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Innate and learned predator recognition in four strains of captive-bred atlantic salmon, Salmo salar

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Abstract

Innate and learned anti-predator responses can be important determinants of survival in natural environments. However, few studies have examined population differences in these anti-predator responses. My study measured innate and learned anti-predator responses in four strains of Atlantic salmon, *Salmo salar*, which had varying captive breeding histories. All four strains of salmon tested had an innate anti-predator response to alarm cue and no response to a visual predator cue. Following training in which the alarm cue and predator cue were paired, I found that one of the four strains (Sebago), developed a learned anti-predator responses as indicated by reduced activity in response to the predator cue. The duration of captive breeding could not explain why only the Sebago strain showed an ability to learn, suggesting that other factors affect the evolution of learned anti-predator responses. Understanding population variability in learning ability may be important when selecting populations for reintroduction efforts.

Keywords

Atlantic salmon, anti-predator response, learning, captive breeding
Co-Authorship Statement

I plan to submit a version of this thesis for publication with Bryan Neff, Shawn Garner, and Chris Wilson as co-authors. I contributed to the experimental design, performed the experiment, and drafted the manuscript. Bryan Neff contributed to the study design and assisted in drafting the manuscript. Shawn Garner contributed to the design and provided insight regarding data analysis and the manuscript. Chris Wilson assisted with the collection and rearing of the fish as well as contributing to the experimental design of the experiment.
Acknowledgments

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1 Introduction

1.1 Effect of predation on prey populations

Predation, which occurs when an animal kills and eats another animal, is a major source of mortality that can have large impacts on prey populations (Elton, 1924). For example, Atlegrim (1989) studied the effect of excluding avian predators from populations of larval sawflies (Hymenoptera: Symphyta) and geometrids (Geometridae). Atlegrim found that when predators were excluded from a habitat, the populations of both prey species were larger than in habitats that included predators because of the lack of mortality due to predation. Predator and prey population sizes also tend to co-vary. In a classic example, Elton (1924) used historic data from Hudson’s bay fur trappers to estimate the population size for Canada lynx (Lynx canadensis) and snowshoe hare (Lepus americanus) over a 200-year period. Elton found that there was a consistent 10-year cycle in the population sizes of both the predatory lynx and the prey hare; when the hare population increased the lynx population increased shortly after, followed by a decline in the hare population, which was associated with a decline of the lynx population. Predators can also cause strong selection on prey species to avoid predation. In many species, the selection resulting from predation has lead to the evolution of specialized defense mechanisms that allow prey species to better avoid predation (Alcock, 1993).

1.2 Predator defense mechanisms

To avoid predation, many prey have evolved physiological or behavioural defence mechanisms (Alcock, 1993). One such defence mechanism is chemical defense, where individuals become toxic to predators and are subsequently avoided. For example, a
tropical frog (*Dendrobates pumilio*) produces steroidal alkaloids, which are toxic to many predators, is one mechanism these frogs have evolved to resist predation (Daly & Myers, 1967). Chemical defenses can also be linked with a change in the physical appearance of prey species that signals the presence of toxic chemicals (referred to as aposematism; Alcock, 1993). Schuler and Hesse (1985) showed that warning colourations are effective at deterring predation by examining predation by chickens (*Gallus gallus domesticus*) on mealworms (*Tenevrio molitor*) of different colours. They found that chickens avoided black and yellow coloured mealworms, colours typically associated with toxicity, and preferred to eat olive coloured mealworms. These colourations can be so effective at deterring predators that some prey species will mimic the colouration of other toxic species, without producing the associated defense chemical. For example, species of toxic and non-toxic butterflies (*Lepidoptera* spp.) that occupy similar habitats can have similar colour patterns and this is a result of the non-toxic species mimicking toxic species colouration as a defense mechanism to predators (Mallet & Joron, 1999). Changes in colouration and morphology can also help prey species blend into their environments so as to camouflage themselves from predators. Kettlewell (1956) studied pepper moths (*Biston betularia*) in England at the dawn of industrialization in that country. Pepper moths were originally light in colour, similar to the trees on which they were found. However, as England became more industrialized, soot began darkening trees, leading to a rise in a form of moth with a darker colouration. Kettlewell found that in forests closest to industrial centers, where there was the greatest build up of soot, the darker coloured moths had higher survival than the light coloured moths and this difference was attributed to increased detection by predators of light moths on the now dark tree bark. Prey may
also react behaviourally to predators and one of the most common ways to reduce mortality due to predation is to flee when a predator is detected (Lima & Dill, 1990; Lima 1998). Prey species can also avoid predators on a larger scale by changing their habitat use either spatially or temporally. For example, Heithaus and Dill (2006) examined habitat use of bottlenose dolphins (*Tursiops aduncus*) in the presence and absence of predatory tiger sharks (*Galeocerdo cuvier*). Heithaus and Dill found that when tiger sharks were present, dolphins preferentially used less productive but safer foraging sites. Prey species can also combine colouration and behaviour to reduce mortality due to predation. For example, yellow-legged frogs (*Rana muscosa*) are conspicuous while sun-bathing on top of a rock out of the water, so when confronted with a potential predator the frogs dive into the water and stay motionless, blending into the yellow-green coloured algae found on submerged rocks (Norris & Lowe, 1964).

1.3 Energetic costs of anti-predator behaviours

The evolution of anti-predator behaviours are often associated with an energetic cost, and these costs can result in a trade-off between growth and survival (Werner & Anholt, 1993). For example, Bryant and Julkunen-Titto (1995) examined growth rates in related species of birch trees (*Betula* spp.) that do and do not produce chemical defenses. They found that species with defense chemicals had slower rates of growth when compared to species that did not produce the chemicals. Similarly, the production of cryptic colouration has been shown to be costly. Talloen et al. (2004) fed satyrine butterflies (*Pararge aegeria*) on either a low or high nutrient diet. They found that when butterflies were fed a low nutrient diet they produced paler, less cryptic wing colours. The authors suggest that the butterflies on the low nutrient diet had insufficient resources available to
produce melanin, the compound that results in the cryptic wings. Defense mechanisms can also have more direct effects on growth by reducing time available for foraging or the efficiency of foraging. By fleeing or hiding from predators, prey have less time to allocate to foraging. Foraging efficiency can also be reduced if prey species adopt less efficient but safer foraging strategies. For example, when under predation risk, gray squirrels (*Sciurus carolinensis*) adopted foraging strategies that maximized the time spent in cover, sacrificing foraging efficiency to maximize survival (Lima & Valone, 1986).

1.4 Innate and learned anti-predator behaviour

Individuals can respond to signals of predation either through innate or learned mechanisms. I defined an innate anti-predator response, such as fleeing or motionlessness, as one that is expressed in its entirety upon the first exposure to the signal of predation; subsequent expression of the behaviour is not modified or otherwise enhanced (e.g. Jackson & Brown, 2011). An innate response to predators has been shown in a wide variety of taxa. For example, Japanese monkeys (*Macaca fuscata*) that had never experienced predation by snakes innately identified and avoided snakes (Shibasaki & Kawai, 2009). Atlantic salmon (*Salmo salar*) that had never encountered an avian predator increased the time spent in shelter in response to great blue herons (*Ardea herodias*; de Mestral & Herbinger, 2013). Prey may also innately respond to conspecific signals of threat that are associated with the presence of a predator or predation event; these signals are called alarm cues (Brown, 2003). For example, juvenile white-browed scrubwrens (*Sericornis frontalis*) suppress calling in response to their first exposure to alarm calls from conspecifics (Haff & Magrath, 2012). Responding to alarm cues appears to be adaptive. For example, mortality caused by northern pike (*Esox lucius*) predation
was reduced in fathead minnows (*Pimephales promelas*) that were exposed to alarm cue compared to those that were not exposed to alarm cue (Mathis & Smith, 1993).

A learned behavioural anti-predator response is one that is not expressed in its entirety after the first exposure but is instead modified over subsequent expression of this behaviour (Alcock, 1993). A common example of a learned anti-predator response is the development of an association between an alarm cue that elicits an innate response and a predator cue that does not elicit an innate response (Chivers & Smith, 1998). This type of learned anti-predator response has been shown in a wide range of taxa. For example, robins (*Petroica australis*) have been shown to learn to reduce feeding and movement in response to stoats (*Mustela erminea*) after a presentation of a model stoat with an alarm call (Maloney & McLean, 1995). Rainbow trout (*Oncorhynchus mykiss*) reduce foraging and stay motionless in response to northern pike after repeated pairing of northern pike odour with an alarm cue (Brown & Smith, 1998). Learned anti-predator responses presumably confer additional survival benefits above innate responses, likely due to increased specificity of the signal of predation (Mirza & Chivers, 2000; Chivers et al., 2002). Mirza and Chivers (2000) examined the survival of brook trout (*Salvelinus fontinalis*) when exposed to chain pickerel (*Essox niger*). They found that brook trout that were first exposed to a combination of alarm cue and pickerel odour had greater survival when encountering chain pickerel than brook trout that were previously exposed to either alarm cue or pickerel odour alone.

### 1.5 Evolution of innate and learned anti-predator behaviour

There are many factors that might affect the expression and evolution of anti-predator defense mechanisms. I expect that innate anti-predator responses to predator cues and
alarm cues would evolve when these cues are reliable and consistent signals of predation across multiple generations. For example, damage-induced alarm cues, which are released after mechanical damage to the skin, are reliable signals of predation risk (Pfeiffer, 1977). Rainbow trout are a species with damage-induced alarm cues and the response to alarm cue has been shown to be maintained over 100 years in the absence of predation in some populations (Scheurer et al., 2007). One potential explanation for the maintenance of alarm cue in rainbow trout is that because of the mechanism of release, damage-induced alarm cues are a reliable signal of risk that is consistent across generations regardless of predation and there is little selection to weaken the response to alarm cue. I would expect the response to predator cues to be more variable, as predator cues can be less reliable and less consistent than alarm cues across generations, as predator communities have been shown to change over time (Parody et al., 2001). In fact, an innate response to odour cues of a predatory fish has been found in some studies of salmonids (Hawkins et al., 2004; Jackson & Brown, 2011), but not in others (Brown & Smith, 1998; Ferrari et al., 2010a). In some species there are even population differences in response to predator cues. For example, O’Steen et al. (2002) found that guppies (Poecilia reticulata) from populations that experienced predation had an innate response to predator cues, while guppies from populations that never experienced predation did not respond to the same predator cues. Additionally, populations of guppies transplanted from streams without predators to streams with predators evolved an innate anti-predator response to predator cues within 35 years of the transplant (Magurran et al., 1992; O’Steen et al., 2002).
I expect a learned response to be effective when predator communities are variable both within and across generations and by modifying behaviour based on changes in the predator community, prey species increases the effectiveness their anti-predator response. In fact, brown trout (*Salmo trutta*) have shown the ability to learn to recognize many different types predators, but only have innate anti-predator response to a select few (reviewed by: Brown et al., 2013). Migratory species in particular have a strong potential for encountering multiple discrete predator communities as populations move from one location to another. Anadromous species in particular may experience vast changes in the predator communities experienced as individuals migrate from natal streams to oceans and I expect learning to be an effective anti-predator mechanism in anadromous species. Atlantic salmon are an anadromous species that have shown the ability to learn to respond to predators (Hawkins et al., 2008; Houde et al., 2010; de Mestral & Herbinger, 2013) and it is likely that the variability in the predator community resulting from migration contributed to the evolution of the ability to learn in Atlantic salmon (Brown & Chivers, 2005).

1.6 Anti-predator behaviours in aquatic ecosystems

Aquatic ecosystems provide an excellent system for studying alarm cues. Alarm cues have been observed in a wide range of aquatic taxa, including coral (*Discophyton rudyi*; Goddard, 2006), larval mayfly (*Siphlonurus lacustris*; Huryn & Chivers, 1999), and Atlantic salmon (Hawkins et al., 2004). In fact, Pfeiffer (1977) found that 64% of all freshwater fishes use alarm cue as a defense mechanism. Alarm cue is a reliable signal in aquatic environments where visual and auditory cues can be obscured and it is likely that Pfeiffer underestimated the prevalence of alarm cue in fishes as subsequent studies have
continued to identify new species that use alarm cue in their anti-predator response (reviewed by: Ferrari et al., 2010b). In fishes, damage-induced alarm cues are the most common form of alarm cue (reviewed by: Chivers & Smith, 1998). Alarm cue is stored in specialized cells in the skin, which have the sole function of releasing alarm cue when the skin is broken (Smith, 1982). Little is known about the chemical composition of alarm cues in aquatic systems, however Ferland et al. (2010) investigated the composition of alarm cue in bullfrog tadpoles (*Rana catesbeiana*) and found that alarm cue is likely a large, complex carbon chain that contains a single sulphur molecule. After alarm cue is released fish that detect this signal typically decrease movement, seek shelter, increase shoal cohesion, and reduce foraging (Chivers & Smith, 1998).

There are three main theories for the evolution of alarm cue in fishes. The first is that kin selection is the major driving force on the evolution of alarm cue and the cost of producing and storing alarm cue is compensated by the increased survival of nearby conspecifics (Smith, 1982). The second theory is that individuals directly benefit from damage released alarm cues by attracting secondary predators (Smith, 1982). Chivers et al. (1996) found that alarm cue released from a fathead minnow attracted northern pike and that when multiple pike were present each pike interfered with the others increasing the chance of survival for the fathead minnow. A third alternative is that there is a survival benefit to both the releaser and receiver of alarm cue. Previous work on innate anti-predator response to alarm cue has shown that several factors, such as concentration of alarm cue, developmental stage, and condition can affect the innate response to alarm cue (Hawkins et al., 2004; Brown et al., 2006; Binning et al., 2014). For example, Binning et al. (2014) examined the innate anti-predator response of parasitized and non-
parasitized coral reef fish (*Scolopsis bilineata*). They found that when individuals were parasitized they took longer to flee from predators than non-parasitized individuals. Innately responding to an alarm cue is a common anti-predator mechanism that has been thoroughly studied in aquatic systems.

Learned anti-predator responses are a topic of growing interest in aquatic ecosystems. How learned anti-predator responses develop and evolve is less well known than how innate anti-predator responses have evolved. This is likely due to the complex nature of learned anti-predator responses; not only is a learned response reliant on the presence of an innate response but learned responses also require more complicated testing procedures to study. To measure an innate response, an individual is exposed to a stimulus and their behaviour recorded. In contrast, to measure a learned response, an individual must form an association between a stimulus that innately elicits a behavioural response and a stimulus that does not innately elicit a behavioural response, then the individual must be tested to evaluate if this association is retained over time. However, a number of aspects of the learned anti-predator response have been investigated. For example, Ferrari et al. (2010a) investigated the retention time of juvenile rainbow trout that had been trained to recognise pumpkinseed sunfish (*Lepomis gibbosus*) as predators and found that higher concentrations of alarm cue during training increased the length of time the learned response was retained. Brown et al. (2011) studied the retention of a learned response in rainbow trout and found that faster growing fish had shorter retention times than slower growing fish.
1.7 Effect of captive breeding on anti-predator response

Captive breeding in fishes provides an excellent system for studying the development and evolution of innate anti-predator responses. Captive breeding programs remove the effect of predation during much of the life cycle, and this artificial selection could have large impacts on fitness when captive-bred individuals are released in natural environments (Fleming & Gross, 1993; Neff et al., 2011). Captive-bred species have been shown to have lower survival than wild-caught fish after exposure to a live predator (Fritts et al., 2007), and this difference likely occurs because captive breeding is associated with a reduction in the innate response to both predator cues and alarm cues. For example, Houde et al. (2010) compared the innate response of Atlantic salmon to an avian predator cue across groups differing in the proportion of wild and hatchery ancestry and found that the response to predator cue decreased as hatchery ancestry increased. In a comparison of the innate response to an avian predator model in first- and second-generation captive-bred Atlantic salmon, de Mestral and Herbinger (2013) found that the second-generation salmon displayed more risk-taking behaviours than the first-generation salmon. Captive breeding programs provide an opportunity to study the effect of removing populations from predation and previous work has shown a decrease in innate anti-predator response after even one generation of captive breeding.

Captive breeding programs similarly offer an excellent opportunity to study learned anti-predator responses in populations that have been removed from the effects of predation. Captive-bred populations are known to have the ability to learn to recognise predators, even after several generations in captivity (Mirza & Chivers, 2000; Ferrari et al., 2010a; Brown et al., 2013b). For example, Brown et al. (2013b) showed that captive-bred
rainbow trout were able to learn to recognise pumpkinseed sunfish as predators. Although individuals from captive-bred populations have shown a learned anti-predator response, no study has explicitly examined the effect of captive breeding on the learned anti-predator response. I would expect that the removal from predation would reduce the ability of individuals to be able to learn to recognize predators. My study will be the first to investigate the effect of captive breeding on the learned anti-predator response.

1.8 Reintroduction of Atlantic salmon into Lake Ontario

My thesis will use Atlantic salmon populations targeted for reintroduction into Lake Ontario to study the effect of captive breeding on innate and learned anti-predator responses. Lake Ontario once supported an abundant Atlantic salmon population and a thriving freshwater fishery (Whitcher & Venning, 1869), but Atlantic salmon were extirpated from Lake Ontario by 1898 (Crawford, 2001). There are four strains of Atlantic salmon with different captive breeding histories that are currently being reintroduced into Lake Ontario: the LaHave River strain, the Sebago Lake strain, the Lac-Saint-Jean strain, and the Mersey-LaHave strain. The LaHave strain has been propagated in the hatchery system for five generations, the Sebago strain for two generations, and the Saint-Jean strain for two generations. The Mersey-LaHave strain is a hybrid strain that was created in 2014 by crossing wild Mersey females and hatchery LaHave males. The Mersey-LaHave strain was created to revitalize the LaHave strain by interbreeding the captive-bred strain with a wild population; the Mersey population was used due to the lack of wild LaHave salmon and the close proximity of the Mersey and LaHave rivers. Despite this reintroduction effort, a self-sustaining population of Atlantic salmon has not yet been established in Lake Ontario. One factor that may be affecting the current
reintroduction efforts is a reduction in the anti-predator response of the strains being released. Specifically, predation by belted kingfishers (*Megaceryle alcyon*) and common mergansers (*Mergus merganser*) is estimated to cause between 21% and 45% of all mortality in 0-2 year old Atlantic salmon (Cairns, 2001), and a reduction in the response to belted kingfishers could have large impacts on the survival of salmon after release into natural streams. The variation in captive breeding history of these four strains provides an opportunity to study changes in innate and learned anti-predator responses to belted kingfishers due to captive breeding.

1.9 The classical conditioning paradigm

In my study I used a classical conditioning paradigm to evaluate the innate and learned anti-predator responses of captive-bred Atlantic salmon (Rescorla, 1967). Salmon were exposed to either an alarm cue (unconditioned stimulus), a predator cue (conditioned stimulus) or both cues together. The first exposure to these cues was used to evaluate the innate response of salmon. Salmon were then exposed to either a paired or unpaired training treatment. In the paired treatment the alarm cue and predator cue were presented simultaneously, which simulates an environment in which the predator cue is a reliable signal of short-term predation risk. In the unpaired treatment the alarm cue and predator cue were presented at different times, which simulates an environment in which the predator cue is not a reliable signal of short-term predation risk. Following training, salmon were exposed to the predator cue alone to evaluate their learned response. If captive breeding has an effect on anti-predator response, then I predict that the Mersey-LaHave strain will have stronger innate and learned anti-predator responses than the
LaHave strain. Additionally, I predict that the Saint-Jean and Sebago strains would both have stronger anti-predator responses than the LaHave strain.

2 Methods

2.1 Experimental fish

Four Atlantic salmon strains were examined in this study: the LaHave River strain from Nova Scotia (44.3°N, 64.4°W), the Sebago Lake strain from Maine (43.8°N, 70.5°W), the Lac-Saint-Jean strain from Quebec (48.6°N, 72.0°W), and the Mersey-LaHave strain from Nova Scotia (Mersey River: 44.0°N, 64.7°W). These strains differ in the length of time they have been maintained in captivity (in the Ontario Ministry of Natural Resources hatchery system). The LaHave strain has been propagated in the hatchery system since 1995, the Sebago strain since 2006, and the Saint-Jean strain since 2007. The Mersey-LaHave strain is a hybrid strain that was created in 2014 by crossing wild Mersey females and hatchery LaHave males.

The individuals used in this study were from mixed family production stock from the Normandale fish hatchery (42.7°N, 80.4°W). The individuals were raised at Normandale fish hatchery until they became free swimming and at least 1 g in mass, then they were brought to the University of Western Ontario. Upon arrival to the university, the salmon were placed into strain specific 50 L flow through housing tanks that were maintained at 11°C throughout the experiment. Salmon were fed pelleted floating food (Corey Foods) *ad libitum* and maintained on a 12:12 hour light:dark cycle. My experiment began when salmon reached 8 months of age, between August and December in 2014 and 2015.
2.2 Experimental trials

Predator recognition training and behavioural observation took place in $60 \times 40 \times 20$ cm experimental tanks (Figure 2.1). Experimental tanks had a fresh water flow of 100 mL/min to maintain water quality. Tanks included a gravel substrate and a 9 cm long refuge made of 3.8 cm diameter PVC pipe placed on the gravel opposite the inflow. A blind was placed around and above the experimental tanks to obscure the researcher and the digital cameras used to record behaviour.

![Experimental tank](image)

**Figure 2.1** Experimental tank used for behavioural observation and predator recognition training of Atlantic salmon, *Salmo salar*.

At the start of each trial, two salmon from the same strain were randomly selected and moved from a housing tank into an experimental tank (day 0). On days 1-3, salmon were fed 100 mg of floating food (Corey Foods) between 10:00 and 11:00 AM. On day 4, salmon were not fed to ensure that hunger levels were similar during the observations.
The first observations were collected on day 5. Between 10:00 and 11:00 AM, a digital video camera was positioned above each experimental tank and individuals were given 10 minutes to acclimate to the presence of the video cameras. Individuals were then recorded for 5 minutes, during which time they were provided with 50 mg of floating food. Individuals were then exposed to alarm cue alone, predator cue alone, or both cues together. The alarm cue was derived from the skin of juvenile salmon and the predator cue was a belted kingfisher model (described in detail below). When exposed to the alarm cue, 10 mL of cue was injected over 1 second into the tank via a fixed piece of tubing located above the tank inflow. When exposed to the predator cue, the model was presented at the upper edge of the tank for 5 minutes. Individuals were recorded for 5 minutes starting at the beginning of the presentation of a cue, during which time they were provided with 50 mg of floating food. I term the first 5-minute recording, before the presentation of any cue, the first observation pre-cue block, and the second 5-minute recording, during the presentation of cues, the first observation post-cue block. The pre-cue block measured baseline behaviour and the post-cue block measured the behavioural response to cues (behavioural measures are described below).

Predator recognition training began on day 5 and lasted a total of 3 days (i.e. from day 5 until day 7 of the experiment). Individuals that were exposed to the alarm cue and predator cue together during the first behavioural observations were assigned to the paired treatment, whereas individuals that were exposed to either cue alone were assigned to the unpaired treatment. Individuals in the paired treatment were exposed to the predator cue and alarm cue simultaneously, twice a day, at random intervals no less than 60 minutes apart. Individuals in the unpaired treatment were exposed to the predator cue
and alarm cue at separate times, twice a day for each cue, at random intervals no less than 60 minutes apart (Figure 2.2). All cues were presented between 10am and 5pm. On the day after the training period (day 8), all fish were given 24 hours during which no cues were presented and they were not fed.

Figure 2.2 Example schedules for paired and unpaired treatments of predator recognition training of Atlantic salmon, *Salmo salar*.

On day 9, there was a second observation day, in which behaviour was recorded for 5 minutes in the pre-cue block as described above. All salmon were then exposed to the predator cue alone, and behaviour was recorded for 5 minutes in the post-cue block as described above. I term these recordings the second observation pre-cue block and second observation post-cue block. Salmon were then euthanized with an overdose of MS-222 and their body mass and fork length were measured.
A summary of the training and observation timeline is included in Table 2.1. In total, I examined 40 salmon from the LaHave strain (22 unpaired, 18 paired), 28 salmon from the Sebago strain (14 unpaired, 14 paired), 28 salmon from the Saint-Jean strain (14 unpaired, 14 paired), and 18 salmon from the Mersey-LaHave strain (10 unpaired, 8 paired).

**Table 2.1** Timeline of predator recognition training and behavioural observations in Atlantic salmon, *Salmo salar*.

<table>
<thead>
<tr>
<th>Day</th>
<th>Period</th>
<th>Action by experimenter</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>Start of trial</td>
<td>Two salmon from the same strain moved into an experimental tank</td>
</tr>
<tr>
<td>1-3</td>
<td>Acclimation</td>
<td>No cue presented. Salmon fed</td>
</tr>
<tr>
<td>4</td>
<td>Observation preparation</td>
<td>No cue presented. Salmon not fed</td>
</tr>
<tr>
<td>5</td>
<td>Behavioural observation</td>
<td>Salmon fed and recorded for 5-minutes (pre-cue), first exposure to predator cue, alarm cue or both cues, salmon fed and recorded for 5-minutes (post-cue)</td>
</tr>
<tr>
<td>5-7</td>
<td>Predator recognition training</td>
<td>Salmon exposed to paired or unpaired treatments</td>
</tr>
<tr>
<td>8</td>
<td>Observation preparation</td>
<td>No cue presented. Salmon not fed</td>
</tr>
<tr>
<td>9</td>
<td>Behavioural observation</td>
<td>Salmon fed and recorded for 5-minutes (pre-cue), final exposure to predator cue, salmon fed and recorded for 5-minutes (post-cue), termination of trial</td>
</tr>
</tbody>
</table>

### 2.3 Behavioural measures

Each 5-minute block (first observation pre-cue, first observation post-cue, second observation pre-cue, second observation post-cue) was scored for anti-predator response by an observer who was blind to the timing and treatment using a stopwatch and tally counter. Behaviour was scored separately for each fish in a pair. Four behaviours commonly associated with anti-predator response were examined for each block: time spent in shelter, time spent motionless, number of feeding acts, and number of aggressive acts (Brown & Smith 1998, Leduc et al. 2007, de Mestral & Herbinger 2013). Time spent
in shelter was calculated as the total amount of time within a 5-minute block that a salmon spent with at least half of its body within the refuge. Time spent motionless was calculated as the total amount of time within a 5-minute block that a salmon spent stationary on the gravel bottom of a tank or within the refuge. Number of feeding acts was calculated as the total number of floating food pellets consumed within a 5-minute block. Number of aggressive acts was calculated as the total number of rapid movements or biting motions directed towards another salmon within a 5-minute block.

2.4 Preparation of cues
As in previous studies on salmonids, alarm cue was derived from the skin of juvenile Atlantic salmon. Salmon were euthanized using an overdose of MS-222; the skin was removed, homogenized, and filtered through cheesecloth into water collected from the housing tanks to a concentration of 10 mL water per 1 cm² of skin (Ferrari et al., 2010a). Alarm cue was prepared in 400 mL batches by pooling alarm cue produced by individuals from all 4 strains and then frozen in 50 mL aliquots at -20°C until needed (Brown et al., 2013b).

The predator cue was a belted kingfisher model that was 20 cm tall. When presented to salmon in an experimental tank, the model was placed on a raised stand such that the top 12 cm of the model (most of the body and head) was visible over the edge of the tank (depicted in Figure 2.3).
2.5 Statistical analysis

Body mass and fork length were examined using a linear model with strain and treatment as fixed factors. I then calculated overall condition of individuals using the residual from a regression of body mass and fork length. Condition was then also examined in a linear model with strain and treatment as fixed factors. For both models, a Tukey’s HSD test was used to investigate differences in among populations.

The first observation was used to determine the naïve response to the cues. Each of the four behaviours (time spent in shelter, time spent motionless, number of feeding acts, and number of aggressive acts) were examined using a linear model that included as fixed
factors: strain (LaHave, Sebago, Saint-Jean, and Mersey-LaHave), treatment (alarm cue, predator cue, or both cues), the treatment × strain, and as a covariate: body mass. The same model was used for both first observation pre- and post-cue behaviours. Similar models were used to examine the behavioural response to predator cue following training, for the second observation pre- and post-cue blocks, differing only in that the treatments were paired and unpaired. In all models pair ID, a unique identifier for each pair of fish, was included, nested within treatment and strain, to control for any interaction between two fish in an experimental tank (results not presented). For all models the number of feeding acts and number of aggressive acts were logarithm transformed log_{10}(number +1) to achieve normality.

Additionally, the four behavioural measures were combined into a single variable to capture overall activity. To do so, I first calculated a Z-score value for each behaviour based on combined observations across all time periods. These Z-scores were combined into an index of overall activity using Z-score(log(number of feeding acts+1)) + Z-score(log(number of aggressive acts+1)) – Z-score(time spent in shelter) – Z-score(time spent motionless). In general, positive activity values were associated with high feeding and aggression and low time spent in shelter or motionless. The same linear models described above were used to examine the activity values during each observation block.
3 Results

3.1 Physical differences among salmon

Body mass, fork length, and overall condition of each Atlantic salmon was recorded following the final exposure to the predator cue (Table 3.1). There were significant differences in body mass among strains ($F_{3,67} = 18.34, p < 0.01$). Sebago salmon were on average 112% heavier than the other three strains. There was also a significant difference in fork length among strains, with the Sebago salmon significantly longer than LaHave salmon, and Saint-Jean and Mersey-LaHave salmon intermediate in length ($F_{3,67} = 3.07, p = 0.03$). On average Sebago salmon were 15% longer than LaHave salmon. Sebago salmon were also in significantly better condition than the three other strains of salmon ($F_{3,67} = 17.64, p < 0.01$). There were no significant differences in body mass, fork length, or condition for salmon assigned to different treatments (all $p > 0.05$).

**Table 3.1** Descriptive statistics of body mass, fork length, and overall condition as calculated as the residuals of the regression of body mass and fork length of four Atlantic salmon strains, *Salmo salar*. Means in each row followed by the same letter are not significantly different according to Tukey’s HSD ($p < 0.05$).

<table>
<thead>
<tr>
<th></th>
<th>LaHave</th>
<th>Sebago</th>
<th>Lac-Saint-Jean</th>
<th>Mersey-LaHave</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>40</td>
<td>28</td>
<td>28</td>
<td>18</td>
</tr>
<tr>
<td>Body mass (g)</td>
<td>2.8 ± 0.2 b</td>
<td>5.4 ± 0.5 a</td>
<td>2.4 ± 0.2 b</td>
<td>2.6 ± 0.2 b</td>
</tr>
<tr>
<td>Fork Length (cm)</td>
<td>6.5 ± 0.1 b</td>
<td>7.4 ± 0.3 a</td>
<td>7.1 ± 0.3 ab</td>
<td>7.1 ± 0.2 ab</td>
</tr>
<tr>
<td>Overall condition</td>
<td>-0.4 ± 0.2 b</td>
<td>1.8 ± 0.4 a</td>
<td>-1.1 ± 0.3 b</td>
<td>-0.9 ± 0.2 b</td>
</tr>
</tbody>
</table>
3.2 Innate response to cues

Examining overall Atlantic salmon activity on the first observation day during the pre-cue block, there was a significant effect of strain on activity (Table 3.2; Figure 3.1). Mersey-LaHave salmon had lower activity levels than all other strains. As expected, there was no significant difference in activity among salmon assigned to different treatments prior to the onset of those treatments (i.e. before the first presentation of the cues). To investigate the innate anti-predator response and confirm the presence of an innate response to alarm cue, the post-cue block on the first observation day was examined. There was a significant effect of treatment on activity (Table 3.2; Figure 3.1). Exposure to alarm cue either alone or paired with the predator cue resulted in lower activity levels than exposure to the predator cue alone, indicating an innate response to the alarm cue but not to the predator cue (Table 3.2; Figure 3.1). There was a significant effect of strain on activity. Mirroring the trends in the pre-cue block, Mersey-LaHave salmon were less active after exposure to any cue than the other three strains (Table 3.2; Figure 3.1).
Table 3.2 Statistical analysis of behaviour in four Atlantic salmon, *Salmo salar*, strains on the day of the first exposure to predator and alarm cues.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Factor</th>
<th>Pre-cue</th>
<th>Post-Cue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>df</td>
</tr>
<tr>
<td>Time spent in shelter</td>
<td>Strain</td>
<td>1.82</td>
<td>3, 67</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>0.39</td>
<td>2, 67</td>
</tr>
<tr>
<td></td>
<td>Strain × Treatment</td>
<td>0.74</td>
<td>6, 67</td>
</tr>
<tr>
<td></td>
<td>Mass</td>
<td>5.15</td>
<td>1, 67</td>
</tr>
<tr>
<td>Time spent motionless</td>
<td>Strain</td>
<td>13.74</td>
<td>3, 67</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>2.10</td>
<td>2, 67</td>
</tr>
<tr>
<td></td>
<td>Strain × Treatment</td>
<td>0.95</td>
<td>6, 67</td>
</tr>
<tr>
<td></td>
<td>Mass</td>
<td>0.13</td>
<td>1, 67</td>
</tr>
<tr>
<td>Number of feeding acts</td>
<td>Strain</td>
<td>16.44</td>
<td>3, 67</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>2.87</td>
<td>2, 67</td>
</tr>
<tr>
<td></td>
<td>Strain × Treatment</td>
<td>2.69</td>
<td>6, 67</td>
</tr>
<tr>
<td></td>
<td>Mass</td>
<td>0.14</td>
<td>1, 67</td>
</tr>
<tr>
<td>Number of aggressive acts</td>
<td>Strain</td>
<td>0.42</td>
<td>3, 67</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>1.45</td>
<td>2, 67</td>
</tr>
<tr>
<td></td>
<td>Strain × Treatment</td>
<td>1.11</td>
<td>6, 67</td>
</tr>
<tr>
<td></td>
<td>Mass</td>
<td>4.22</td>
<td>1, 67</td>
</tr>
<tr>
<td>Activity</td>
<td>Strain</td>
<td>11.63</td>
<td>3, 67</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>2.64</td>
<td>2, 67</td>
</tr>
<tr>
<td></td>
<td>Strain × Treatment</td>
<td>1.68</td>
<td>6, 67</td>
</tr>
<tr>
<td></td>
<td>Mass</td>
<td>4.64</td>
<td>1, 67</td>
</tr>
</tbody>
</table>
Figure 3.1 Innate and learned anti-predator response of Atlantic salmon, *Salmo salar*, as measured by activity levels. Atlantic salmon, *Salmo salar*, activity as calculated through combined Z-score of four behavioural measures (time spent motionless, time spent in shelter, number of feeding acts, and number of aggressive acts) for each individual. Activity is presented on the first observation day when salmon were first exposed to alarm cue, predator cue, or both cues in the (a) pre-cue block and (b)
post-cue block. Also presented is activity on the second observation day when salmon were exposed to the predator cue following a five day predator recognition training protocol in the (c) pre-cue block and (d) post-cue block. Mersey-Lave salmon were significantly less active than all other strains. After the first exposure to cues, salmon exposed to alarm cue had significantly lower activity levels than salmon exposed to the predator cue, demonstrating an innate response to alarm cue. Following training, salmon from the Sebago strain that had received paired training had significantly lower activity than Sebago salmon from the unpaired training, indicating that only Sebago salmon had acquired a learned anti-predator response.

Examining the behaviour of the Atlantic salmon on the first observation day during the pre-cue block, there was a significant effect of strain on time spent motionless and number of feeding acts (Table 3.1; Figure 3.2). Mersey-LaHave salmon generally spent more time motionless and made fewer food strikes than all other strains. The Mersey-LaHave salmon were motionless for an average of 113 seconds longer per block and made 0 feeding acts compared to the average of 8 feeding acts per block in the other strains. There was no significant effect of strain on time spent in shelter or number of aggressive acts. There was a significant effect of body mass on number of aggressive acts and activity, with larger salmon showing more aggressive acts and more activity than smaller salmon. There was no significant effect of body mass on time spent in shelter, time spent motionless, and number of feeding acts. There was no significant interaction between strain and treatment for any behaviour. As expected, there were no significant behavioural differences among salmon assigned to different treatments prior to the onset of those treatments (i.e. before the first presentation of the cues).
**Figure 3.2 Four measures of Atlantic salmon, *Salmo salar*, innate anti-predator response.** Atlantic salmon, *Salmo salar*, behaviour on the first observation day when salmon were first exposed to alarm cue, predator cue or both cues. Presented are the per individual mean (± SE) of four measures in both the pre- and post-cue block: (a) pre-cue time spent in shelter, (b) post-cue time spent in shelter, (c) pre-cue time spent motionless, (d) post-cue time spent motionless (e) pre-cue number of feeding acts, (f) post-cue number of feeding acts, (g) pre-cue number of aggressive acts, (h) post-cue number of aggressive acts. Mersey-LaHave salmon spent significantly more time motionless and made fewer feeding acts compared to all other strains. After exposure to alarm cue, there were significantly fewer feeding acts than after exposure to the predator cue, indicating and innate response to alarm cue but not the predator cue.

To investigate the innate behavioural anti-predator response and confirm the presence of an innate response to alarm cue, the post-cue block on the first observation day was examined. There was a significant effect of treatment on number of feeding acts (Figure 3.2). Exposure to alarm cue either alone or paired with the predator cue resulted in significantly fewer feeding acts than exposure to the predator cue alone (Table 3.2; Figure 3.2). After exposure to alarm cue, either paired or alone, salmon made an average of 2.1 food strikes per block, compared to the average of 6.7 food strikes per block after exposure to the predator cue alone. This reduction in feeding represents a 74% decrease in the number of feeding acts relative to the pre-cue block and indicates that the alarm cue elicited an innate behavioural response. In contrast, after exposure to the predator cue there was only a 16% decrease in number of feeding acts per block, indicating that the predator cue did not elicit an innate behavioural response. There was a significant effect
of strain on time spent motionless, and number of feedings acts. Mirroring the trends in the pre-cue block, Mersey-LaHave salmon spent significantly more time motionless and fed less after exposure to any cue than the other three strains (Table 3.2; Figure 3.2). Mersey-LaHave salmon were motionless for an average of 132 seconds longer per block and made 0 feeding acts compared to the average 4 feeding acts per block in the other strains. For time spent motionless, there was no significant effect of either treatment or body mass and no significant interaction between strain and treatment. There was no significant effect of body mass and no significant interaction between strain and treatment for number of feeding acts. For activity, there was no significant effect of body mass and no significant interaction between strain and treatment. There were no significant factors in the model examining both time spent in shelter and number of aggressive acts.

3.3 Learned response to predator cue

Examining overall Atlantic salmon activity on the second observation day, following training, during the pre-cue block there was a significant effect of strain on activity. Mersey-LaHave salmon were less active than all other strains (Table 3.3; Figure 3.1). There was a significant effect of body mass on activity; specifically, larger salmon were more active (Table 3.3). There was no significant interaction between strain and treatment for activity. As expected, there was no behavioural difference in activity levels among salmon assigned to different treatments before exposure to the predator cue. To test the hypothesis that captive breeding affected the learned anti-predator response, the post-cue block on the second observation day was examined. There was a significant effect of strain on activity (Table 3.3). Similar to the pre-cue block, the effect was driven
by the Mersey-LaHave strain being less active than all other strains. There was also a significant effect of body mass on activity, where larger salmon were more active than smaller salmon. There was a significant interaction between strain and treatment on activity (Table 3.3). Specifically, based on pairwise comparisons the only significant difference between the paired and unpaired treatments occurred in the Sebago strain \([F_{1, 67} = 4.62, p = 0.039]\) where salmon were more active in the unpaired treatment than in the paired treatment, although two of the other three strains also showed a trend in which activity was lower after exposure to the predator cue in the paired treatment than in the unpaired treatment (Figure 3.1). This reduction in activity indicates that the Sebago strain was the only strain that showed an ability to display a learned response to the predator cue.
Table 3.3 Statistical analysis of behaviour in four Atlantic salmon, *Salmo salar*, strains on the day of the final exposure to the predator cue.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Factor</th>
<th>Pre-cue</th>
<th></th>
<th></th>
<th>Post-Cue</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>df</td>
<td>p</td>
<td>F</td>
<td>df</td>
</tr>
<tr>
<td>Time spent in shelter</td>
<td>Strain</td>
<td>1.21</td>
<td>3, 67</td>
<td>0.31</td>
<td>1.32</td>
<td>3, 67</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>0.26</td>
<td>2, 67</td>
<td>0.61</td>
<td>0.38</td>
<td>1, 67</td>
</tr>
<tr>
<td></td>
<td>Strain × Treatment</td>
<td>0.44</td>
<td>6, 67</td>
<td>0.72</td>
<td>0.71</td>
<td>6, 67</td>
</tr>
<tr>
<td></td>
<td>Mass</td>
<td>3.57</td>
<td>1, 67</td>
<td>0.064</td>
<td>0.43</td>
<td>1, 67</td>
</tr>
<tr>
<td>Time spent motionless</td>
<td>Strain</td>
<td>16.55</td>
<td>3, 67</td>
<td>&lt;0.001</td>
<td>2.09</td>
<td>3, 67</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>1.99</td>
<td>2, 67</td>
<td>0.16</td>
<td>0.97</td>
<td>1, 67</td>
</tr>
<tr>
<td></td>
<td>Strain × Treatment</td>
<td>2.35</td>
<td>6, 67</td>
<td>0.082</td>
<td>1.44</td>
<td>6, 67</td>
</tr>
<tr>
<td></td>
<td>Mass</td>
<td>0.97</td>
<td>1, 67</td>
<td>0.33</td>
<td>0.16</td>
<td>1, 67</td>
</tr>
<tr>
<td>Number of feeding acts</td>
<td>Strain</td>
<td>12.44</td>
<td>3, 67</td>
<td>&lt;0.001</td>
<td>12.57</td>
<td>3, 67</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>1.06</td>
<td>2, 67</td>
<td>0.31</td>
<td>5.24</td>
<td>1, 67</td>
</tr>
<tr>
<td></td>
<td>Strain × Treatment</td>
<td>1.28</td>
<td>6, 67</td>
<td>0.29</td>
<td>1.91</td>
<td>6, 67</td>
</tr>
<tr>
<td></td>
<td>Mass</td>
<td>0.001</td>
<td>1, 67</td>
<td>0.98</td>
<td>1.66</td>
<td>1, 67</td>
</tr>
<tr>
<td>Number of aggressive acts</td>
<td>Strain</td>
<td>4.41</td>
<td>3, 67</td>
<td>0.008</td>
<td>1.24</td>
<td>3, 67</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>0.001</td>
<td>2, 67</td>
<td>0.98</td>
<td>0.25</td>
<td>1, 67</td>
</tr>
<tr>
<td></td>
<td>Strain × Treatment</td>
<td>0.41</td>
<td>6, 67</td>
<td>0.74</td>
<td>1.00</td>
<td>6, 67</td>
</tr>
<tr>
<td></td>
<td>Mass</td>
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<td>0.014</td>
<td>5.95</td>
<td>1, 67</td>
</tr>
<tr>
<td>Activity</td>
<td>Strain</td>
<td>8.04</td>
<td>3, 67</td>
<td>&lt;0.001</td>
<td>14.88</td>
<td>3, 67</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>0.20</td>
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<td>0.46</td>
<td>0.83</td>
<td>1, 67</td>
</tr>
<tr>
<td></td>
<td>Strain × Treatment</td>
<td>1.53</td>
<td>6, 67</td>
<td>0.053</td>
<td>4.14</td>
<td>6, 67</td>
</tr>
<tr>
<td></td>
<td>Mass</td>
<td>3.48</td>
<td>1, 67</td>
<td>0.008</td>
<td>7.81</td>
<td>1, 67</td>
</tr>
</tbody>
</table>

Examining the behaviour of Atlantic salmon on the second observation day, following training, during the pre-cue block there was a significant effect of strain on time spent motionless, number of feeding acts, and number of aggressive acts. Mersey-LaHave salmon spent more time motionless, fed less, and made fewer aggressive acts than all other strains (Table 3.3; Figure 3.3). Compared to the other strains, Mersey-LaHave salmon were motionless for an average of 23 seconds longer per block, made 12 fewer feedings acts per block and made 0 aggressive acts compared to the average 0.2 aggressive acts per block of the other strains. There was a significant effect of body mass
on number of aggressive acts; specifically, larger salmon made more aggressive acts (Table 3.3). There was no significant effect of body mass and no significant interaction between strain and treatment for time spent motionless and number of feedings acts. There was no significant interaction between strain and treatment for activity and no significant effects for time spent in shelter. As expected, there were no behavioural differences among salmon assigned to different treatments before exposure to the predator cue.
Figure 3.3 Four measures of Atlantic salmon, *Salmo salar*, learned anti-predator response. Atlantic salmon, *Salmo salar*, behaviour on the second observation day when salmon were exposed to the predator cue following a five day predator recognition training protocol. Presented are the per individual mean (± SE) for four measures in both the pre- and post-cue block: (a) pre-cue time spent in shelter, (b) post-cue time spent in shelter, (c) pre-cue time spent motionless, (d) post-cue time spent motionless (e) pre-cue number of feeding acts, (f) post-cue number of feeding acts, (g) pre-cue number of aggressive acts, (h) post-cue number of aggressive acts. Mersey-LaHave salmon spent significantly more time motionless, made fewer feeding acts, and fewer aggressive acts than all other strains. After the paired training treatment, salmon exposed to the predator cue displayed significantly fewer feeding acts compared to salmon from the unpaired training treatment, indicating a learned anti-predator response.

Examining the behaviour of Atlantic salmon during the post-cue block on the second observation day there was a significant effect of strain on number of feeding acts (Table 3.3). Similar to the pre-cue block, the effect was driven by the Mersey-LaHave strain feeding less than all other strains, making an average of 7 fewer feedings acts per block than the other strains (Table 3.3). There was also a significant effect of body mass on number of aggressive acts, where larger salmon made more aggressive acts than smaller salmon. There was a significant effect of treatment on number of feeding acts; exposure to the predator cue resulted in fewer feeding acts in the paired treatment but not the unpaired treatment (Table 3.3; Figure 3.3). In the paired treatment salmon made an average of 7 feedings strikes per block compared to 12 feeding strikes per block made in the unpaired treatment; the reduction in feeding in the post-cue paired treatment
represented a 50% reduction in number of feeding acts per block made in the pre-cue block. This reduction in feeding indicates that after the paired training treatment salmon learned to respond to a predator cue that previously did not elicit a response. In contrast, after the unpaired treatment there was only a 14% reduction in number of feeding acts, indicating that there was no learning after the unpaired treatment. There was no significant effect of body mass and no significant interaction between strain and treatment for number of feedings acts. Apart from body mass, there were no significant effects for number of aggressive acts and there were no significant effects for time spent motionless and time spent in shelter.
4 Discussion

4.1 Innate anti-predator response

Exposure to alarm cue commonly elicits an innate behavioural response, often resulting in decreased movement and feedings (Brown, 2003). Here, I found that an alarm cue derived from skin homogenates elicited an innate response in four strains of Atlantic salmon. This is consistent with my prediction that, due to the mechanism of release, damage-induced alarm cues have been a consistent signal of threat over an evolutionary timescale and that the response would be maintained despite captive breeding. Other studies in salmonids have similarly shown that an innate response to skin-derived alarm cue is conserved across multiple generations (reviewed by: Ferrari et al., 2010b), including in hatchery-raised salmonids (Brown & Smith, 1998; Berejikian et al., 2003; Jackson & Brown, 2011). Indeed, one study of rainbow trout found an innate response to alarm cue even after 100 years (~15 generations) in a predator free environment (Scheurer et al., 2007). In my study, presentation of alarm cue was associated with a reduction in the number of feeding acts and overall activity, but there was no effect of the cue on time spent in shelter, time spent motionless, or number of aggressive acts. Previous studies of the innate response to alarm cue in Atlantic salmon have shown that time spent in shelter and time spent motionless are associated with an anti-predator response (Houde et al., 2010; Jackson & Brown, 2011). It is possible that feeding behaviour shows more drastic changes in the anti-predator response and is a more consistent response across salmonids than movement and social interactions, which may be more subtly affected.
Innate responses to direct predator cues are generally more variable among species than the innate response to alarm cues (Hawkins et al., 2004; Brown & Smith, 1998; Ferrari et al., 2010a). For example, an innate response to odour cues of a predatory fish has been observed in some studies of fishes (Hawkins et al., 2004; Jackson & Brown, 2011), but not in others (Brown & Smith, 1998; Ferrari et al., 2010a). I predicted that due to the migration of anadromous fish and natural shifts in predator communities over time, predator cues would not be a consistent signal of threat, both within and between generations, leading to the absence of an innate response to direct predator cues in Atlantic salmon. Consistent with this prediction, I found no innate response to an avian predator cue in four strains of Atlantic salmon. Interestingly, avian predator cues were associated with an innate anti-predator response in previous studies of Atlantic salmon (Houde et al., 2010; de Mestral & Herbinger, 2013). However, these studies of avian predators had the predator model strike the surface of the water, integrating a disturbance component to the presentation of the avian predator cue that was not present in my study. Taken with my data, I suggest the disturbance and not the visual cue of the bird actually elicited the behavioural response in the Atlantic salmon. As predicted, I found that there was an innate behavioural response to the alarm cue but no innate response to the predator cue.

4.2 Effect of captive breeding on the learned anti-predator response

Most studies on learned anti-predator responses pair a stimulus that elicits an innate behavioural response (e.g. an alarm cue), with a stimulus that does not elicit an innate behavioural response (e.g. a predator cue; Brown, 2003). Learning is demonstrated when
the presentation of a predator cue that previously did not elicit a response subsequently elicits the original innate behavioural response. The consistent differences in innate response to alarm cue and predator cue that I observed allowed me to assess learning across the four strains of Atlantic salmon that I used. I predicted that after multiple generations without predation, captive-bred Atlantic salmon would have reduced ability to learn to respond to predators. However, I found no differences among the four strains of Atlantic salmon that could be attributed to captive breeding history. Specifically, the Mersey-LaHave strain did not show an increased ability to learn compared to the LaHave strain, and while the Sebago strain did show a greater ability to learn than the LaHave strain the Saint-Jean strain did not differ from the LaHave strain. Additionally, the Sebago strain only showed evidence of learning when examining overall activity and feeding behaviour, further reinforcing the idea that foraging behaviour is more drastically changed in the anti-predator response than movement and social interactions. My data represent the first explicit test of the effect of captive breeding on the learned anti-predator response, although a number of studies that have shown a learned anti-predator response have been performed using captive-bred salmonids (e.g. Berejikian et al., 2003; Hawkins et al., 2008; Brown et al., 2013a). It is surprising that although learning has been shown in captive-bred salmonids with a wide range in number of generations spent in captivity only one of the strains studied showed an ability to learn to recognise predators. It is possible that the effect of captive breeding on anti-predator learning depends on the nature of the predator cue. Previous demonstrations of learning in captive-bred salmonids all used an odour cue of a predator (e.g. Jackson & Brown, 2011); in contrast, my study used an overhead visual cue of a predator. It is possible that the regular overhead cues
associated with hatchery feeding lead to a reduced sensitivity to these cues, as for example appears to be the case in a study that showed that captive breeding reduced the innate response of Atlantic salmon to overhead predator cues (de Mestral & Herbinger, 2013). Alternatively, the Mersey-LaHave strain was only recently brought into captive environments and any difference in learning ability may have been masked by a disruption to normal behaviour caused by the captive environment. Similarly, Metcalfe et al. (2003) found that after the first generation of captive breeding, Atlantic salmon were less active than both wild-caught and second-generation captive-bred salmon. It is possible that the low baseline activity in the Mersey-LaHave strain masked the anti-predator response. Overall, it remains unclear if captive breeding has an effect on the ability of individuals to learn to respond to predators.

4.3 Other causes of population differences in the learned anti-predator response

Factors other than captive breeding might also affect cross-population differences in the ability to learn. Such factors are implicated in my study, because the Sebago strain showed a greater capacity to learn than the Saint-Jean strain, despite comparable captive breeding histories. If anti-predator learning depends on body size or developmental stage, then the greater body mass and condition of the Sebago strain compared to the Saint-Jean strain could explain the difference in learning ability. However, in a study of rainbow trout the acquisition of a learned anti-predator response was not linked to growth rate or body size, and in fact, larger fish had lower retention of the learned response (Brown et al., 2011). Previous work investigating the ontogeny of the learned anti-predator response in Atlantic salmon similarly showed that the ability to learn was unrelated to body size,
although learning was age dependant and occurred only when salmon were at least four-months old (Hawkins et al., 2008). All the fish used in my experiment were older than four months, and based on previous studies body size itself is unlikely to explain the differences in learning ability I observed among populations. If body size cannot explain cross-population differences in the ability to learn, then other factors must be considered.

Another factor that might explain the difference in learned anti-predator responses between the Sebago and Saint-Jean strains could result from population-level differences in the predator communities that these two populations have evolved with. Bird counts from the eBird Survey (Sullivan et al., 2009), which spans North America and uses volunteer reporting of bird sightings, show no major differences in the summer abundance of major avian predators of Atlantic salmon (banded kingfishers and common mergansers) across the populations used in my study. However, the Saint-Jean and Sebago populations do show temporal differences in the abundance of banded kingfishers. Sebago Lake is farther south than Lac-Saint-Jean, and banded kingfishers are present at Sebago Lake year-round whereas banded kingfishers are only present at Lac-Saint-Jean between April and October (Sullivan et al., 2009). Salmon are smallest and most vulnerable to avian predation early in the year (Cairns, 2001) and the greater abundance of banded kingfishers at Sebago Lake during this time of year could lead to a predisposition of Sebago salmon to respond to banded kingfisher cues. Indeed, previous work in mammals has shown that individuals are innately more skilled at detecting visual cues of native predators than of neutral objects (Shibasaki & Kawai, 2009). Regardless of the cause, I present some of the first evidence that predator recognition learning differs among populations within a species.
4.4 Implications for reintroductions

When captive-bred animals are released into novel environments, as is the case in reintroduction programs, the choice of source population may be an important determinant of a program’s success (reviewed by Houde et al., 2015a). The strains investigated in this study are all being considered for reintroduction into Lake Ontario and previous studies have suggested that the Sebago strain is the most likely to establish a self-sustaining population, based on greater growth and survival when in competition with non-native salmonids present in Lake Ontario streams (e.g. Van Zwol et al., 2012; Houde et al., 2015b). Here, I found that the Sebago strain also had the greatest ability to develop a learned association between alarm cue and a signal of predation, which may reduce mortality due to predation after release into natural habitats. Indeed, recent work shows that individuals from the Sebago population have the highest survival in natural streams feeding into Lake Ontario (Houde et al., 2015b). This survival advantage could be due to a greater ability to learn and respond to novel predators, although more work is needed to understand the effect of learning ability on post-release survival. Overall, my results add to the growing evidence that the Sebago population is best suited for reintroduction to Lake Ontario streams.

4.5 Caveats of the research

In the scope of my Masters there were a few things that could not be included, which would be interesting directions for future studies. For example, I could not entirely separate the effects of population-level differences in learning ability from the effects of captive breeding history due to a lack of wild populations that could be paired for each captive-bred population. Wild populations would ideally be used to measure baseline
learning ability for each captive-bred population in a paired design and would allow a more detailed and powerful investigation that could partition differences in learning to effects of the population, captive breeding, and the interaction between those factors. Second, the populations I used were not selected for ecological differences in predator communities; however, it appears as though predator communities may be an important factor in the learned anti-predator response. Belted kingfishers are an important predator for all three populations studied and are abundant across all three populations. To investigate the effects of predator community on learning, a different predator could be used that is more variable in its distribution across the populations studied. Of particular interest, if a predator cue from an introduced species was used (e.g. brown trout), then the anti-predator response could be linked to known and variable co-evolutionary histories between the predator and prey. Finally, it would be interesting to look at the ability of these populations to learn to recognize stimuli that are not ecologically relevant. There may be enhanced recognition of an ecologically relevant stimulus, like a belted kingfisher, that affects the ability of salmon to learn. By including non-relevant stimuli as a control we could further investigate differences in the absolute learning potential between captive-bred and wild populations of salmon.

4.6 Impact on the field of learned anti-predator behaviour

In my Masters, I contributed two main findings to the field of learned anti-predator behaviour, providing tests of the effects of between population differences and captive breeding on the ability to demonstrate a learned anti-predator response. Previous work on the learned anti-predator response has focused on within population differences and factors that affect the acquisition and retention of the learned anti-predator response. For
example, Hawkins et al. (2008) investigated a single population of Atlantic salmon and found that the ability to learn to recognize northern pike as predators was only present after the salmon were four months old. I have provided some of the first evidence that populations differ in the ability to learn, which suggests a widespread limitation for a field that typically uses a single population to test hypotheses. Previous research has shown a consistent decline in the innate anti-predator response following captive breeding (Houde et al., 2010; Jackson & Brown, 2011; de Mestral & Herbinger, 2013). However, in my Masters I found no evidence that the learned anti-predator response is affected by captive breeding. It is possible that, while innate anti-predator responses are reduced after multiple generations of captive breeding, the learned response can be maintained through multiple generations, even in the absence of predation. This effect had not been tested prior to my thesis.

4.7 Future research

Future research could expand on the two main findings of my Masters. First, further investigation into cross-population differences could examine how factors that effect the acquisition and retention of the learned anti-predator response vary among populations. For example, comparing populations with different timing of major predation events (such as the arrival date of a migratory predator) could reveal differences in age-dependant learning. Alternatively, comparing populations with different predator communities could reveal pre-dispositions of individuals to learn to respond to ecologically relevant stimuli. Second, further work comparing the learned anti-predator response within a single population across varying numbers of generations spent in captive breeding programs is needed. There is a lack of knowledge about the evolution of
the learned anti-predator response and if it can be maintained through captive breeding this would have large impacts on reintroduction efforts. Additionally, studies of the effect of captive breeding on the learned anti-predator response across different populations would provide valuable knowledge into the evolution of the learned anti-predator response. Comparing the learned anti-predator response of wild and captive-bred individuals from multiple populations with different predator communities could provide insight on the evolution of the learned anti-predator response.
References


Appendices

Appendix A: Ethics Statement.
All experiments followed ethical guidelines from the Canadian Council on Animal Care as reviewed and approved by the Animal Use Subcommittees at the University of Western Ontario (protocol # 2010-214).

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