Induced neophobic predator avoidance in hatchery-reared juvenile Atlantic salmon (*Salmo salar*)

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ABSTRACT

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Hatchery-reared fishes, especially salmonids, are routinely stocked into natural waterways as a part of population enhancement and recovery programs, and conservation efforts. These initiatives, however, are often met with limited success due to poor post-stocking survival of hatchery fish. It has been suggested that a failure to recognize predators by hatchery-reared fish leads to disproportionately low post-stocking survival rates. A commonly advocated approach to enhancing post-stocking survival is to condition hatchery fish to recognize and avoid relevant threats through 'life skills training'. However, such approaches have yielded decidedly mixed results. Here, I tested the prediction that phenotypically plastic neophobic predator avoidance (the avoidance of novel cues) can be induced in hatchery reared Atlantic salmon. This response may lead to the enhancement of post-stocking survival among hatchery reared juvenile Atlantic salmon; making it a new approach to life skills training. Initially, I tested the prediction that hatchery reared juvenile salmon subjected to alarm cue (a known, reliable cue indicating a predation event) for a prolonged period of time would elicit a neophobic response (defined as a significant decrease in time spent on the substrate and foraging attempts) to a novel cue during testing. During the treatment phases of three or six days, juvenile hatchery Atlantic salmon were given either alarm cue (high risk) or a water control (low risk). Following the treatment phases, fish from both treatment conditions were given a stimulus of either novel odour or a water control. The test fish's time on substrate and foraging attempts were recorded pre- and post-stimulus, allowing me to investigate changes in behaviour caused by the presence of the novel stimuli. My results suggested that juvenile Atlantic salmon pre-exposed to alarm cue for three days did not demonstrate neophobic predator avoidance behaviour, however those pre-exposed for six days did exhibit an increase in time on substrate and a reduction in foraging attempts. Secondly, I tested the prediction that a neophobic response induced in hatchery reared fish over a six-day treatment phase would be retained throughout a transportation and stocking event.

Conditioned salmon were transported to Catamaran Brook (Catamaran Research Centre), and tested for their response to a novel cue vs. water control. My results suggest that juvenile Atlantic salmon pre-exposed to alarm cue for six days and transported and released into a semi-natural environment did not demonstrate neophobic behaviour towards a novel cue. This suggests that the neophobic response was not retained throughout a transportation and stocking event. Finally, I tested the prediction that neophobic predator avoidance could be induced in hatchery reared and wild juvenile Atlantic salmon in a semi-natural environment. Wild and hatchery fish were subjected to alarm cue or water (control) for six days in a semi-natural environment after which their behaviour towards a novel cue was tested (in the same fashion as in experiment one) in the semi-natural environment. My results suggested that hatchery-reared fish given alarm cue for six days in a semi-natural environment demonstrated a neophobic predator avoidance response to a novel cue during behavioural testing in the semi-natural environment; however wild fish in the same conditions did not.

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Contribution of Authors

As first author of this thesis, I was responsible for most of the writing, design, set up, data collection and analysis. This thesis is co-authored by my supervisor Dr. Grant E. Brown. He also came up with the general concept of this thesis and work.

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1. Introduction

Many economically relevant fishes are currently facing extinction (Naylor et al., 2000; Heithaus et al., 2008). Between 1970 and 2010, declines of at least 39 % has been observed in aquatic marine fishes population numbers and 79% decline has been observed in freshwater fishes (Living Planet Report, 2014). This decline in population sizes can be linked to an variety of non-mutually exclusive factors such as habitat degradation, overexploitation and global climate change (Cambray & Bianco, 1998; Brander, 2007). Loss of fishes in our oceans and rivers leads not only to environmental consequences such as decreased biomass and unknown resulting consequences such as loss of productivity and stability (Tilman, 1999), but also economic losses. In Canada alone, the recreational fishing industry brings in over eight billion dollars of economic impact annually (Bailey et al., 2012). Marine commercial fishing also has strong economic impacts; bringing in over 17 billion dollars of indirect and direct profits to the US in 2004 (IGFA, 2006). For ecological and economic reasons, conservation of fish species has become a priority.

Conservation practices such as reintroduction (stocking individuals into their native range where their population had been depleted), reinforcement (stocking individuals into their native range where there is an existing but declining population; Swan et al., 2016) and ecological replacement (stocking individuals into a suitable area outside of their native range; Hunter et al., 2012) are used as tools to boost diminishing wild aquatic populations. These conservation practices often require captive rearing in which individuals of a locally endangered species are bred in captivity and released (stocked) into suitable wild habitats at an optimal age or size for survival (Cowx, 1994). Stock enhancement programs involving captive breeding are believed to be a successful method to restore stable populations of endangered species (Patrick et al., 2006), and are for that reason, are numerous.

Conservation efforts for depleting fish populations typically rely on hatchery programs. Hatcheries rear fish in captivity and stock them into natural waterways as part of fish population recovery efforts (Patrick et al., 2006). As of 2002, over 300 different fish species (including 290 freshwater species) are raised in hatcheries and released as part of conservation efforts globally (Patrick et al., 2006). This costs governments and investors estimated billions of dollars annually (Brown & Day, 2002). Although expensive, they are efficient at releasing fish into the wild. For

example, more than five billion Atlantic salmon *(Salmo salar)* individuals are released into the wild from hatcheries per year (Brown & Day, 2002).

Hatchery programs, however, do have the downfall of producing individuals with low post-stocking survival rates (Olla et al., 1998). Low survival following the release of hatchery reared fish into the wild has been seen in a range of fish species, and is most notable in at risk or endangered Salmonid species (Olla et al., 1998). Low post-stocking survival has been observed in trout parr (*Salmo trutta)* stocked in the Guden River tributaries (Berg & Jorgensen, 1991), and in muskellunge (*Esox masquinongy*), which only live up to 60 days following their release (Margenau, 1992). This low post-stocking survival trend has also been seen in cutthroat trout (*Oncorhynchus clarkii*); two-year-old hatchery reared cutthroat trout die within two weeks of being released as a result of starvation and inability to compete with the native populations (Miller, 1952).

Although low post-stocking survival rates may be caused by a variety of factors, high levels of predation on hatchery reared fish directly following stocking is argued to be a major effect (Howell, 1994). An Atlantic salmon stocking event in Massachusetts resulted in a 48.6% mortality caused by predation on the salmon fry within two days of release (Henderson & Letcher, 2003). This same study found that the percent of stocked salmon eaten substantially decreased after two days. Furthermore, hatchery released Atlantic salmon smolts equipped with acoustic transmitters were found to be heavily preyed upon following release. Within just days after release, at least 75% of hatchery Atlantic salmon were lost to predation; 15% in the river systems and 71% following their entrance to a marine environment (Archavala et al., 2011). Another study found that gut contents of the predatory squawfish (*Ptychocheilus oregonensis)* differed greatly before and after the release of 1.1 million yearling chinook hatchery salmon into their Clearwater River system in Idaho. Within 24 hours of the hatchery salmon's release, 54% of the squawfish gut content by weight consisted of hatchery chinook salmon. After five days this jumped to 78 %, and after seven days it went up to 86 % (Shively et al., 1996).

In a wild environment, Atlantic salmon must be able to successfully avoid a wide range of predators across all ontogenetic stages. Not only must they manage fish predators living in their immediate environment, like pike (*Esox lucius)* (Kekäläinen et al, 2007) and cod (*Gadus morhua*) (Hvidsten & Møkkelgjerd, 1987), they must also avoid avian predators. Common avian

Atlantic salmon predators include the heron (*Ardeidae*) (Johnsson et al., 2001) and the kingfisher (*Ceryle alcyon*) (Gotceitas et al., 1991).

The threat of predation on hatchery reared fish following release decreases with time. Stocked chinook salmon (*Oncorhynchus tshawytscha*) that acclimatized to their release site in predator free enclosures for three days prior to release had a higher likelihood of survival following their release than non-acclimated fish (Brennan et al., 2006). The high mortality rates recorded in released hatchery fish, largely caused by predation events, suggests a lack of anti-predator behaviours (i.e. a failure to recognize relevant predation threats) in these fish compared to their wild counterparts. Fish living under natural conditions learn directly and indirectly through experience with their environment to recognize and avoid predators.

This lack in predator avoidance survival skills can be attributed to the lack of complexity and ecologically relevant cues in the hatchery system (Kellison et al., 2000); leading to lack of predator avoidance skills and other context appropriate behaviours (foraging strategies and spatial use) in hatchery fish (Salvanes & Braithwaite, 2006). Cultured flounder (*Pseudopleuronectus americanus*), for example, were found to take two days following their release to gain burying skills and 90 days for their colour to adapt to the sediment (components of the natural predator avoidance response). Cultured flounder take significantly longer to adopt these survival skills than their wild counterparts (Fairchild & Howell, 2004). Studies have further shown that a lack of experience plays into decreases in survival skills in hatchery fish. For example; hatchery reared coho salmon (*Oncorhynchus kisutch*) smolts subjected to stimuli associated to a predation event (visual, chemical and tactile cues) demonstrated a decrease in mortality due to predation following their release into the wild compared to conspecifics that did not receive the predation stimuli prior to release (Olla et al., 1998). Furthermore, in Atlantic salmon, an improvement in post-stocking foraging success was seen when fish were subjected to live prey in the hatchery system (Brown et al., 2003). This suggests that hatchery fish lack experience with predation and foraging cues leading to a lack of survival skills.

These lack of survival skills can also be attributed to the relaxation of selective forces leading to inadvertent selection of maladaptive traits caused by differential experiences in the hatchery (Olla et al., 1998). The hatchery system is very stable when compared to wild habitats; there is little change in abiotic environmental factors such as temperature, precipitation, habitat complexity, water quality and oxygen levels (New & Valentin, 1999). There is also a lack of

biotic variation and stimulation such as predation and competition (due to the large amount of a consistent and predictable food; Álvarez & Nicieza, 2003). This lack in variation of conditions in the sheltered hatchery setting leads to the biotic relaxation of natural selective pressures that occur in the wild (Kostow, 2004); which can lead to an inadvertent selection for traits beneficial in the hatchery setting, yet maladaptive in the fish's native range (Olla et al., 1998).

A lack of ecologically relevant experience in the stable, predictable hatchery system selects for bold behavioural phenotypes. Conversely, unpredictable, high predation wild environments select for shy behavioural phenotypes (Sundström et al., 2004). Bold phenotypes are selected for in the hatchery system since predation threats are non-existent. Fish can afford to forage and utilize all of the water column since increased spatial use and foraging cannot increase the chance of predation. Furthermore, hatchery fish that exhibit a bold behavioural phenotype will have access to the most food. This suggests a difference in investments towards predator avoidance in stable predictable environments in comparison to more unpredictable, high predation environments (Jackson & Brown, 2011). In contrast, wild fish with bolder phenotypes that forage at higher rates than shyer individuals are at a higher risk for predation (Biro et al., 2004). The adoption of shy behavioural phenotype comes from the need invest more in predator avoidance at the cost of other activities, such as foraging and courting under high predation conditions, because the cost of failing to appropriately react to a predation threat in a high predation environment is high (Johnson et al., 2013). Therefore, wild Atlantic salmon from high predation sites demonstrate strong responses to predation cues where as second generation (F2) young-of-the-year Atlantic salmon from hatchery environments (in which no predators are present) show only a weak response to predation cues (Jackson & Brown, 2011).

In order to favour the adoption of anti-predator behaviour in the bolder hatchery reared fish (compared to their shyer wild counterparts), researchers have implemented life skills training. Life skills training works on the assumption that if fish can recognize predators, they can better balance trade-offs between foraging (and other costly activities) and predator avoidance following their release into the wild.

1.1. Life Skills Training

Hatchery fish can be conditioned to exhibit context-specific behaviours (recognition of a specific predator, recognition of foraging opportunities) through life skills training (Brown & Day, 2002; Brown et al., 2013). Life skills training is being used as a tool in the hatchery system to increase predator avoidance in post-stocked hatchery reared fish (Brown et al., 2013). Rainbow trout (*Oncorhynchus mykiss*), for example, will react appropriately to a predator's odour after being conditioned to associate a predator odour with a predation event (Brown & Smith, 1998). This type of learning occurs when conspecific chemical alarm cues (Chivers & Smith, 1994) are paired with predator chemical cues. Alarm cues are found in the skin of many aquatic species and are reliable indicators of predation (Chivers & Smith, 1994; Brown, 2003). Although promising in laboratory settings, life skills training projects involving learned behaviours have shown decidedly mixed results and have yet to translate into a method to increase post-stocking survival (Brown et al., 2013).

The life skills training approach is argued to allow fish to behave appropriately in very specific contexts (when subjected to recognized cues) (Brown & Smith, 1998). However, for life skills training to be successful, fish would have to retain learned information. Likewise, life skills training would have to include all ecologically relevant associations required for their survival following their release into their native environment. Fish released from a hatchery system are exposed to a large number of novel cues; making such a task unfeasible. Instead of teaching fish to recognize specific cues, researchers should focus their efforts on changing the fish's overall behaviour in a way that will minimize the costs of the fish's behavioural decision making once released into the wild.

1.2. Neophobia as a Novel Approach to Life Skills Training

As an alternative to acquired predator recognition approaches (Brown et al., 2013), phenotypically plastic neophobic predator avoidance may serve as a life skills training tool. Neophobia is broadly defined as an avoidance of novel cues (Brown et al., 2015). Neophobic predator avoidance is argued to reduce the costs associated with the initial encounter with a predator (i.e. learning; Brown et al., 2013). Prey exposed to elevated or unpredictable predation risks exhibit increased antipredator responses towards novel cues (Brown et al., 2013). For

example, convict cichlids (*Amatitlania nigrofasciatus*), woodfrog tadpoles (*Lithobates sylvaticus*) and Trinidadian guppies (*Poecilia reticulata*) have been shown to exhibit induced neophobic predator avoidance (Brown et al., 2013; Brown et al. 2015). This plastic response allows prey to respond to variable or elevated predation threats while reducing the initial cost of learning to recognize specific predator types. However, continued neophobic responses in the absence of an actual threat are potentially costly in terms of, for example, lost foraging opportunities. In the absence of acute threats, the neophobic response to novel cues will wane (Brown et al. 2015). As such, induced neophobic predator avoidance is potentially beneficial as it reduces the costs of learning while still allowing for sufficient behavioural flexibility to respond to unknown predation threats (Brown et al. 2013). Thus, phenotypically plastic neophobic predator avoidance is argued to be an adaptive response to elevated and/or unpredictable predation risk.

1.3. Predictions

The main objective of this study was to test if neophobic predator avoidance could be induced in hatchery-reared juvenile Atlantic salmon. A neophobic predator avoidance response in this study was defined as a significant interaction between condition during treatment (experimental and control) and cue given during testing (novel odour or water control). It was predicted that fish under the experimental condition during treatment (subjected to alarm cue) would demonstrate a neophobic predator avoidance response when given a novel cue. A neophobic predator avoidance response in this study is defined as a significant decrease in foraging attempts and a significant increase in time spent on the substrate following the contact of the fish with a novel odour.

1.3.1. Experiment one

I predicted that if hatchery reared juvenile salmon were subjected to alarm cue for three days, then they would elicit a neophobic response to a novel cue. I predicted an interaction between treatment condition and cue in pre-exposed fish to alarm cue for three days in response to a given novel cue; based on past lab and field studies (Brown et al., 2015; McCormick et al., 2017; Crane & Ferrari, 2016). Likewise, I also predicted that if hatchery reared juvenile salmon were subjected to alarm cue for six days, then those experimental fish would also elicit a neophobic predator avoidance response to a novel cue. I predicted a strong interaction between

treatment condition and cue given during testing. Finally, I predicted that if juvenile hatchery reared Atlantic salmon were successfully induced with neophobic predator avoidance in the hatchery, then the behavioural response will be retained during a transportation and stocking event. I predicted a significant interaction between treatment condition and cue given during testing in a semi-natural environment following a transportation and stocking event.

1.3.2. Experiment two

I hypothesized that if wild juvenile Atlantic salmon were exposed to alarm cue in a semi-natural environment, then they would demonstrate a neophobic predator avoidance response to a novel cue. I predicted an interaction between treatment condition and cue given during testing in pre-exposed wild juvenile Atlantic salmon in a semi-natural environment. I also predicted that if hatchery juvenile Atlantic salmon were exposed to alarm cue in a semi-natural environment, then they would also demonstrate a neophobic predator avoidance response to a novel cue. I predicted an interaction between treatment condition and cue given during testing in pre-exposed hatchery reared juvenile Atlantic salmon in a semi-natural environment.

1.4. Study subjects

Atlantic salmon were a suitable subject for this study, as conservation of this species is of great interest. Extirpation of the species has been confirmed in most of their southern native range and population numbers continue to diminish along their central native range (Parrish et al., 1998). Also, the wild Atlantic salmon is a fish that holds not only economic value, but also cultural significance to Canadians (Mills, 2003) and plays an important ecological role in the Atlantic Canadian shore ecosystem (Hendry & Cragg-Hine, 2003).

2. Methods

2.1. Treatment/Testing Locations and Facilities

2.1.1. Hatchery facility

The hatchery facility utilized in this study was the Miramichi Salmon Conservation Centre, South Esk, New Brunswick. This hatchery breeds young-of-the-year Atlantic salmon from wild adults caught directly from the Miramichi river system. The young-of-the-year hatchery reared Atlantic salmon used in this study were housed in the Miramichi Salmon Association hatchery in fibreglass raceways at a high density of ~3000 fish m^{-3} in brook water at a temperature of 16 to 18°C. Oxygen levels were monitored by the hatchery staff and was never lower than 11 ppm (mg L^{-1}). This study used young-of-the-year Little South-West strain Atlantic salmon raised by the Miramichi Salmon Conservation Centre. The hatchery staff fed the juvenile salmon 0.7 gr Optimum™ (Corey Tec) flake food every 45 minutes between eight am and four pm. The hatchery staff put these fish on preventative antibiotics (Tribrisson™) at a dose of 75 mg kg^{-1}, based on the total biomass per race-way during the first ten days of this study.

I used modified 20 L translucent bins to hold the fish during induction and testing periods of this study. Bins measured 52 x 25 x 32 cm (L x W x H). I replaced the front and back of the bins with wired five mm mesh squares to allow water to flow through. These mesh square cut-outs were 18 cm (length) x 15 cm (height) and were attached onto the bins using white aquarium grade non-toxic silicone. Juvenile salmon were placed into either an experimental holding bin at the downstream end of the raceway or a control holding bin at the up stream end of the raceway (see figure 1.1.). I separated experimental and control holding bins by at least four meters, to prevent cross-exposure. This distance was found to be sufficient when testing with florescent dye. A water depth of 11 cm was maintained within holding bins.

2.1.2. Field site

Behavioural observations (see below) under semi-natural conditions were conducted at Catamaran Brook at the Catamaran Brook Research centre (for maps and exact location of the site please refer to Leduc et al., 2006). While in the field, I kept the juvenile salmon in the bins described above. I placed these bins into the Little South West River and weighed them down with rocks. I placed the bins inside a four by one-meter mesh enclosure (see Figure 1.2.). Flow

rates varied daily, but were consistent across all conditions. Temperature of the water fluctuated from 16-21°C. Salmon were transported to the Catamaran research centre (approximately a one-hour drive) in aerated 50 L buckets. I placed the juvenile salmon in translucent blue bags filled with hatchery raceway water. This method of transport resulted in a 100% survival rate.

2.2. Preparing chemical cues

I prepared alarm cue using hatchery reared juvenile Atlantic salmon of approximately 2.5 cm in standard length. I euthanized the donors by giving a single blow to the head in accordance to Concordia University Animal Care Committee Protocol AREC #30000255. I made a whole body homogenate alarm cue using distilled water. The alarm cue concentration was calculated using the cm^2 of skin on the fish used in the whole body homogenate, since the chemical compound need is found in the skin. The final alarm cue concentration was 0.168 cm^2mL^{-1} of fish skin into distilled water. A stock solution was made and separated and packaged into 60 mL aliquots. A concentration of 0.1 of alarm cue has been shown to elicit a neophobic predator avoidance response in juvenile cichlids (Brown et al., 2015; Wisenden & Sargent, 1997) and a concentration of 0.15 $cm^2\ mL^{-1}$ has been shown to be sufficient for juvenile and parr Atlantic salmon to detect the alarm cue under wild conditions (Leduc et al., 2006). The alarm cue solution used in this study was up to 60% more concentrated than alarm cue concentrations used in past studies. I increased the concentration used to ensure detection under flowing water conditions.

I prepared a novel odour each test day using grocery store No Name™ brand almond extract and stream or hatchery water (depending where testing took place) at a concentration of six mL of novel odour per 300 mL of water. This concentration of novel odour has been shown to elicit a neophobic predator avoidance response in the experimental fish (Brown et al., 2015).

2.3. Experiment One: Treating hatchery reared fish with alarm cue in a hatchery environment

I placed groups of 250 juvenile hatchery Atlantic salmon into two separate holding bins within a raceway in the hatchery. These fish were haphazardly taken from the same brood stock controlling for differences in size across treatments (Figure 1.3.). I placed the control holding bin into the hatchery raceway upstream and the experimental bin downstream.

The juvenile salmon were treated with alarm cue or water (control) for three or six days at the hatchery. During the treatment phase, I placed 20 mL of alarm cue or 20 mL of water into the experimental and control bin respectively at unpredictable times, three times per day (no more than three and a half hours apart and never more than once within an hour). I administered the alarm cue or water into the bins using syringes that excreted water or alarm cue in front of the mesh squares of the holding bins. The alarm cue or water flowed through the mesh front of the bins, through and out of the bins at a rate of ~ 4.5 cm sec^{-1}. A treatment period of three or six days was followed by behavioural tests on a subset of experimental and control fish in which two behavioural measures (time on substrate and foraging attempts) were recorded before and after the fish were exposed to a given cue (novel odour or a water control).

During testing, juvenile salmon were haphazardly taken from either the control or the experimental bin and placed into an identical bin for testing. I made sure that the condition during the treatment phase and cue given to the fish during testing was unknown to the data collectors (i.e. blind to treatment) during testing. Once placed into the testing bin, juvenile salmon were given a five-minute acclimation period after which the fish's initial behaviour was tested. Time spent on substrate was recorded using stop watches. Foraging attempts were recorded using hand counters. Flake food was given every 45 minutes by hatchery staff. The flow through brook water in the hatchery also provided drift that the hatchery fish fed on during testing. Flow through drift was also constantly available to fish tested in the semi-natural environment.

Following the five-minute initial behavioural observation, juvenile salmon received 20 mL of water or a novel odour and were tested for another five minutes. I administered the either novel odour or a water control using two 10 mL syringes that released the stimulus in front of the front mesh panel of the testing bin. The flow of the water brought the stimulus into and through the bin at a rate of ~ 4.5 cm sec^{-1}. I tested approximately 20 juvenile salmon from both bins (the first bin holding fish that received alarm cue and the second holding fish given a water control during the treatment phase) each testing day. Of the 20 fish tested from each bin, ten were given a water control; and ten were given novel odour; giving four different conditions resulting in a targeted n of 10 per block of testing (on some testing days more than 10 fish were tested per condition). I conducted two testing blocks, resulting in a total of at least 40 fish. Standard length for each fish tested was recorded (Table 1.1.).

In order to study the retention of the neophobic response, a subset of the juvenile salmon (25 fish from the control condition and 25 fish from the experimental condition) treated for six days (and not tested in the hatchery) were transported and held in a semi-natural environment at the field site. The holding site consisted of two bins, one bin housing fish given alarm cue for six days during the treatment phase prior to transport and the other for fish given water during the treatment phase prior to transport. Holding bins in the semi-natural environment were identical to bins used during the treatment phase in the hatchery and were placed within a four-by-one-meter net enclosure. Juvenile hatchery reared salmon acclimated to their new bins in the semi-natural environment for 24 hours after which behavioural testing was conducted in the semi-natural environment. The response to novel cue vs. water control was tested as described above.

2.4. Experiment Two: Treating hatchery and wild fish with alarm cue in a semi-natural environment.

Wild fish used in this study were caught using dip nets in the Catamaran Brook. A total of 42 Atlantic salmon juveniles were caught on July 14th (used for block one of testing), and another 42 fish were caught on July 20th and 21st (used for block two of testing). Once caught, fish were placed in 20 L holding buckets on site and then transported down the brook to their holding bins. Fish were divided into either experimental or control fish and placed into their respected bins for treatment (Figure 1.3.). The experimental holding bin was placed downstream, where as the control bin was placed upstream (bins were identical to those used in experiment one) within a four by one-meter mesh enclosure in the semi-natural environment. The wild fish acclimated for at least 24 hours and no more than 48 hours (based on date caught) in their given bins before a six-day treatment period began.

Hatchery juvenile salmon (54 per testing block) were also transported to the semi-natural environment (using the same transport protocol as experiment one) where they too were divided across experimental (downstream) and control (upstream) holding bins identical to those used in the first experiment within the same four by one-meter mesh enclosure. The fish acclimated for 48 hours prior to a six-day treatment period.

The flow rate in the semi-natural environment was reduced when alarm cue was administered. I reduced the flow rate to resemble the flow in the hatchery by placing a plastic sheet in front of the mesh at the front of the bins for five minutes. I also reduced the flow rate at

three other random times during the day so that the fish did not associate the reduction in flow rate to the alarm cue administered.

During testing in the field, I haphazardly took a juvenile salmon out of either the control or the experimental bins within the net enclosures and placed the fish into an identical bin outside the net enclosure. The testing bins were placed in parallel in the brook at the same depth as the bins inside the net enclosures. I made sure that the treatment condition and cue given to the fish during testing was unknown to the data collectors. Once placed into a testing bin, I gave the juvenile salmon a five-minute acclimation period after which the fish's initial behaviour was tested (time on substrate and forage attempts). The flow of the brook was reduced during testing by placing a plastic sheet in front of the mesh at the front of the bins. Time spent on substrate was recorded using stop watches, foraging attempts were recorded using hand counters. The brook water provided drift that the fish fed on during testing.

Following the five-minute initial behavioural observation, the juvenile salmon received 20 mL of either a water control or a novel odour and were tested for another five minutes. I administered the either novel odour or water using two 10 mL syringes that released the stimulus in front of the front mesh panel of the testing bin. The flow of the water brought the stimulus into and through the bins at a rate of ~ 4.5 cm sec^{-1}. I tested approximately 20 juvenile salmon (weather not always permitting) from both the experimental and control treatment condition each testing day. I tested the behaviour of ten fish from each condition after giving a water control; and ten from each condition after giving novel odour; giving four different conditions resulting in n = 10 per block of testing. I conducted two testing blocks, resulting in a total of 40 fish (weather permitting). The standard length of both hatchery and wild fish were recorded (Table 1.1. & Table 2.1.).

2.5. Statistical Analysis

All analyses were conducted using IBM SPSS Statistics version 24. The behavioural measures in this study were time spent on substrate and foraging attempts. For each behavioural measure, I calculated the change in behaviour between the pre- and post-cue observations and used these difference scores as dependent variables. I checked these difference values for all data collection days for normality using the Shapiro-Wilks test that plots MANOVA residuals against the predicted value. Difference values from each test day did not meet the assumptions of

normality under the Shapiro-Wilks test ($P < 0.05$). As a result, I rank-transformed the difference values. I tested the rank transformed data using the Box's test of equality of covariance matrices to insure homogeneity ($P > 0.05$). I performed two way MANOVAs and ANOVAs (if MANOVAs gave significant outputs) for each hypothesis test in which the dependent variables were time on substrate and foraging attempts and the independent variables were risk (high and low) and cue (novel odour and water).

I disregarded 39 juvenile salmon (ten from the three day and 29 fish from the six-day induction) in the first experiment out of the 207 hatchery reared Atlantic salmon tested across trials from our analysis due to inactivity during testing (no activity in both pre and post cue). Final sample sizes and mean standard lengths (mm) of the tested Atlantic salmon in each treatment was recorded (Table 1.1). The t tests of differences in the standard lengths from block one and two show no significant difference ($P = 0.583$ for the six-day induction blocks, $P = 0.568$ for three-day induction blocks) in size of fish between blocks and so lengths for both blocks are presented together. No significant difference was observed across blocks ($P > 0.05$) and so blocks has been dropped as a factor from the analysis.

I disregarded 20 out of 136 juvenile salmon tested in the second experiment across trials due to inactivity during both pre and post stimulus injection periods. Final sample sizes and mean standard lengths (mm) of the tested Atlantic salmon in each treatment was recorded (Table 2.1). The t tests show no significant difference in size between blocks in both the hatchery and wild Atlantic salmon ($P = 0.354$ for wild fish blocks, $P = 0.978$ for hatchery blocks) and so mean standard lengths for each block are presented together. No significant difference is observed across blocks ($P > 0.05$) and and block was dropped as a factor from the analysis.

3. Results

3.1 Experiment One: Inducing neophobia in a hatchery setting

3.1.1. Does three days of exposure to alarm cue result in a neophobic response?

Contrary to my initial hypothesis, I found no evidence of induced neophobia among young-of-the-year Atlantic salmon exposed to alarm cue for three days in a hatchery setting. The overall MANOVA results found no significant main effects of pre-exposure treatment, test stimulus or an interaction between condition during treatment and cue given during testing ($P > 0.05$ for all). The change in both time on substrate and number of foraging attempts in response to a novel cue when compared to a water control was similar for salmon pre-exposed to the experimental and control treatments (Figure 1.4. & Figure 1.5.). Likewise, novel odour vs. water as a test stimulus appeared to have no effect on the change in foraging behaviour. Thus, the results suggest that pre-exposure to elevated risk for three days is not sufficient to induce a neophobic predator avoidance response in juvenile hatchery reared Atlantic salmon in a hatchery setting.

3.1.2. Does six days of exposure to alarm cue result in a neophobic response?

Consistent with my initial hypothesis, salmon pre-exposed to alarm cue for six days under hatchery conditions did show evidence of induced neophobia. The overall MANOVA (Table 1.2.) revealed a significant main effect of risk and a marginally significant interaction between pre-exposure (alarm cue vs water control) and test stimulus (novel odour vs. water control). For the change in number of foraging attempts (Figure 1.6.), I found a significant effect of risk (ANOVA, $P = 0.003$) and a significant cue x risk interaction (ANOVA, $P = 0.016$). For the change in time on substrate (Figure 1.7.), I found a significant effect of cue (novel odour vs. water, ANOVA, $P = 0.023$). The results suggest that pre-exposure to alarm cue for six days is sufficient to induce a neophobic predator avoidance response in juvenile hatchery reared Atlantic salmon in a hatchery setting.

3.1.3. Is a neophobic response induced in the hatchery retained throughout a transportation event?

Contrary to my initial hypothesis, I found no support for the retention of induced neophobia throughout transportation and stocking among young-of-the-year Atlantic salmon exposed to alarm cue for six days in a hatchery setting (Figure 1.8. & Figure 1.9.). The overall MANOVA results found no significant main effects of pre-exposure treatment, test stimulus or an interaction between the main effects ($P > 0.05$ for all). Thus, the results suggest that an induced neophobic predator avoidance response induced in hatchery reared Atlantic salmon in a hatchery setting is not retained throughout transport and stocking.

3.2. Experiment Two: Inducing neophobia in the field

3.2.1. Can hatchery reared Atlantic salmon be induced with neophobia in a semi-natural environment?

Consistent with my initial prediction, hatchery reared salmon pre-exposed to alarm cue for six days under semi-natural conditions did show evidence of induced neophobia. The overall MANOVA (Table 2.2) revealed a significant main effect of risk ($P = 0.020$) and a significant interaction ($P = 0.018$) between pre-exposure (high vs. low risk) and test stimulus (novel odour vs. water). For the change in number of foraging attempts (Figure 2.1.), I found a significant effect of risk (ANOVA, $P = 0.007$) and a cue x risk interaction (ANOVA, $P = 0.022$), suggesting salmon pre-exposed to high background risk (but not low background risk) showed an anti-predator response towards a novel cue. For the change in time on substrate (Figure 2.2.), I found a marginally significant effect of cue x risk interaction (ANOVA, $P = 0.052$) but no effect of risk nor cue (Table 2.2). Thus, the results suggest that pre-exposure to alarm cue for six days in a semi-natural environment is sufficient to induce a neophobic predator avoidance response in hatchery reared Atlantic salmon.

3.2.2. Can neophobia be induced in wild salmon?

Contrary with my initial predictions, I found no support for the retention of induced neophobia throughout transportation and stocking among young-of-the-year Atlantic salmon exposed to alarm cue for six days in a hatchery setting (Figure 2.3. & Figure 2.4.). The overall

MANOVA results found no significant main effects of pre-exposure treatment, test stimulus or an interaction between the main effects ($P > 0.05$ for all).

4. Discussion

4.1. Major Findings:

The main objective of this study was to test the prediction that neophobic predator avoidance behaviour could be induced using alarm cue in hatchery reared Atlantic salmon. I observed no indication of an induced behavioural response after three days of exposure to alarm cue at a hatchery setting in hatchery reared Atlantic salmon. This three-day timeline was based off of past studies that had successfully induced neophobia over relatively short time frames. Studies with juvenile convict cichlids showed that three days of exposure to alarm cue was enough to induce neophobic predator avoidance behaviour (Brown et al., 2015). Other work has also induced neophobic predator avoidance in a similar timeline in different fish species; for example, four days of induction was found to elicit the neophobic predator avoidance behavioural response in juvenile whitetail damselfish (*Pomacentrus chrysurus)* (McCormick et al., 2017) and in fathead minnows (*Pimephales promelas)* (Crane & Ferrari, 2016). Although no neophobic predator avoidance response was demonstrated by Atlantic salmon exposed to alarm cue for three days, I did find evidence of an induced neophobic predator avoidance behavioural response in hatchery reared Atlantic salmon after six days of treatment in a hatchery setting. This demonstrates the ability to induce a neophobic predator avoidance response to a novel cue in Atlantic salmon in a hatchery setting. However, the exact length of exposure time to alarm cue necessary to elicit this behavioural response remains unknown and should be further investigated.

Second, I found no support for the retention of the induced neophobic predator avoidance behaviour in hatchery reared Atlantic salmon following transportation and stocking. Brown et al. (2015) looked at the retention of an induced neophobic predator avoidance behaviour in convict cichlids. They demonstrated that background risk level determined the strength and retention of the induced behavioural response, and that fish from a high risk condition were found to retain the induced response for 14 days. Differing from Brown et al., (2015)'s study, my study included a transport and stocking event between treatment and testing. There is a possibility that stress caused by transport and stocking in my study may have influenced the retention of the induced response. This is a concern because in this particular application of induced neophobic predator avoidance, the retention of the induced behaviour throughout transport and stocking is necessary for it to be a useful tool to help maximize survival following the hatchery fish's release. Future

work should focus on retention of this induced behaviour, since retention of the response is an indicator of the possible success of this life skills training tool.

Thirdly, my study suggests that hatchery fish can also be induced under semi-natural conditions. This suggests that hatchery fish are responsive to the alarm cue treatment for neophobic predator avoidance behaviour in a semi-natural environment following a transportation and stocking event. This is relevant because it opens up the possibility of reinforcing the neophobic predator avoidance behaviour after transport and stocking, but prior to the fish's release. This would eliminate the need for the behaviour to be fully retained during transportation and stocking. Finally, my study demonstrated that wild fish did not demonstrate neophobic predator avoidance behaviour after a six-day treatment with alarm cue in a semi-natural environment. This is believed to be a result of size effects.

4.2. Possible reasons for the long treatment period needed in Atlantic salmon

A number of non-mutually exclusive factors, including the continuous flow of water, the selection of bold phenotypes, high investments in growth in juvenile Atlantic salmon, and the high density of fish during the treatment phase influenced the success of the treatment during this study. Prior studies that have successfully induced neophobic predator avoidance in a short period of time (Brown et al., 2015; McCormick et al., 2017; Crane & Ferrari, 2016). However, past studies induced fish in a closed system in which the fish were held in static water. This differs greatly form the hatchery system and semi-natural environment setup. In this study, the fish were placed in flow through bins with the flow rate of ~4.5 cm per second (calculated by measuring the time taken for the plume of a florescent dye to flow through a bin). The continuous cycling of water through the holding bins in during the treatment phase may have limited the contact between the fish and the alarm cue, muting the resulting observed behaviour.

Secondly, the predictable, low predation environment of the hatchery selects for bold behavioural phenotypes (Sundström et al., 2004). Bold fish tend to engage in more risk taking behaviour (Berejikian, 1995), which can minimize the hatchery fish's response to alarm cue during the induction period; since bold fish are less likely respond to predator cues (Chiba et al., 2007).

Brown et al., (in press) demonstrated that personality shapes retention in Trinidadian guppies. Shy guppies will respond to a novel odour at the same intensity as bold guppies; yet shy

guppies are found to retain the response for a longer period during extinction trials. This again suggests that the selection for bold fish may decrease the potential retention of an induced neophobic predator avoidance behaviour in a hatchery setting, since boldness has been negatively correlated to the length of retention of a neophobic avoidance behaviour (Brown et al., in press).

Thirdly, investments towards growth at the juvenile stage may have limited the influence of the alarm cue on hatchery Atlantic salmon during the treatment phase of this study. A fish is more likely to grow if it can utilize resources efficiently (Metcalfe, 1998). Bold, hatchery fish live in an ideal environment in which resources are easily utilized, resulting in faster growth (Biro & Post, 2008). Hatchery reared Atlantic salmon may be more likely to invest in growth over predator avoidance behaviour due to the drive to grow quickly and a lack of cues to suggest danger to do so in the hatchery setting (Kellison et al., 2000); thus making them potentially more resilient to adopting neophobic predator avoidance behaviour than other fish. On top of hatchery selected traits favouring fast early growth, Atlantic salmon as a species are required to engage in quick growth in early life in order to improve their likelihood to survive long term (Rye et al., 1990; Friedland et al., 2000).

A selection for quick growth at early life stages may lead to a different energy allocation strategy in the Atlantic salmon than fish used in previous neophobia studies. Energy allocation is the trade-off between the production of soma cells (leading to increased biomass and observed growth in fish) and energy storage as lipids (which can later be used for activities such as reproduction or predator avoidance behaviour) (Biro et al., 2005). Brook trout (*Salvelinus fontinalis*) were observed at age-0 to allocate almost all energy to the production of soma (growth), regardless of background risk in order to minimize time spent at vulnerable sizes (Biro et al., 2005). These same fish grew at maximum rates in the month of July (Biro et al., 2005), the month in which this study took place.

Atlantic salmon need to grow quickly in early life due to the need to reach certain size thresholds at given times of the year in order to mature to their next life history stage (Metcalfe, 1998). Atlantic salmon do not mature to their next life stage or smolt if they do not meet a certain growth requirement at a given time of year since small smolts have low survival rates, and maturation at a small size is very costly (Hutchings, 1994). This further encourages fast growth in the early life of Atlantic salmon. There is also a strong correlation between size and survival in Atlantic salmon. This suggests that fish that grow to a given size early are more likely to survive

(Rye et al., 1990; Friedland et al., 2000), especially when faced with difficult climate years in later life (Saloniemi et al., 2004).

The high density of hatchery fish during housing and induction could have also influenced the treatment phase of this study. Social learning has been shown to play a significant role in life skills training and is influenced by density (Chapman et al., 2008), since fish often look at conspecifics to gain information of their surroundings (Brown & Laland, 2001). The density of fish in the system might influence if social learning will promote or minimize neophobic predator behaviour. Chapman et al., (2008) examined social learning in guppies and demonstrated that fish reared in lower densities relied more on social learning than those reared at high densities. Low density fish were able to navigate quicker through a maze when placed with a trained demonstrator. This suggests that the density of fish in the system might influence if social learning will promote or minimize neophobic predator avoidance behaviour.

Atlantic salmon, especially bold hatchery Atlantic salmon are motivated to engage in behaviour that corresponds to growth rather than engaging in any form of predator recognition or avoidance, which could have influenced the adoption of neophobic predator avoidance behaviour in this study. Also, the flow through design and high densities found in the hatchery system may limit alarm cue effect, which may have also affected the treatment phase of this study.

4.3. Retention of acquired associations and induced behaviour in Atlantic salmon

Retention of an acquired association seems to differ depending on the species. An experiment by Chivers and Smith, (1994) showed that fathead minnows correctly responded to learned predator cue two months after being conditioned to the novel predator odour coupled with minnow alarm cue. Berejikian et al., (1999) showed that Chinook salmon responded to a learned predator cue three days after being conditioned to the novel predator odour of cutthroat trout paired with Chinook alarm cue, but showed no response to the cutthroat trout odour ten days after induction.

Two factors are known to influence the retention of a neophobic response; background level of predation risk and frequency of exposure to a novel cue (Brown et al., 2015). In convict cichlids, those induced with neophobia under a medium risk condition (medial amounts of alarm cue) showed little retention of their neophobic response, yet those induced under a high risk (high amounts of alarm cue) condition showed stronger levels of retention (Brown et al., 2015). Also,

cichlids exposed to a novel cue repeatedly showed weaker retention of a neophobic response than cichlids exposed to a novel cue once. (Brown et al., 2015). Retention diminishes as cues are experienced with no associated risk.

Future studies will need to consider the limitations of retention when looking to use neophobic predator avoidance as a life skills training tool. As mentioned above, the results of this study suggest that induction of neophobic predator avoidance behaviour can be done in the field. With this knowledge and with what has been suggested in past literature on the concept of reinforcing predator avoidance behaviour (Brown et al., 2015), reinforcement of the induced neophobic predator avoidance behaviour just prior to the hatchery fish's release should be investigated.

4.4. Caveats in the wild vs. hatchery fish induction

Unlike hatchery Atlantic salmon treated with alarm cue in the semi-natural environment, wild Atlantic salmon did not demonstrate neophobic behaviour to a novel cue following treatment. I believe that this can be linked to the size of the wild fish during treatment compared to the hatchery fish as well as the differences in past background predation levels in the wild vs. hatchery environments.

Wild fish in this study were significantly larger ($p < 0.05$) than the hatchery reared fish. This leads to the possibility of size playing a role in the treatment period. Hawkins et al., (2008) found that larger Atlantic salmon showed a lower innate opercula response to predator odours. A difference in responses to predator cues due to size provides support for the possibility that Atlantic salmon of different sizes may respond to alarm cue (a reliable indication of a predation event) differently.

Also, wild fish are reared in an unpredictable, high predation environment when compared to hatchery reared fish. It has been shown that fish from a low predation environment like the hatchery setting, demonstrate a stronger anti-predator response than would fish from a high risk environment (Brown et al., 2006). This suggests that the hatchery reared fish induced in the semi-natural condition may respond to the alarm cue during induction at a higher intensity than the wild Atlantic salmon.

These two factors may have altered the strength of the induced response in the wild fish when compared to hatchery fish induced under the same conditions. Further research should look at the effects of size on treatment of neophobic predator avoidance in Atlantic salmon.

4.5. Suggestions to Hatchery systems; avenues for future research:

I believe that neophobic predator avoidance can and should be incorporated into life skills training within hatchery systems. This project showed neophobic predator avoidance's potential to greatly alter a fish's behaviour in a way that can positively impact a fish's survival in a novel, uncertain environment.

Hatcheries should invest in finding an induction method that is optimal for the Atlantic salmon in a hatchery system. This new method should maximize duration of contact with the alarm cue, which is difficult when working with a flow through system. Perhaps the solution is to alter the concentrations of alarm cue given, increase the amount of induction events per day, minimize flow rates during induction, or lower densities. Future studies should also look at the possibility of promoting social learning during induction by placing a neophobic fish with the next cohort of bold hatchery fish to promote the neophobic predator avoidance behaviour.

Hatcheries should look at finding transportation methods that minimize stress for the transported neophobic fish. Carmichael et al., (1984) looked at factors leading to mortality and stress during largemouth bass (*Micropterus salmoides)* transport and found that stress and mortality is reduced when fish are treated for disease, not given food for 72 hours and were anesthetized before transport. It is also beneficial for the fish to be held in cool temperatures in physiological concentrations of salts with mild antibiotics and a mild anesthetic.

Hatcheries should also look into methods for optimizing the retention of the induced neophobic predator avoidance behaviour. Perhaps a solution to the lack of retention in fish used in this study is to reinforce the neophobic response during or after transport. My finding that neophobic predator avoidance can be induced in the wild also allows for the possibility of reinforcing the induced behavioural response right before release. This would counteract the loss of retention of the response during transport and stocking.

In summary, this study has provided support for the implementation of neophobic behaviours into life skills training within hatchery systems. Although retention of the neophobic response is an issue, I believe that an optimal treatment method can be found within a hatchery

system that can lead to a stronger neophobic response in the hatchery fish. The induced response will then allow hatchery reared fish to display context appropriate behavioural patterns once stocked into the wild, thus minimizing their chances of mortality and elevating post-stocking survival rates in hatchery reared fish.

4.5.1. A financial solution for hatcheries

A novel approach to life skills training is important not only for the ecological benefits (Heithaus et al., 2008) of stabilizing declining fish populations but also for the cost efficiency of hatchery systems. As it stands, hatchery systems are not cost-effective (Patrick et al., 2006). The costs associated with captive breeding include: facilities, personnel, collection of brood stock and transportation (Patrick et al., 2006). As it stands, most stock enhancement efforts are not yielding enough viable biomass to be cost effective. Using striped bass as an example (*Morone saxatilis);* the cost to rear one fish is about $1.94. In order to be cost effective, it was calculated that the recapture rate by anglers of these captive bread fish following their release would have to be at a conservative 68.8%. Yet, recapture rates are found to be more around the 2.5 % range (Patrick et al., 2006).

With the insight gained throughout this study, I believe that I have demonstrated that induced neophobic predator avoidance can be induced in hatchery reared Atlantic salmon. Implicating this treatment of neophobic predator avoidance as part of life skills training within hatchery systems has the potential to greatly increase the survival of hatchery fish following their release into a native environment thus helping with the management of declining fish populations and the efficacy of hatchery systems. This being said, more work must be done to insure that induced neophobic predator avoidance as a tool for life skills training is optimized to it's full potential.

4.6. Implications

This study tested if neophobic predator avoidance could be induced in hatchery reared Atlantic salmon. This may be a viable way to improve current life skills training within hatchery systems. This could yield hatchery fish that engage in behaviours favouring higher post-stocking survival. If hatcheries become more efficient at rearing fish that survive following their release into the wild, then wild fish populations are more likely to stabilize over time, making hatcheries a more useful and successful conservation tool.

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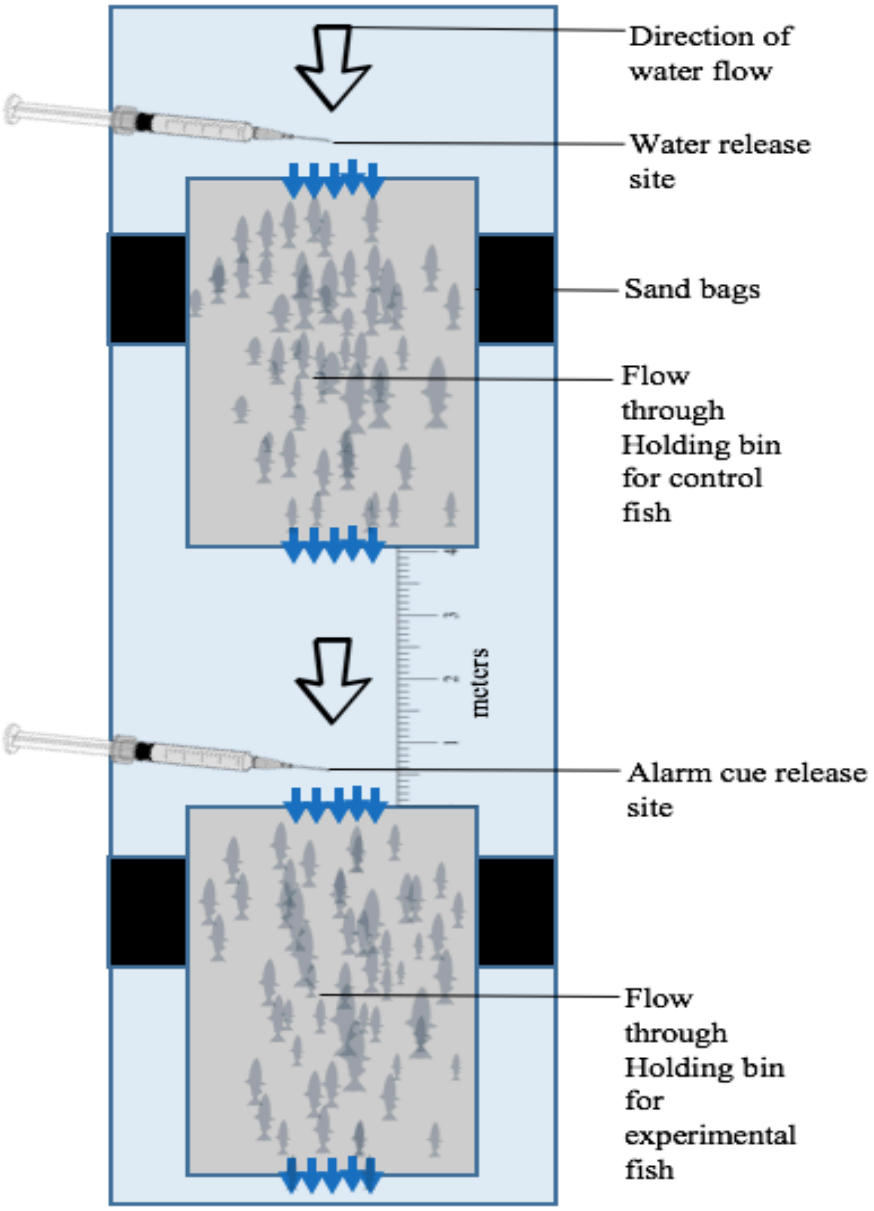

Figure 1.1. Diagram of the treatment phase set-up within a hatchery raceway.

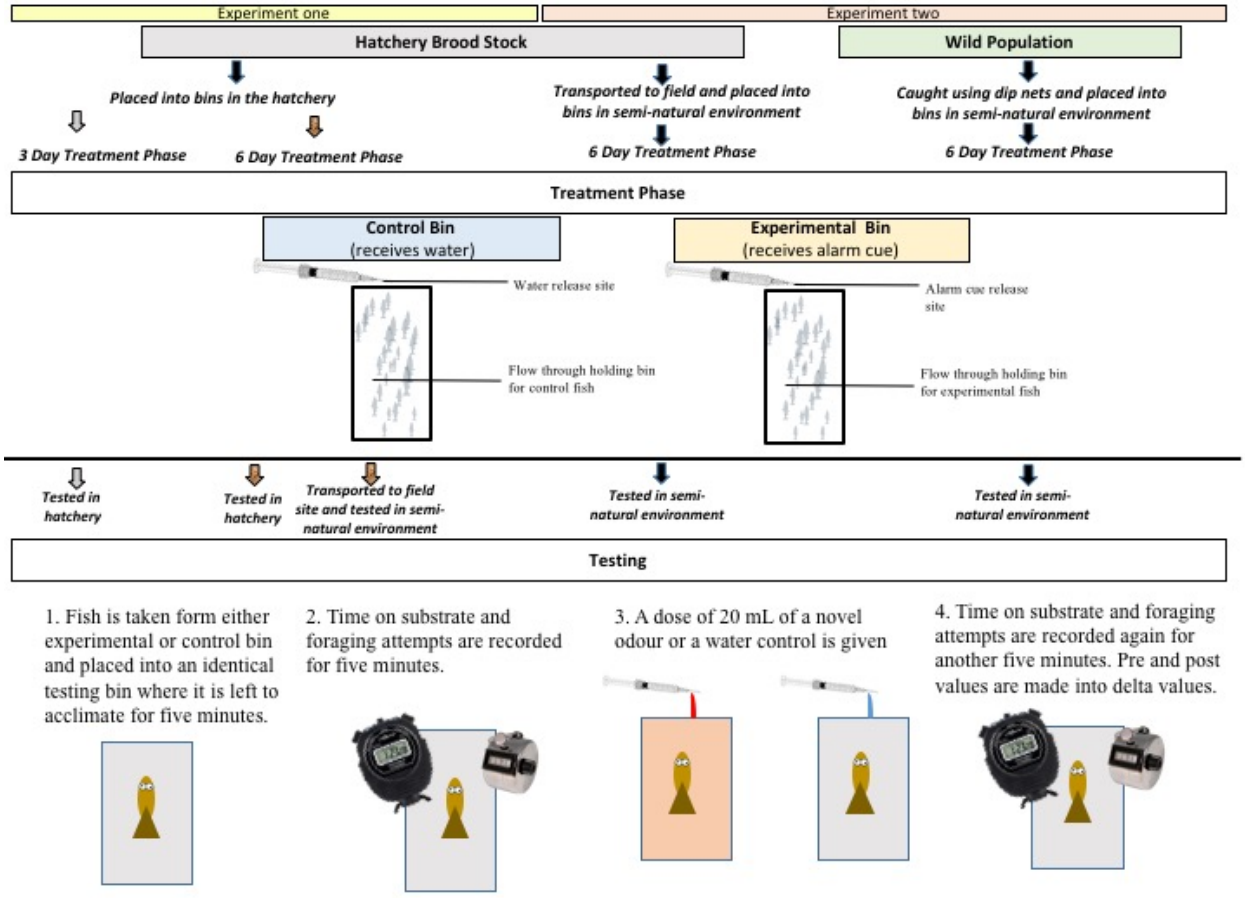

Figure 1.2. Flow chart of experiment one and two including method for treatment phase and testing.

Figure 1.3. Experimental set-up in the semi-natural environment at the field site.

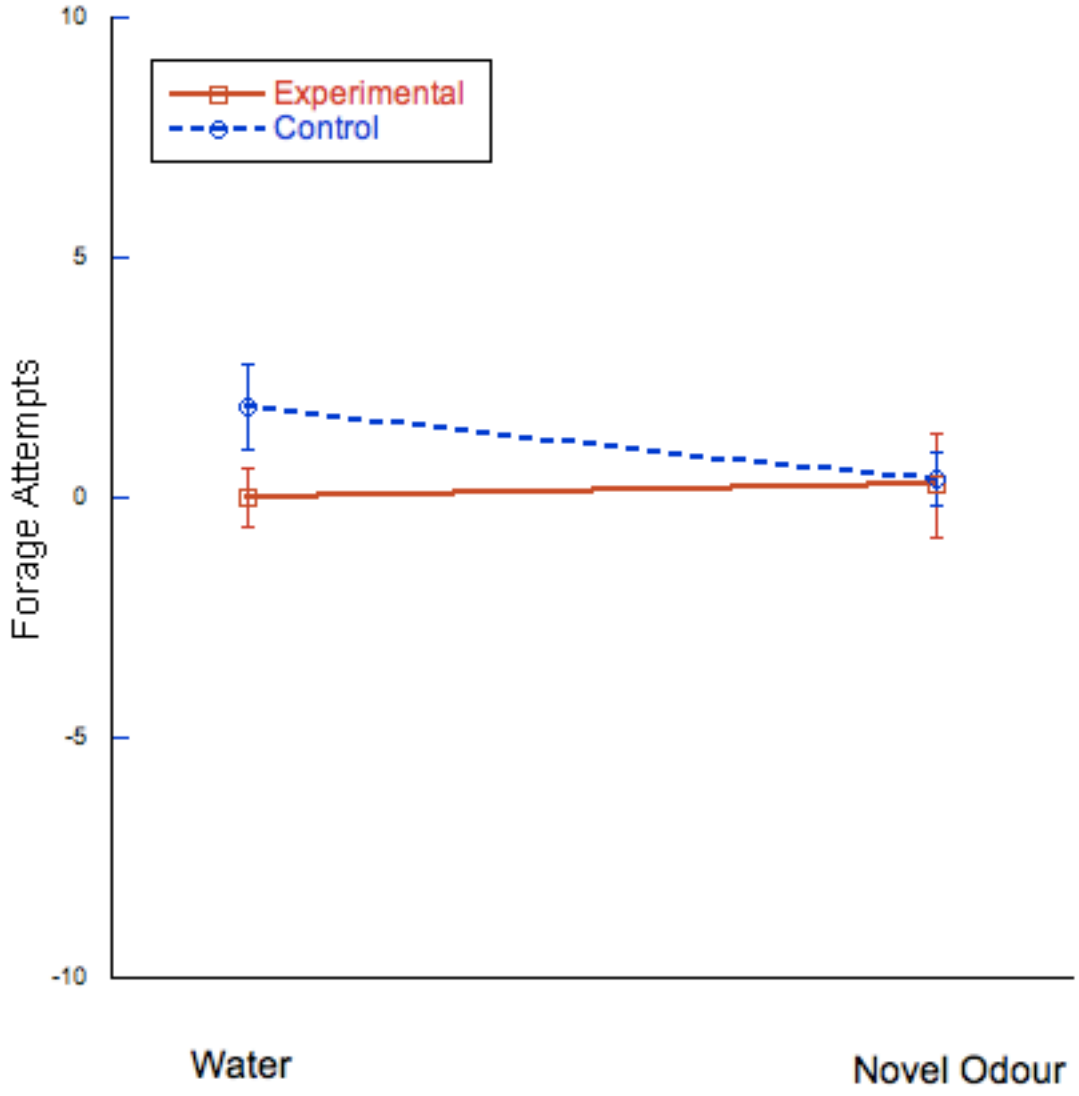

Figure 1.4. Interaction plot of forage attempts data (deltas) taken from experimental and control treatment hatchery juvenile Atlantic salmon following a three-day treatment phase in the hatchery.

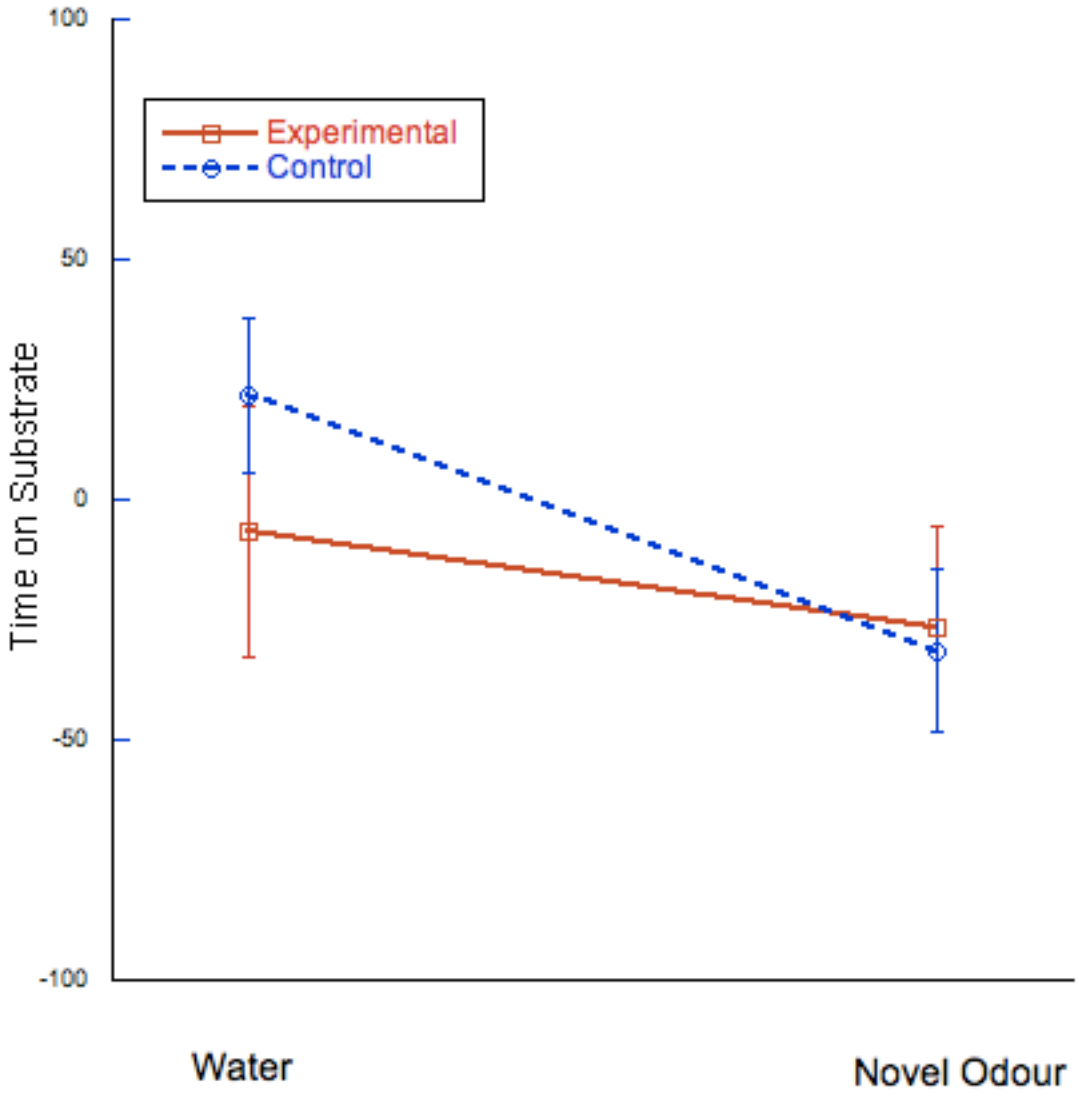

Figure 1.5. Interaction plot of time on substrate data (deltas) taken from experimental and control treatment hatchery juvenile Atlantic salmon following a three-day treatment phase in the hatchery.

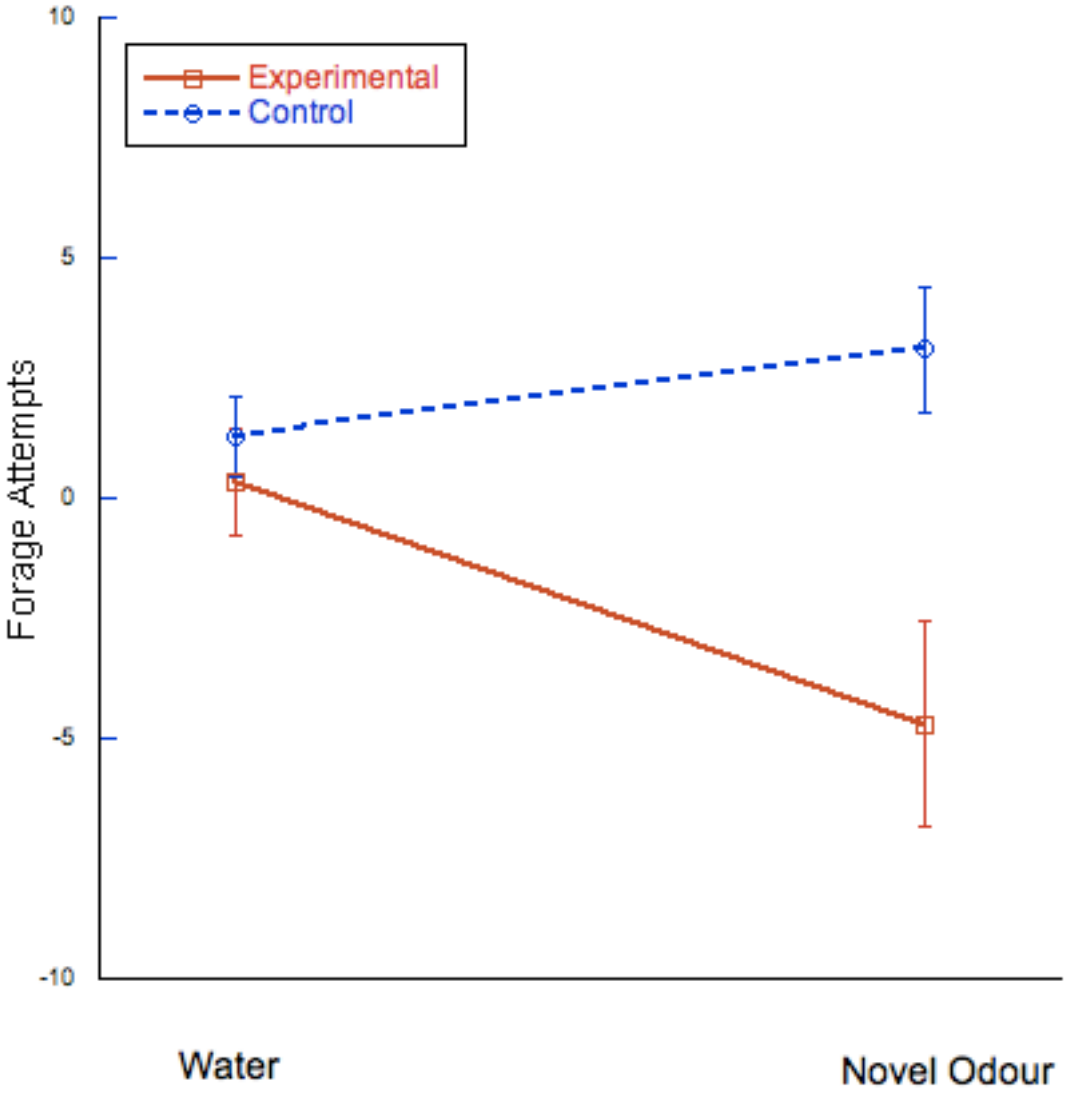

Figure 1.6. Interaction plot of foraging attempts data (deltas) taken from experimental and control treatment hatchery juvenile Atlantic salmon following a six-day treatment phase in the hatchery.

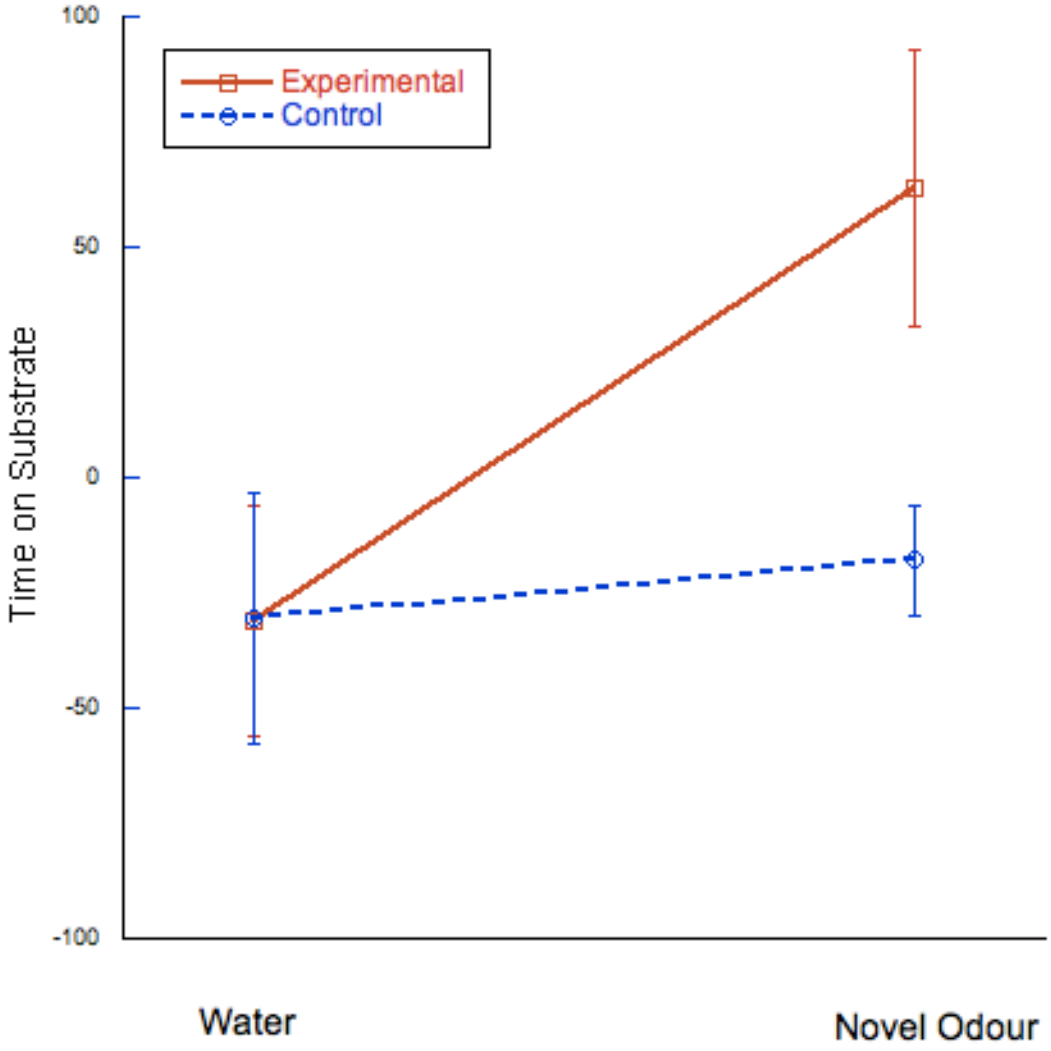

Figure 1.7. Interaction plot of time on substrate data (deltas) taken from experimental and control treatment hatchery juvenile Atlantic salmon following a six-day treatment phase in the hatchery.

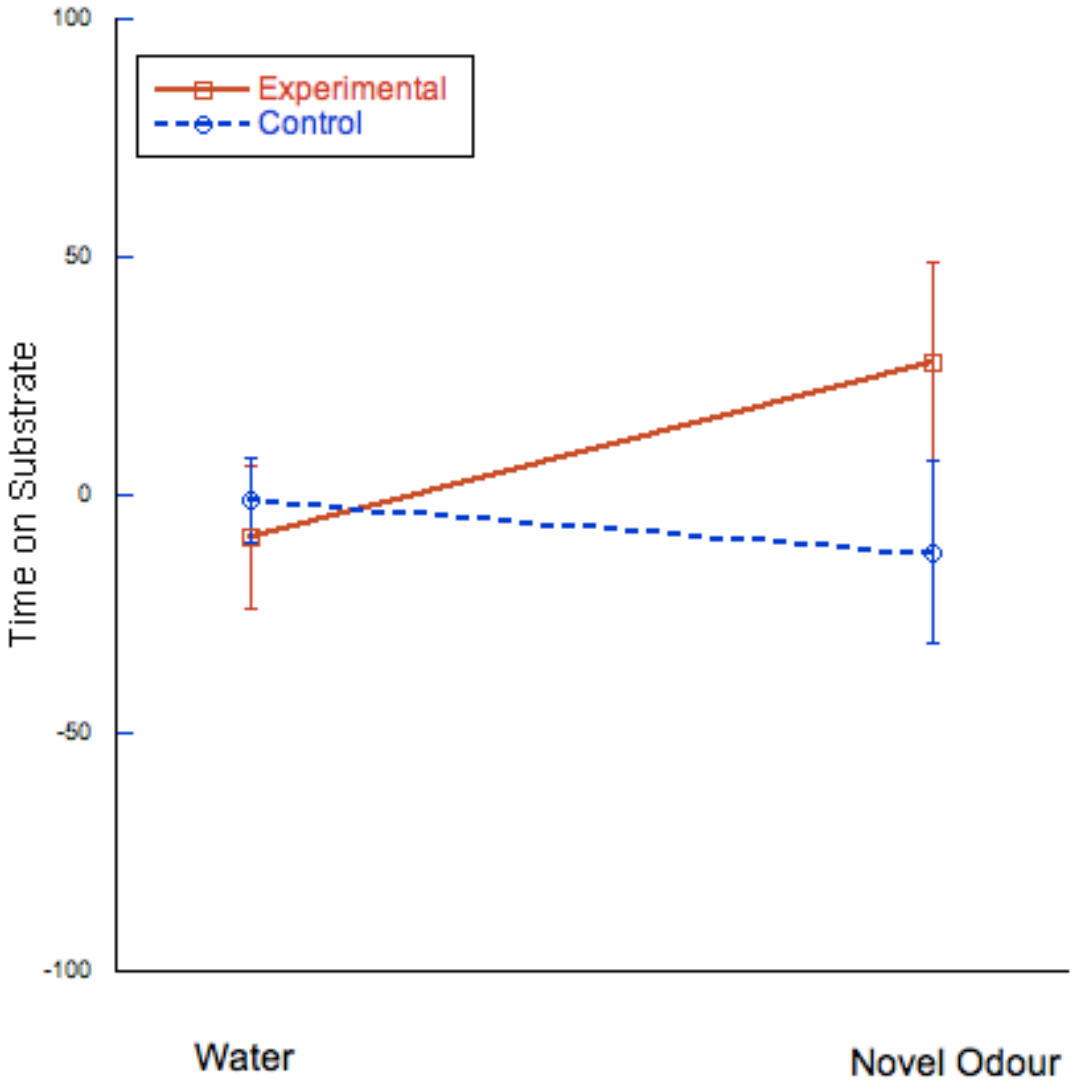

Figure 1.8. Interaction plot of time on substrate data (deltas) taken from experimental and control treatment hatchery juvenile Atlantic salmon following a six-day treatment phase in the hatchery and a transportation and stocking event. Fish were tested in a semi-natural environment.

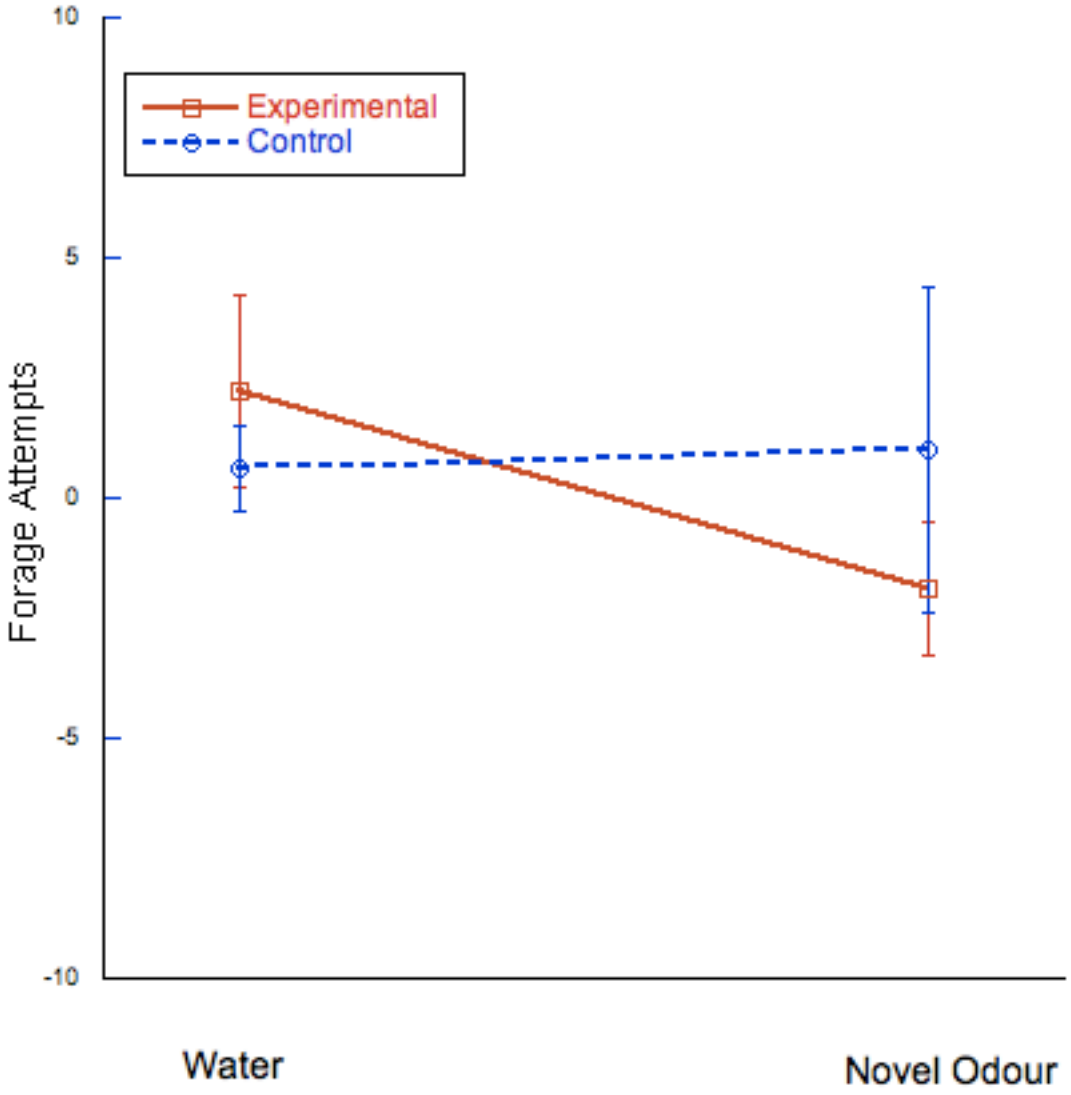

Figure 1.9. Interaction plot of foraging data (deltas) taken from experimental and control treatment hatchery juvenile Atlantic salmon following a six-day treatment phase in the hatchery and a transportation and stocking event. Fish were tested in a semi-natural environment.

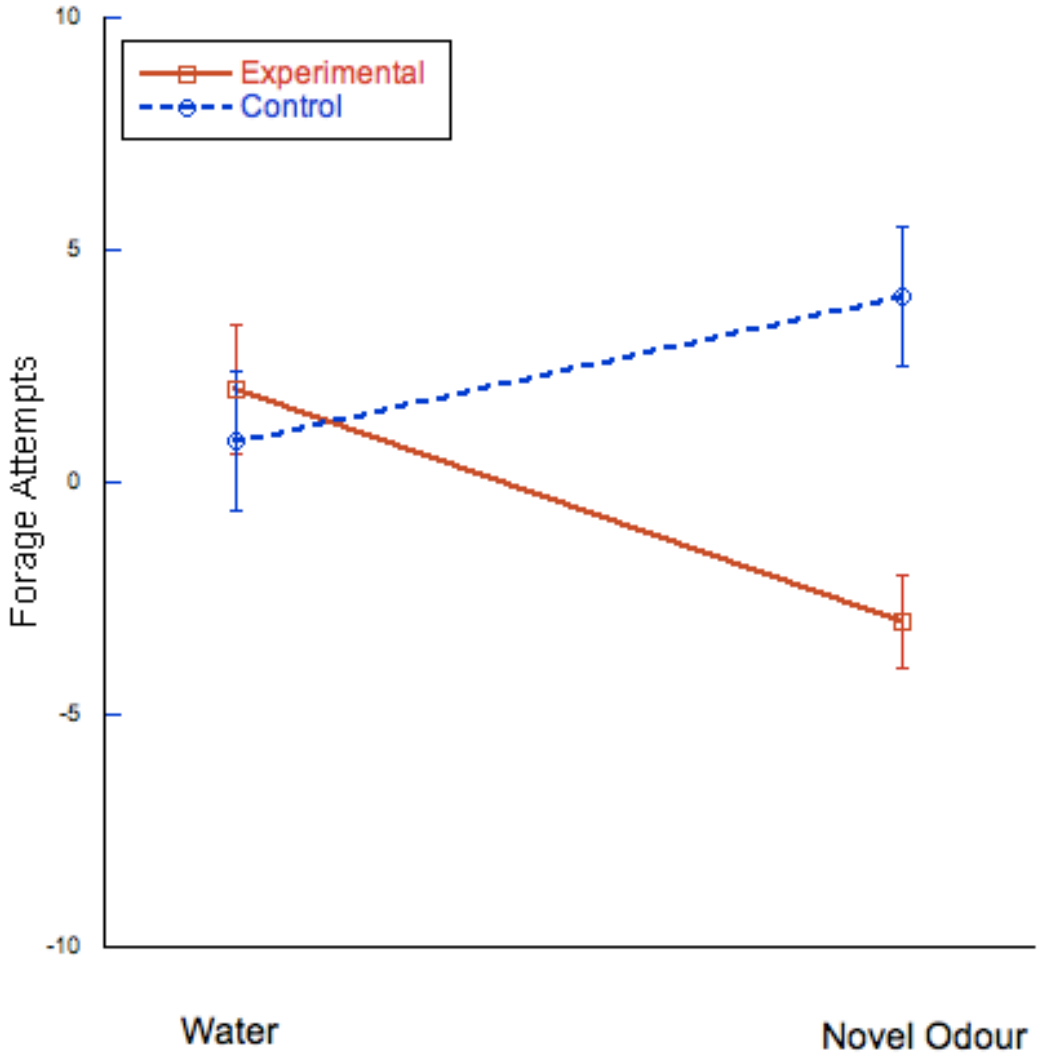

Figure 2.1. Interaction plot of foraging attempts data (deltas) taken from experimental and control treatment hatchery juvenile Atlantic salmon following a six-day treatment phase in a semi-natural environment. Fish were tested in a semi-natural environment.

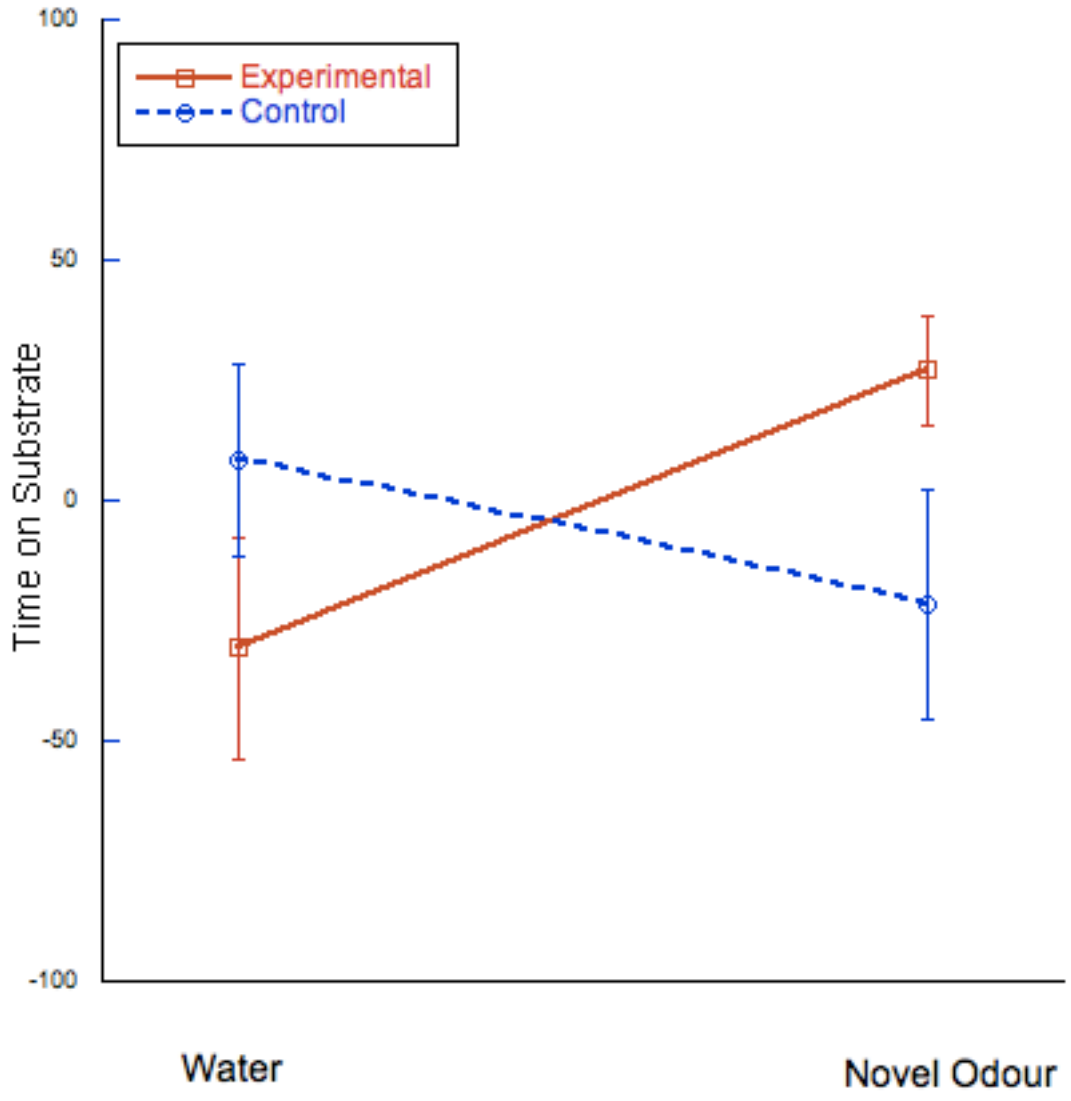

Figure 2.2. Interaction plot of time on substrate data (deltas) taken from experimental and control treatment hatchery juvenile Atlantic salmon following a six-day treatment phase in a semi-natural environment. Fish were tested in a semi-natural environment.

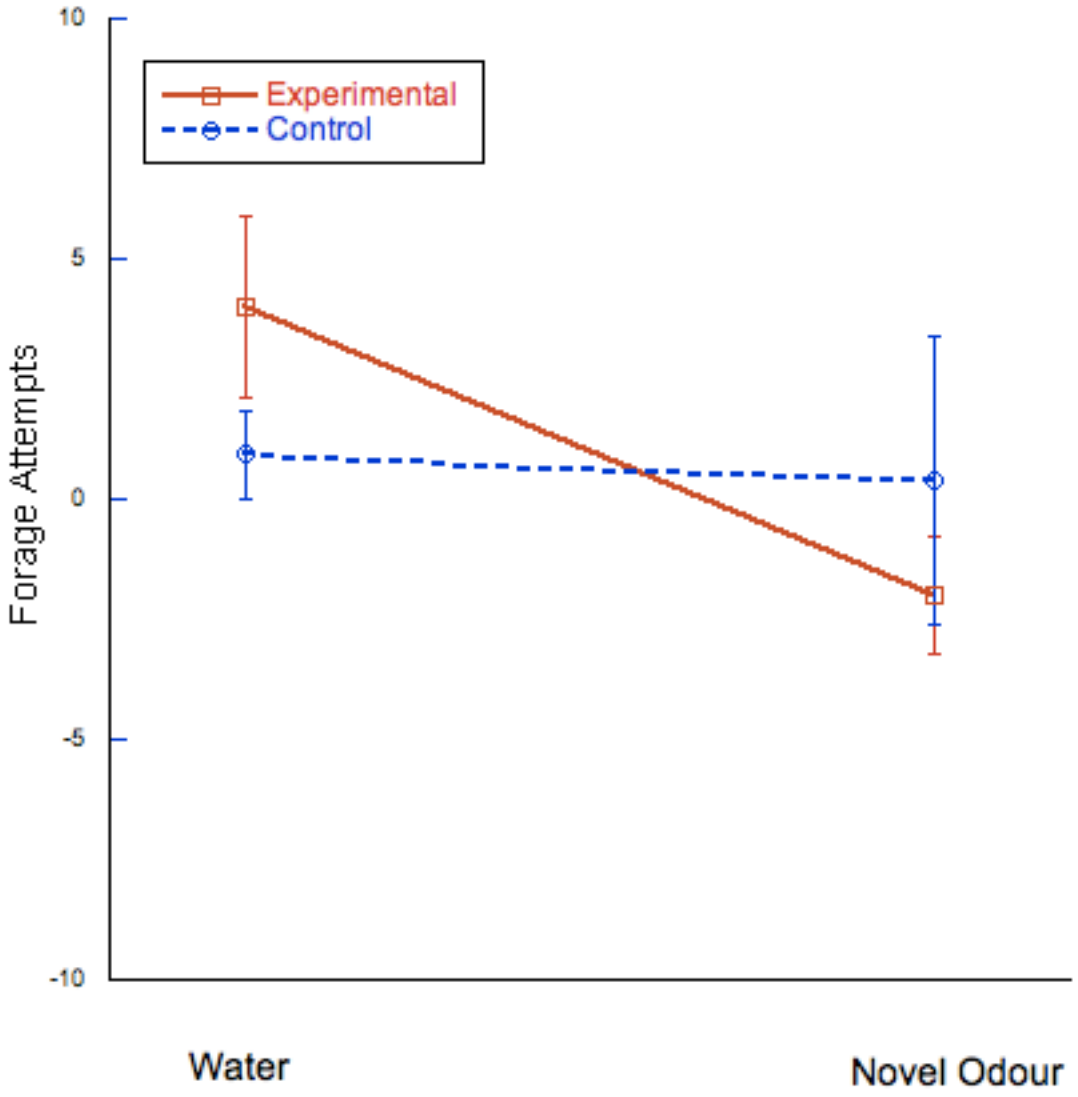

Figure 2.3. Interaction plot of foraging attempts data (deltas) taken from experimental and control treatment wild juvenile Atlantic salmon following a six-day treatment phase in a semi-natural environment. Fish were tested in a semi-natural environment.

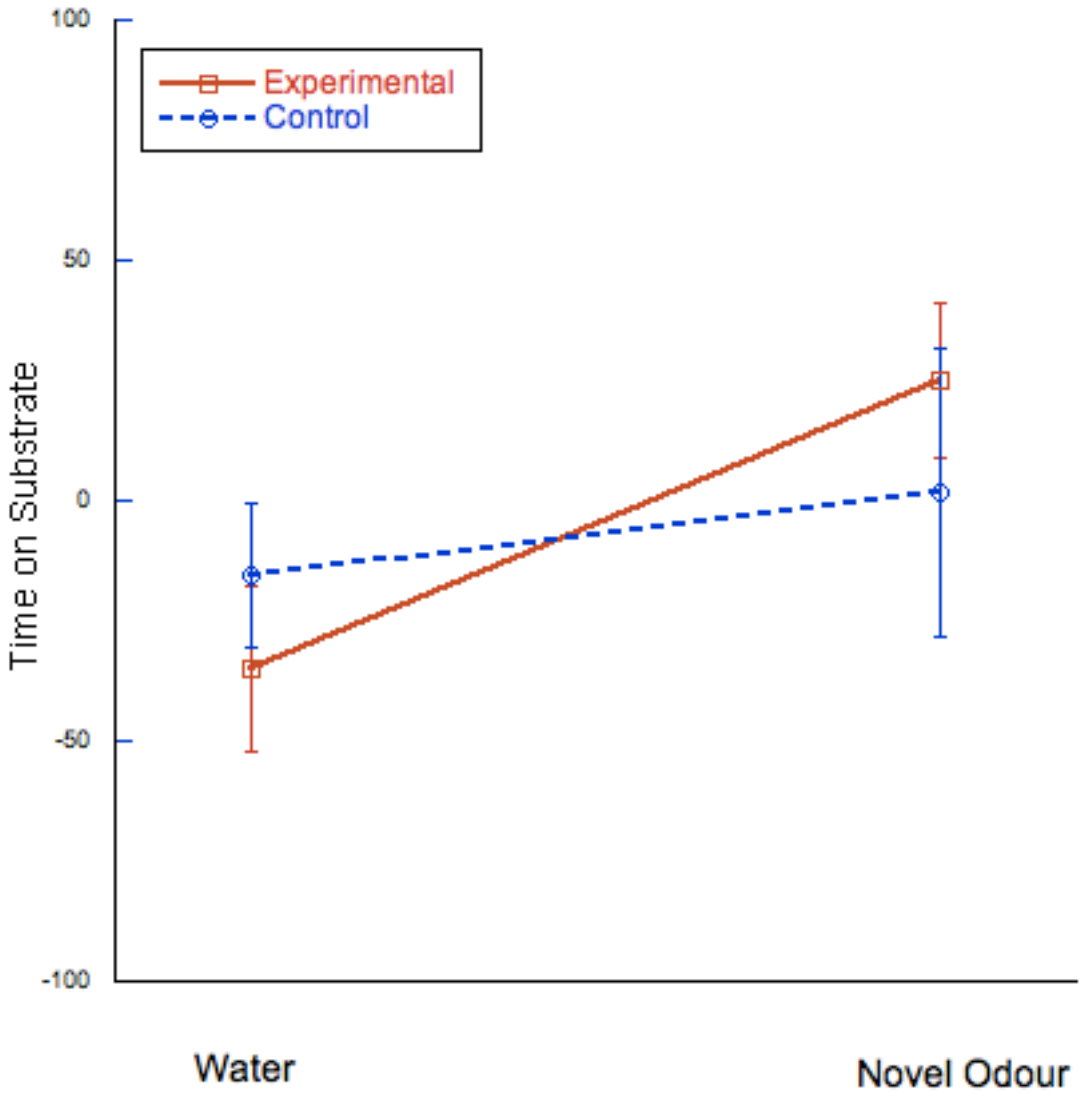

Figure 2.4. Interaction plot of time on substrate data (deltas) taken from experimental and control treatment wild juvenile Atlantic salmon following a six-day treatment phase in a semi-natural environment. Fish were tested in a semi-natural environment.

Table 1.1. Sample size and mean standard length of juvenile Atlantic salmon used per treatment for experiment one tests.

Experiment One Test		Size Standard Length (mm)	Sample size
Testing for neophobic behaviour following three days of induction	High Risk		
	Novel Odour	31.0 ± 14.1	15
	Water	30.3 ± 8.05	15
	Low Risk		
	Novel Odour	29.2 ± 4.57	15
	Water	30.1 ± 11.7	15
Testing for neophobic behaviour following six days induction	High Risk		
	Novel Odour	31.1 ± 4.45	15
	Water	31.1 ± 5.70	15
	Low Risk		
	Novel Odour	29.0 ± 7.85	15
	Water	28.4 ± 8.01	15
Retention test in the field following transport	High Risk		
	Novel Odour	29.0 ± 13.9	12
	Water	29.1 ± 13.0	12
	Low Risk		
	Novel Odour	27.0 ± 7.27	12
	Water	29.7 ± 22.0	12

Table 1.2. Statistical test results following six days of induction on hatchery juvenile Atlantic salmon in a hatchery setting.

Effect		F	DF	p
MANOVA				
	Cue	2.746	2, 55	0.073
	Risk	4.773	2, 55	0.012*
	Cue x Risk	3.049	2, 55	0.055
ANOVA				
Foraging Attempts				
	Cue	1.052	1, 56	0.310
	Risk	9.716	1, 56	0.003*
	Cue x Risk	6.121	1, 56	0.016*
Time on Substrate				
	Cue	5.427	1, 56	0.023*
	Risk	0.832	1, 56	0.366
	Cue x Risk	0.917	1, 56	0.342

Table 2.1. Sample size and mean standard length of wild and hatchery juvenile Atlantic salmon used per treatment for experiment two tests.

Experiment Two Test		Size Standard Length (mm)	Sample size
Testing for neophobic behaviour in wild fish induced in a semi-natural condition	High Risk		
	Novel Odour	38.7 ± 3.70	14
	Water	36.8 ± 1.28	14
	Low Risk		
	Novel Odour	35.5 ± 14.8	14
	Water	36.6 ± 13.3	14
Testing for neophobic behaviour in hatchery fish induced in a semi-natural condition	High Risk		
	Novel Odour	31.1 ± 12.3	15
	Water	30.3 ± 3.23	15
	Low Risk		
	Novel Odour	30.9 ± 12.3	15
	Water	30.8 ± 11.5	15

Table 2.2. Statistical test results following six days of induction on hatchery juvenile Atlantic salmon under semi-natural conditions.

Effect		F	DF	p
MANOVA				
	Cue	1.538	2, 55	0.224
	Risk	4.223	2, 55	0.020*
	Cue x Risk	4.335	2, 55	0.018*
ANOVA				
Foraging Attempts				
	Cue	2.448	1, 56	0.123
	Risk	7.711	1, 56	0.007*
	Cue x Risk	5.566	1, 56	0.022*
Time on Substrate				
	Cue	0.901	1, 56	0.347
	Risk	1.349	1, 56	0.250
	Cue x Risk	3.957	1, 56	0.052