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Chemically mediated learning in juvenile Atlantic salmon (*Salmo salar*); testing the limits of acquired predator recognition under laboratory conditions and in the wild

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ABSTRACT

Chemically Mediated Learning in Juvenile Atlantic Salmon (*Salmo salar*); Testing the Limits of Acquired Predator Recognition under Laboratory Conditions and in the Wild.

Camille Macnaughton

The assessment of predation risk is crucial to the survival of a prey individuals and the ability to gauge risk accurately will consequently be shaped by a suite of behavioural trade-offs. In salmonids, risk may be assessed through the detection of damage-released chemical cues. When these chemical cues are paired with a novel odour, covert antipredator responses are elicited upon subsequent exposure to the novel odour and learning occurs. My research focuses on the retention of newly acquired information (lemon odour), through sequential exposure to this same novel odour in both laboratory-reared and wild populations of juvenile Atlantic salmon (*Salmo salar*). Laboratory and field experiments consisted of a single conditioning day (AC + NO) followed by three recognition days (NO), in which antipredator responses were measured from the change in behaviour observed between the five minute pre-stimulus and post-stimulus observation periods. Significant short-term antipredator responses in the laboratory population were observed at the conditioning day, while they were absent at all subsequent recognition days. In particular, the foraging rate and the time spent moving decreased in response to the alarm cue treatment at the conditioning phase, but responses were not significantly different between treatments during any of the succeeding recognition phases. These results suggest that fish respond immediately and overtly to chemical cues, but may treat the information as irrelevant without subsequent exposure to the pairing of chemical cues with a novel odour. Conversely, my field experiment failed

to confirm the laboratory results. Further work is required to elucidate any ecological processes that affect the learning mechanism in the current experiments.

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Introduction:

Predation is an important and ubiquitous selection force shaping the morphology, life history and behaviour of many prey individuals (Lima & Dill, 1990; Kats & Dill, 1998; Lima & Bednekoff, 1999). Predator avoidance is crucial to the survival of a prey species and the ability to accurately assess local predation risk and respond to such threats in a threat-sensitive fashion should greatly decrease the probability of being captured during an encounter with a predator. This notion of making efficient antipredator decisions stems from apparent trade-offs between the cost of predation and the benefits to be gained from engaging in any given fitness-related activity (Helfman, 1989; Lima & Dill, 1990). In fact, prey that exhibit an antipredator response when faced with a non-predator, waste valuable time and energy that would otherwise be available for other activities, such as foraging and reproduction (Lima & Dill, 1990). As a result, prey that can recognize potential risk, display antipredator responses with an intensity that matches their risk of predation, and make accurate threat-sensitive decisions, should gain a fitness advantage over prey individuals that do not recognize this risk (Mirza & Chivers, 2001b, 2003, Ferrari et al., 2005).

Indicators of predation risk operate in many sensory modalities, with two or more sensory modes relaying often complementary sources of information (Wisenden & Millard, 2001; Lima & Steury, 2005). In aquatic environments, the perception of predation threat is highly variable, both temporally and spatially. Therefore, prey animals must rely on many sensory inputs in order to avoid predation in a fashion that is sensitive to the current level of predation risk (Lima & Dill, 1990; Smith & Belk, 2001). Visual cues are thought to be spatially and temporally reliable but very risky, as the prey and

predator must be within close proximity (Brown et al., 2004). Furthermore, evidence suggests that visual cues alone are potentially unreliable as they can be easily manipulated by predators by means of predator behaviour and posture (Smith, 1997; Brown & Godin, 1999; Brown et al., 2000). Thus, the use of chemosensory cues as an additional information source should increase the accuracy of the assessment of local predation threats and facilitate the assessment of predation threats in a variable environment (Smith, 1997; Smith, 1999; Smith & Belk, 2001).

A wide variety of taxonomically diverse aquatic organisms rely on chemosensory information to assess and avoid local predation risks; from arthropods (Hazlett, 1994, 2003; Wisenden, 2000; Wisensen & Millard, 2001; Wisenden et al., 2004), amphibians (Kiesecker et al., 1999; Woody & Mathis, 1998; Fraker, 2008) to various fishes including ostariophysans, salmonids, gobies, poeciliids, sticklebacks, and percids (Smith, 1992; reviewed in Chivers & Smith, 1998; Brown & Chivers, 2005). These cues are produced and/or stored in the skin and released following mechanical damage to the skin, as would likely occur during a predation event (Chivers & Smith, 1998; Smith, 1999). When detected by nearby fish of the same species (conspecifics) or of a different species, but living within the same habitat (sympatric heterospecifics), dramatic, innate, short-term increases in antipredator behaviours may be elicited (Chivers & Smith 1998; Smith, 1999). Such overt and immediate behavioural responses have been well documented and include: increased shoal cohesion, area avoidance, dashing, freezing and reduced foraging and mating (Chivers & Smith, 1998; Smith, 1999, Mirza & Chivers, 2001a). In addition to this conspicuous behaviour, covert responses, such as induced morphological and life history changes (Chivers et al., 2008), social learning (Mathis et al.,1996b;

reviewed in Brown & Laland, 2003) and of particular interest to this study, acquired recognition of novel odours, are also elicited (reviewed in Kelley & Magurran, 2003; Brown, 2003; Brown & Chivers, 2005; Leduc, 2008). Acquired predator recognition, is based on the pairing of alarm cues with the visual and/or chemical cues of the predator (Chivers & Smith, 1998; Smith, 1999; Kelley & Magurran, 2003). Given the passive nature of their dispersal and the behavioural responses they trigger, chemical alarm cues should enable the association of an originally neutral stimulus (novel predator odour) as a potential predation threat (reviewed in Brown, 2003; Brown & Chivers, 2005). Since chemical cues provide reliable and honest information regarding local predation risk, their role in facilitating learning is expected to be important.

There exists considerable evidence for the antipredator function of alarm cues. The failure of a prey animal to recognize and respond to predation risk is presumably very costly during an encounter with a predator (Lima & Dill, 1990). It stands to reason that prey fishes, based on a dynamic feedback mechanism (Krebs & Davies, 1993) between foraging, body reserves, and predator avoidance, can reliably maximize their fitness (Lima & Bednekoff, 1999). In other words, fish are able to base their decision to seek fitness-related benefits on a continuous feedback mechanism that weighs an individual's energetic needs against the perception of immediate predation risk. Evidence clearly shows that throughout their lives, prey animals continually modify their responses to predation risk. Whether the perceived risk changes as prey individuals grow in size (size-dependent predation risk; Brömark & Miner, 1992; Brown et al., 2001c; Relyea, 2005) or is subject to temporal (i.e. seasonal) fluctuations in biotic and abiotic conditions (Dahl et al., 1998; Gilliam & Fraser, 2001; Leduc et al. 2007b), individuals that are able

to distinguish between these subtleties of predation risk and adjust their predator avoidance decisions accordingly, should be at a selective advantage (reviewed in Brown & Chivers, 2005). For example, food deprivation or hunger level has been shown to temper antipredator responses because the cost of starvation is greater than that of perceived predation (Hobson's choice, Clark, 1994; Harvey, 2005). The flexibility of antipredator behaviour is further supported in a laboratory study, whereby convict cichlids (*Archocentrus nigrofasciatus)* integrated threat-sensitive antipredator responses within variable background levels of predation risk experienced (Brown et al., 2006). More recently, wild-caught Trinidadian guppies *(Poecilia reticulata)* have been shown to adopt threat-sensitive antipredator behaviours in response to provenance or predation level (Brown et al., in press). Moreover, fish that can make subtle and adaptive adjustments to their antipredator repertoire through learning, both individual and social and may benefit from this survival tool (Kelley & Magurran, 2003). This implies that learning with recent experience rather than the use of innate, fixed behaviours is responsible for the plasticity in the response to potential predation (reviewed in Brown, 2003). In fact, widespread population differences in response to the variability in predation pressure support this notion of learned response patterns versus genetically inherited antipredator responses (Brown & Chivers, 2005).

When making decisions whether to forage or to avoid predators, fish may utilize information that is produced by others, either passively through the release of damage-released alarm cues or through active displays. Gaining information from others as opposed to from risky personal experience is termed 'social learning' (Brown & Laland, 2003). Learning of context-appropriate responses by means of ecologically relevant

stimuli will therefore require that the perceived information is reliable, that behavioural patterns are flexible to optimize threat-sensitive trade-offs, and that learning association and retention between relevant cues and appropriate antipredator responses occurs (reviewed in Brown & Chivers, 2005). Evidence suggests that innate recognition of predators is absent in some prey fishes (Mathis & Smith, 1993a; Chivers & Smith, 1994) and that learned recognition mediated through visual and chemical cues confers direct survival benefits for the receivers (Mirza & Chivers, 2001b, Chivers et al., 2002; Darwish et al., 2005; Leduc, 2008). One mechanism of chemosensory learning that has been the focus of much research is that of the acquired recognition of predators, which enables prey to recognize potential predators through the pairing of alarm cues with the chemical cues of the predator (Suboski, 1990; Smith, 1999) or with a biologically irrelevant or neutral stimulus (Yunker et al., 1999).

Learning of a novel predator odour has been considered to have occurred if overt, short-term antipredator behavioural responses characterized by reduced movement or activity, decreased foraging and mating attempts, increased use of shelter and increased area avoidance, are elicited upon subsequent recognition trials. However, studies have shown that prey are able to detect and learn to recognize the chemical identity of a previously novel predator paired with concentrations of chemical alarm cues well below the concentration required to elicit an overt behavioural response (Brown et al., 2001a; Mirza & Chivers, 2003). In other words, prey are able to learn via this associative pairing of alarm cues and novel odours, despite the absence of conspicuous antipredator behaviours at the conditioning phase (reviewed in Brown & Chivers, 2005). This assessment of local predation risk even at sub-threshold concentrations of chemical alarm

cues indicates that individuals are likely able to maximize the threat-sensitive trade-offs between predator avoidance and other fitness related activities by means of a graded response to varying levels of risk (Helfman, 1989). Ferrari et al. (2005) demonstrated that individuals respond more intensely to predator cues associated with high risk (i.e. predator odour paired with high versus low concentrations of alarm cue), thus further supporting the notion of a graded response that matches the level of local threat. Likewise, fathead minnows (*Pimephales promelas*) learned to recognize either high or low concentrations of predator odour (northern pike, *Esox lucius*), in such a way that they matched the intensity of their response with the relative threat posed by the predator (Ferrari et al., 2006b). Despite this evidence supporting the notion of variability in antipredator responses, Brown and Chivers (2005) have alluded to certain conditions where one might expect more fixed responses. As previously mentioned, one's hunger level has been shown to modify responses, triggering either a fixed suite of antipredator behaviours or potentially nothing at all (risk prone) when prey are satiated, regardless of the level of predation threat.

Many laboratory experiments have shown that prey acquire predator recognition of a chemical and/or visual cue of a novel predator and develop threat-sensitive predator avoidance (Chivers & Smith, 1998; Kats & Dill, 1998; Ferrari et al., 2005; Wisenden et al., 2004). Laboratory reared fathead minnows, for example, which do not exhibit innate recognition of either visual and chemical predatory cues (Chivers and Smith, 1994; Mirza & Chivers, 2000), acquire the recognition of a novel predator after a single exposure to the predator cue paired with conspecific alarm cues (Magurran, 1989; Mathis & Smith, 1993a; Chivers and Smith, 1994). In similar studies, several species of salmonids,

including juvenile Chinook salmon (*Oncorhynchus tshawytscha*; Berejikian et al., 2003), brook trout (*Salvelinus fontinalis*; Mirza & Chivers, 2000, 2001b), and rainbow trout (*Oncorhynchus mykiss*; Brown & Smith, 1998; Mirza & Chivers, 2001a; Leduc et al., 2004a) all demonstrate an increase in conditioned behavioural fright responses, such as reduced territorial aggression and foraging attempts and increased time sheltering, following exposure to a novel predator odour. To date, much of our understanding of chemosensory learning in fish is restricted to laboratory or semi-natural trapping studies (Brown & Smith, 1998; Mirza & Chivers, 2000; Berejikian et al., 1999; Berejikian et al., 2003). However, some studies have tested the mechanism of chemically mediated learning under fully natural conditions and results thus far support the laboratory findings. In fact, field studies conducted by Leduc et al. (2004b, 2006) demonstrated that juvenile salmonids (brook trout, rainbow trout and Atlantic salmon, (*Salmo salar*) are able to detect conspecific alarm cues under neutral conditions and that in Atlantic salmon, relatively small changes in ambient pH can influence alarm responses under natural conditions. In addition, Kim et al. (in press) have demonstrated the combined effect of chemical and visual information in eliciting antipredator behaviour in wild juvenile Atlantic salmon.

While much of the current research supports this mechanism of chemosensory learning in both artificial and wild conditions, there remains some individual variation in the amount and strength of antipredator responses on the part of salmonids. Previous studies have shown that predator-naive fish show complex responses to predators, but the strength of the responses is population specific (Vilhunen & Hirvonen, 2003). Likewise, Vilhunen & Hirvonen (2003) observed variability of innate antipredator responses in

Arctic charr (*Salvelinus alpinus*), as a result of predator species and their diet. Ontogeny, state level (hunger level), social status and prior experience have also been shown to impact learning in fishes by influencing the strength of predator avoidance (Brown et al., 2002, Kelley & Magurran, 2003). Under natural conditions, prey are likely to be repeatedly exposed to both direct and indirect learning opportunities, which allow for continuous reinforcement of biologically relevant cues (Brown & Chivers, 2005). This is particularly important for species that undergo ontogenetic niche shifts. In both laboratory and field studies, juvenile largemouth bass (*Micropterus salmoides*) undergo an ontogenetic change in their response to alarm cues of a heterospecific prey guild member, shifting from antipredator to foraging behaviour when prey individuals are larger (Brown et al., 2001c, 2002). In a field experiment, Kim et al. (in press) have demonstrated that age or size influences risk assessment as well as their responses to an increased perceived predation risk in juvenile salmon. In a final attempt to explain how the variability of antipredator responses can occur in the wild, it is clear that physical limitations such as water velocity, temperature and pH among others, can constrain learning (Dahl et al., 1998; Leduc et al., 2007a, 2007b).

Salmon aquaculture is now a major industry in Canada, operating as the world's fourth-largest farmed-salmon producer, after Norway, Chile and the United Kingdom (Agriculture and Agri-Food Canada website). Juvenile salmon are subject to the greatest predation pressure, with reports of 4-49% mortality within the first two days of experimentally stocking juvenile Atlantic salmon (Henderson & Letcher, 2003). Given the vulnerability of emergent predator-naïve Atlantic salmon fry to predation, artificially 'teaching' fry to adopt threat-sensitive antipredator behaviours through the acquisition of

learned recognition of novel predator cues should greatly increase their survival post-stocking. In fact, evidence has clearly demonstrated that an individual's ability to respond to chemical cues should translate into greater survival for prey individuals (Mirza & Chivers, 2000, 2001b, 2003). In a wild study conducted on fathead minnows, Brown et al. (1997a) revealed that acquired predator recognition in a wild population can occur very quickly, between two and four days, and that individuals retain valuable acquired predator information up to two months after a single exposure to paired alarm cues and predator odour (Chivers & Smith, 1994). Likewise, Brown and Smith (1998) demonstrated that rainbow trout were able to retain acquired predator recognition for at least 21 days but observed a decrease in the intensity of behavioural responses to predator odour over time, if not reinforced with additional associative learning events. Under natural conditions however, it is expected that the recognition of acquired predator odours would be continually reinforced through direct exposure to predators, leading to enhanced retention of relevant information (reviewed in Brown & Chivers, 2005).

Studies have verified the mechanism of acquired predator recognition under both laboratory and fully natural conditions, but further verification is required to assess the retention of newly acquired information through repeat exposure to novel stimuli. Laboratory conditions lack the ecological relevance of the challenges that prey fishes are faced with in their natural habitat. As a result, critics fail to accept the validity of some laboratory-based learning results and insinuate that significant observations stem from laboratory artifact rather than an accurate depiction of how learning occurs in the wild (Magurran et al., 1996; Brown & Godin, 1999). Nevertheless, laboratory studies have been ideal for the purpose of verifying the mechanism of acquired predator recognition

under controlled conditions, as well as suggest the direction of any expected behavioural trends when evaluating the parameters of chemically-mediated learning, which remain unknown in Atlantic salmon.

Our research focuses on the assessment of predation risk mediated by damage-released alarm chemical cues and in particular, the limitations of chemosensory learning in laboratory and field populations of juvenile Atlantic salmon. In the current study, we hope to test learning retention, and replicate previous learning-based work conducted in the field at Catamaran Brook (Leduc, 2008). Our objectives are twofold and address the questions: 1) does acquired predator recognition occur in a laboratory setting and how does it compare to the field and 2) how long does the acquired predator recognition of novel predators last?

Predictions:

The main objective of this study is to address the limits of acquired predator recognition under both laboratory and fully natural conditions. Given that learning retention potential has been demonstrated on numerous occasions under artificial or semi-natural conditions, we expect to see a similar trend with juvenile Atlantic salmon in both lab and field experiments. In particular, we predict that acquired predator recognition would be retained throughout the duration of the experiment but decrease in the intensity of behavioural responses to the novel odour (NO) over time, if not reinforced with additional associative learning events. Assuming that acquired predator recognition is successful, an anticipated decrease in the intensity of antipredator behaviour as time elapses is expected and would translate graphically in a gradual reduction of the mean

differences (post-stimulus – pre-stimulus total scores) in antipredator behaviours, for all the variables studied over time. Eventually, the mean differences between pre and post-stimulus antipredator responses for individuals exposed to the alarm cue (AC) treatment should match those seen for our control treatment, which would indicate a loss of recognition of the predator or absence of learning retention.

With respect to the field experiment, it is hypothesized that since the wild population is continually bombarded with a plenitude of chemical cues arising from the environment (ie. other paired learning associations), we predict that without reinforcement of the paired AC + NO (repeat conditioning), as would likely occur in natural situations, the retention potential would be less than that seen in laboratory-reared fish in an artificial setting. In other words, the mean differences in all behavioural measures between treatments at the conditioning phase should be equivalent both for the lab and field experiments, but would differ in intensity over time, with a quicker loss of learning retention from day 1 to day 3, to day 5 and then to day 7 in wild populations. This is attributed to the absence of re-exposure to the pairing of AC with NO, which has rendered the learned information perhaps irrelevant to merit retention. Moreover, we speculate that environmental factors such as flow will constrain learning, by means of impeding proper conditioning or by bombarding the focal fish to a greater number of other cues and pairings due to increased flow.

Materials and methods: laboratory trials

Test fish and set-up:

Newly emerged, hatchery-reared YOY Atlantic salmon originating from the Little Southwest Miramichi River stock were placed in pairs in the test tanks and fed *ad libitum* with 0.7GR (grain size) floating pellet fish food. Fish were left in test tanks for the duration of the experiment and on testing days, fed one hour prior to testing then again at the completion of our observations. Each pair consisted of a focal fish (mean standard length ± SE = 26.55 ± 0.19 mm; mean depth ± SE = 3.48 ± 0.11 mm) and a tagged fish that served as a companion (mean standard length ± SE = 26.21 ± 0.25 mm; mean depth ± SE = 3.47 ± 0.26 mm). Tagged companions were introduced to reduce apparent stress on the focal fish, and were excluded from all behavioural observations. Companion fish were tagged with visible implant elastomer (VIE) tags placed in the caudal fin and caudal peduncle to facilitate their differentiation from the focal individuals (tagging protocol followed that of Steingrimssom & Grant, 2003). Our observations were restricted to the untagged fish in order to preclude any behavioural repercussions from handling stress during the tagging procedure.

Test tanks consisted of 11.3L clear plastic Rubbermaid™ bins, filled with approximately 11L of brook water from the Miramichi Salmon Association (MSA) hatchery in South Esk, New Brunswick. Prior to placement of test fish, the bottom of each tank was covered with ~ 2 cm of white and sand coloured gravel to stimulate a natural substrate and placed in continuous-flow stream channels in order to keep the test tanks at a constant cool temperature (mean ± Std. Dev. = 16.2 ± 1.12 °C). Air stones attached to single output air pumps provided oxygen to the tank water for the duration of

the experiment and extra airline tubing (50 cm) used to inject the stimuli was attached to the airstone to facilitate diffusion without disturbing the fish. Throughout experimentation, the photoperiod was adjusted to reflect natural seasonal conditions with approximately 14h light, 10h dark and was not altered over the course of experimentation.

Stimulus preparation:

1. Conspecific skin extract or Alarm cue (AC):

Laboratory-reared juvenile YOY Atlantic salmon donors were obtained from the Little Southwest Miramichi River stock at the MSA hatchery in Miramichi, New Brunswick. Donor fish were killed with a blunt blow to the head in accordance with Concordia University Animal Research Ethics protocol (#AREC-2008-BROW). Heads and tails were severed immediately behind the pectoral fin and at the caudal peduncle, respectively, and the remaining bodies were then placed into 50 mL of brook water, homogenized and filtered through polyester filter floss. The filtrate was subsequently diluted to the desired final volume with the addition of brook water. In order to control for possible age-specificity in behavioural responses to chemical alarm cues, YOY donors were exclusively selected to make the skin extract rather than Parr (1+).

We used a total of 35 YOY Atlantic salmon (mean ± SE standard length = 2.52 ± 0.031 cm, mean ± SE width= 0.37 ± 0.012 cm) as alarm cue donors for a total skin area of 39.78 cm^2, and a total volume of 398.2 mL. The final concentration of skin extract (~0.1 cm^2 mL^{-1}) of pH = 6.95 was prepared in a similar fashion to that used in previous studies with salmonids and has been found to be adequate to elicit antipredator

behavioural responses in juvenile Atlantic salmon (Leduc et al., 2006, 2007a, b). Skin extract was subsequently frozen in 22 mL aliquots at –20 °C and thawed at room temperature when needed for the conditioning phase of the experiment.

2. Novel odour (NO):

A solution of lemon oil and stream water served as the novel odour. The solution was produced as needed with a dilution factor 1:45, lemon oil to brook water, after Leduc et al. (2007a, 2007b). A lemon oil dilution was used because none of the fish had prior experience with the stimulus, thus making it truly novel.

Experimental procedure:

A focal and companion test fish were placed together in a series of 40 test tanks (20 experimental and 20 control) and left to acclimate at least 24 hours prior to experimentation. The protocol consisted of a single conditioning phase (C or Day1) and three subsequent recognition phases (R1, R2, R3 or Day 3, 5 and 7 respectively), each divided into a five minute pre-stimulus and a five minute post-stimulus observation periods. The pre-stimulus observation periods serve as individual baseline activity levels to compare to the respective post-stimulus levels. The conditioning phase followed a similar protocol as in Leduc et al. (2004, 2006, 2007a), whereby equal amounts of either alarm cue (AC) or a brook water control (SW) were injected immediately prior to the novel lemon odour (NO). Prior to commencing an observation, we withdrew and discarded 60mL of tank water through the stimulus injection tube to remove any debris. We then withdrew and retained an additional 60 mL of tank water. Following the pre-

stimulus observation period for the conditioning phase, we injected 10 mL of alarm cue or stream water control, flushed the line with 30 mL from the syringe and then injected the lemon odour followed by the remainder of the 30 mL from the same syringe of tank water. The fish were then observed for an additional five minute post-stimulus period. The same general protocol was observed for the recognition phases, except that for both experimental and control groups, stimulus injections consisted only of 10 mL of NO, followed by 60 mL of tank water in order to achieve the same level of mechanical disturbance as fish were exposed to during the conditioning phase. Once testing was complete, partial water changes were administered to reduce the risk that fish may be exposed to different amounts (concentrations) of stimuli, thus affecting behaviour.

During both pre- and post- stimulus observation periods, we recorded: 1) an index of area use, 2) time spent moving, 3) total number of foraging attempts, 4) total counts of aggressive interactions and 5) total incidents of dashing behaviour. Area use is a measure of the position of focal fish within the milieu, with 1 = on the substrate and 2 = hovering over the substrate or swimming around the tank. The positions of focal fish were recorded every 15 seconds in order to calculate the mean area use during each observation. Time moving was recorded as the total amount of time that the focal fish spent moving in any direction from its initial position within a 5 minute block. A foraging attempt was defined as a directed lunge towards a food item, in the water column or at the surface. Aggressive interactions were defined as a chase or flee caused by either fish, while dashing was a sudden burst of apparently disoriented swimming. As seen in previous work, a reduction in area use, foraging attempts, total time moving and overall aggression are all indicative of an antipredator response in juvenile Atlantic salmon. By

contrast, an increase in dashing is evidence of predator- evasive behaviour in the same species (Leduc et al. 2006, Leduc, 2007a). Trials commenced June 16th, 2008 on site and ran for a total of 11 days, resulting in 20 replicates each of experimental and control treatments, i.e. N = 20.

Statistical analysis:

This experiment tests the null hypothesis that learned recognition of antipredator responses is the same over time, without further reinforcement. Pre- and post- stimulus behaviour was scored and the differences between the pre- and post-stimulus observation periods (post – pre-stimulus totals) were calculated for each behavioural measure. Once compiled, all data were tested for normal distribution using the skewness and kurtosis indices, and those measures (area use, aggression and dashing) that were not homoscedastic or normally distributed, were ranked with SPSS 16.0. This enabled us to proceed with parametric tests as opposed to the non-parametric equivalent (Scheirer et al., 1976). We used one-way ANOVAs to test for changes in each behavioural measure in response to the different treatments (AC or SW control). We then used a repeated-measures analysis, with the differences in mean behavioural measures treated separately for each phase (C = conditioning phase, R1 = first recognition phase, R2 = second recognition phase, R3 = third recognition phase) as the within subject factor (the repeated measure) and treatment (AC or SW control) as the between-subject factor. The repeated-measures ANOVA allows for the partitioning of variability by individual from the main treatment effects, with a resulting loss of degrees of freedom for the testing the main treatment effect. Consequently, the repeated-measures is appropriate when one has low

individual variability with a relative small treatment effect, as may be the case when observing an individual repeatedly over a short time period (7 days in the current study). Pre- stimulus and post- stimulus intervals for each phase (observation period as the repeated measure) were analyzed using repeated-measures ANOVA, with treatment as the between-subjects factor. Finally, to test for the interaction between behavioural measures, foraging and time spent moving at the conditioning phase and the first recognition phase (phases taken separately), a repeated-measures MANOVA (MANOVAR) analysis was conducted. All statistics reported were calculated using SPSS version 16.0.

Materials and methods: field studies

Study area:

Field experiments were conducted in Catamaran Brook, a third order tributary of the Little Southwest Miramichi River, New Brunswick, Canada (approximately 46°52·7′N, 66°06·0′W). The 13 study sites selected were located between the mouth of Catamaran and a point ~ 400 m upstream. On average, experimental sites measured 13.15 ± SE = 1.43 m in length and 10.37 ± SE = 0.50 m in width (Figure 1). In order to ensure that contamination by means of treatment or from displaced upstream focal fish did not occur, a minimum distance of 5 m between sites was maintained.

Catamaran Brook has been well studied in various multidisciplinary studies (Cunjak et al., 1993; Steingrímsson & Grant, 2003; Breau et al., 2007; Leduc et al., 2006, 2007a, 2007b) and information pertaining to annual physical and biotic characteristics of the various reaches of the brook is readily available to compare our current measurements

with those collected previous years. Throughout our field seasons (2007-2008), pH measurements (mean pH ± SE = 7.26 ± 0.025) were comparable to prior measurements in 2005, (7.14 ± 0.09) (Leduc et al., 2007a). Other physical variables collected throughout the experiments, at each observation period and at a predetermined marked site (flagged stone), included water temperature, current speed (at~ 50% from the bottom of the total water depth), substrate complexity, habitat type (flat, pool, riffle, run, back water), cloud cover and canopy cover after Leduc et al., 2006, 2007a, 2007b.

Stimulus preparation:

Chemical alarm cues were prepared from hatchery- reared juvenile (parr) Atlantic salmon donors from the Miramichi Salmon Association hatchery in South Esk, New Brunswick, in late June 2007 and early June 2008. Using a total of 12 parr (1+) Atlantic salmon skin fillets (mean ± SE standard length= 8.98 ± 0.31 cm, mean ± SE width= 2.19 ± 0.0091 cm), a total skin area of 226.61 cm^2 for a total volume of 2267.08 mL was adjusted to reach a final concentration (~0.1 cm^2 mL^{-1}) as described in the laboratory section. The protocol for preparing the novel odour was identical to that for the laboratory component.

Test fish:

We used wild juvenile Atlantic salmon (YOY and 1+) to test for the ability to acquire the recognition of a novel odour from a single pairing with conspecific chemical alarm cues and to assess the retention of this newly acquired information. Focal fish were approached from downstream while snorkeling and captured using two small commercial

dip nets. Focal fish measured on average, standard length ± SE = 4.14 ± 0.072 cm and mean depth ± SE = 0.80 ± 0.023 cm for the YOY and for the parr (1+), standard length ± SE = 6.64 ± 0.16 cm and depth ± SE = 1.35 ± 0.027 cm. Individuals were anesthetized using a solution of ethanol (70%) and clove oil in water and tagged using a visible implant elastomer (VIE) device as described in Steingrímsson & Grant, (2003) and Breau et al., (2007). Due to the limited size, each focal fish was tagged in one to three locations: the caudal fin and/or peduncle and/or in both opercula. For larger juvenile fish (1+), up to three tags were placed in the tail and caudal peduncle to increase the number of individual markings for a specific site. According to the manufacturer, when the placement of the implant is done correctly, the tagging method is non-invasive and does not affect fish behaviour or movement (Frederick, 1997). Furthermore, markings and tag retention improved over the course of the field season, with an initial retention of approximately 50% in June 2007 to nearly 80% in August 2007 and summer 2008. This is partly attributed to both ease of manipulation with practice, but mostly due to an increase in average size of focal individuals, which resulted in less mortality or displacement upon reentry to the stream. In addition to the VIE tags, stones wrapped in flagging tape were placed to mark the location of each tagged fish and used to indicate the area where each physical measurement was to be taken. Site fidelity in individually tagged YOY and 1+ Atlantic salmon is shown to be strong amongst these fish at this particular site, with recorded movements of less than 1m up- or down- stream throughout the study season (28-74 days) (Steingrímsson & Grant, 2003; Breau et al., 2007). Once tagged, the fish were released back into its territory and allowed to recover from any

handling stress that may have occurred during the tagging process (generally 24 hours) before observations began.

Experimental procedure:

Trials for the first field season commenced June 28th, 2007 and ran for approximately 8 weeks, with some delay in testing due to difficult weather conditions. The second field season started July 12th and finished August 28th 2008. The experiment was divided into a single conditioning phase (C or Day1) followed by three recognition phases (R1, R2, R3 or Days 3, 5, and 7), each divided into a five minute pre-stimulus and a five minute post-stimulus injection observation periods. On the conditioning day, one of the 2 stimuli either 20 ml of AC + 20 ml of NO or 20 ml of stream water + 20 ml of NO was injected immediately upstream from the test individual, following the pre-stimulus observation period. To test for the loss of response, recognition trials were conducted on days 3, 5 and 7 and consisted of the same observation periods as described for the C phase (pre- and post- stimulus), with 20 ml of novel odour alone given to focal fish after the pre-stimulus phase. To ensure that stimuli were detected by our focal individuals and given that fish orient themselves upstream, the syringe was positioned ~ 20 cm upstream of the fish, on the same vertical plane as the subject and within 10° of the axis of stream current. Milk tests were conducted in early field observations to ensure that our injected stimuli reached our focal fish.

Behavioural data for the 2007 field season were collected using methods described by Leduc et al. (2006), which involved an underwater Sea View camera positioned downstream and to the side of the focal test fish. Small-scale behavioural

responses were recorded in real time and reviewed at a later date to assess the intensity of an alarm response from exposure to a stimulus (AC or SW). The behavioural measures observed and coded from the tapes included: 1) the number of foraging attempts, 2) the number of aggressive interactions, 3) the total time spent moving (seconds), 4) the total time spent motionless on the substrate (seconds) and 5) the total time spent absent from the screen (seconds). In previous experiments using the underwater Sea View camera, the camera was positioned 1-1.5 m from the focal fish (Leduc et al., 2006), however due to the complexity of the habitat, turbidity of the water and the mean size of the test fish, distance between the camera and fish was reduced to less than 30cm, increasing the chance that fish would be visually disturbed upon testing. To counter the potential skewing of the results by means of visual disturbance, focal fish were allowed to acclimate to the observer's presence prior to each taped trial and recordings only commenced once the test fish had resumed normal activities such as foraging and moving about its habitat.

The experimental protocol for the second field season (2008) differed from that of the first in that behavioural observations were recorded and coded live, *in situ*, as opposed to relying on recordings. This modification to the protocol meant that some of the behavioural measures recorded during the first season were omitted or modified. Behavioural measures recorded during each of the observation periods in 2008 were identical to those coded for in the laboratory section (refer to experimental protocol in the laboratory section for behaviour definitions). As seen in previous work, a reduction in area use, foraging attempts, total time moving and overall aggression and an increase in dashing are indicative of an antipredator response in juvenile Atlantic salmon (Leduc et

al. 2006, 2007a, 2007b). Population density was too low for aggression between individuals to occur with any regularity. Therefore, we omitted the measure altogether and relied on the other remaining behavioural parameters for our analysis.

Statistical analysis

Analyses conducted on field behavioural measures were similar to those described in the laboratory section. Ranked and non-ranked data were initially analyzed by way of multiple two-way ANOVAs, which tested for the effect of treatment (AC or SW control), summer (2007 or 2008) and their interaction on the change of antipredator behaviour. Preliminary analyses (1-way ANOVA) testing the physical variables independently revealed significant differences in flow, depth and water temperature in response to treatment and summer separately, which meant that flow and water temperature were factored into the subsequent analyses of behaviour. Data were then analyzed using multiple repeated- measures ANOVAs, assessing the effect of treatment (the between-subject factor) on the change in each behavioural measure, taken at each phase (C, R1, R2 and R3) as the repeated measure and flow or water temperature as a covariate. This was then repeated for pre-stimulus and post-stimulus intervals (observation period as the repeated measure), at each phase. Repeated-measures MANOVA were conducted, testing the effect of treatment on behaviours, foraging and time moving together with respect to pre and post-stimulus data at the conditioning phase. The same statistical tests were used when looking for an effect of treatment on behaviour with respect to age class (YOY or 1+). All analyses of ranked data followed the Scheirer-Ray-Hare method of analysis,

which is an extension of the Kruskal-Wallis ranks tests (Scheirer et al., 1976) and all results reported were calculated using SPSS version 16.0.

Acquired predator recognition and learning retention

Results: laboratory experiment

There is a significant effect of treatment on foraging behaviour during the conditioning phase (C, Day 1) ($F_{1,41} = 11.47$, $p = 0.002$; Table 1 and Figure 2A). More specifically, we observed a decrease in mean change in foraging attempts with respect to the experimental treatment by comparison to the control group data. On the first recognition day (R1, Day 3), there is a significant effect of treatment on area use, with experimental fish spending a greater amount of time spent on the substrate than the control group ($F_{1,41} = 5.71$, $p = 0.022$; Table 1 and Figure 2C).

Repeated-measures ANOVAs were conducted in order to examine the change in behaviour over time (C, R1, R2, R3), then at each phase separately (pre-stimulus vs. post-stimulus) (Table 2). There is a significant effect of treatment on foraging behaviour over all phases, but in particular at the conditioning phase ($F_{3,35} = 4.084$, $p = 0.014$ and $F_{1,41} = 16.42$, $p < 0.05$ respectively; Table 2). The change in mean time spent moving at the conditioning phase did not yield a significant effect in response to treatment, but a notable decrease in time spent moving with respect to the experimental treatment was observed ($F_{1,41} = 4.0$, $p = 0.052$; Table 2).

The repeated-measures MANOVA testing for an interaction between foraging and time moving behaviours in response to treatment at the conditioning day (C, Day 1) yields a significant result ($F_{2,40} = 5.95$, $p = 0.005$; Table 3), due to the significant effect of treatment and the change in foraging behaviour ($F_{1,41} = 11.45$, $p = 0.002$; Table 3). *Post-hoc* power analysis were conducted on the foraging and time moving variables at the first

recognition phase (R1) as well as on area use behaviour at the conditioning phase (C). Given a sample size of N = 21, actual power was: 5.3%, 5.4% and 6.3%, respectively.

Results: field experiments

The mean change in behaviour, number of foraging attempts, time spent moving, area use and time spent motionless, is not significantly different between treatments at any phase of the experiment (refer to Table 4 for statistical results and Figures 3A and 3B). In fact, foraging rates pre- to post- stimulus are not significantly different with respect to treatment, but foraging activity remains greater with individuals exposed to chemical cues versus stream water (Figure 4). When comparing our foraging data with that collected during previous field seasons, clearly our results are within the range of values expected (Table 5), with the exception that the variability in our data is greater than observed in previous studies. An *a priori* power analysis, computing the sample size required in order to achieve an actual power of 95% as observed by Leduc (unpublished data from 2005 at Catamaran Brook), suggested a minimum necessary sample size of 2672. Finally, a *post-hoc* power analysis was tabulated for time moving data at the C phase and our resulting power given a sample size of N = 16, is 5.8%.

Discussion: laboratory experiment

Laboratory-reared juvenile Atlantic salmon appeared to respond to the initial association of alarm cue and novel odour stimuli in a species-typical fashion, but we were unable to detect a response to the acquired information 48 hours after this conditioning event. In particular, experimental fish responded with an increase in antipredator

behaviour at the conditioning phase that stemmed mainly from a decrease in foraging rate. The change in time spent moving in response to alarm cues by experimental fish did decrease, but this change was not significantly different from that of the control fish. Moreover, the average change in area use at the conditioning phase decreased as expected, but this change in mean behaviour was not significantly different from that of the control group. However, at the first recognition phase, the mean change in area use was the only variable to yield significant results with respect to treatment. The absence of significant results for area use at the conditioning phase as well as for all other variables across phases (except foraging at Day 1), support the notion that any observed difference in area use observed at the first recognition day, may not be attributed to retention of any learned association, but rather a product of variability in our data and/or other unexplained ecological or experimental processes.

Numerous studies have shown that several salmonid species respond with a suite of species-specific antipredator behaviours when exposed to conspecific chemical alarm cues (Brown & Smith, 1997b, 1998; Mirza & Chivers, 2001a, Leduc et al., 2007a, 2007b). Thus, our results are consistent with previous research and lend support to the notion that individuals adjust their activity level based on an immediate assessment of local predation risk (Brown & Chivers, 2005). Despite these findings, all but one antipredator behaviour (decreased foraging) did not reveal a significant effect of treatment, suggesting that perhaps individuals are adjusting their behaviour less intensely as predicted by the threat-sensitive predator avoidance hypothesis (Helfman, 1989).

Reliance on chemosensory cues alone to gauge the risk of predation is unlikely in this study. Although we have not directly examined the combined effects of chemical and

visual information on the assessment of perceived threat, we can speculate that our fish may be using multiple cues about predators in an additive and complementary manner (Smith & Belk, 2001). This suggests that our test fish were exhibiting an antipredator response to the conspecific alarm cues that was proportional to the immediate perceived threat: information stemming from both the absence of visual threat and a chemosensory source. In a field study conducted on pumpkinseeds (*Lepomis gibbosus*), habitat complexity plays a significant role in influencing the threat-sensitive use of these chemical cues (Pollock & Chivers, 2003; Denno et al., 2005; Golub et al., 2005). Fish are faced with conflicting sources of information about immediate predation threat and are perhaps responding to alarm cues more subtly than if they were tested in more structured habitats (high level of habitat complexity) or paired with visual stimuli. In fact, it is thought that the presence of a companion fish may have dissuaded the test fish from responding needlessly to chemical cues (i.e., reduced perceived level of risk).

Antipredator behaviours improve with experience and the use of socially transmitted information (social learning) enables individuals to respond to threats without having to verify the presence of danger independently (Suboski & Templeton, 1989; Kelley & Magurran, 2003). Rapid information transfer via vision and the lateral-line system is very common in fish and predator- naïve fish (like both our focal and companion fish) can learn through cultural transmission when following a demonstrator fish (Mathis et al. 1996b; Brown & Laland, 2003). Pairs of test focal fish and companion fish used in the experiment were placed simultaneously and left in their respective testing tanks throughout the experiment. This precludes any doubt that companion fish were 'knowledgeable' in the level of predation threat at the start and throughout the

experiment. However, the presence of another individual perhaps minimized the perception of threat, which resulted in fish responding more subtly with respect to alarm cues.

An individual's hunger state may also temper any potential antipredator responses that would be elicited if the benefits gained from adopting predator aversive behaviours outweighed those of foraging (Harvey, 2005). It has been shown that fish that are well fed either engage in threat-sensitive antipredator behaviour or conversely, with risk prone responses (Harvey, 2005). Since our fish were fed *ad libitum,* we think that they were simply not responding adequately to chemical alarm cues at the conditioning phase or at all in response to subsequent exposure to novel odours. Therefore, we believe that threat-sensitive behavioural decisions may be variable and 'fine-tuned' to incorporate an individual's physiological state, hunger and energy stores (reviewed in Brown, 2003), as well as visual cues, be it the absence of perceived visual threats and/or the presence of "public information" about risk (Mathis et al., 1996 b; Lima & Steury, 2005).

The absence of subsequent recognition of the novel odour across most behavioural measures was unexpected, as it contradicts prior studies assessing the learned recognition of predators and learning retention in fishes. Conditioned predator recognition after a single exposure to the predator cue paired with conspecific alarm cues has been observed in minnows (Magurran, 1989; Mathis & Smith, 1993a; Chivers & Smith, 1994) and in salmonids (Berejikian et al., 2003; Brown & Chivers, 2006; Leduc et al. 2004b). The short- term retention (24 hours) of the learned information has been shown in Atlantic salmon (Leduc et al. 2006), but the lack of reinforcement, perhaps rendered this newly acquired information irrelevant and most likely lost within 24 to 48

hours. Likewise, Brown and Smith (1998) demonstrated that rainbow trout were able to retain acquired predator recognition for at least 21 days under laboratory conditions, but noticed a decrease in the intensity of learned behavioural responses to predator odour with time elapsed since initial conditioning, if not reinforced with additional associative learning events. However, the absence of antipredator responses at any recognition phase, notably with respect to foraging and moving behaviours, indicates that perhaps learning was not successful let alone retained. Recent studies suggest that learning can occur in the absence of an overt antipredator response (Brown & Smith, 1996; Brown et al. 2001a; Mirza & Chivers, 2003), and in fact, we found that area use did decrease significantly in response to the novel odour on R1. However, since the conditioning event for this particular behavioural measure was deemed unsuccessful and our foraging results show that both experimental and control fish increased their foraging behaviour (number of foraging attempts) at the first and second recognition phases, this significant decrease in area use at the first recognition phase may be attributed to the individual variability in the data rather than covert recognition of an acquired odour. In fact, the variance for all measured behaviours was large across phases and not significantly different between treatments (Levene's F test for homogeneity of variance).

Another plausible, though purely speculative explanation for the inconsistencies between our results and that of published work on salmonids, is that of age, rearing and their interaction with other ecological processes, all of which are biological constraints on learning. The fish used for this study were two weeks post-hatching and may have been exposed to many stressors throughout the duration of the experiment, as well as prior to use in this study. Skepticism about the validity of our results arises when newly-hatched

fish rather than older individuals are used. Granted, our fish were small (standard length ± S.E. = 2.65 ± 0.019 cm and depth ± S.E. = 0.35 ± 0.011 cm), but they were feeding normally on pellet food prior to the start of the experiment. Our test fish were younger, therefore smaller than those used in previous learning studies on salmonids (Berejikian et al., 2003; Leduc et al., 2007a, 2007b), but learning of possible predators as early as at the embryonic stage has been shown (Mathis et al., 2008). Indeed, ontogenetic shifts in antipredator behaviour have been reported in fishes (Brown et al., 2002), but studies have not specifically examined how ontogenetic changes influence the ability to learn about predators (Kelley & Magurran, 2003). The impact of age, provenance (hatchery-rearing), competitive interactions and abiotic factors (water temperature) have on learning of juvenile Atlantic salmon were beyond the scope of this experiment and require substantially more work.

Discussion: field experiment

Given the absence of a response to the initial pairing of chemical alarm cues with a novel odour and the failure to respond to the acquired information at all recognition phases throughout our experiment, we can confidently refute our initial predictions about learning in wild populations of juvenile Atlantic salmon. Contrary to prior research conducted at Catamaran Brook, chemical alarm cues did not elicit any overt or covert antipredator behaviour at the conditioning and at the first recognition phases respectively. Moreover, these results are consistent when age class (YOY and 1+), study summer (2007, 2008) and water velocity as a covariate are factored into our analyses. When comparing all the measured environmental variables between treatments over time, only

water temperature at the R1 and R3 (third recognition phase) phases varied significantly between treatments, even though the range in the water temperature measurements combining both treatments was within a suitable range for normal to peak activity levels in Atlantic salmon (16- 23°C for YOY and up to 21°C for 1+) (Breau et al., 2007). As one would expect from conducting field research over the course of two summers, all environmental factors differed significantly from 2007 to 2008, which were taken into account when conducting the analyses on the behavioural measures between treatments over both summers (Table 2).

In the discussion of the laboratory results, we alluded to the possible effects of habitat type on the perception of predation threat. Likewise in field studies, ecological constraints on learning such as habitat complexity and differences in rates of diffusion, which stems either from different rates of dilution within slow-moving or static water systems (i.e. side pools) and/or dispersal rates in faster flowing water (streams, rivers), may pose a significant barrier to learning in fishes (Brown & Chivers, 2005). In other words, fish located downstream from a predation event may likely have been exposed to one but not both type of cues (chemical alarm and predator cues), because of subtle differences in microcurrents, coupled with differential dispersal rates and differential breakdown rates of predator and prey cues (Hazlett, 2003). In our case, we encountered very high and fast flowing water during our field seasons due to substantial flooding in New Brunswick in late July to early August 2007 and 2008. Consequently, we could speculate that learning may have been constrained because one or both cues were 'lost' while injecting the stimuli. In the field, proximity to the predation event would likely dictate whether fish perceive threat and consequently, learn from it. In our early field

observations, milk tests were conducted to assure proper delivery of the stimuli, but one would have to test with milk prior to every observation in order to minimize possible loss of stimuli from increased dilution rates due to higher water flow. For logistical reasons this was not done and the incurred chance that stimuli were lost may have posed a significant problem to our experiment.

Weather conditions, gauged through the combined changes in water flow, water temperature and precipitation measurements varied greatly from 2007 to 2008, as well as from prior field studies at Catamaran Brook. This led us to question whether our observations were done accurately and/or atypical weather ultimately influenced overall fish behaviour at our sites. In order to gain some insight on this matter, we have compared our field results, looking at foraging rate behaviour between treatments over the years 2005 to 2008 (Table 3). Much of the field work conducted at Catamaran Brook has focused on assessing the effects of chemical information in eliciting antipredator behaviour on wild juvenile Atlantic salmon (Leduc et al., 2006, 2007a, 2007b; Kim et al., in press). When comparing our mean foraging rates for the YOY and the 1+ in 2007 and 2008 with those of Kim et al. (in press) and Leduc (unpublished data from field season 2005), clearly foraging behaviour varied between age class and between field seasons. Of particular interest is the discrepancy in mean foraging rates between age classes, observed in 2007, 2008 and in 2005-2006 (Kim et al., in press). Experimental YOY fish in 2005, 2005-2006 and 2008 decreased their foraging activity by comparison to the control fish, while in 2007 YOY increased their foraging activity in response to the alarm cue treatment. In addition, an increase in foraging behaviour is observed, for juvenile Atlantic salmon exposed to the stream water controls. This may be explained by the

notion that a greater number of prey organisms were dislodged into the drift upon release of the stimulus (Kim et al., in press). Excluding foraging rates in experimental fish in 2005, mean foraging rates pre- to post stimulus, for both YOY and 1+ age classes do not vary greatly between treatments as they are within the range of variance (all rates are within 3.11 and 4.41 no. of foraging attempts/minute). These observations suggest that perhaps antipredator behaviour in juvenile Atlantic salmon is more context-dependant and that a more comprehensive approach to examining learning in wild populations of fish is in order. For example, common garden experiments with the use of enclosures may eliminate some of the environmental factors (water velocity) and impacts resulting from exploitative competition, which may have constrained learning in the field experiment. Furthermore, testing hatchery-reared fish that have undergone a strict feeding regimen and have been selected for size may eliminate any possible effects of size and hunger level on the decision to forage or adopt risk-sensitive behaviour. Given the laboratory bias surrounding the knowledge of predator- prey interactions in fishes, the need for field studies is apparent. Perhaps fully natural experimental designs are ideal in theory, logistically; they may pose more problems than answer questions.

General Conclusions:

The results presented in this thesis suggest that under strict laboratory conditions, individuals exhibited antipredator behaviours in response to the chemical alarm cues paired with a novel odour, but did not exhibit any response to the subsequent exposure to the conditioned novel odour 48 hours after the initial association. As for our field component, the absence of a response across all phases and between treatments was not

expected and suggest that variability in the data and the various biological and ecological processes involved perhaps constrained learning of relevant stimuli.

Given the importance of predation pressures and the potentially severe impacts engendered by the failure to recognize and accurately respond to predators on survivability in wild salmon populations, the need to fully understand how ecological variability hinders learning is obvious. Making efficient threat-sensitive decisions is complex and dependant not only on a suite of trade-offs between predation costs and the benefits gained by engaging in fitness-related activities, but on context-dependant cues such as exploitative competition, habitat complexity and a myriad of physical variables, of which, flow and the resulting water volume is a major contributor influencing antipredator behaviour in our field experiments. Moreover, latent inhibition and learned irrelevance have also been significant barriers preventing individuals from acquiring biologically relevant information in the wild (Ferrari & Chivers, 2006; Brown & Chivers, 2005). Thus, our knowledge in predator-prey interactions in the field is lacking. With such remarkable laboratory evidence supporting the efficiency of acquired-predator recognition across various fishes, the focus should shift from laboratory experiments to designing a multidimensional field experiment, which would factor in temporal variability when assessing the influence of chemical alarm cues in the context of predator-prey interactions. Further research is also required in order to address questions relating to the limitations of this learning mechanism in hatchery-reared and wild populations of Atlantic salmon. While our experiments may not have yielded many significant results, the fact remains that variance was great throughout years and across treatments, suggesting that perhaps fish would only respond to alarm cues in a species-

specific fashion only under optimal conditions. Regardless of the outcome of our results, the variability in our data alludes to the need of conducting more research, both under lab and field conditions, in order to reliably make generalities about learning in fish. Only once our knowledge on these topics is improved, can we apply them to enhance the techniques used in fish management and the conservation of endangered salmonid populations.

Figure 1: Location of the Catamaran field site in New Brunswick, Canada. The inset map shows the location of the study area in New Brunswick in Canada (square) as well as the location of our study site within Northumberland County.

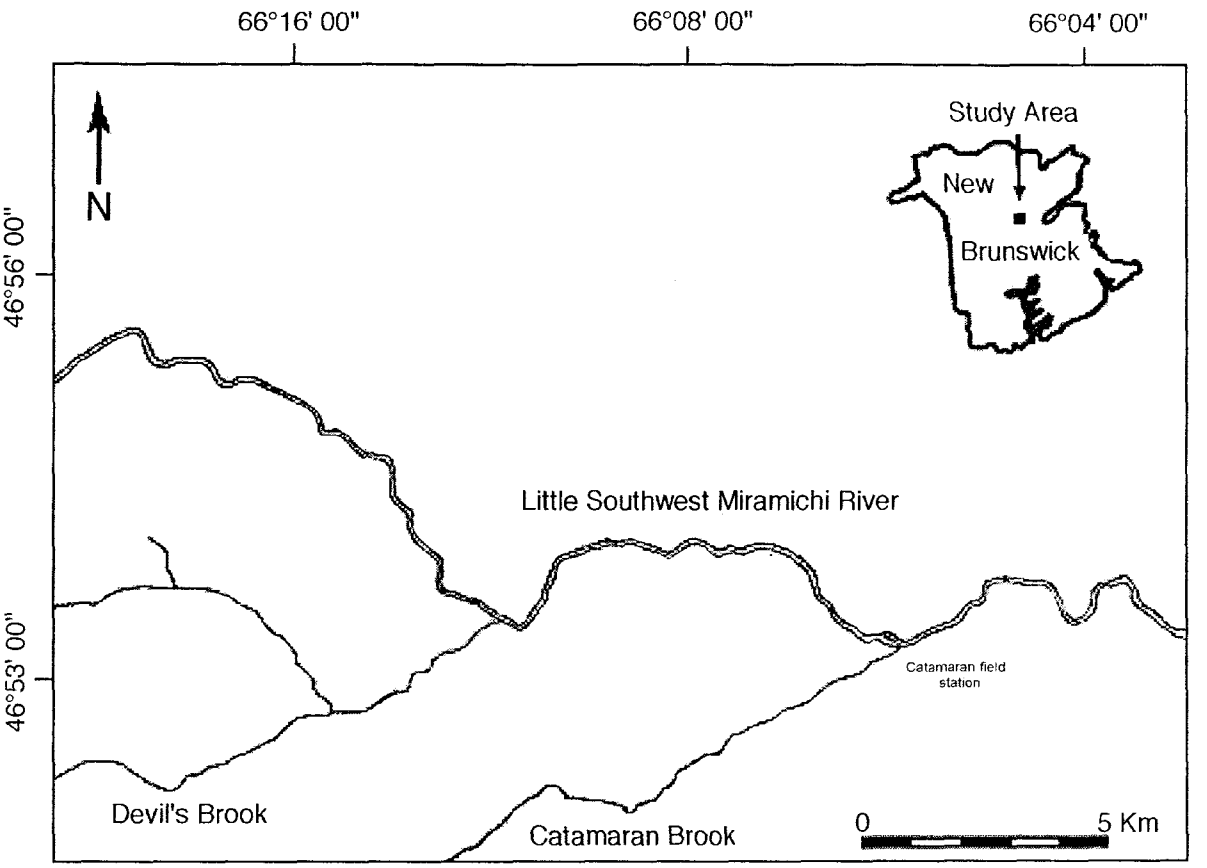
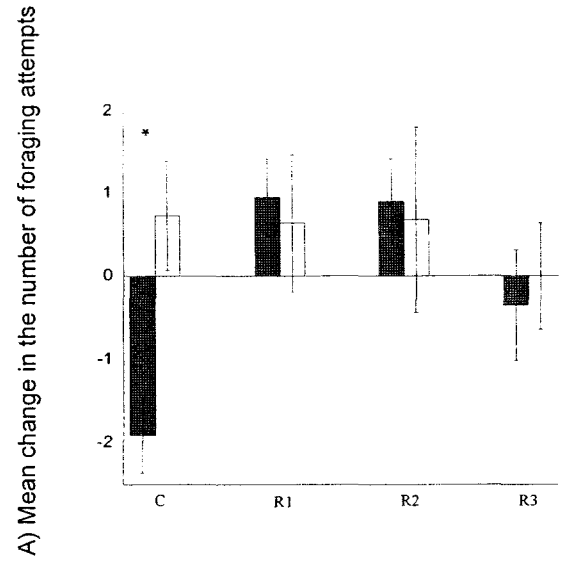
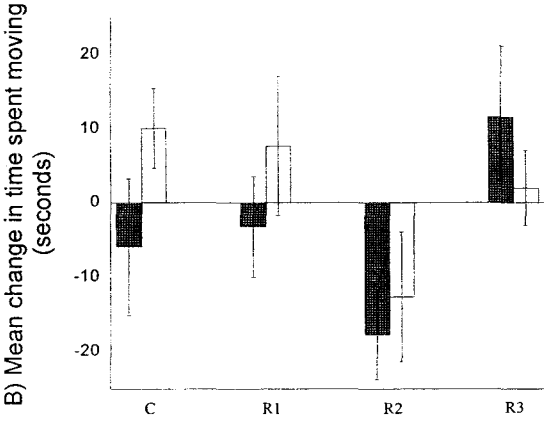

66°16' 00"
66°08' 00"
66°04' 00"
46°56' 00"
46°53' 00"
N
Study Area
New
Brunswick
Little Southwest Miramichi River
Catamaran field station
Devil's Brook
Catamaran Brook
0
5 Km

Figure 2: Mean (±SE) change in antipredator behaviour in A) the number of foraging attempts, B) time spent moving (seconds), and C) area use in laboratory-reared juvenile Atlantic salmon in response to alarm cue (grey bars) or stream water control (white bars) treatments at each phase. N=21 for the conditioning (C) and first recognition (R1) phases, N= 20 for the second (R2) and third recognition (R3) phases, for both treatments.


A) Mean change in the number of foraging attempts
2
1
0
-1
-2
*
C
R1
R2
R3


B) Mean change in time spent moving (seconds)
20
10
0
-10
-20
C
R1
R2
R3

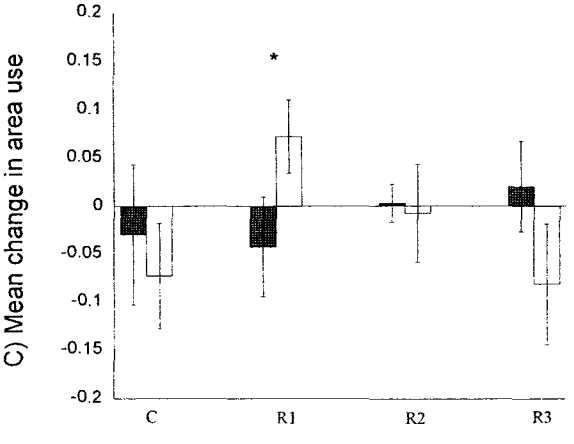
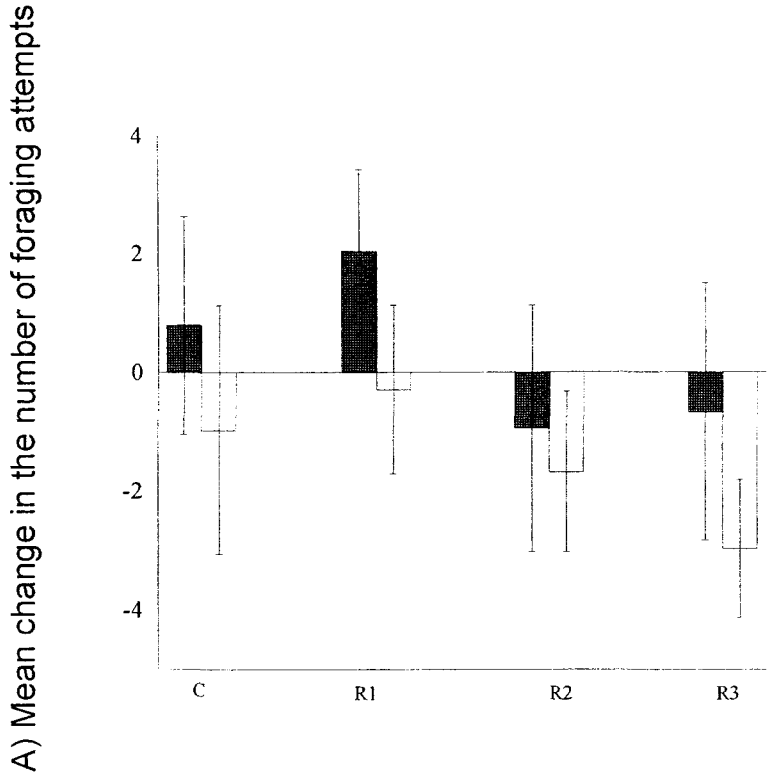

C) Mean change in area use
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
*
C
R1
R2
R3

Figure 3: Mean (±SE) change in antipredator behaviour in A) the number of foraging attempts and B) time spent moving (seconds), of wild juvenile Atlantic salmon in response to alarm cue (grey bars) or stream water control (white bars) treatments at each phase. N=16 for the conditioning (C) and first recognition (R1) phases, N= 14 for the second (R2) and third recognition (R3) phases.


A) Mean change in the number of foraging attempts
4
2
0
-2
-4
C
R1
R2
R3

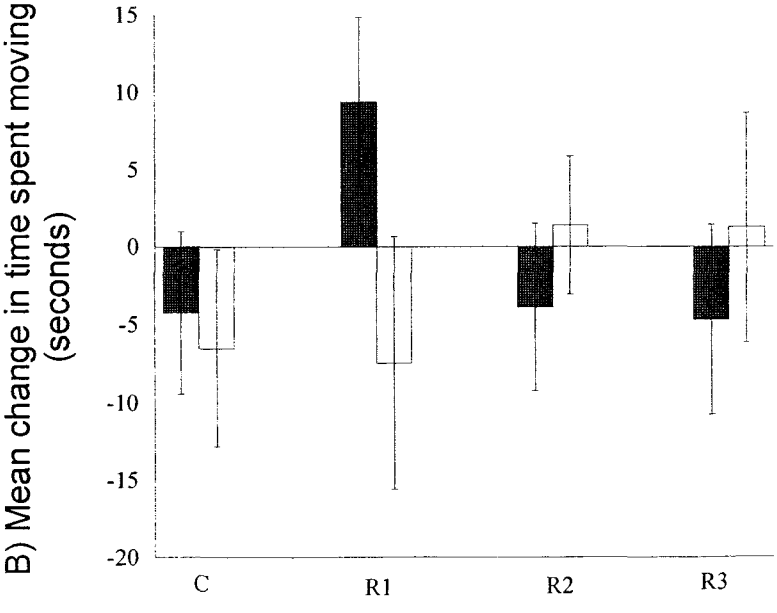

B) Mean change in time spent moving (seconds)
15
10
5
0
-5
-10
-15
-20
C
R1
R2
R3

Figure 4: Mean (± SE) foraging rate (no. attempts/minute), pre- to post-stimulus in response to alarm cue (solid line) and stream water (hatched line) control treatments at the conditioning phase in wild juvenile Atlantic salmon. N= 16.

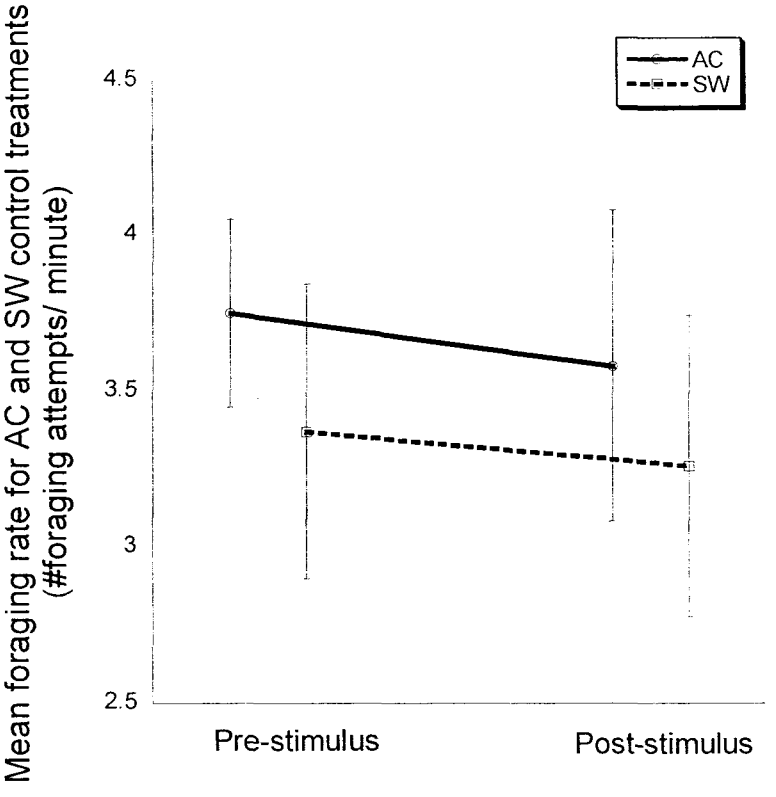

Mean foraging rate for AC and SW control treatments
(#foraging attempts/ minute)
4.5
4
3.5
3
2.5
AC
SW
Pre-stimulus
Post-stimulus

Table 1: Results of the 1-way ANOVAs, assessing the effect of treatment (alarm cue) or stream water (control) at the various phases (C, R1, R2, R3), for overall and specific behavioural measures observed in lab-reared juvenile Atlantic salmon.

Effect	F	Df	p
Foraging attempts			
Diff. Cond.	11.465	1, 41	**0.002**
Diff. R1	0.116	1, 41	0.735
Diff. R2	0.877	1, 37	0.355
Diff. R3	0.141	1, 41	0.709
Time moving			
Diff. Cond.	2.467	1,41	0.124
Diff. R1	0.946	1,37	0.337
Diff. R2	0.227	1,37	0.636
Diff. R3	0.783	1,37	0.382
Area use			
Diff. Cond.	0.612	1, 41	0.438
Diff. R1	5.707	1, 41	**0.022**
Diff. R2	0.002	1, 37	0.962
Diff. R3	0.443	1, 37	0.51
Dashing			
Diff. Cond.	0.214	1, 40	0.646
Diff. R1	0.1	1, 40	0.754
Diff. R2	0.012	1, 36	0.914
Diff. R3	0.001	1, 36	0.971
Aggressive interactions			
Diff. Cond.	0.887	1, 34	0.353
Diff. R1	0.758	1, 35	0.39
Diff. R2	0.258	1, 32	0.615
Diff. R3	0.024	1, 35	0.878

Significance was established when p≤ 0.05.

Table 2: Results of the repeated- measures ANOVA for the laboratory experiment, assessing the effect of each behavioural measure (pre-stimulus to post-stimulus) at all phases (C, R1, R2, R3), between treatments alarm cue and the stream water control.

Effect	F	Df	p
foraging attempts			
phase (C, R1, R2, R3)	0.674	3, 35	0.574
phase * treatment	4.084	3, 35	**0.014**
Conditioning	0.009	1, 41	0.925
Cond. * treatment	16.42	1, 41	**<0.05**
Recogniton 1	0.001	1, 41	0.978
R1 * treatment	1.432	1,41	0.238
Recogniton 2	1.639	1, 37	0.208
R2 * treatment	0.03	1, 37	0.862
Recognition 3	0.141	1,37	0.709
R3 * treatment	0.141	1,37	0.709
time spent moving			
phase (C, R1, R2, R3)	3.862	3, 35	**0.017**
phase * treatment	0.867	3, 35	0.468
Conditioning	0.002	1, 41	0.963
Cond. * treatment	3.996	1, 41	0.052
Recognition 1	0	1, 41	0.994
R1 * treatment	0.118	1, 41	0.733
Recognition 2	8. 134	1, 37	**0.007**
R2 * treatment	0.227	1, 37	0.636
Recognition 3	2.166	1, 37	0.15
R3 * treatment	0.493	1, 37	0.487

Significance was established when $p \leq 0.05$.

Table 2 continued

Effect	F	Df	p
area use			
phase (C, R1, R2, R3)	0.413	3, 35	0.745
phase * treatment	2.045	3, 35	0.125
Conditioning	0	1, 41	0.983
Cond. * treatment	0.886	1, 41	0.352
Recognition 1	0	1, 41	0.986
R1 * treatment	0.576	1, 41	0.452
Recognition 2	0	1, 37	0.986
R2 * treatment	0.462	1, 37	0.501
Recognition 3	0.001	1, 37	0.975
R3 * treatment	1.545	1, 37	0.222
dashing events			
phase (C, R1, R2, R3)	1.204	3, 34	0.323
phase * treatment	0.061	3, 34	0.98
Conditioning	0.002	1, 40	0.969
Cond. * treatment	0.685	1, 40	0.413
Recognition 1	0	1, 40	0.985
R1 * treatment	0.152	1, 40	0.699
Recognition 2	0	1, 36	1
R2 * treatment	0.012	1, 36	0.914
Recognition 3	0	1, 36	1
R3 * treatment	0.001	1, 36	0.971

Significance was established when $p \leq 0.05$.

Table 2 continued

Effect	F	Df	p
aggressive interactions			
phase (C, R1, R2, R3)	0.528	3, 20	0.668
phase * treatment	1.318	3, 20	0.296
Conditioning	0	1, 36	0.984
Cond. * treatment	0.152	1, 36	0.699
Recognition 1	0	1, 35	0.992
R1 * treatment	0.125	1, 35	0.726
Recognition 2	0	1, 33	0.994
R2 * treatment	0.08	1, 33	0.779
Recognition 3	0	1, 35	0.994
R3 * treatment	0.086	1, 35	0.771

Significance was established when $p \leq 0.05$.

Table 3: Results of the repeated-measures MANOVA, assessing the interaction of foraging attempts and time moving behaviours between (treatments alarm cue and stream water), A) at the conditioning phase only, then B) at the conditioning and first recognition phases.

A) factor = foraging * time moving (pre vs. post stimulus at C phase)

Effect	F	Df	p
Factor	1.379	2, 40	0.264
Factor * treatment	5.948	2, 40	**0.005**
Factor (foraging)	2.295	1	0.137
Factor (moving)	0.151	1	0.7
Factor * treat. (foraging)	11.465	1	**0.002**
Factor * treat. (moving)	2.425	1	0.127

Significance was established when p≤ 0.05.

B) factor = change in foraging * change in time moving (C, R1)

Effect	F	Df	p
Factor	2.925	2, 40	0.065
Factor * treatment	3.196	2, 40	0.052
Factor (foraging)	5.73	1	**0.021**
Factor (moving)	0.001	1	0.981
Factor * treat. (foraging)	6.507	1	**0.015**
Factor * treat. (moving)	0.125	1	0.725

Significance was established when p≤ 0.05.

Table 4: Results of the 1-way ANOVAs assessing the effect of treatment (alarm cue) or stream water (control), at the various phases (C, R1, R2, R3) for all behavioural measures observed in wild juvenile Atlantic salmon in 2007 and 2008.

Effect	F	Df	p
Foraging attempts			
Diff. Cond.	0.401	1, 70	0.529
Diff. R1	1.365	1, 60	0.247
Diff. R2	0.086	1, 54	0.771
Diff. R3	0.927	1, 44	0.341
Time moving			
Diff. Cond.	0.19	1, 70	0.664
Diff. R1	0.121	1, 60	0.729
Diff. R2	0.963	1, 54	0.331
Diff. R3	1.05	1, 44	0.311
Area use			
Diff. Cond.	0.357	1, 32	0.554
Diff. R1	1.22	1, 30	0.279
Diff. R2	1.358	1, 24	0.255
Diff. R3	1.426	1, 24	0.244
Time immobile			
Diff. Cond.	0.019	1, 36	0.89
Diff. R1	0.015	1, 27	0.903
Diff. R2	0.525	1, 28	0.344
Diff. R3	0.52	1, 18	0.48

Significance was established when p≤ 0.05.

Table 5: Mean foraging rates (± S.E.) between AC and SW control treatments, for wild juvenile Atlantic salmon (YOY and 1+), for field seasons 2005 to 2008, at Catamaran Brook.

			Foraging rate (#attempts/ minute) ± S.E.		
Year	Age class	Treatment	pre-stimulus	post-stimulus	differences
2005[1]	YOY	AC	3.6 ± 0.70	2.0 ± 0.39	N/A
		SW	3.6 ± 0.91	3.85 ± 1.062	N/A
2005-2006[2]	1+	AC	2.17 ± 0.87	1.62 ± 0.51	N/A
		SW	1.64 ± 0.70	2.20 ± 0.58	N/A
2005-2006[2]	YOY	AC	4.41 ± 1.44	3.72 ± 1.48	N/A
		SW	3.8 ± 1.37	3.11 ± 1.46	N/A
2007[3]	YOY	AC	N/A	N/A	0.28 ± 0.49
		SW	N/A	N/A	-0.056 ± 0.75
2007[4]	YOY	AC	N/A	N/A	0.22 ± 0.46
		SW	N/A	N/A	0.79 ± 0.57
2008[3]	YOY	AC	3.98 ± 0.36	3.43 ± 0.70	-0.55 ± 0.68
		SW	3.13 ± 0.49	3.35 ± 0.61	0.22 ± 0.36
2008[3]	1+	AC	3.53 ± 0.48	3.73 ± 0.75	0.2 ± 0.85
		SW	3.83 ± 1.08	3.07 ± 0.87	-0.77 ± 0.80

1) Leduc et al. (unpublished data); foraging rates of YOY when exposed to the paired AC or SW with NO.
2) Kim et al. (in press); foraging rates of YOY and 1+ when exposed to AC or SW alone.
3) Current data; foraging rates when exposed to the paired AC or SW with NO.
4) Current data; foraging rates when exposed to AC or SW alone.

Table 6: Mean value (± Std. dev.) of the physical variables for Catamaran Brook, between alarm cue and stream water control treatments and between summers 2007 and 2008.

	mean ± Std. dev.	
Variable	AC treatment	
	2007	2008
flow (m/s)	0.35 ± 0.19	0.26 ± 0.13
depth (cm)	27.5 ± 6.83	32.83 ± 12.96
water temperature (C)	16.33 ± 1.10	17.88 ± 1.05
air temperature (C)	19.61 ± 2.27	22.82 ± 1.97
cloud cover (%)	59.9 ± 26.31	40.43 ± 22.78
canopy cover (%)	16.46 ± 18.07	45.12 ± 37.01

Variable	SW treatment	
	2007	2008
flow (m/s)	0.39 ± 0.10	0.27 ± 0.083
depth (cm)	39.86 ± 12.95	28.33 ± 7.98
water temperature (C)	16.59 ± 1.57	17.52 ± 1.31
air temperature (C)	20.38 ± 2.65	21.78 ± 3.03
cloud cover (%)	60.13 ± 26.61	47.74 ± 33.33
canopy cover (%)	22.92 ± 17.68	45.08 ± 18.84
pH	7.26 ± 0.11	

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