each of the testing periods (conditioning and testing). I then used the differences in behavioural parameters between pre- and post-stimulus injection periods as response variables in subsequent analyses. I performed a 1-way ANOVA on the change in line crosses and change in vertical use during conditioning trials, and again on the change in behavioural parameters during recognition trials. Data met parametric assumptions.

2.3. Results

Conditioning trials

The results of the 1-way ANOVA indicate that there is no significant effect of conditioning cue on pre-stimulus values for line crosses ($F_{4,97} = 0.1$, $P = 1.0$) or vertical use ($F_{4,97} = 0.4$, $P = 0.8$).

Results of the 1-way ANOVA on conditioning trials show a significant effect of conditioning treatment on change in activity ($F_{4,97} = 14.8$, $P < 0.001$, Figure 2.2) and no significant difference of change in vertical use ($F_{4,97} = 0.1$, $P = 0.4$, Figure 2.3). Post-hoc comparisons using the Tukey HSD test indicated that the mean change in line crosses for fish conditioned with alarm cue paired with predator odour was significantly different than all other stimuli, and the other treatments did not differ from each other (Figure 2.2 and 2.3).

Recognition trials

The results of the 1-way ANOVA indicate that there is no significant effect of conditioning treatment on pre-stimulus values for line crosses ($F_{3,78} = 0.1$, $P = 1.0$) or vertical use ($F_{3,78} = 0.8$, $P = 0.6$). The results of the 1-way ANOVA indicate no effect of conditioning treatment on either the change in activity ($F_{3,78} = 1.0$, $P = 0.4$, Figure 2.4) or the change in vertical use ($F_{3,78} = 0.9$, $P = 0.4$, Figure 2.5) of the sturgeon.
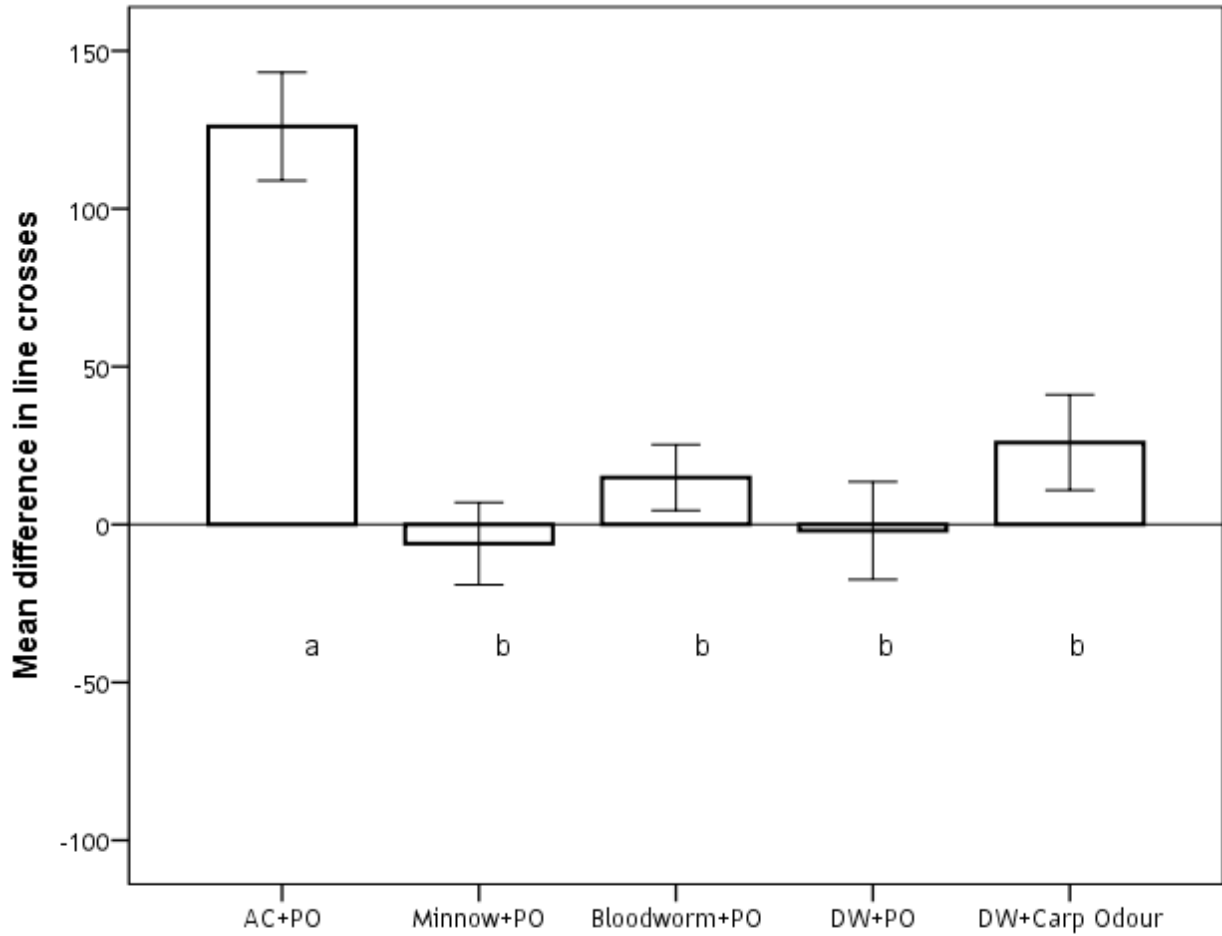
Figure 2.2: Mean (± S.E.) change in line crosses from the pre-stimulus baseline of Rainy River juvenile sturgeon during conditioning trials, exposed to either skin-derived conspecific alarm cue paired with pike odour (AC+PO), minnow alarm cue paired with pike odour, bloodworm cue paired with pike odour, distilled water paired with pike odour (DW+PO), or distilled water paired with carp odour (DW+ carp odour) (N = 20/treatment). Group "a" represents a statistically different group than group "b" from post-hoc comparisons (α = 0.05).
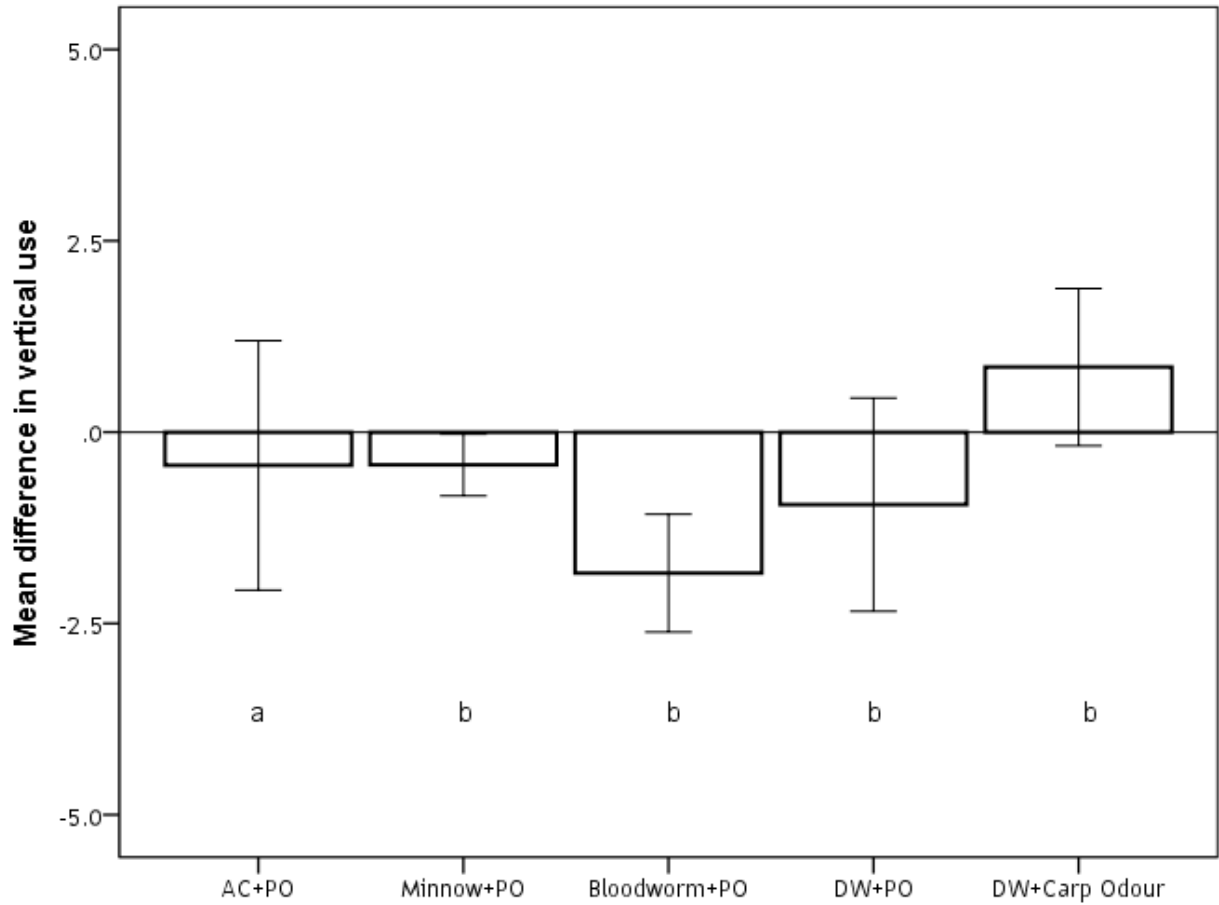
Figure 2.3: Mean (± S.E.) change in vertical use from the pre-stimulus baseline of Rainy River juvenile sturgeon during conditioning trials, exposed to either skin-derived conspecific alarm cue paired with pike odour (AC+PO), minnow alarm cue paired with pike odour, bloodworm cue paired with pike odour, distilled water paired with pike odour (DW+PO), or carp odour paired with distilled water (DW+Carp Odour) (N = 20/treatment).
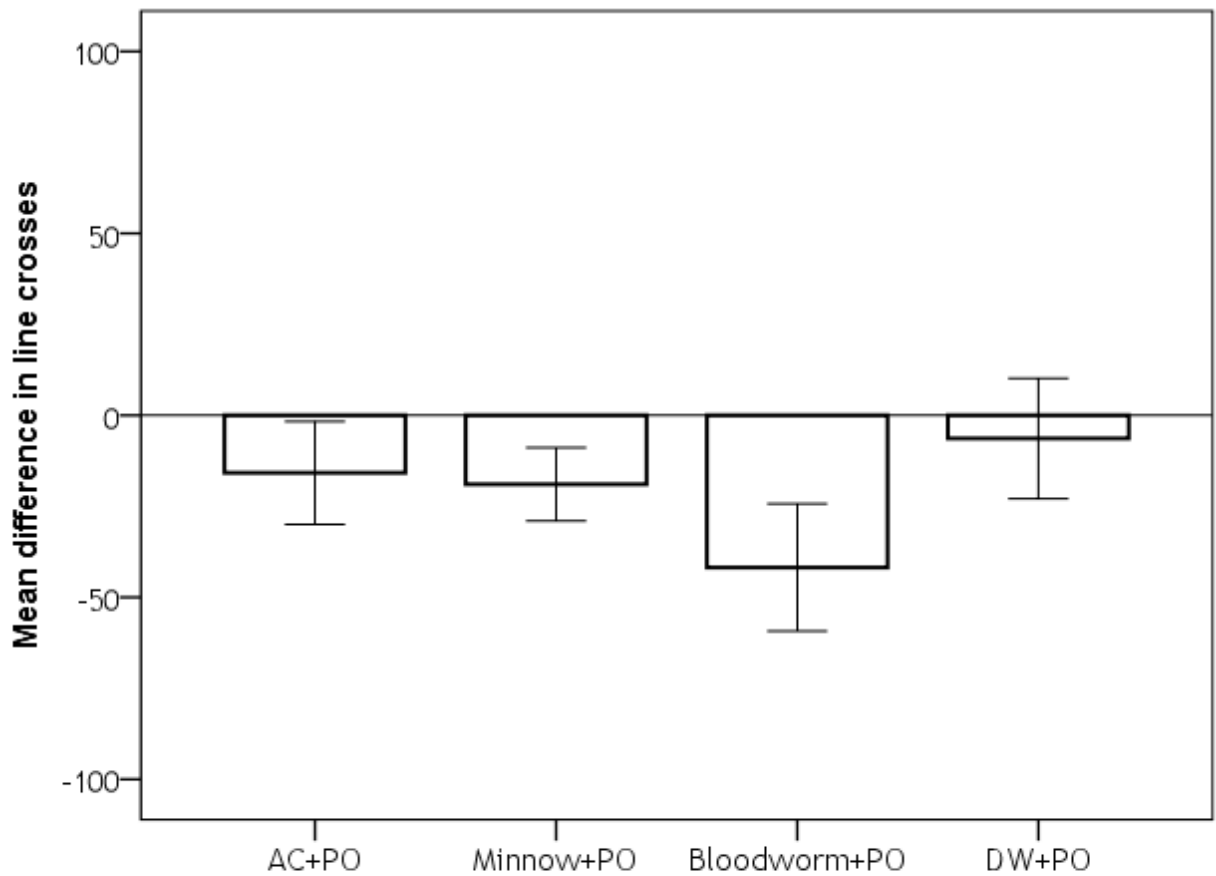
Figure 2.4: Mean (± S.E.) change in line crosses from the pre-stimulus baseline of Rainy River juvenile sturgeon exposed to either skin-derived conspecific alarm cue paired with pike odour (AC+PO), minnow alarm cue paired with pike odour, bloodworm cue paired with pike odour, or, distilled water paired with pike odour (DW+PO) during conditioning, and tested for recognition 24 hours later with pike odour alone (N = 20/treatment).
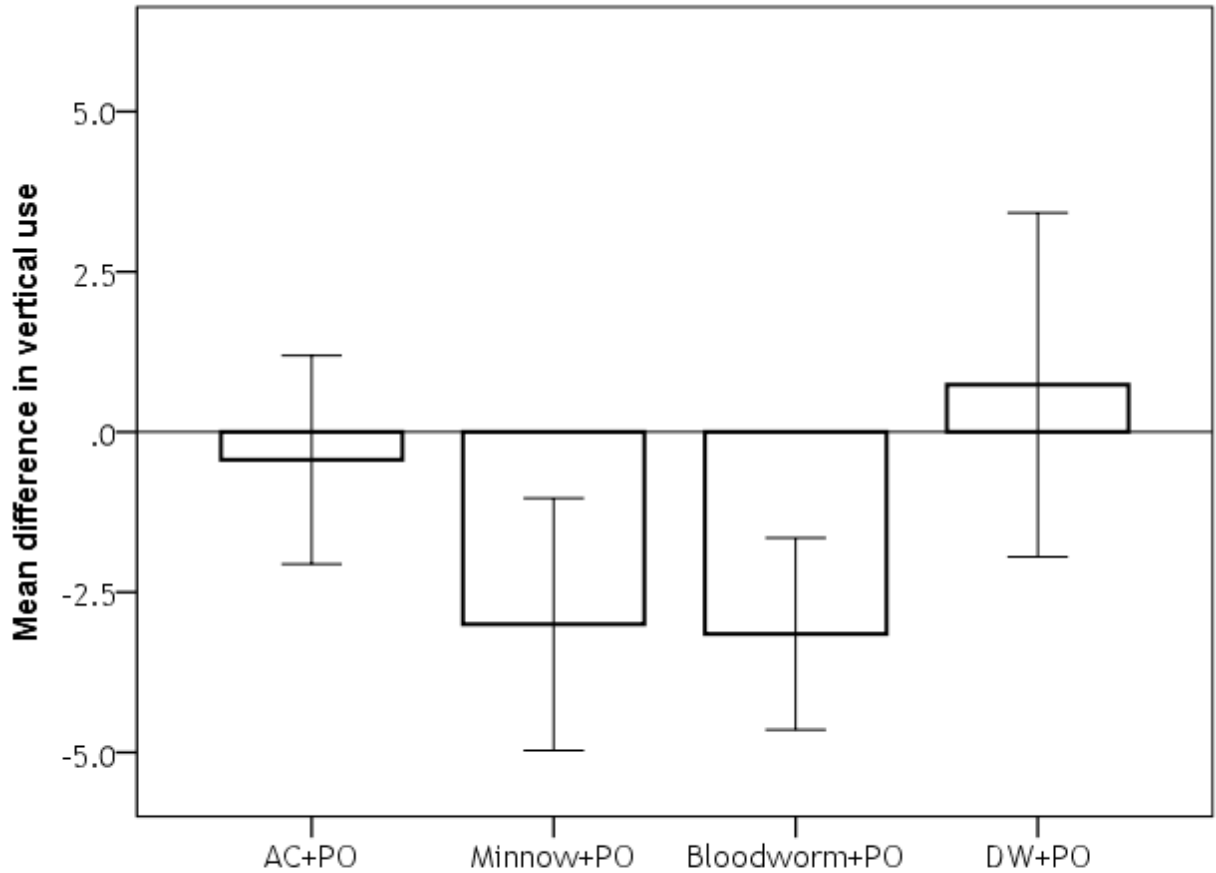
Figure 2.5: Mean (± S.E.) change in vertical use from the pre-stimulus baseline of Rainy River juvenile sturgeon exposed to either skin-derived conspecific alarm cue paired with pike odour (AC+PO), minnow alarm cue paired with pike odour, bloodworm cue paired with pike odour, or, distilled water paired with pike odour (DW+PO) during conditioning, and tested for recognition 24 hours later with pike odour alone (N = 20/treatment).

2.4. Discussion

The results of the conditioning trials indicate that juvenile lake sturgeon dramatically increase activity in response to conspecific skin extract paired with pike odour, but do not change their activity pattern in response to heterospecific (minnow) skin extract, food cues or novel odours like pike or carp. This is the first documentation of the response of juvenile sturgeon to conspecific skin extract as no work on sturgeon's antipredator response had been published at the

start of my thesis work. This response to conspecific skin extract has since then been confirmed (Wishingrad *et al* 2014a, b, c). Interestingly, the observed response is an increase in activity. The majority of reported antipredator responses in fishes involves reduction in activity to increase crypsis (reviewed by Chivers and Smith 1998), but a few increase activity to increase their chances of escaping a predator attack (i.e., a fleeing response in minnows (review Chivers and Smith 1998)). To ensure that this behaviour did indeed belong to the antipredator repertoire of the fish, I added a number of controls to verify that a similar behaviour would not be observed in a foraging or exploratory context. If exposure of prey to a novel stimulus results in fear or avoidance behaviour, the reaction is termed neophobia – the avoidance or fear of any novel cues. Under conditions of highly variable risk, prey that respond vigilantly to novel stimuli have a better chance of avoiding threats. However, prey able to associate the threat of predation with appropriate stimuli through recognition learning can avoid wasting time and energy linked to the response to irrelevant cues. Sturgeon did not display an innate fear response when exposed to pike odour or carp odour paired with distilled water during conditioning, indicating that sturgeon do not respond to novel odours with a neophobic response.

Cultured sturgeon are typically provided live food, commonly brine shrimp nauplii, at the initiation of exogenous feeding. Sturgeon have been found to imprint on food items, and switches to formulated diets have resulted in over 80% mortality for this reason (Monaco *et al* 1981). During these trials, sturgeon were fed a diet of blood worm (Family Chironomidae) twice daily. Sturgeon rely on tactile and olfactory systems for feeding and detecting environmental cues rather than visual stimulus (Kasumyan and Kazhlayev 1993). When the sturgeon were presented with the extract of their daily food item, blood worm, as well as a novel potential food cue, minnow extract, differences in behaviour were not observed.

Certain *Acipenseriformes* that are commonly raised in aquaculture programs, such as white sturgeon and Siberian sturgeon (*Acipenser baerii),* are known to cannibalize conspecifics when fed low quality diets, or when there is a large size variation in rearing tanks (Deng, *et al* 2003; Dabrowski, Kaushik, and Fauconneau 1985). Although cannibalism among larvae and juveniles is described as rare or opportunistic in many species of sturgeon, it has been observed in higher frequency in other species, such as Russian sturgeon (*Acipenser gueldenstaedtii)* in aquaculture environments (Memiş *et al* 2009). As the behavioural response of juvenile sturgeon to conspecific skin extract was significantly different than the response to food cues, the increase in activity is not accounted for by a feeding response.

The results of the recognition trials indicated that the sturgeon did not appear to associate pike odour with the risk of predation after exposure to alarm cue paired with pike odour. While the cue elicited an antipredator response during conditioning, it is possible that the skin-derived alarm cue provided an unreliable indicator of predation risk, and thus did not facilitate learning. Similar results have been found with disturbance cues emitted from disturbed or startled prey. It may be possible that sturgeon are exposed to skin-derived conspecific alarm cue frequently outside of the context of predation. For example, juvenile sturgeon commonly occupy habitats containing rough sand or rocky substrate and may experience frequent skin abrasion that would result in the release of alarm cue from the skin. Thus, a more reliable indicator of risk might be a whole-body derived alarm cue. I address this possibility in the next chapter.

Chapter 3: Conditioned predator recognition using whole body extract

3.1. Introduction

In Chapter 2, sturgeon displayed fright behaviour in response to skin extracts; however, they did not learn to recognize the predator when the predator odour was associated with alarm cues. I hypothesized that skin extract alone may not indicate a high enough level of risk for associative learning to result in this armoured species. Given that sturgeon juveniles likely hide in interstitial spaces of rocky substrates or sandy bottom, the detection of injured skin cue may not always indicate that a predation event has occurred. However, whole-body extract—the cues released from badly-injured conspecifics—would provide a more accurate indicator of predation. A decrease in activity, or dramatic fleeing in response to predator odour during recognition trials, would indicate successful learned association between sturgeon alarm cue and the predator odour. As sturgeon may also spend more time in the upper water column once they are disturbed (Kynard *et al* 2010), I predicted that an increase in vertical use would indicate an appropriate antipredator response.

3.2. Methodology

3.2.1 Test Species

In these experiment, the Rainy River population from Chapter 2, as well as an additional population of lake sturgeon from the Wolf River system in Wisconsin, were used for testing. The Wolf River lake sturgeon were donated from the sturgeon stocking program out of the Genoa National Fish Hatchery in Genoa, Wisconsin. The fish arrived in the laboratory on August 29, 2012 as fingerlings and were held in a 6000-L flow-through circular tank at 15 ± 1 ℃. All

juvenile sturgeon were fed bloodworms twice daily to satiation. The photoperiod was held

constant at 14L:10D hr in both holding tanks and experimental tanks. The two populations of

fish were of different ages/sizes at the time of testing, but there was considerable temporal

overlap in the testing times. For the Rainy River fish, data collection was carried out from

August 23 to December 17, 2012, when the sturgeon were between 87 and 203 days post hatch.

For the Wolf River sturgeon, data collection was carried from October 1 to December 20, when

the fish were between 168 and 248 days post hatch. Rainy River fish were (mean ± SD total

length) 5.7 ± 0.4 cm at the time of testing, while Wolf River fish were 11.0 ± 1.3 cm.

3.2.2 Stimulus collection

Skin extracts used in this experiment were collected from five juvenile lake sturgeon

(Rainy River: 6.5 ± 0.4 cm; Wolf River: 11.1 ± 1.4 cm). Sturgeon were killed by cervical

dislocation (in accordance with the Canadian Council on Animal Care) and the whole sturgeon

were placed in 100 mL of distilled water. The tissue and distilled water were homogenized using

a Polytron homogenizer (Brinkman Instruments, Rexdale ON), and then filtered through glass

wool to remove remaining tissue. Tissue was collected and diluted to create a solution containing

~ 3.2 g of tissue per L of distilled water for both populations. Skin extracts were frozen in 60 mL

aliquots at −20 ℃ to preserve the functional properties of the chemical alarm cue until required

(Lawrence and Smith, 1989). Pike odour was prepared during Experiment 2, and used

throughout this experiment.

3.2.3 Experimental protocol

This experiment consisted of two phases: conditioning trials followed by recognition

trials. During conditioning trials, sturgeon were exposed to one of two treatments: body-derived

alarm cues paired with pike odour or distilled water paired with pike odour. During the testing

phase, the fish were exposed to either pike odour, to test whether they had learned to

behaviourally respond to pike odour, or a distilled water control. Testing trials took place 24 h

after the conditioning trials. As in Chapter 2, fish were tested in groups of 3.

Conditioning trials

The experimental set-up and stimulus injection protocol was identical to that used in

Chapter 2. All trials were performed blind and the order of stimulus injection was randomized.

Since my goal was to see whether learning had occurred, no observations were carried during the

conditioning phase. I injected either 20 mL of sturgeon extract, or 20 mL distilled water paired

with 20 mL pike odour into the tank.

Recognition trials

One hour after the end of the conditioning trials, the fish were moved to a similar 37-L

tank filled with clean dechlorinated tap water. After 24 h, recognition trials were performed. The

protocol was the same used for the testing trials from Chapter 2 except that only pike odour (20

mL) or distilled water (20 mL) was injected in the tank following the pre-stimulus period. All

trials were performed blind and the order of testing was randomized. As a measure of response to

novel stimuli, the vertical use index, as well as the number of line crosses of the focal fish was

scored every 15 sec.

3.2.4 Statistical analysis

To test if any behavioural differences existed among treatment groups prior to injecting

the stimulus, I first performed a 2×2 ANOVA to test the effect of conditioning cue (alarm cue

vs water) and testing cue (pike odour vs water) on the pre-stimulus values. I then calculated the

change in line crosses and vertical use index from the pre-stimulus baseline. While the data for

change in vertical use were normally distributed and homoscedatic, the data for change in line crosses during recognition trials in Rainy River (but not in Wolf River) sturgeon was not normally distributed, although variances among treatments were homogenous. Given that ANOVAs are robust against violation of normality assumptions (Sokal and Rohlf 2012), I still performed 2×2 ANOVA on each of the variables for each of the population.

3.3. Results

3.3.1 Rainy River sturgeon

The results of the 2-way ANOVA indicate that there is no significant effect of conditioning cue ($F_{1,73} < 0.1$, $P = 1.0$), testing cue ($F_{1,73} = 1.4$, $P = 0.2$), or any interaction between the two factors ($F_{1,73} = 0.7$, $P = 0.4$) on pre-stimulus values for line crosses. For vertical use, similar results were found, with no significant effect of conditioning cue ($F_{1,73} < 0.1$, $P = 0.9$) or testing cue ($F_{1,73} = 0.9$, $P = 0.5$) on pre-stimulus values. However, there was a significant effect of interaction between the two factors ($F_{1,73} = 4.6$, $P = 0.036$, Figure 3.1) on pre-stimulus values.

The results of the 2-way ANOVA revealed a significant interaction between conditioning and testing cues on line crosses ($F_{1,73} = 4.0$, $P = 0.049$, Figure 3.2), however this interaction was not present when I looked at vertical use ($F_{1,73} = 0.1$, $P = 0.1$, Figure 3.3). The above interaction comes from the fact that sturgeon pseudo-conditioned with water paired with pike odour did not differ in their response to water and pike odour ($P > 0.9$). However, sturgeon conditioned with body-derived alarm cues significantly decreased their activity compared to water ($P = 0.024$), indicating that Rainy River juvenile sturgeon have learned to recognize pike odour as a threat when conditioned with body-derived alarm cues.

34
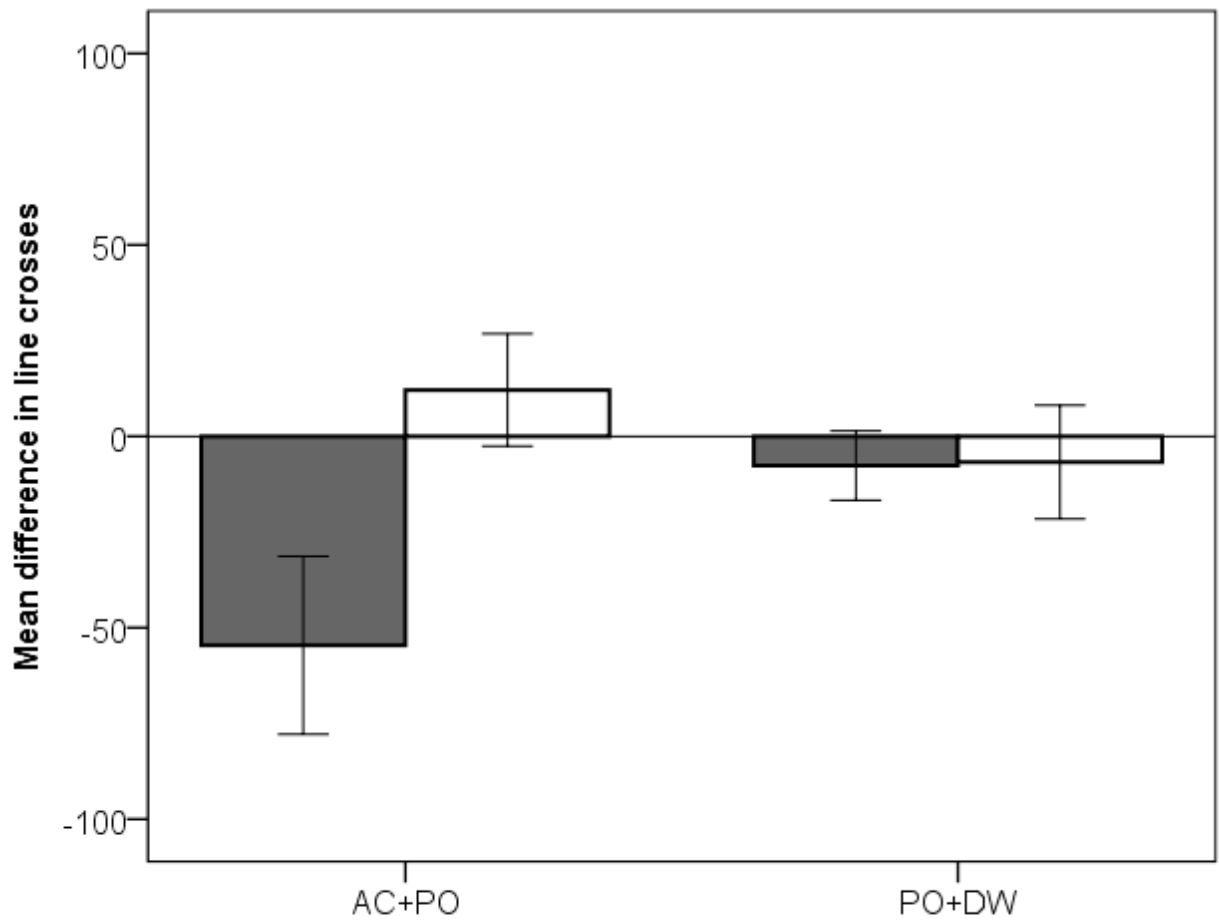
***Figure 3.1: Mean (± S.E.) change in line crosses from the pre-stimulus baseline for Rainy River juvenile
sturgeon initially exposed to either conspecific alarm cue paired with predator odour, or distilled water paired
with predator odour during conditioning, and tested for recognition 24 hours later with either distilled water
(white bars) or predator odour alone (grey bars) (N = 20/treatments).***
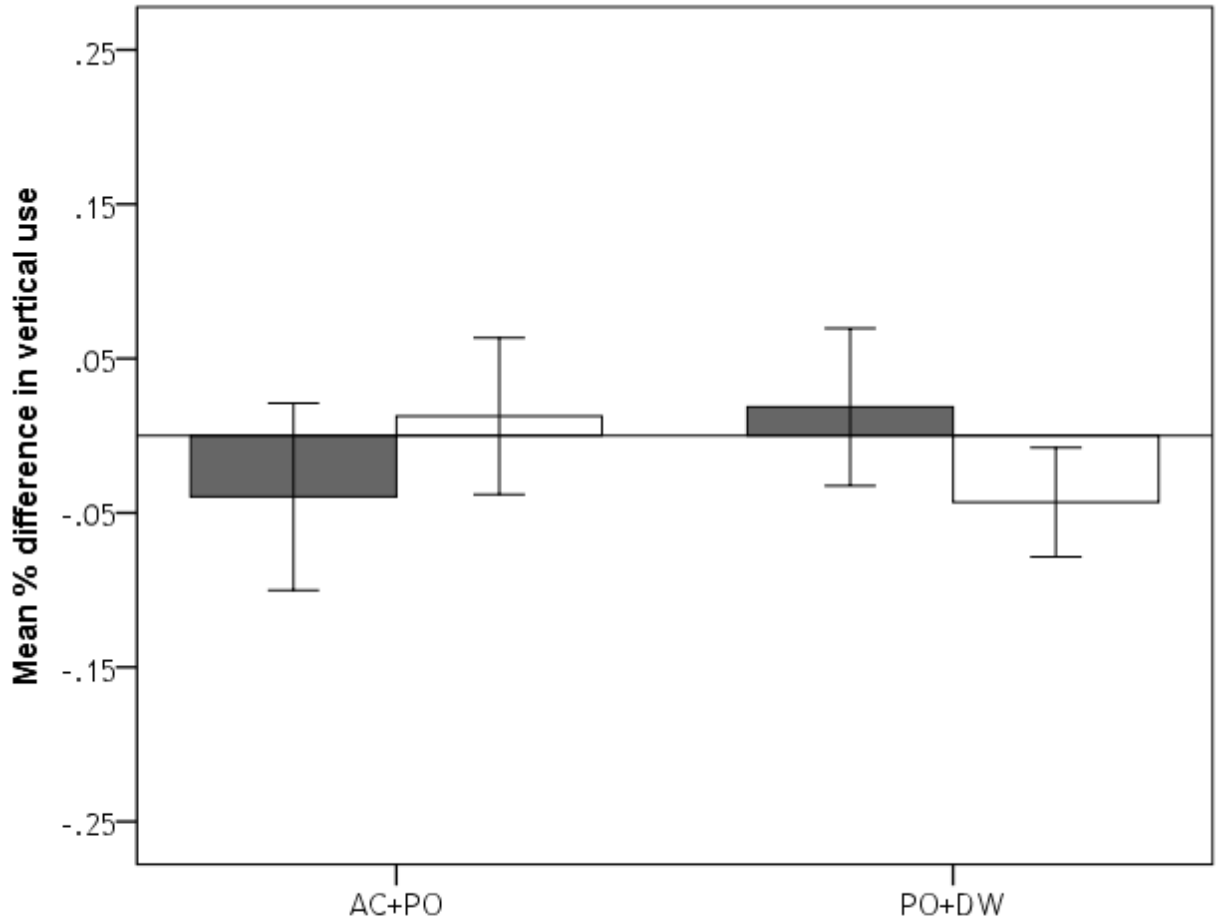
Figure 3.2: Mean (± S.E.) percent difference in vertical use from the pre-stimulus baseline for Rainy River juvenile sturgeon initially exposed to either conspecific alarm cue paired with predator odour, or distilled water paired with predator odour during conditioning trials, and tested for recognition 24 hours later with either distilled water (white bars) or predator odour alone (grey bars) (N = 20/treatments).

3.3.2 Wolf River sturgeon

The results of the 2-way ANOVA indicate that there is no significant effect of conditioning cue ($F_{1,70} = 0.2$, $P = 0.7$), testing cue ($F_{1,70} < 0.1$, $P = 0.9$), or any interaction between the two factors ($F_{1,70} = 0.4$, $P = 0.5$) on pre-stimulus values for line crosses. Likewise, for vertical use, the results of the 2-way ANOVA indicate no significant effect of conditioning cue ($F_{1,70} = 1.2$, $P = 0.5$), testing cue ($F_{1,70} = 0.7$, $P = 0.6$), or any interaction between the two factors ($F_{1,70} = 3.9$, $P = 0.053$) on pre-stimulus values.

36

The results of the 2-way ANOVA indicated no significant interaction between conditioning and recognition treatments for the number of line crosses ($F_{1,70} < 0.1$, $P = 1.0$, Figure 3.3) or vertical use ($F_{1,70} = 0.5$, $P = 0.5$, Figure 3.4). However, in all cases we saw an antipredator response to predator odour from the Wolf River fish. Their reduction in activity was comparable in magnitude to that of the learned recognition by Rainy River fish. This suggests the possibility of innate recognition of pike by this population of sturgeon.
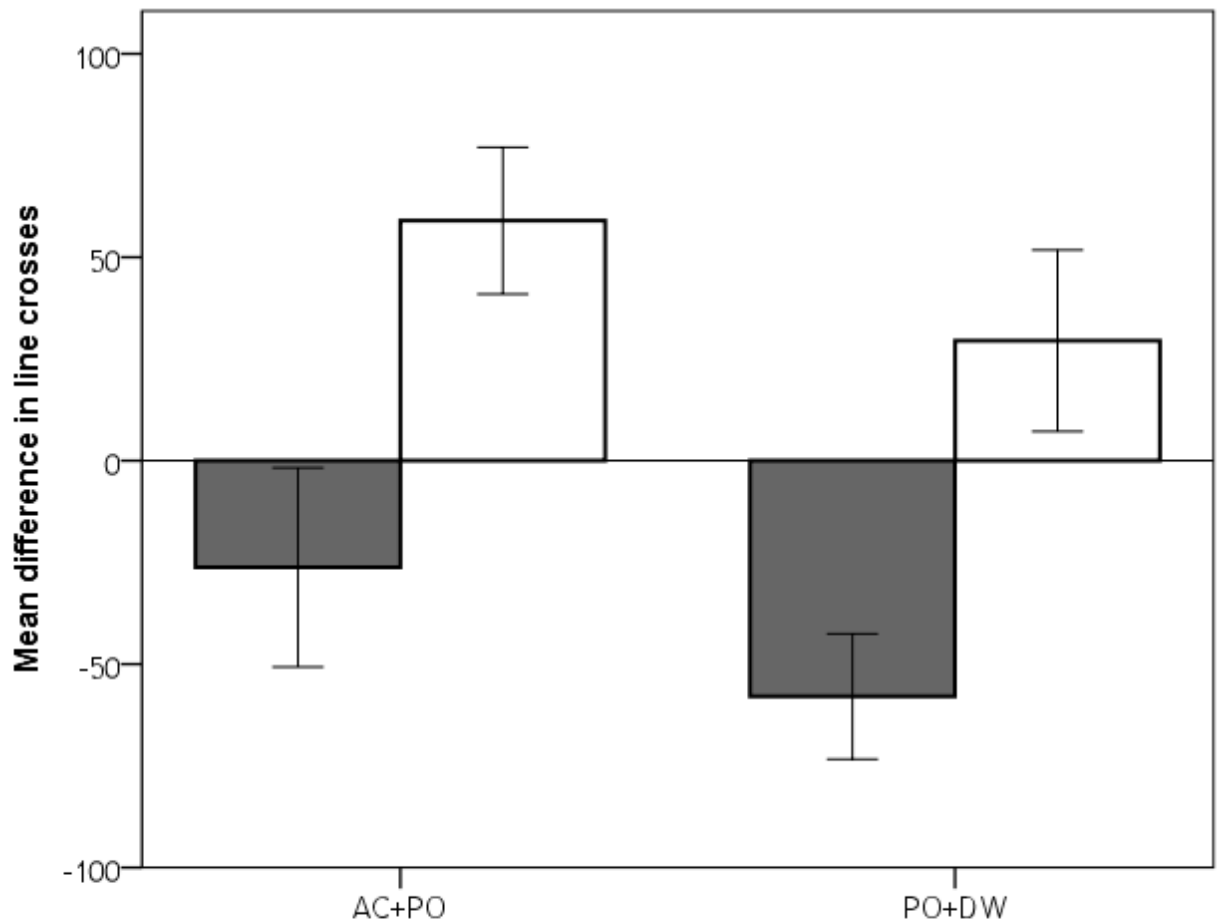
Figure 3.3: Mean (± S.E.) change in line crosses from the pre-stimulus baseline for Wolf River juvenile sturgeon initially exposed to either conspecific alarm cue paired with predator odour, or distilled water paired with predator odour during conditioning trials, and tested for recognition 24 hours later with either distilled water (white bars) or predator odour alone (grey bars)(N = 20/treatments).
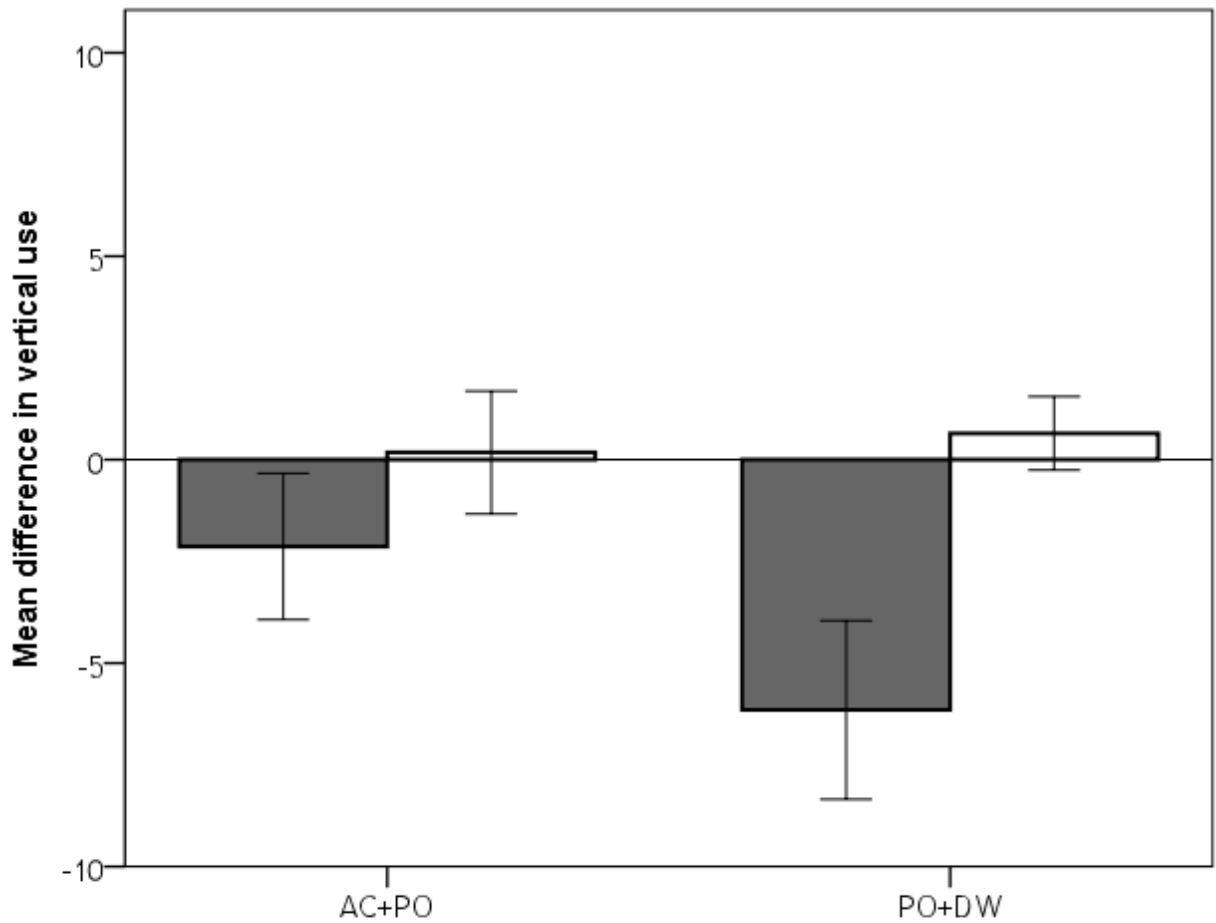
Figure 3.4: Mean (± S.E.) Change in vertical use from the pre-stimulus baseline for Wolf River juvenile sturgeon initially exposed to either conspecific alarm cue paired with predator odour, or distilled water paired with predator odour during conditioning trials, and tested for recognition 24 hours later with either distilled water (white bars) or predator odour alone (grey bars)(N = 20/treatments).

3.4. Discussion

3.4.1 Rainy River

The results of this experiment demonstrate that juvenile sturgeon from the Rainy River

population can learn to recognize pike odour as a predation cue when it is paired with whole-

body conspecific extract during a single conditioning event. This recognition is achieved through a process of releaser-induced recognition learning (Suboski 1990). Rainy River lake sturgeon increase activity when exposed to conspecific alarm cue. However, sturgeon later displayed a significant decrease in activity when exposed to a conditioned stimulus that indicates risk of predation. The results of the experiments on Rainy River sturgeon suggest that the antipredator behaviour of juvenile sturgeon upon recognition of a predator is to decrease activity. These data also seem to suggest that learned predator recognition of sturgeon is dependent on whole body alarm cue, rather than the skin-only alarm cue used in Chapter 2. Note that the concentration of tissue in the solution was similar between Chapter 2 and 3, indicating that the difference likely does not stem from a concentration problem. These results (along with those presented in chapter 2) also suggest that vertical use is not a reliable measure of predator avoidance for this population, despite what Kynard *et al* (2010) reported on the swim-up behaviour of disturbed juvenile sturgeon.

Avoidance and escape responses to conspecific alarm cues has recently been documented in European sturgeon (*Acipenser sturio)* (M.L. Acolas, personal communication) as well as shovelnose sturgeon (*Scaphirhynchus platorynchus*), that will actively avoid channel catfish when they are placed together in a partitioned tank (Hintz *et al* 2013). In my system, when sturgeon were initially exposed to the alarm cue paired with predator odour, they increased their activity. However, sturgeon that demonstrate a conditioned response to predator odour decrease their activity. The differential responses are rather fascinating. Perhaps different strategies may be employed under varying degrees of perceived risk. The presence of alarm cues may indicate that the individual is living in a high risk environment and as such dispersing may be adaptive. Indeed, injured fish cues (alarm cues from prey fish) have been shown to be a potent foraging

cue for a number of piscivores, with large numbers of predators appearing in the vicinity of the alarm cue release location within minutes (Lonnstedt *et al* 2012). Thus, it is possible that the smell of injured sturgeon would lead to an immediate recruitment of potential predators (Lonnstedt *et al* 2012; Chivers *et al* 1996; Mathis *et al* 1995), and hence, the best strategy for nearby conspecifics would be to leave the area. However, once a known predator has been detected, fleeing may be less desirable unless the exact location of the predator is known.

3.4.2 Wolf River

These data suggest that conditioning Wolf River sturgeon does not influence the intensity of the antipredator response they exhibit when exposed to pike odour. This could indicate that the sturgeon were unable to recognize pike odour as a predation threat during a single exposure when it is paired with whole body grinds. Nevertheless, we need caution with this interpretation, as there was an indication that the sturgeon already recognized the pike as a threat.

Chemically-mediated predator recognition learning can allow a high degree of behavioural plasticity (Brown et al 2013a). A number of intrinsic (behavioural trade-offs and ontogenetic switches) and extrinsic (concentration of chemical cues, water chemistry, context, and habitat) factors can affect how prey respond to alarm cue, whether a learned association is possible, and how long it may persist (Ferrari *et al* 2010a). It is possible that an ontogenetic shift would occur that would also prevent older sturgeon from responding to non-threatening cues. Responding to cues by avoiding the area, hiding or dashing may be a costly behaviour, as that time could be better allocated to essential activities such as feeding or reproduction (Ferrari and Chivers 2006). Dewitt *et al* (1999) found consistent differences among individual snails in their response to predation risk. Behavioural differences may be accounted for by size effects, previous experience, as well as genetic variation. Rainy River and Wolf River sturgeon are naïve

to predation; however, as Rainy River sturgeon were raised entirely in the laboratory and Wolf River sturgeon were raised for several months in a recirculating aquaculture facility, I cannot entirely omit the possibility that Wolf River sturgeon were previously exposed to predation cues through the process water at the Genoa National Fish Hatchery.

My results may indicate that while sturgeon are able to respond behaviourally to conspecific alarm cue and demonstrate associative learning, they may rely more heavily on morphological defenses of cryptic colouration and sharp bony scutes to minimize risk. Differences in size and morphology between the two populations of sturgeon may account for behavioural differences. Wolf River sturgeon hatched on April 17th, 2012 and starting feeding exogenously on April 29th, 2012 while Rainy River sturgeon arrived in the laboratory as newly hatched larvae on May 29th, 2012 and started feeding exogenously on June 13, 2012. Since the Wolf River fish came from a hatchery production facility, whereas Rainy River sturgeon were reared in laboratory conditions, feeding schedules for Wolf River sturgeon were optimized for maximum growth. Rainy River sturgeon were conditioned as early as 87 days post hatch, whereas Wolf River sturgeon were tested at a minimum age of 168 days post hatch. In laboratory conditions, predation on white sturgeon by channel catfish and northern pikeminnow was nearly absent once sturgeon reach a total length of approximately 130 mm, in the presence of alternative prey (Gadomski and Parsley 2005). The fish tested in these experiments exceeded a total length of 130 mm. Predation on sturgeon decreases as a function of their size, thus, Wolf River sturgeon are less vulnerable than the smaller, younger Rainy River sturgeon. Furthermore, bony scutes not only increase the energetic costs of swimming due to increased drag, but are energetically costly to produce (Peterson *et al* 2007). Investment of energy into morphological defenses of size and

scutes, rather than investment of energy into learning and behavioural defense, might explain why Wolf River sturgeon do not respond to paired cue conditioning.

Morphological and behavioural defenses may act in accordance with one another. A particular morphology may increase the effectiveness of antipredator behaviours, decrease the need for antipredator behaviour, or even work in conjunction with the behaviour to provide a specialized form of defense (Dewitt *et al* 2000). For example, morphological defenses, such as narrow apertures in freshwater snails, can impede a predator's ability to handle prey thus providing more time for snails to invoke behavioural defenses (Dewitt *et al* 2000). Prolonged handling time of prey decreases the energetic value of the prey item for the predator (Stephens and Krebs 1986), and may also facilitate the escape of nearby future prey if damage-released alarm cues alert other conspecifics of the risk of predation (Dewitt *et al* 2000).

The degree or intensity of antipredator responses performed by prey may also be highly variable, owing to underlying connections with other defense traits such as morphological defenses (Dewitt *et al* 1999). The vulnerability of prey animals can be influenced by size, reproductive status and sex (Stein 1977; Sih 1982). Vulnerable prey are more likely to engage in antipredator behaviours than well-defended prey, with prey in good condition better able to employ more costly defense mechanisms (Damsgaard and Dill 1998). Understanding how antipredator behaviours and antipredator traits correlate may provide information on the natural selection of behaviour (Dewitt *et al* 1999). If morphological or chemical defenses of prey are effective defense mechanisms, it is possible that behavioural responses may appear to be weak or suboptimal (Dewitt *et al* 1999). According to Dewitt *et al* (1999) the four types of mechanisms that can generate correlations between morphology and behaviour are codependence, complementation, compensation and cospecialization. When traits are mechanically linked, and

prey depend upon particular morphology to enact the behaviour, the correlation is called codependence. Complementation results when behaviour and morphology are mechanically independent, but may still be used in combination for an effective antipredator response. When prey exhibit a weak morphological defense, they may display stronger antipredator behaviour, resulting in a compensation correlation between traits. Cospecialization refers to situations when behavioural and morphological traits are each effective on their own. Recently, Wishingrad *et al* (2014a) found escape behaviour and body size in larval lake sturgeon to be codependent traits, with larger fish able to show stronger escape behaviour. Cryptically-coloured fish (mottled colouration) did not flee in response to risk, indicating a trait compensation correlation between colour morphology and escape behaviour. Cover-seeking behaviour presented a more complex relationship between particular traits, with smaller fish most often seeking cover (i.e. trait compensation) and larger fish using rocky mircrohabitats following exposure to risk (i.e. trait cospecialization between cover-seeking and escape behaviour). In the same study, scute size was not found to have a compensation relationship with antipredator behaviour, likely due to scutes being unable to provide sufficient defense against predation at the larval stage.

Another possible reason for why the Wolf River sturgeon failed to learn may be due to maladaptive behavioural traits as a result of their hatchery rearing environment. Fish hatcheries are designed to produce maximum growth and survival, void of any threats of predation. In order to achieve this goal, fish are provided adequate feed for optimal growth in conditions controlled for water chemistry, lighting, and habitat. Fishes reared in these unnatural environments lack direct experience with foraging, predator avoidance, and environmental cues, resulting in poorly developed behavioural phenotypes (Brown and Day 2002; Brown *et al* 2013a). Additionally, hatchery conditions do not select for traits that would be beneficial to fish in the wild. For

43

example, juvenile Atlantic salmon (*Salmo salar)* of parents that had spent one generation under hatchery conditions produced weaker predator avoidance behaviour than their wild-caught counterparts (Jackson and Brown 2011). A number of studies have shown that brain structures often reflect the manner in which a species has adapted to a particular environment (Masai *et al* 1982*;* Marchetti and Nevitt 2003). The effect of rearing environment on behavioural abnormalities of hatchery raised fish is an emerging field. Wild and hatchery-reared rainbow trout (*Oncorhynchus mykiss)* were used by Marchetti and Nevitt (2003) as a model for comparing brain structure development to explain maladaptive behaviours of hatchery-reared fish. Marchetti and Nevitt (2003) found that the most profound differences existed in the optic tectum and the telencephalon. These brain structures are often linked to behaviours of aggression, feeding and reproduction, and deficiencies in these areas can account for changes in growth, gaining access to mates, as well as changes in predator avoidance. These behaviours are particularly problematic when they occur in fish that are stocked as part of conservation effort. Fortunately, environmental enrichment has been shown to promote neural growth, which is particularly important for fish that undergo neurogenesis throughout life (Kotrschal *et al* 1998). Although hatchery-reared lake sturgeon are produced from wild-caught brood stock, hatchery rearing conditions can still contribute to dulled cognitive abilities. Thus, multiple training events may be necessary to promote learned predator recognition in hatchery-reared sturgeon.

Chapter 4: Multiple-event conditioning and predator recognition of Wolf River sturgeon

4.1. Introduction

A learned association between a conditioned and unconditioned stimulus can result after a single exposure event; however, multiple exposures would seemingly allow prey to gather more information about potential predators and increase the certainty that the perceived threat poses a predation risk that is worth responding to in future encounters. In Chapter 3, I showed that Wolf River sturgeon showed the same response to the predator odour regardless of whether or not they were conditioned. This could indicate that the fish were unable to learn via a single pairing of alarm cues and predator odour. This conclusion needs to be taken with caution as I also had an indication the fish may have been exhibiting an innate response to the pike odour. Regardless of whether the fish have an innate response or not, the training had no impact on altering the intensity of the response. It is possible that multiple instances of conditioning could result in learning or could enhance the already existing predator recognition, therefore, I hypothesized that reinforced conditioning events would result in a significant enhancement of the antipredator response.

4.2. Methodology

4.2.1 Experimental protocol

This experiment consisted of 6 conditioning events over a three-day period followed by recognition trials on the fourth day. During conditioning trials, groups of three sturgeon were exposed to northern pike odour paired with body-derived conspecific alarm cue, or with distilled

water. The fish were conditioned twice daily, at 1030 hours and 1530 hours. The fish were exposed to either pike odour or distilled water 24 h later during recognition trials. The experiments were carried out from April 15 to April 20, 2013 when the Wolf River sturgeon were between 364 and 369 days post hatch.

Conditioning trials

The experimental tank, and general setup used in this experiment are the same as those used in the previous experiments. Again, sturgeon were acclimated for a 24-h period in their test tanks (water at 19.0ºC, 14:10 h light:dark cycle) prior to the start of the experiment.

All conditioning injections were performed at 1030 h and 1530 h following a similar procedure described in previous experiments. I injected either 20 mL of sturgeon extract paired with 20 mL of pike odour, or 20 mL distilled water paired with 20 mL pike odour into the tank. A complete water change was performed at 1700 h each day. No observations were carried during this phase.

Recognition trials

One hour after the end of the conditioning trials, the fish were moved to a similar 37-L tank filled with clean dechlorinated tap water. After 24 h, recognition trials were performed. All trials were conducted between 1000 and 2200 h. The injection protocol was the same used for the conditioning trials except that only pike odour (20 mL) or distilled water (20 mL) was injected in the tank following the pre-stimulus period. All trials were performed blind and the order of testing was randomized. I tested 64 sturgeon 14.0 ± 0.4 cm in total with $N = 16$ per treatment. Consistent with the previous experiments, I recorded the vertical use index of the focal fish every 15 sec and the number of line crosses for one randomly selected fish.

4.2.2 Statistical analysis

For recognition trials, I first checked to see if any pre-stimulus differences existed among treatment groups. The data for line crosses were heteroscedastic, so I used log-transformed data for the analysis. The transformation allowed the data to satisfy the assumptions of parametric analysis. I then calculated the change in line crosses and vertical use from the pre-stimulus baseline. However, once again, the data did not meet the assumption of homoscedasticity. Given that the presence of both positive and negative values prevented the use of common mathematical transformations, I rank-transformed the data and ran a non-parametric 2-way analysis of variance, using the Scheirer-Ray-Hare extension of the Kruskal-Wallis test (Sokal and Rohlf 2012). A 2×2 ANOVA was used to test the effect of conditioning cue and testing cue on the vertical use.

4.3. Results

The 2×2 ANOVA revealed no effect of conditioning cue ($F_{1,60} = 3.3$, $P = 0.07$; $F_{1,60} = 0.24$, $P = 0.6$), testing cue ($F_{1,60} = 0.2$, $P = 0.7$; $F_{1,60} = 1.3$, $P = 0.3$) or any interaction ($F_{1,60} = 0.01$, $P = 0.9$; $F_{1,60} = 2.6$, $P = 0.1$) on the prestimulus value for line crosses and vertical use, respectively. The results of the 2-way Kruskal-Wallis revealed a significant effect of conditioning cue ($F_{1,60} = 5.4$, $P = 0.035$) and testing cue ($F_{1,60} = 5.4$, $P = 0.023$) but no interaction ($F_{1,60} = 0.6$, $P = 0.5$) on the change in line crosses. Namely, the average change in activity was greater for conditioned than pseudo-conditioned fish, and this change tended to be greater in response to water than in response to pike odour (Figure 4.1).

For vertical use, the 2-way ANOVA revealed a significant interaction between conditioning and testing cue ($F_{1,60} = 5.0$, $P = 0.029$). Namely, fish that were pseudoconditioned with water did not differ in their response to water and pike odour ($P = 0.78$). However, those juveniles that underwent the multiple conditioning procedure did reduce vertical use significantly more in response to pike odour than water ($P = 0.011$).
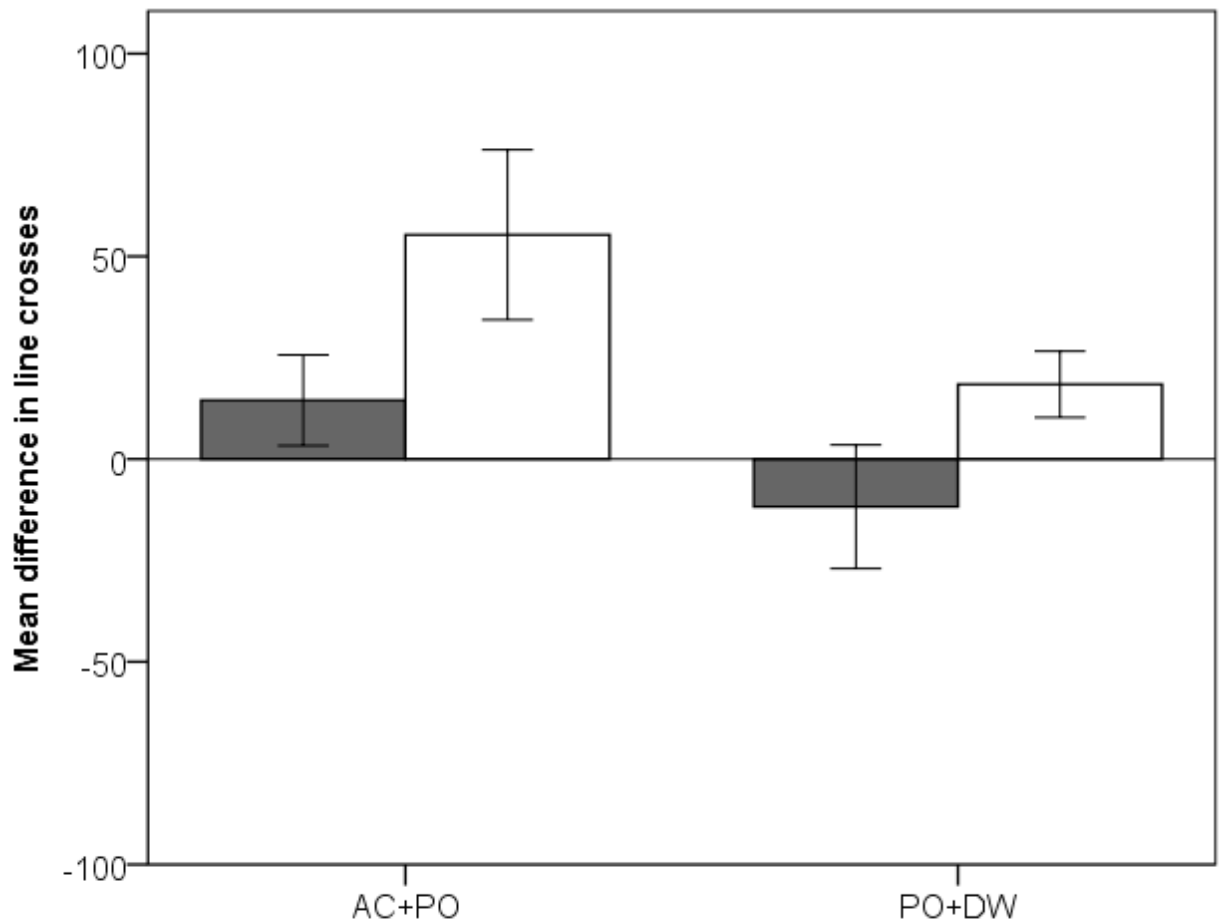
Figure 4.1: Mean (± S.E.) change in line crosses from the pre-stimulus baseline for Wolf River juvenile sturgeon during recognition trials. Sturgeon were previously exposed six times to either conspecific alarm cue paired with predator odour, or distilled water paired with predator odour, and subsequently tested for their response to distilled water (white bars) or predator odour alone (grey bars)(N = 16/treatments).
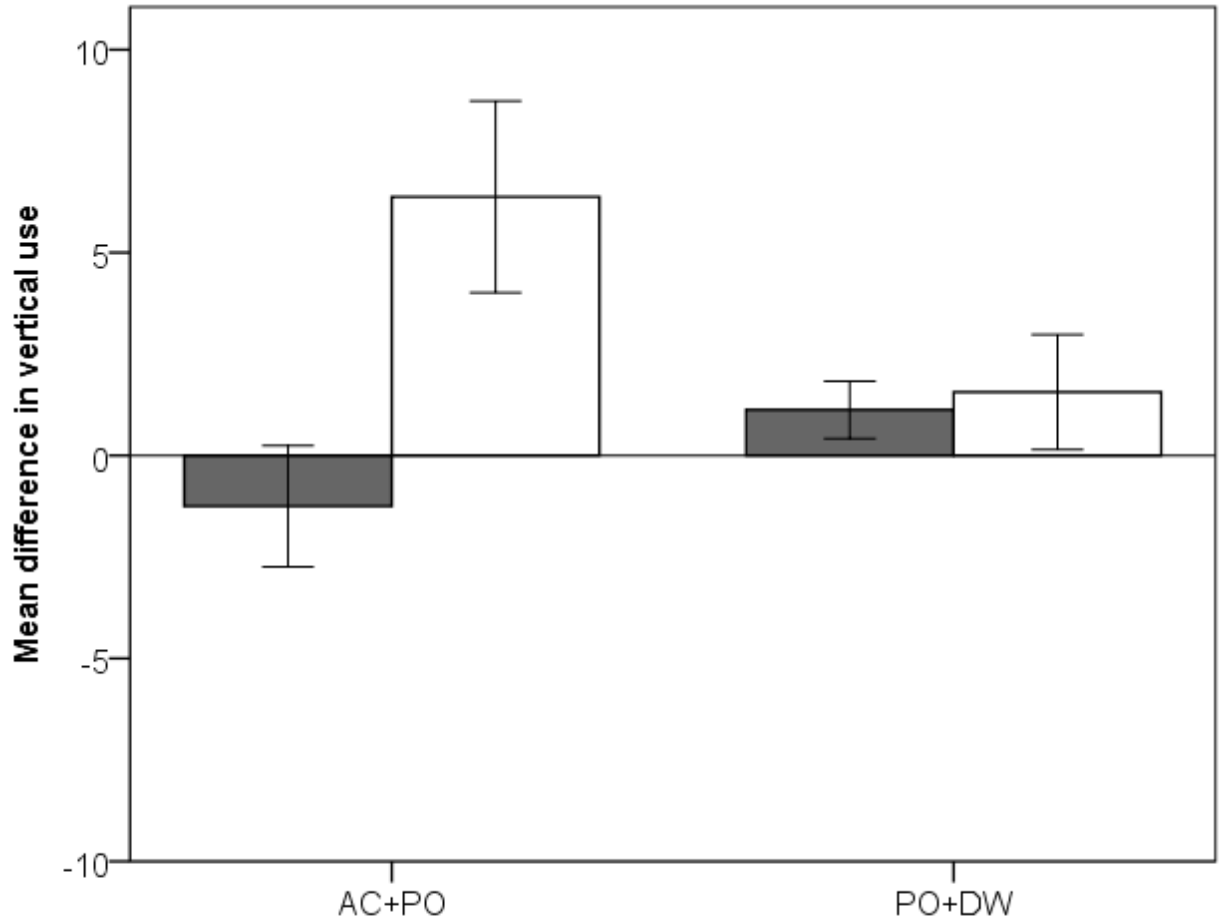
Figure 4.2: Mean (± S.E.) change in line vertical use from the pre-stimulus baseline for Wolf River juvenile sturgeon during recognition trials. Sturgeon were previously exposed six times to either conspecific alarm cue paired with predator odour, or distilled water paired with predator odour, and subsequently tested for their response to distilled water (white bars) or predator odour alone (grey bars)(N = 16/treatments).

4.4. Discussion

I found that the average change in activity was greater for conditioned than pseudo-conditioned fish. This pattern is what I would expect if there was predator learning; however, the difference arose because the fish increased activity to the control water as opposed to reducing activity to the predator odour. In fact, that response of conditioned and pseudo-conditioned fish

to pike odour was almost exactly the same. This makes it very difficult for me to conclude that the conditioning enhanced predator recognition.

In contrast, if I consider change in vertical area use, there was a dramatic increase in vertical area use in response to predator training. Conditioned fish fled up into the water column in response to predator odour, while pseudo-conditioned fish did not. Fleeing into the water column is consistent with the results of Kynard et al (2010) who reported on the swim-up behaviour of disturbed juvenile sturgeon. Interestingly, I did not find evidence that Rainy River fish in Chapter 3 fled into the water column in response to risk. Instead, they showed a dramatic reduction in activity.

Unlike the results of Chapter 3, in this experiment I had no evidence of innate recognition of pike odour by Wolf River sturgeon. If there was innate recognition of the pike by sturgeon, then how do I explain the lack of response in the current study? There was a significant difference in age, with Wolf River sturgeon being on average 158 days older, and 3 cm larger than sturgeon tested in Experiment 3. Perhaps sturgeon go through an ontogenetic shift in response to pike odour. As they grow larger, morphological responses could take over and be of greater importance, making behavioural responses unnecessary even if there was recognition of pike as a danger.

The Wolf River juvenile sturgeon used in this experiment were fast-growing fish with mottled colouration. Although no direct studies of predation have been performed on lake sturgeon juveniles, at the time of these experiments, the Wolf River sturgeon were approximately the size of white sturgeon that were not ingested during laboratory trials (>134mm TL, Gadomski and Parsley 2005a). Thus, while smaller juvenile sturgeon may have relied more

heavily on escape behaviour to avoid predation threats, larger juveniles may be able to conserve

energy and rely on the defense provided by size and scutes. This could explain why vertical use

can indicate a learned response to predator odour in this group of fish, whereas it was a poor

indicator in earlier trials.

Chapter 5: General Discussion

On account of their ancient origins, unique life history and threatened status worldwide, Acipenseriforms are a charismatic family that has drawn many fisheries managers to evaluate their status in recent years. However, at the time I began my research, little information was available on the most vulnerable stages of a sturgeon's life history (larval and juvenile) and their susceptibility to predation. My thesis work represents the first attempt to document releaser-induced recognition learning in juvenile sturgeon as well as early work in determining an antipredator behavioural response to conspecific alarm cue. Additionally, my work provides preliminary insight into the role of morphological and behavioural defenses throughout a sturgeon's life history.

I first investigated the ability of juvenile sturgeon to recognize a potential predator through releaser-induced recognition learning. In Chapter 3, I showed that fish from the Rainy River population could be successfully conditioned to recognize novel northern pike odour as a predator after a single pairing event, while sturgeon from the Wolf River population could not. Prey can respond to predation threats differently according to their state of vulnerability. The observed difference between Rainy River and Wolf River populations of sturgeon may be due to a number of underlying traits not limited to age, size, and population variances. Based on the results from Chapter 3, I performed an experiment (Experiment 4) to determine whether Wolf River juvenile sturgeon could demonstrate a conditioned response to predator odour if the associative learning was reinforced by multiple conditioning events. Learned predator recognition was evident in the significant difference in vertical use of the Wolf River population of sturgeon.

My results indicate that the response of lake sturgeon to alarm cue, and the conditioned response to novel predator odour, are antipredator behavioural responses. In Chapter 2, I demonstrated that sturgeon do not exhibit similar behaviours in response to other novel odours, (pike or carp odour), nor do they behave similarly to the addition of foraging cues (blood worm). For some aquatic prey animals in highly variable, high risk environments, novel stimuli may induce fright responses prior to any learned association. In Chapter 2, I observed the response of naïve juvenile Rainy River sturgeon to novel pike odour, as well as novel carp odour to determine whether if a fear response was evident. The response of juvenile sturgeon to novel pike odour demonstrated that a fright response towards pike odour alone only resulted after a paired conditioning event of alarm cue and pike odour.

These experiments suggest that lake sturgeon demonstrate releaser-induced recognition learning during the vulnerable juvenile life stages. As sturgeon populations become increasingly threatened worldwide, conservation stocking programs will continue to gain momentum. This initial work contributes a small, but important, piece of information to a large body of research on behaviour of artificially reared fish, and the effects of artificial selection, environment, and training.

5.1. Predator recognition continuum hypothesis

For prey animals, although the ultimate cost of a predator-prey interaction is loss of life, there is also a trade-off made between the benefits and costs of antipredator responses (Ferrari, Capitania-Kwok and Chivers 2006). Helfman (1989) developed a hypothesis for threat-sensitive predator avoidance, stating that prey should respond to a predation threat with an intensity that matches the risk posed by the predator. Prey, when exposed to a great variety of predator species

that are unpredictable in their probability of attack, should benefit more from learned predator recognition (Ferrari, Messier and Chivers 2008). Conversely, prey inhabiting environments where predictability of attack by a predator is high and predator diversity low, are likely to benefit more from innate predator recognition. These concepts are a part of the Predator Recognition Continuum Hypothesis (Ferrari *et al* 2007) in which it is proposed that the recognition of predators is dependent on (but not limited to) the temporal and spatial predictability of predation and the diversity of the predators. Moreover, prey behave differently based on a number of underlying traits and conditions related to their vulnerability (Dewitt *et al* 1999). More vulnerable prey may rely more on antipredator behaviour than well-defended prey (Sih 1982, 1986), with prey in poor condition less likely to use costly defences. Abrahams (1995) for example, showed that brook stickleback (*Culea inconstans*) with their sharp dorsal spines do not demonstrate strong antipredator behaviour in response to predators, while the behavioural response of unarmoured fathead minnows (*Pimephales promelas*) is significantly greater. The results of my thesis indicate that the sturgeon do not rely solely on morphological defense, but also behavioural defense to provide a more effective antipredator response.

5.2. Conservation applications of learned predator recognition

Hatchery rearing environments are highly artificial systems that are void of predation threats, habitat variability, as well as environmental cues. Thus, hatchery-reared fish may develop maladaptive behavioural traits that put them at a disadvantage compared to their wild counterparts who encounter natural environmental cues, threat of predation, and selective pressures (Huntingford 2004; Fernö *et al* 2011; Brown *et al* 2013a). Rearing environments that lack enrichment may also impact neural plasticity and development directly (Marchetti and Nevitt, 2003). Hatchery-reared fish that are unable to forage and avoid predators as well as wild

propagated fish are particularly problematic when they are being stocked as a part of a conservation program to assist declining populations. To avoid maladaptive behaviours commonly found in fish reared in artificial environments, many fish culturists are developing techniques and environments to produce fish with more wild-type characteristics (Crossman *et al* 2011). Fish hatcheries are often designed as production facilities, producing large quantities of fast-growing fish in a cost and time efficient manner. Conservation stocking programs should have a goal of producing high quality individuals that do not create a genetic divergence between wild and hatchery-reared fish. Raising fish in environments that imitate natural environments can increase survival upon release (Maynard *et al* 2003), and higher tank complexity, such as the addition of substrate, can improve growth and survival (Crossman 2008; Boucher 2012; Crossman *et al* 2011). A method currently used for hatchery-raised sturgeon is the implementation of stream-side hatcheries. Streamside hatcheries utilize water directly from natal streams, allowing fish to become exposed to natural daily and seasonal fluctuations, as well as chemical information, such as pheromones, dissolved organics, and predator cues (Crossman *et al* 2011). Another method that has been tested for sturgeon conservation culture is termed the 'head start' method of stocking. Naturally spawned sturgeon larvae are collected from the wild, and transferred to a protective hatchery environment, where they are reared for release into their natal streams once they have outgrown their most vulnerable stages of life (Peterson *et al* 2007). This process utilizes selection from natural spawning events, thus avoiding artificial selection inadvertently caused by artificial spawning. Hatchery-reared fish that are fed a diet consisting of natural food sources demonstrate a higher survival (Anderson 1984), and avoid imprinting on hatchery feed. DiLauro *et al* (1998) found that larval sturgeon would not consume a different diet after imprinting on brine shrimp, a typical diet provided to hatchery-reared sturgeon.

Through "life skills training" (Suboski and Templeton 1989; Brown and Smith 1998; C. Brown and Laland 2001; Brown, *et al* 2013a), a variety of processes may be employed for exposing hatchery-reared fish to natural foraging and predator cues in order to prepare them for release. Studies on the effectiveness of "life skills training" in promoting increased survival of hatchery-reared fish have produced mixed results (Brown *et al* 2013a). Atlantic salmon (*Salmo salar)* conditioned to recognize northern pike do not show improved survival when stocked into lakes with pike present (Hawkins *et al* 2007). Chinook salmon (*Oncorhynchus tshawytscha)* also did not experience improved survival when conditioned to recognize an ecologically relevant predator prior to release (Berejikian *et al* 1999). Conversely, conditioning white seabream (*Diplodus sargus*) prior to release increases survival rates by two-fold (D'Anna *et al* 2012), while murray cod (*Maccullochella peelii)* post-stocking survival rates are quadrupled (Hutchinson *et al* 2012). Conditioned learning, however, is a complex mechanism that can be influenced by social learning, visual reinforcement, and even feeding history which can in turn influence covarying factors of body condition and morphology (Lönnstedt *et al* 2012). Thus while early exposure to predation risk is critical for the retention of predator learning and recognition (Lönnstedt *et al* 2012), life skills training may require further reinforcement, through the addition of visual cues, in order to effectively increase post-release survival. Although conditioned predator recognition learning alone does not guarantee improved survival rates post-release, the potential for cultivating neuronal development through chemosensory stimulation may improve upon other maladaptive behavioural traits commonly described in hatchery-reared fish. While predator recognition learning is only newly described for a single population of lake sturgeon, ample opportunities for determining how kairomones affect post-stocking survival

rates of sturgeon exist through current conservation stocking programs for a number of species and populations.

5.3. Future directions

The relevance of this research demonstrating releaser-induced recognition learning in juvenile lake sturgeon as it applies to life skills training of hatchery reared fish, is dependent on further research related to the retention of learned information by sturgeon. Retention of learned predator recognition varies widely among prey fishes (Ferrari et al 2010b), and many factors, such as growth, level of perceived risk (Ferrari et al 2010b), and perhaps even population origin (Brown et al 2013a; Mackney and Hughes 1995) may contribute to how long learned information may persist. Due to unfortunate circumstances of sturgeon mortality, a direct comparison of behaviour between Rainy river sturgeon and Wolf river sturgeon was not possible. Experiments focusing on determining whether the observed differences were an effect of age, size, or population should be completed to help realize the full potential of alarm cue recognition learning in sturgeon.

With the imminent extinction of particular sturgeon populations and sub-populations, with many more populations becoming increasingly threatened (Paragamian *et al* 2005), conservation stocking programs are sure to become a popular technique for increasing recruitment. However, the emphasis must not be placed on sheer production capacity for supplementing collapsed populations. While hatcheries are an important tool in sturgeon conservation, hatchery-released fish must be regarded experimentally with emphasis on understanding post-release behaviours and demographic characteristics (Secor *et al* 2002). An understanding of early life-histories and the importance of enrichment and chemosensory

information as it relates to neurological development and behavioural ecology is essential to the

preservation of these ancient animals.

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