

Behavioural response of adult sea lamprey (*Petromyzon marinus*) to predator and conspecific alarm cues: evidence of additive effects

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## **Abstract**

Behavioural response of adult sea lamprey (*Petromyzon marinus*) to predator and conspecific alarm cues: evidence of additive effects

Richard T. Di Rocco

Sea lampreys (*Petromyzon marinus*), an invasive pest in the Upper Great Lakes, avoid odours that represent danger in their habitat. These odours include damage-released alarm cues from conspecifics and predator cues, like 2-phenylethylamine hydrochloride (PEA HCl), a promising predator cue found in the urine of mammalian predators. The objectives of this experimental study were: (1) determine if the avoidance response of sea lamprey to PEA HCl is graded or hypersensitive, (2) determine if the avoidance response to the combination of a predator cue (PEA HCl) and damage-released alarm cue is synergistic or additive. To meet the first objective, groups of ten sea lampreys were placed in an artificial stream channel and presented with stepwise concentrations of PEA HCl ranging from  $5 \times 10^{-8}$  to  $5 \times 10^{-10}$  M and a deionized water control. Sea lampreys exhibited graded avoidance behaviour in response to PEA HCl. To meet the second objective, sea lampreys were exposed to PEA HCl, conspecific damage-released alarm cue, and a combination of the two. Sea lamprey responded to the combination of predator cue and damage-released alarm cue in an additive manner, as the response to the combination of cues did not trigger a significantly greater response than the sum of their separate effects.

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## Introduction

Invasive species are non-indigenous species that arrive in a new habitat and cause severe environmental damage (Mack et al. 2000). Few non-indigenous species are able to naturalize in a new habitat (Jeschke and Strayer 2005) and fewer still have negative impacts for indigenous species (Mack et al. 2000). One of the main goals of invasion ecology is to predict which species have the potential to become invasive but so far, invasion ecology has failed to reliably make these predictions (Williamson 1999). The field of animal behaviour could help predict how invasive species spread and compete in novel environments and aid in control efforts (Holway & Suarez 1999). Recent studies, for example, investigating the behaviour of invasive species have found them to be more flexible (Sol et al. 2002) and more aggressive (Duckworth and Budyayev 2007) than native competitors.

The behavioural interactions between invaders and novel habitats, which may contain new prey, predators, parasites and/or abiotic conditions (Sakai et al. 2001), are a key factor in the success of an invader. It may be possible to exploit the behaviour of an invasive species to manage the population but it is important to consider that the behaviour of the invaders may be different than the population they have dispersed from. In general, there are two ways an invasive species could access a new habitat: 1) random dispersal; by chance, a random group of individuals from one habitat arrive in a new habitat (e.g. zebra mussels (*Dreissena polymorpha*) released with the ballast water of ships) and 2) non-random dispersal; given the opportunity to access new habitat, a non-random group of individuals moves into another habitat. In random dispersal, the success of the invader is largely based on chance because the individuals that are transferred may not be suitable for the new habitat. Therefore, increasing the number of dispersal events, dispersal sources, and number of individuals transferred will increase the likelihood of



the non-indigenous species becoming established in a new habitat (Saikai et al. 2001). In non-random dispersal, the individuals may differ phenotypically (e.g. naked mole-rat, *Heterocephalus glaber*, O’Riain et al. 1996), genetically (e.g. pygmy grasshopper, *Tetrix subulata*, Berggren et al. 2012) or behaviourally (e.g. Trinidadian killifish, *Anablepsoides hartii*, Fraser et al. 2001) from individuals in their source population. This difference between dispersers and their source population partially explains why they sometimes become invasive. For example, dispersal is coupled with ecological relevant personality (i.e. risk-taking) traits, such as boldness, aggressiveness and high activity (Sih et al. 2004). These correlated traits exhibited at the population or species level are referred to as behavioural syndromes (Sih et al. 2004). The behavioural syndromes that are more likely to lead to dispersal are also more likely to negatively impact invaded communities (Sih et al. 2004). Since colonization of a new habitat can result in a population bottleneck (Sakai et al. 2001), there is often less genetic diversity in new populations as compared to the same species in an older population (Barrett & Kohn, 1991). So, invasive species may have fewer behavioural types than the indigenous populations they are competing with, potentially amplifying the negative impacts of invasive species.

### ***Risk assessment in aquatic habitats***

Aquatic vertebrate and invertebrate species rely on a variety of sensory inputs to assess local predation threats. In particular, prey fishes commonly utilize a combination of visual, chemical, auditory, electric and tactile information as sources of risk assessment information (Emde et al. 2004). Fishes will exhibit antipredator behaviour, such as crypsis, area avoidance and (or) movement to shelter in response to information indicating a predator or predation event nearby (Smith 1992). Aquatic environments are abundant in chemical information, which describe surrounding biological interactions (Wisenden and Chivers 2006). This chemical

information is publicly available (Wisenden and Chivers 2006), persists in aquatic environments longer than other sources of information (Wisenden 2003), is low risk to acquire (Brown 2003), and reliably indicates nearby predators (Kats and Dill 1998). As a result, chemical information is the dominant form of communication in aquatic environments (Ferrari et al. 2010). Chemical information can originate from a variety of sources but the first discovered was conspecific tissue extract by von Frisch in 1938 (Magurran et al. 1996). These conspecific tissue extracts, or damage-released alarm cues, are released into the environment when a predator injures prey. The strong selection pressure to detect cues that indicate local predation threats has led to virtually all aquatic animals responding independent of previous experience to conspecific alarm cue with antipredator behaviour (Wisenden and Chivers 2006). Another type of chemosensory cue are predator cues or kairomones, the natural odours released by predators (Wisenden and Chivers 2006). These predator cues are arguably the most widespread chemosensory cue in predator-prey interactions (Wisenden 2003) as they are available even if no recent predation events have occurred. Prey have evolved to detect a predator prior to a predation event by recognizing the natural odours released by predators innately (Dixson et al 2010). A third type of chemosensory cue are disturbance cues. These cues are released by prey when disturbed (not injured), which warns conspecifics of nearby danger (Chivers et al. 2012).

Although the antipredator response to chemosensory cues will reduce the risk of an individual being preyed upon, the behaviour also consumes time and energy (Brown et al. 2011) and may cause non-consumptive effects on prey such as reduced foraging, lower mating success or emigration (Preisser et al. 2005). The threat-sensitive predator avoidance hypothesis (Helfman 1989) expects prey to match the degree of antipredator behaviour to the level of risk detected. A common criticism of risk-assessment research is that much of the experiments occur in the

laboratory (Magurran et al. 1996) where there is relatively little sensory information available. Recent research has investigated the effect of multiple cues and has mostly focused on multiple modalities of cues, such as a visual cue paired with a chemical cue (Brown and Magnavacca 2003, McCormick and Manassa 2008, Ward and Mehner 2010, Holmes and McCormick 2011). Notably, Mikheev et al. (2006) found that a predator odour alone did not affect the feeding rate of European perch (*Perca fluviatilis*) but a combination of predator odour with a visual predator cue caused a significantly stronger effect than the visual cue alone. To our knowledge, only Ferrari et al. (2008) has paired two odours, a disturbance cue with a damage-released alarm cue, to determine how prey would interpret the combinative effect of these supporting cues and found the combination to have an additive effect. In a scenario where only a damage-released alarm cue is present, no information about a predator has been transmitted. If only a predator cue is present, a potential predator is in the area but may not be consuming conspecifics of the detector for a variety of potential reasons, such as gap limitation or state of satiation. The combination of predator cue and alarm cue can have a much larger impact on antipredator defences than either cue alone (Schoeppner and Relyea 2005) as the increase in chemical information reduces uncertainty about the environment. In some species of snail, for example, both a predator cue and damage-released alarm cues are required to cause any avoidance behaviour (Alexander and Covich 1991, Jacobsen and Stabell 2004).

### ***Sea lamprey as an invasive pest***

In Canada, costs associated with invasive species are estimated to be between \$13.3 and 34.5 billion CDN per year (Colautii et al. 2004). Approximately \$22 million is spent on management and research to control sea lampreys (Colautii et al. 2004), an invasive species in the Great Lakes Basin (Smith and Tibbles 1980). It is assumed that sea lampreys, indigenous to

Lake Ontario (Waldman et al. 2004, Bryan et al. 2005), were able to bypass the natural barrier of the Niagara Escarpment using the Welland Canal (Daniels 2001). A sea lamprey was found in Lake Erie in 1921 (Dymond 1922) and eventually spread throughout the Upper Great Lakes. Historically, sea lampreys in Lake Ontario were referred to as lake lamprey (*Petromyzon marinus unicolor*; Dymond 1922). These ‘lake lamprey’, which occupy freshwater habitats their entire life, differ phenotypically from the marine sea lamprey as the adults are approximately half the size (Applegate 1950). In addition, the invasive sea lampreys of Lake Superior have a reduced number of haplotypes when compared with the Lake Ontario population indicating there was a genetic bottleneck caused by dispersal through the Welland Canal (Waldman et al. 2004). As a result, the invasive sea lamprey population of the Upper Great Lakes is genetically, phenotypically and potentially behaviourally unique. The population bottlenecks that occurred as a result of the non-random dispersal of this invasive species may have resulted in a population with fewer behavioural types. This possible lack of behavioural diversity in sea lampreys makes them suitable for exploitation.

The Great Lakes Fishery Commission (GLFC), a binational group charged with eradicating or minimizing sea lamprey populations in the Great Lakes, relies heavily on lampricide treatments to minimize sea lamprey populations (Brege et al. 2003). In addition to lampricide, the GLFC uses physical barriers to deny migratory-phase sea lampreys access to spawning habitat but these barriers are controversial because they may impede non-target fishes (McLaughlin et al. 2007). The GLFC has actively been searching for alternatives to lampricides to reduce the cost of the sea lamprey control program (Great Lakes Fishery Commission 2011). One strategy the GLFC has committed to research is the use of repellent-based control methods. Repellents could be used to create a virtual barrier, which would restrict access to spawning

grounds during the spring migration (Imre et al. 2010) while ideally allowing passage for non-target species.

### ***Chemosensory cues as a repellent***

A novel method proposed to control invasive sea lamprey suggested the use of chemosensory cues to act as a repellent to manipulate the movements of migratory-phase sea lampreys (Sorensen and Hanson 1993, Imre et al. 2010). Chemosensory cues are particularly useful for nocturnal animals (Kats and Dill 1998), like sea lamprey. Recent studies have shown that sea lampreys avoid areas containing chemosensory cues that indicate danger in their habitat when in darkness (Wagner et al. 2011, Bals and Wagner 2012, Imre et al. 2014) and even when visual information is available during the day (Di Rocco et al. 2014). A new management plan could pair these chemosensory cues with reproductive pheromones, which attract sea lampreys (Johnson et al. 2013), to implement a push-pull pest management strategy similar to those used to control some insect species (see Cook et al. 2007). Sea lampreys could be ‘pushed’ away from suitable spawning habitat by placing a virtual barrier of chemosensory cues and ‘pulled’ to an area containing unsuitable spawning habitat, traps, or future lampricide treatments using reproductive pheromones.

In semi-natural laboratory conditions, adult sea lampreys avoid the damage-released alarm cues of adult conspecifics (Wagner et al. 2011, Bals and Wagner 2012, Imre et al. 2014) and sea lamprey ammocoetes (Pietrzakowski et al. 2013). Sea lampreys also avoid the predator cues 2-phenylethylamine (PEA), a compound found in mammalian carnivore urine (Ferrero et al. 2011), and human saliva (surrogate for saliva of a mammalian predator) (Imre et al. 2014, Di Rocco et al. 2014). PEA is a chemosensory cue avoided by rats and mice (Ferrero et al. 2011)

and the stable hydrochloride salt (PEA HCl) shows promise as a potential control method for invasive sea lampreys as it is a readily available pure substance.

The synergistic effect of multiple cues demonstrated in Mikheev et al. (2006) could reduce the cost of implementing chemosensory cues into a management strategy to control invasive sea lamprey. Unfortunately, sea lampreys are primarily nocturnal during their migration and do not rely on their sense of vision (Binder and McDonald, 2007), making visual cues ineffective for this purpose. Sea lampreys avoid two types of chemosensory cues, predator cues and alarm cues, alone and in combination (Imre et al. 2014). In this study, we explored the effects of combining a predator cue with an alarm cue. Sea lampreys must avoid cues at an intensity related to the level of threat detected. This graded response (Brown et al. 2009) is required for comparison of cues. If sea lampreys are hypersensitive, meaning they exhibit a non-graded response (Helfman and Winkelman 1997), to one of the cues, it would be impossible to determine how the combination of cues function. Bals and Wagner (2012) found a correlation between mass of extracted tissue and sea lamprey avoidance indicating that their response to damage-released alarm cue is graded but whether the response to PEA HCl is graded or hypersensitive is currently unknown.

The goal of this study was to determine if the combination of predator cues and damage-released alarm cues function synergistically or additively in sea lampreys. To accomplish this, we first conducted an experiment to determine if sea lampreys avoid a predator cue, PEA HCl, in a graded manner. In a second experiment, we exposed sea lamprey to PEA HCl, sea lamprey tissue extract and their combination ( $\frac{1}{2}$  PEA HCl +  $\frac{1}{2}$  sea lamprey tissue extract). If the combination of cues induces a significantly stronger response than both cues individually, the combination will function synergistically. Otherwise, the combination will function in an

additive manner, as the sum of the combined effect is not greater than the individual effects. We hypothesized sea lampreys will avoid PEA HCl in a graded manner, as predicted by the threat-sensitivity hypothesis. We also hypothesized the response to a combination of damage-released alarm cues and predator cues will synergize.

## **Materials and methods**

### ***Experimental subjects***

The sea lampreys used in this experiment were captured from the St. Marys River by Fisheries and Oceans Canada in July 2014 and transported to the Hammond Bay Biological Station in Millersburg, MI. The lampreys were separated by sex and held in 1000 L flow-through tanks, which received 180 L of Lake Huron water per hour. To maintain the natural photoperiod of the subjects, the holding tanks were equipped with artificial lights on a timer set to a 15L:9D photoperiod with the lights turning off at 21:00 h. During the PEA HCl concentration gradient experiment, water temperature in the holding tanks was (mean  $\pm$  SD)  $12.0 \pm 2.3$  °C, the mean wet mass of subjects was  $237 \pm 48$  g and their mean total length was  $484 \pm 33$  mm. During the synergy experiment, the water temperature in the holding tanks was  $11.9 \pm 0.9$  °C, the mean wet mass of subjects was  $230 \pm 45$  g and the mean total length was  $475 \pm 33$  mm. Sexual maturity was determined by applying gentle pressure to the abdomen. If an individual released sperm or eggs, they were classified as sexually mature (Siefkes et al., 2003). The weight, length and sexual maturity were measured after observations to reduce handling stress. Individuals were only exposed to a chemosensory cue once before being passed on for scientific communication purposes or further research.

## ***Stimulus preparation***

To determine if the response to 2-phenylethylamine hydrochloride is graded or hypersensitive, concentration levels of PEA HCl were selected in a stepwise manner based on preliminary experiments. Three levels of decreasing magnitude were selected at  $5 \times 10^{-8}$ ,  $5 \times 10^{-9}$ , and  $5 \times 10^{-10}$  M. An intermediate step was added between the two lowest concentrations at  $3 \times 10^{-9}$  M. These molar concentrations represent the in-stream concentration and were achieved by adding PEA HCl to the stream channel (for specific amounts, see table 1) via a 400 mL delivery solution.

The sea lamprey tissue needed for tissue extract preparation for the synergy experiment was obtained from 10 donor animals. Use of experimental and tissue donor subjects was approved by the Algoma University Animal Care Committee (AUP No. 2014-II-001). Tissue extract was prepared using a modified procedure originally described by Di Rocco et al. (2014) and Imre et al. (2014). Donors were killed with a swift blow to the head before being decapitated. The remaining body was separated lengthwise into five approximately equal segments and 8 g of tissue cross sections were removed from one of the segments. This step was repeated for all ten donors (males: N = 5, mean total length =  $455 \pm 17$  mm, mean wet mass =  $194 \pm 26$  g, proportion sexually mature = 0%; females: N = 5, mean total length =  $478 \pm 37$  mm, mean wet mass =  $241 \pm 72$  g, proportion sexually mature = 0%) with tissue being removed once from all five segments for both sexes. The 80 g of tissue was homogenized in 750 mL of deionized water before being filtered through 100% cotton cheese cloth. Additional deionized water was added to the filtrate to bring the final volume to 1 L. The filtrate was stored at  $-10^\circ\text{C}$  in 50 ml aliquots until further use. Sea lamprey extract (20 mL or 10 mL depending on treatment, table 1) was further diluted to 400 mL to create the delivery solution.



The concentrations of chemosensory cue used in the second experiment were selected to correspond with intermediate levels of threat. For PEA HCl,  $3 \times 10^{-9}$  M resulted in ~10% of sea lampreys avoiding the scented side of the artificial stream channel. Based on previous research (Imre et al. 2014) 20 mL of sea lamprey whole body extract (corresponding to approximately 1.6 g of tissue) delivered over 20 minutes caused an avoidance response of similar intensity. For the combination of cues, half of each cue was delivered (table 1).

### ***Experimental protocol***

To measure the behavioural response of sea lampreys to these chemosensory cues, experiments in two artificial stream channels were conducted at the Hammond Bay Biological Station in Millersburg, MI. For both experiments, ten groups of ten sea lampreys (five groups of males and five groups of females) were exposed to one of the treatments. Ten replicates were run each night with every treatment being delivered at least twice per night. This was to reduce potential differences in water temperature between treatments. The PEA HCl concentration experiment was conducted from July 16 to July 22, 2014 and the synergy experiment was conducted from July 31 to August 4, 2014. The artificial stream channels were parallel and had a water depth of 20 cm and a discharge of  $0.01 \text{ m}^3/\text{s}$ . The groups of sea lampreys were confined to baskets at the head of the artificial stream channel for one hour to acclimate. After the acclimation period, the basket was gently slid down the artificial stream and overturned in the middle of the experimental arena. The experimental arena (figure 1) was a  $6.23 \times 1.84$  m area with removable gates. The group of lamprey were given 30 minutes to acclimate to the arena before video cameras (model: Axis Q1604 Network Camera) directly above the stream channels began recording. Each recording was 30 minutes in length and split into three, 10 minute observation periods: the pre-stimulus period, the transition period, and the stimulus period. At

the beginning of the transition period, peristaltic pumps (model: MasterFlex L/S 7533-20) at the head of the experimental arena were turned on to supply the 400 mL delivery solution at 20 mL/min to half of the stream channel. The final 10 minutes of the recording was the ‘stimulus period’, when avoidance behaviour to the stimulus being presented was observed. Afterwards, the lampreys were removed from the arena to have their wet mass (to the nearest g), total length (to the nearest mm) sexual maturity (mature or immature) recorded while the next group of lamprey (which had been acclimating upstream for the previous hour) were released to acclimate in the experimental arena. All observations occurred between 21:00 h and 05:00 h. The experimental schedules were designed to ensure that groups of males and females were exposed to all treatments each day and they encountered treatments on both the left and right side of the artificial stream channel.

### ***Data collection and statistical analysis***

During the pre-stimulus and stimulus observation periods, the proportion of sea lampreys on the scented side of the stream channel was recorded every 30 seconds, resulting in 20 observations per observation period. The mean of these 20 observations was calculated for each replicate, resulting in the mean proportion on the scented side during the pre-stimulus and stimulus observation period. A line on the bottom of the stream channel (figure 1) was used to determine if individual sea lamprey were on the scented or unscented side. A sea lamprey was considered to be on the scented side if the head had crossed the line into the scented half of the stream channel. An ANCOVA was used to analyze the PEA HCl concentration experiment. The dependent variable was the proportion of sea lampreys on the scented side, sex was a fixed factor, and the  $\log_{10}$  transformed molar concentration of PEA HCl was included as a covariate. If there was no significant sex effect, the analysis was repeated as a linear regression. To include

the control treatment in the analysis, the linear regression was repeated with  $\log_{10}(\text{molar concentration of PEA} + A)$  as the independent variable where A was a constant selected to match the slope of the regression without the control included. The synergy experiment was analyzed using a three-way ANOVA with proportion of sea lampreys on the scented side as the dependent variable and sex, sea lamprey extract, and PEA HCl as fixed factors. Each fixed factor had two levels (male/female for sex and present/absent for PEA HCl and sea lamprey extract). Interactions were excluded if they were not significant with the exception of PEA HCl  $\times$  sea lamprey extract interaction.

For both experiments, a two-way ANOVA with sex and treatment as fixed factors was performed on the pre-stimulus observation period, to ensure there was no side preference prior to treatments being delivered. All proportion data was normally distributed (Shapiro-Wilk,  $P > 0.05$ ). One-way ANOVAs were used to verify that there was no difference in length, weight, sexual maturity or water temperature between treatments. All statistical analyses were performed using SPSS Statistics 21.0 (IBM Corp. 2012). The significance level was set at  $\alpha = 0.05$ .

## Results

### ***PEA HCl concentration experiment:***

Our experiment demonstrated that sea lampreys avoid the scented half of the stream channel in a graded manner as the molar concentration of PEA HCl increased (linear regression:  $\log_{10}[\text{molar concentration of PEA HCl}] \times \text{proportion on scented side}$ ,  $P < 0.001$ ,  $R^2 = 0.30$ ,  $y = -0.083 \log_{10}x - 0.333$ ). Including the control in the analysis by adding a constant to all values was also significant (linear regression:  $\log_{10}[\text{molar concentration of PEA HCl} + 1 \times 10^{-10}] \times$

proportion on scented side,  $P < 0.001$ ,  $R^2 = 0.40$ ,  $y = -0.080 \log_{10} [x + 1 \times 10^{-10}] - 0.312$ ) (figure 2).

There was no difference in the proportion of sea lampreys on the scented side during the pre-stimulus period (two-way ANOVA: treatment  $F_{4,40} = 0.691$ ,  $P = 0.60$ , sex  $F_{1,40} = 0.293$ ,  $P = 0.59$ , treatment  $\times$  sex  $F_{4,40} = 1.444$ ,  $P = 0.238$ ). There was no significant difference in total length (ANOVA:  $F_{4,45} = 0.397$ ,  $P = 0.81$ ), wet mass (ANOVA:  $F_{4,45} = 0.582$ ,  $P = 0.68$ ), proportion sexually mature (ANOVA:  $F_{4,45} = 2.550$ ,  $P = 0.052$ ) or water temperature (ANOVA:  $F_{4,45} = 0.349$ ,  $P = 0.84$ ) between treatments. None of the female sea lampreys used in this experiment were sexually mature and 1.6% of males were spermiating.

### ***Synergy experiment:***

The sea lamprey extract caused significant avoidance of the scented side of the stream channel but sex, PEA HCl and the PEA HCl  $\times$  sea lamprey extract interaction had no significant effect (three-way ANOVA: sea lamprey extract  $F_{1,35} = 14.543$ ,  $P = 0.001$ , sex  $F_{1,35} = 0.129$ ,  $P = 0.72$ , PEA HCl  $F_{1,35} = 2.703$ ,  $P = 0.11$ , PEA HCl  $\times$  sea lamprey extract  $F_{1,35} = 0.351$ ,  $P = 0.56$ ). Since the PEA HCl  $\times$  sea lamprey extract interaction was not statistically significant, the synergy hypothesis is rejected as the cues appear to function additively (figure 3).

There was no difference in the proportion of sea lampreys on the scented side during the pre-stimulus period (two-way ANOVA: treatment  $F_{3,32} = 0.449$ ,  $P = 0.72$ , sex  $F_{1,32} = 0.124$ ,  $P = 0.73$ , treatment  $\times$  sex  $F_{3,32} = 0.529$ ,  $P = 0.67$ ). There was no significant difference in total length (ANOVA:  $F_{3,36} = 2.114$ ,  $P = 0.12$ ), wet mass (ANOVA:  $F_{3,36} = 0.295$ ,  $P = 0.83$ ), proportion sexually mature (ANOVA:  $F_{3,36} = 0.127$ ,  $P = 0.94$ ) or water temperature (ANOVA:  $F_{3,36} = 0.330$ ,  $P = 0.80$ ) between treatments. None of the female sea lamprey used in this experiment were ovulating but 33.5% of males were spermiating.

## Discussion

This is the first study to investigate the combined effects of multiple chemosensory cues on the avoidance behaviour of sea lampreys. Consistent with the threat-sensitivity hypothesis, where the overall intensity of antipredator behaviour is proportional to the level of threat detected, sea lampreys responded to increased concentrations of PEA HCl with increased area avoidance. By presenting sea lampreys with a combination of sea lamprey extract and PEA HCl, we found that the mixture did not induce an avoidance response significantly stronger than either cue alone. This finding does not support our prediction of a synergistic effect as the response to the combination of cues functions in an additive manner.

This study demonstrates sea lamprey avoid predator cues at a level proportional to the perceived risk detected as the strongest concentration of PEA HCl resulted in the strongest response and the weakest concentration of PEA HCl resulted in the weakest response. Previous work has shown sea lamprey also avoid conspecific extract in this manner (Bals and Wagner 2012). The graded response established in sea lamprey is parallel to the graded response of fathead minnows (*Pimephales promelas*) to predator cues (Kusch et al. 2004) and conspecific extract (Ferrari et al. 2005). The response of sea lampreys to mammalian predators in the wild can be predicted by comparing the amount of PEA released in urine to the response found in this experiment. Raccoons (*Procyon lotor*), a sea lamprey predator (Scott and Crossman 1998, Kircheis 2004) with relatively high concentrations of PEA in their urine (Ferrero et al. 2011), urinate approximately 28 mL per day (Lotze and Fleischman 1978). Raccoon urine contains 51.1  $\mu\text{M}$  of PEA (Ferrero et al. 2011), so a raccoon could release approximately 173  $\mu\text{g}$  of PEA in their urine at a given time. This is much less than the 2750  $\mu\text{g}$  of PEA HCl that was released over 20 minutes to achieve  $3 \times 10^{-9}$  M concentration and cause  $\sim 10\%$  of sea lampreys to avoid the

scented side but 173.4  $\mu\text{g}$  would provide enough PEA to elicit a comparable response in 477 L of water. A sea lamprey might detect and avoid the amount of PEA released in the urine of a raccoon but it would be a localized area over a relatively short period of time. It is also important to consider PEA is only one compound in urine. Female sea lampreys are attracted to  $7\alpha$ ,  $12\alpha$ , 24-trihydroxy- $5\alpha$ -cholan-3-one-24-sulfate (3kPZS), a pheromone released by spermiating male lamprey that directs females towards nests (Li et al. 2002), but actual washings from spermiating males were more effective at retaining females at nests (Johnson et al. 2009). This finding suggests that 3kPZS is only one of multiple compounds found in spermiating male washings used by sea lampreys to communicate chemically. As PEA is only one of a plethora of compounds released in the urine of mammalian predators, actual raccoon urine might cause a greater response than the PEA component alone.

Knowing that sea lamprey respond to PEA HCl at  $3 \times 10^{-9}$  M concentration allows for the comparison to known sea lamprey pheromones. Female sea lampreys are able to respond to species-specific 3kPZS at concentrations as low as  $1 \times 10^{-14}$  (Johnson et al. 2009). Migratory-phase sea lampreys can detect migratory pheromones released by larval lamprey at  $\sim 10^{-12}$  M (Li et al. 1995). Both types of pheromones are bile acids so it is not surprising that sea lampreys respond to these species-specific pheromones at low concentrations because the olfactory system of migratory-phase sea lamprey is acutely sensitive to these bile acids (Li et al. 1995). However, a study investigating the chemosensory receptors of sea lampreys found 28 intact single-axon trace amine-associate receptors (Libants et al. 2009). Therefore, it is possible sea lampreys are detecting the PEA HCl, an amine, at concentrations lower than  $3 \times 10^{-9}$  M but are not responding because the lower concentrations are below the minimum behavioural response threshold. Previous studies have shown that fish can detect concentrations of alarm cue even though they

do not overtly respond them (Brown et al. 1996, Brown et al. 2004). To verify this is the case with sea lamprey in response to PEA HCl, a learning experiment similar to Brown et al. (2001) could be conducted where concentrations of PEA HCl below the response threshold are paired with conspecific tissue extract. Later, the same lampreys would be dosed with only the low concentrations of PEA HCl to see if they learn to associate the PEA HCl with a predation event and avoid the area.

The second half of this study investigated the effect of combining PEA HCl with damage-released alarm cues. Since the interaction between sea lamprey extract and PEA HCl was not significant, we must conclude that the effect is additive and not synergistic. The sensory compensation hypothesis (Lima and Steury 2005) expects prey to use multiple sensory modes to detect and respond to prey. By presenting two complementary chemosensory cues of the same sensory modality, the overall uncertainty about the risk of predation in the environment is reduced. Sea lampreys exposed to both cues would make antipredator decisions knowing that a predator is in the area and a conspecific has been injured or killed. Non-consumptive effects of predation, such as area avoidance, are costly and could reduce the chance of reproducing. A synergistic response would indicate an over-reaction to the available chemosensory information at this critical migratory-phase of the sea lamprey life cycle but sea lampreys appear unwilling to expend time and energy avoiding predation at a level greater than they are detecting.

Although there does not appear to be an advantage in pairing these two chemosensory cues to deter migratory sea lampreys, they may still be useful for management purposes. Future research should investigate behavioural habituation to these cues. If sea lampreys stop responding to one cue after a certain amount of time, a second cue could be presented that will reinvigorate the avoidance behaviour. There is also the opportunity to re-evaluate chemosensory cue

synergy/additivity with sea lampreys during the day. During the day, sea lampreys are less sensitive to chemosensory cues (Di Rocco et al. 2014) but it might be possible to boost the effectiveness of these cues with passive visual cues.

## **Future work**

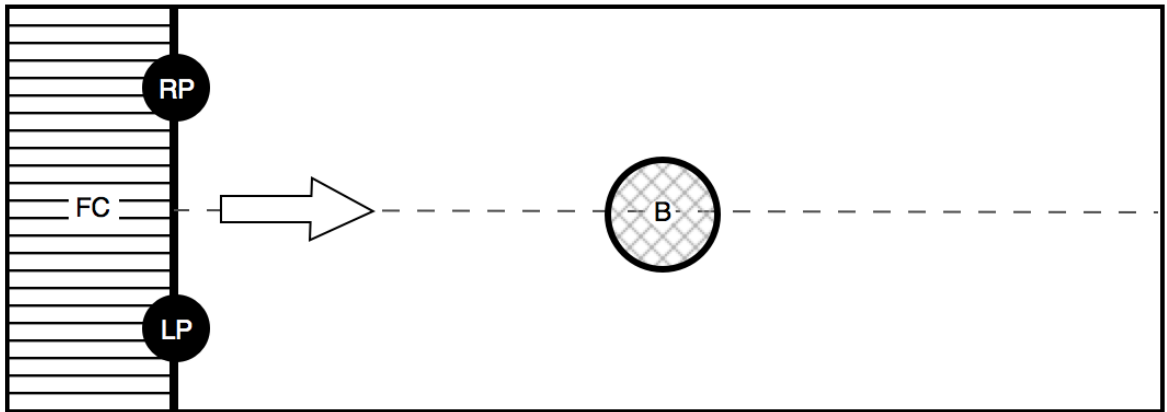
To determine if chemosensory cues can prevent adult sea lampreys from migrating into spawning habitat in the wild, we plan on conducting a field experiment during the 2015 sea lamprey migration season. Passive integrated transponder (PIT) antenna arrays will be placed in the Ocqueoc River (Presque Isle County, Michigan) near the Silver Creek confluence (figure 4). Groups of 40 PIT tagged, female, migratory-phase sea lampreys will be released 500 m downstream of the conflux. Four different treatments will be applied to the Ocqueoc River and Silver Creek conflux (figure 1): 1) no chemosensory cues (control), 2) chemosensory cue released in the Ocqueoc River, 3) chemosensory cue released in Silver Creek, and 4) chemosensory cue released in both Ocqueoc River and Silver Creek. For each treatment, five groups of 40 sea lampreys will be released (200 individuals per treatment). The PIT tagged sea lampreys will be released at 2100 h, because sea lampreys are primarily nocturnal during their upstream migration (Applegate 1951), and monitored using the PIT tag antenna array until 0600h.

To determine the effectiveness of our treatments, we will compare the upstream movements of sea lampreys into Ocqueoc River and Silver Creek during the control (treatment 1) to the movements during treatments 2, 3 and 4. We hypothesize that sea lampreys will migrate up the stream containing the lowest perceived risk of predation (Silver Creek during treatment 2 and Ocqueoc River during treatment 3). Treatment 4 is unlikely to stop all upstream migration because these migratory sea lampreys will die whether they make it to suitable spawning grounds

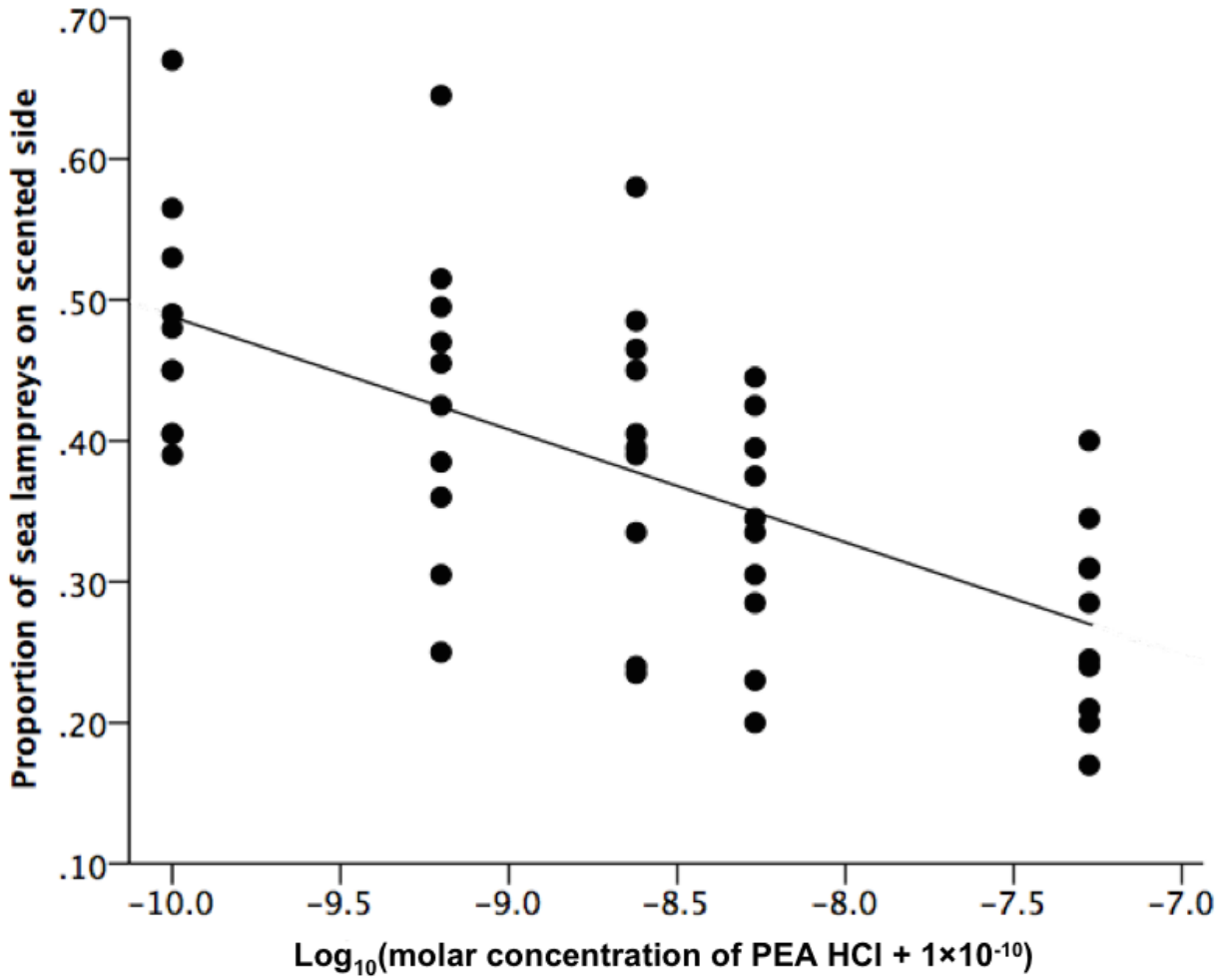


or not. Therefore, they are likely to risk predation for the opportunity to reproduce. This treatment remains in the proposed line-up because if the treatment of both tributaries were able to stop all upstream migrants, it would be a tremendous boon to the integrated lamprey control strategy. Previous experiments have measured the response of groups of sea lampreys to chemosensory cues but with PIT tags, the proportion of adult sea lampreys that respond to or ignore relevant cues during their migration can be quantified.

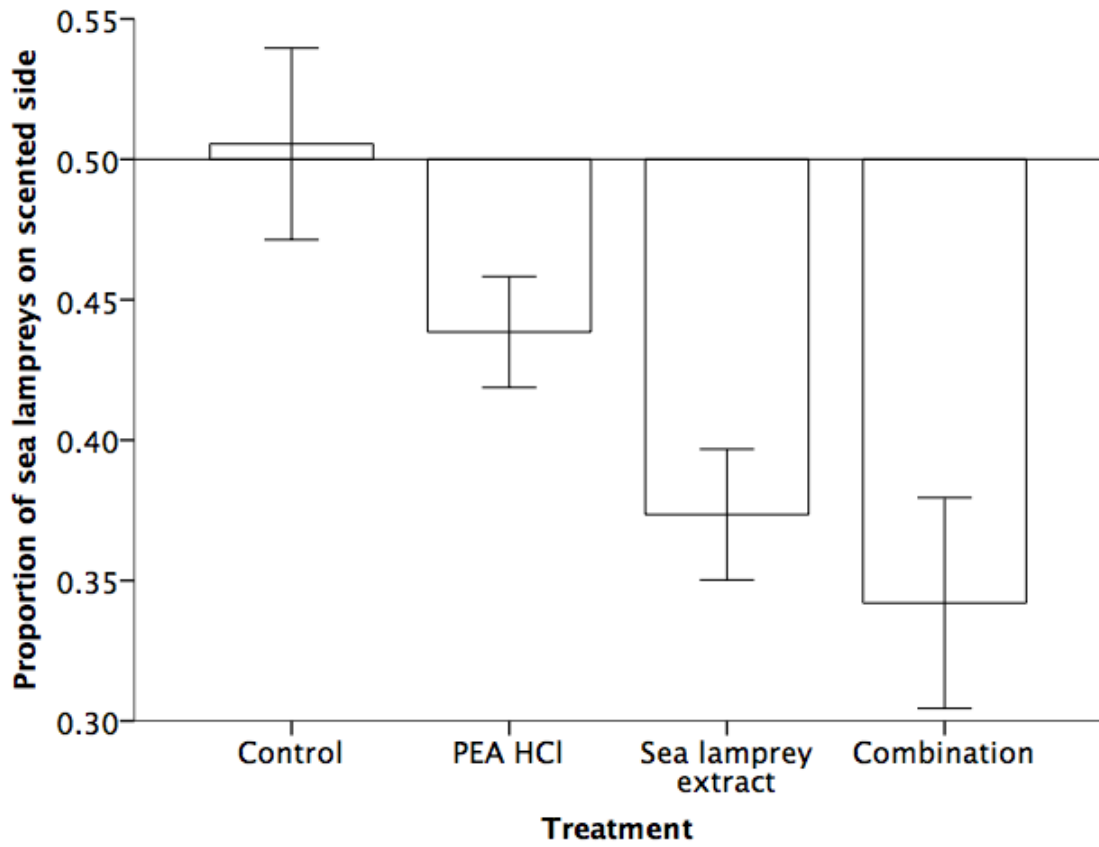
**Figure 1.** View from above a laboratory stream channel. The arrow indicates the direction of flow. The water passed through a flow conditioner (FC) made of PVC pipes to eliminate eddies. Experimental subjects were released from a basket in the center of the stream channel (B) at the beginning of the 30 min acclimation period. Stimuli were dispensed from pumps on the right (RP) or left (LP). Dashed line separates the sides used for data collection.



**Figure 2.** The proportion of sea lampreys on the scented side of the laboratory channel during the PEA HCl concentration experiment. The relationship was fit with a linear regression:  $y = -0.080 \log_{10}(x + 1 \times 10^{-10}) - 0.312$  ( $n = 50$ ,  $R^2 = 0.40$ ,  $P < 0.001$ )



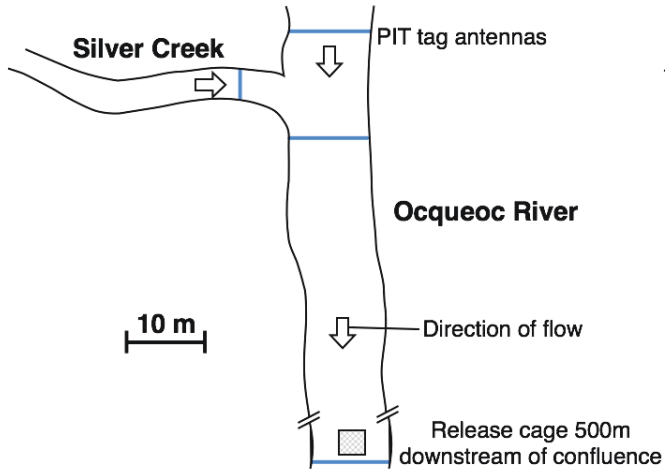
**Figure 3.** The mean ( $\pm 1$  standard error) proportion of sea lampreys on the scented side of the laboratory channel during the synergy experiment.



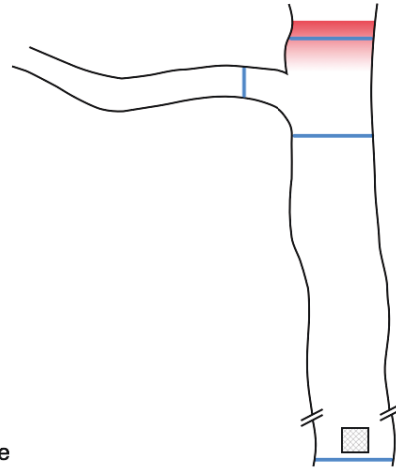
**Figure 4.** Proposed field test to determine if chemosensory cues can block adult sea lampreys from entering spawning streams. **1.** Deionized water applied in both streams; sea lamprey movement into streams expected to be proportional to discharge. **2.** Chemosensory cue applied to Ocqueoc River; all lampreys expected to migrate up the Silver Creek. **3.** Chemosensory cue applied to Silver Creek; all lampreys expected to enter Ocqueoc River. **4.** Chemosensory cue applied to Silver Creek and Ocqueoc River; all lampreys expected to not pass the confluence of Silver Creek and Ocqueoc River.



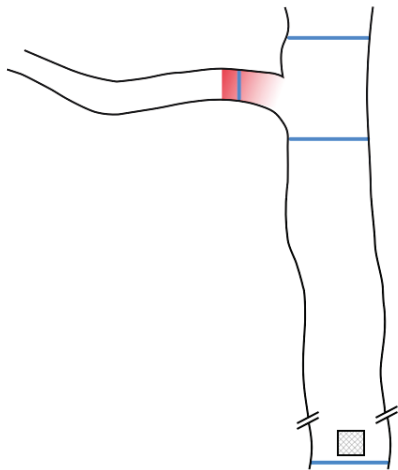
**1. No alarm cue (control)**



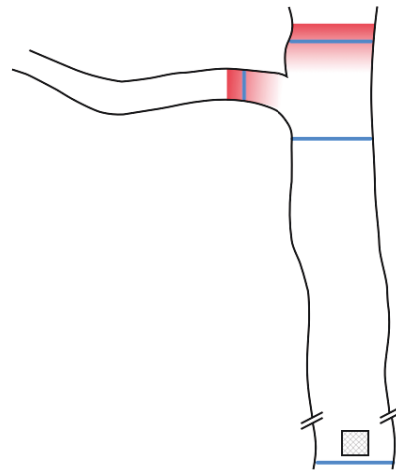
**2. Chemosensory cue applied to Ocqueoc River**



**3. Chemosensory cue applied to Silver Creek**



**4. Chemosensory cue applied to both rivers**



**Table 1.** Amount of chemosensory cue used in each treatment. The concentration was calculated based on 6000L of water passing through the scented half of the stream channel over the 20 minute transition and stimulus period.

Treatment	Amount	Concentration
PEA HCl $5 \times 10^{-8}$	50.0 mg	$5.29 \times 10^{-8}$ M
PEA HCl $5 \times 10^{-9}$	5.00 mg	$5.29 \times 10^{-9}$ M
PEA HCl $3 \times 10^{-9}$	2.75 mg	$2.91 \times 10^{-9}$ M
PEA HCl $5 \times 10^{-10}$	0.500 mg	$5.29 \times 10^{-10}$ M
Sea lamprey extract (SLE)	20 ml	3.3 PPM
Combination	PEA HCl	1.38 mg
	SLE	10 ml
		1.45 $\times 10^{-9}$ M
		1.7 PPM

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