*Effect of Potential Sea Lamprey (*Petromyzon marinus*) Repellents on the Distribution of Juvenile Rainbow Trout (*Oncorhynchus mykiss*) in a Laboratory Environment*

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Abstract

Recent studies have begun to consider the use of chemosensory alarm cues as potential repellents of the invasive sea lamprey (*Petromyzon marinus*) in the Great Lakes Basin. An important factor in determining the efficacy of potential *P. marinus* repellents is whether they are species-specific. Using laboratory stream channels, this study investigated whether a non-target species, the rainbow trout (*Oncorhynchus mykiss*), would change their distribution in response to damage-released *P. marinus* alarm cues, potential mammalian predator cues, and damage-released conspecific cues. In groups of 10 individuals per replicate, with 10 replicates per stimulus type, subjects were exposed to one of the following treatment types: deionized water (control), *P. marinus* extract (heterospecific alarm cue), *O. mykiss* extract (conspecific alarm cue), 2-phenylethylamine hydrochloride (potential predator cue), and human saliva (potential predator cue). None of the stimuli induced a significant avoidance response during the stimulus observation period as compared to the control. These findings provide preliminary support for the species-specific nature of these proposed alternative *P. marinus* control measures. This is an important step in the use of chemosensory alarm cues in the control of invasive *P. marinus* in the Great Lakes.

Keywords: chemosensory alarm cues, non-target species, *Petromyzon marinus* control

Introduction

The Great Lakes, located in Canada and the United States, constitute a tremendous natural resource for the over 60 million individuals who inhabit their surrounding area (Smith & Tibbles, 1980). The Great Lakes are also a major economic resource for Canada and the United States, with the Great Lakes' non-farm economy contributing approximately 30% to the gross domestic product of both countries combined (Krantzberg & de Boer, 2006). The increasing problem of invasive species poses a substantial conservation challenge for the governments of Canada and the United States.

A species of particular interest to environmental bodies around the Great Lakes is the sea lamprey. *P. marinus* is an invasive ectoparasitic species that represents a serious threat to fish species in the upper Great Lakes (Smith & Tibbles, 1980). The primary method of *P. marinus* control in the Great Lakes Basin is the regular use of 3-trifluoromethyl-4-nitrophenol for larval *P. marinus*. In an effort to reduce costs, as well as to reduce the potential for non-target effects of these chemicals, the Great Lakes Fishery Commission (GLFC) has committed to researching and developing alternative *P. marinus* control measures (GLFC, 2011).

One proposed alternative for controlling *P. marinus* populations involves capitalizing on predator avoidance behaviours in response to chemical alarm cues (Imre et

al., 2010). Recent studies have explored the use of conspecific chemical cues and mammalian predator chemical cues to control *P. marinus* (Di Rocco et al., 2014; Imre et al., 2014; Barnett et al., 2016; Di Rocco et al., 2016). Conspecific chemical cues in studies of fishes refer to the conserved alarm substance found in their epidermal cells and released when damaged, which is recognizable via scent by their conspecifics (Chivers et al., 2007).

Chemical alarm cues tend to be the most valuable in fish risk assessment because, unlike visual cues, chemical alarm cues will function in conditions of poor visibility, and many chemicals readily dissolve in water, providing a variety of potential chemical signals (Mirza & Chivers, 2001). These cues enable fish to modify their behaviour, or avoid an area entirely, in order to avoid detection by predators. Recent studies have demonstrated that *P. marinus* exhibit strong avoidance behaviour toward *P. marinus* damage-released alarm cues extracted from fresh and decayed animals as well as putative predator cues, such as a component of mammalian urine (2-phenylethylamine HCl; PEA HCl) and human saliva (Wagner et al., 2011; Bals & Wagner, 2012; Di Rocco et al., 2014; Imre et al., 2014; Barnett et al., 2016; Di Rocco et al., 2016) in the laboratory.

Before the use of chemosensory alarm cues proposed for behavioural manipulation of *P. marinus* populations, it is important to

investigate whether they are species-specific or induce undesirable behavioural responses in sympatric non-target species.

The rainbow trout is an anadromous salmonid species that was intentionally introduced to the Great Lakes (Biette et al., 1981). While not native to the region, *O. mykiss* have been artificially stocked in the Great Lakes since the 1870s (Mills et al., 1994), resulting in the naturalization of *O. mykiss* to the Great Lakes (Krueger & May, 1991). Being sympatric with invasive *P. marinus* populations, *O. mykiss* represent an excellent opportunity to test alternative lamprey repellents against a non-target species of great commercial importance.

Previous studies have typically examined the behaviour of individual *O. mykiss* in aquaria (Brown & Smith, 1997; Mirza & Chivers, 2001, 2003) or in the field (Leduc et al., 2004) exposed to single doses of chemosensory alarm cues. *O. mykiss* exhibits a response to conspecific skin extract by decreasing their use of area, reducing foraging activity, and freezing (Brown & Smith, 1997; Mirza & Chivers, 2001, 2003). Given that proposed chemosensory alarm cues would be released in streams in a continuous manner to manipulate the distribution of migratory *P. marinus* populations, it is important to examine whether this practice might influence groups of *O. mykiss* at a site level and potentially result in the redistribution of *O. mykiss* in

streams. This study is the first to explore whether chemosensory alarm cues used for the control of invasive *P. marinus* will induce undesirable short-term behavioural responses in *O. mykiss*.

This study investigated whether replicate groups of *O. mykiss* would change their channels when exposed continuously for 20 minutes to damage-released alarm cues from *P. marinus* and *O. mykiss*, and potential mammalian predator cues, such as human saliva and PEA HCl. We predicted that a continued exposure to stimuli perceived as alarm cues by *O. mykiss* will result in a larger proportion of non-moving individuals compared to the control treatment.

Methods

Experimental Subjects

The 500 1+ juvenile *O. mykiss* used for this experiment were obtained from the Harrietta Hills Trout Farm and transferred to the Hammond Bay Biological Station (Millersburg, Michigan) on June 5, 2014. Fish were held in 1,000-L flow-through tanks fed with Lake Huron water (water temperature, mean $\pm$ SD = 8.5 ± 0.5 °C) prior to and during the experiment. Experimental subjects were kept at a normal photoperiod (14D:10N) and fed ad libitum daily with Silver Cup Steelhead floating feed (Nelson and Sons, Inc.).

Stimulus Preparation

Animals used for the preparation of the whole-body tissue extracts were sacrificed via a rapid, blunt blow to the head (use of experimental and tissue donor subjects was approved by the Algoma University Animal Care Committee, AUP# 2014-II-01). We harvested 25 g each of *P. marinus* tissue from five males (wet mass, mean $\pm$ SD = 241 $\pm$ 40 g; length, mean $\pm$ SD = 47.1 $\pm$ 4.4 cm) and five females (wet mass, mean $\pm$ SD = 228 $\pm$ 24 g; length, mean $\pm$ SD = 48.0 $\pm$ 14.1 cm). The collected 250 g of *P. marinus* tissue was thoroughly homogenized in 750 ml of deionized water. The homogenate was filtered with 100% cotton cheesecloth, topped up with deionized water to 1 L of the final volume, and stored in 50 ml aliquots in the freezer at -10 °C until further use. As there are no clear morphological differences between male and female *O. mykiss* at this life stage, 10 individuals of varying size (wet mass, mean $\pm$ SD = 53 $\pm$ 22 g; length, mean $\pm$ SD = 16.8 $\pm$ 2.7 cm) were selected for whole-body tissue extract preparation. These concentrations were chosen based on previous laboratory studies that demonstrated their functionality for *P. marinus* (Di Rocco et al., 2014; Imre et al., 2014; Barnett et al., 2016; Di Rocco et al., 2016). The *O. mykiss* extract was prepared exactly the same way as described above.

PEA HCl (purity 99.3%; HPLC assay results supplied by the vendor) was purchased from BulkSupplements.com (Henderson,

Nevada). Human saliva was collected from five individuals (three males and two females) each morning, mixed together, and stored in plastic 50 ml test tubes at room temperature for no more than 8 hours prior to use.

All chemical substances and tissue mixtures were further diluted by combining the following amounts of each stimulus type with Lake Huron water from the experimental arena, to a total of 400 ml of delivery solution for each replicate: 20 g of PEA HCl (final in-stream concentration of 1×10^{-5} M), 20 ml of *O. mykiss* extract and *P. marinus* extract (final in-stream concentration of 2 ppm), 4 ml of human saliva (final in-stream concentration of 0.5 ppm), and 20 ml of deionized water (final in-stream concentration of 2 ppm). These concentrations were selected in order to replicate the stimuli applied to *P. marinus* subjects in prior experiments (Di Rocco et al., 2014; Imre et al., 2014; Barnett et al., 2016; Di Rocco et al., 2016) to observe *O. mykiss* behaviour under the same conditions and test whether these chemical cues at the same chemical concentrations yielded the same response in a non-target species.

Experimental Procedure

The experiment was conducted between June 6-10, 2014 in two parallel stream channels located at the Hammond Bay Biological Station (HBBS) (Millersburg,

Michigan). These stream channels are the same as those used in prior research testing the response of *P. marinus* to the same stimuli (Di Rocco et al., 2014; Imre et al., 2014; Barnett et al., 2016; Di Rocco et al., 2016), allowing the results to be directly comparable to these prior studies. The stream channels were fed with Lake Huron water (water depth = 20 cm; water velocity, mean $\pm$ SD = 0.04 ± 0.01 m/s). Each stream channel harboured an experimental arena delimited by framed screens at the upstream and downstream ends (length x width = 623 cm x 185 cm; see Figure 1).

Ten replicate groups of 10 *O. mykiss* were exposed to five treatments: deionized water (control), *O. mykiss* whole-body extract (conspecific damage-released alarm cue), *P. marinus* whole-body extract (heterospecific damage-released alarm cue), human saliva (mammalian omnivore cue), and PEA HCl (potential predator cue). Two replicates (each of a different

treatment type to account for time effects) were performed simultaneously. Replicate groups of *O. mykiss* were exposed to the different stimuli between 8:00 and 17:00 daily, in such a manner to ensure that replicates of each treatment occurred every day to control for the potential effect of variations in water temperature.

Each replicate group of test subjects was held in holding baskets two meters upstream of the experimental arena for 1 hour to acclimate trout to flow conditions and water temperature. After the acclimation period, the basket containing the experimental subjects was gently slid downstream, into the middle of the experimental arena. All 10 individual *O. mykiss* were released simultaneously from their holding basket in the center of the experimental arena by gently upturning and removing the basket. Upon release into the experimental arena, animals were allowed to acclimate for a 30-minute period. Following this, fish behaviour and

Figure 1. Experimental stream channel, as seen from above.

Arrow indicates the direction of water flow. Horizontal bars indicate the PVC flow conditioners used to decrease turbulence in water flow. Stimuli were released into the experimental stream channel on the right (RP – right pump) or left (LP – left pump) side. B indicates the location where the experimental subjects were released into the experimental stream channel (modified schema from Imre et al., 2014).

distribution were recorded for 10 minutes to provide a baseline level of behaviour prior to the introduction of chemical stimuli. The chemical stimulus was then released on either the left or the right side (“the scented side”) for 20 minutes using peristaltic pumps (Master Flex L/S Easy-Load II, Model: 77200-60, Cole-Parmer Canada Company, Montreal QC) at a discharge rate of 20 ml/min. The pumps were located at the upstream end of the experimental arena, with their stimulus release tubes entering directly into the channel itself. The stimulus release side was regularly alternated (left/right) to control for any potential bias between the two sides of the channel. Rhodamine dye tests prior to the experimental trials indicated that stimulus released on a given side would remain on that side throughout the length of the experimental arena. The first 10 minutes of the stimulus release period were used to allow for homogeneous dispersion of the stimulus on the scented side of the experimental arena. Fish distribution was recorded again for the last 10 minutes of the stimulus release (stimulus observation period).

Water temperature was recorded for each replicate (mean $\pm$ SD = 8.5 ± 0.5 °C), while pH was recorded daily (mean $\pm$ SD = 7.8 ± 0.0). Following each replicate, each *O. mykiss* was weighed to the nearest gram (wet mass, mean $\pm$ SD = 66 ± 38 g), and their fork lengths were recorded to the nearest millimetre (fork length, mean $\pm$ SD = 175 ± 37 mm).

Data Collection

The behaviour and distribution of the experimental fish during the pre-stimulus and stimulus observation period were recorded using video-recording equipment (Axis Q1604 Network Camera) positioned directly above the stream channel. Recordings were stored on magnetic hard drives (Western Digital My Book 3 Tb External Hard Drive). The proportion of all *O. mykiss* and non-moving *O. mykiss* on the scented side of the stream channel was recorded every 30 seconds during the pre-stimulus and stimulus observation periods, respectively. The location of the head of individuals in relation to the black tape separating the two halves of the channel was used to assign the individual to the scented or unscented side (see Figure 1). The proportion data collected during each observation period was used to calculate the mean proportion of all non-moving *O. mykiss* on the scented side for the pre-stimulus and stimulus observation periods, respectively.

Data Analysis

Mean proportion data on the scented side were logit transformed ($\ln(p/(1-p))$) (Warton & Hui, 2011) before statistical analysis. One-way analyses of variance (ANOVA) were used to compare treatments for the pre-stimulus and stimulus observation periods, with a mean proportion of all and non-moving *O. mykiss* on the scented side as the dependent variable and stimulus

type, a fixed factor. If the one-way ANOVA was significant, a one-sided Dunnett's test was used to compare the experimental stimuli to the deionized water control. The analysis was also performed on the pre-stimulus data to ascertain that there was no side preference before stimuli delivery. Water temperature, wet mass, and fork length were analyzed with a one-way ANOVA. The assumption of normality and homogeneity of variances was tested with Shapiro-Wilk's and Levene's tests, respectively. All variables satisfied both assumptions ($p > .05$), except water temperature (non-normal data). All statistical analyses were performed using Statistica Version 12.0 (Statsoft Inc., 2014). The significance level was $\alpha = .05$.

Results

The proportion of all *O. mykiss* that differed between treatments showed a significant difference during the stimulus observation period (Figure 2; one-way ANOVA, $F_{(4, 45)} = 3.095$, $p = .025$).

However, none of the experimental stimuli (*O. mykiss* extract, *P. marinus* extract, PEA HCl, or human saliva) induced a significant change in the distribution of all *O. mykiss* in the stream channel as compared to the control stimuli (deionized water; Dunnett's test, all $p > .05$).

Similarly, there was a significant difference among treatments during the stimulus observation period for non-moving *O. mykiss* (one-way ANOVA, $F_{(4, 45)} = 2.830$, $p = .035$). The proportion of non-

moving *O. mykiss* exposed to conspecific damage-released alarm cues (RTE) on the scented side was larger than the control treatment; however, the difference was not significant (Dunnett's test, $p = .077$). None of the other stimuli induced a significant change as compared to deionized water control (Dunnett's test, all other $p \geq .390$). There was no significant difference between treatments during the pre-stimulus observation period for either all (one-way ANOVA, $F_{(4, 45)} = 1.645$, $p = .180$) or non-moving *O. mykiss* (one-way ANOVA, $F_{(4, 45)} = 2.424$, $p = .062$).

No significant differences were observed between treatments in water temperature (one-way ANOVA, $F_{(4, 45)} = .030$, $p = .998$), wet mass (one-way ANOVA, $F_{(4, 45)} = .786$, $p = .540$), or fork length (one-way ANOVA, $F_{(4, 45)} = .722$, $p = .582$).

Discussion

O. mykiss did not respond to the conspecific alarm cue or to the potential mammalian predator cues by exhibiting avoidance of the scented side of the channel during exposure to any of the stimuli, unlike *P. marinus*, which did exhibit significant avoidance of the same cues in previous experiments (Di Rocco et al., 2014; Imre et al., 2014; Barnett et al., 2016; Di Rocco et al., 2016). Consequently, this study provides preliminary support for the species-specific effect of *P. marinus* extract, PEA HCl, and human saliva as alternative *P. marinus* repellents.

Figure 2. Mean $\pm$ standard error proportion of *O. mykiss* on the scented side of the experimental stream channel during the stimulus observation period exposed to the different stimulus types.

Deionized water (DIW), *P. marinus* extract (SLE), 2-phenylethylamine hydrochloride (PEA), *O. mykiss* extract (RTE), and human saliva (SAL; $N = 10$ for each treatment). Responses to experimental stimuli are not significantly different from the deionized water control (Dunnett's test; all $p > 0.05$). Note that non-transformed proportion data are shown, but the analysis was performed on logit transformed proportion data.

This study is the first to investigate *O. mykiss* response to chemosensory alarm cues on a larger scale in a laboratory stream environment. Prior studies have focused on micro-scale responses of individuals within aquaria, such as freezing behaviours, and decreased feeding and local area use (Brown & Smith, 1997; Mirza & Chivers, 2001). This study examined whether the distribution of the experimental subjects changed when presented with a variety of putative alarm cues and a conspecific damage-released alarm cue. We found that none of the alarm cues induced a significant

change in the distribution of the experimental animals in the laboratory stream channels. There is similar evidence from a study (Kim et al., 2011) that examined the responses of young-of-the-year (YOY) and 1+ Atlantic salmon (*Salmo salar*) presented regularly with conspecific damage-released alarm cues during a 4-week period in a wild stream. The YOY *S. salar* changed their distribution in response to the conspecific alarm cue, resulting in a decrease of their density in the alarm cue sections and an increase of their density in the control and buffer sections. In contrast with YOY *S. salar*, and

consistent with our findings, the experimental manipulation did not affect the density of 1+ *S. salar*.

In previous studies, when exposed to a conspecific alarm stimulus, *O. mykiss* responded by exhibiting significant increases in predator avoidance behaviours, such as freezing in place, reducing the overall time spent swimming and attempting to feed (Brown & Smith, 1997), and an overall reduction in foraging activities (Mirza & Chivers, 2003). We found that there was a trend for a larger proportion of non-moving *O. mykiss* exposed to conspecific damage-released alarm cues on the scented side, as compared to the control treatment. While the finding is not significant, it is in line with similar findings by previous studies. This finding is also consistent with the experimental subjects interpreting a conspecific cue as an alarm cue (Di Rocco et al., 2014; Imre et al., 2014; Barnett et al., 2016; Di Rocco et al., 2016). This finding was not observed for the rest of the putative alarm cues presented. This trend may be of interest to future researchers in this field, given that it is consistent across both *P. marinus* and *O. mykiss*.

Though the experimental conditions replicated those of previous studies with *P. marinus*, this experiment also has implications for the behaviour of fish in the wild (Di Rocco et al., 2014; Imre et al., 2014; Barnett et al., 2016; Di Rocco et al.,

2016). This is because *O. mykiss*, as a non-native, heavily fished species, is constantly being re-stocked into the Great Lakes by government bodies in order to increase population numbers for recreational and commercial fishing purposes. As an example, in Wayne County, New York alone an estimated 20,000 *O. mykiss* are stocked into Lake Ontario each year (New York State Department of Information Technology Services, 2020). The age at which they are restocked is typically 1+ par, the same age cohort used exclusively for this study. As they are replenished each year in this manner, 1+ par are overrepresented in population numbers compared to those expected for a native, naturally replenishing species. The behaviours observed in the individuals used in this study can therefore be expected to carry over to a significant proportion of those found in the wild, since many are of hatchery stock and of a similar age cohort. The results of this study, therefore, indicate that the hatchery-farmed individuals that are regularly stocked into the Great Lakes would not exhibit an avoidance response to the use of the potential *P. marinus* repellents used in this experiment.

In conclusion, *O. mykiss* did not exhibit an observed avoidance of the scented side of the channel to any stimulus (*O. mykiss* extract, *P. marinus* extract, PEA HCl, human saliva) that differed from the control (deionized water). As the first study of its kind to investigate *O. mykiss* response as a

group to chemosensory alarm cues in a laboratory stream environment, this experiment provides preliminary support for the species-specific nature of the proposed alternative *P. marinus* control. More research is needed to confirm these findings, as well as to test the impacts of these proposed *P. marinus* repellents using other species inhabiting the Great Lakes. Based on the findings of this study, there appears to be great potential in the use of these alternative *P. marinus* repellents in invasive *P. marinus* control efforts, and more research in this field stands to greatly benefit both our understanding of fish avoidance behaviour and the preservation of the vital Great Lakes economy.

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