# Application of a putative alarm cue hastens the arrival of invasive sea lamprey (Petromyzon marinus) at a trapping location

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**6 authors**, including:

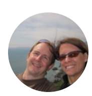

Trevor D Meckley
National Oceanic and Atmospheric Administr…



Nicholas S Johnson
United States Geological Survey



Thomas M Luhring
University of Nebraska at Lincoln



Michael J Siefkes
Michigan State University

# Application of a putative alarm cue hastens the arrival of invasive sea lamprey (*Petromyzon marinus*) at a trapping location

John B. Hume[1, †], Trevor D. Meckley[2], Nicholas S. Johnson[3], Thomas M. Luhring[4], Michael J. Siefkes[5] & C. Michael Wagner[6]

[1, 2, 4, 6] *College of Agriculture & Natural Resources, Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI 48824, U.S.A*; [1]jhume@msu.edu; [2]meckleyt@msu.edu; [4]tluhring@msu.edu, [6]mwagner@msu.edu

[3] *U.S. Geological Survey, Great Lakes Science Center, Hammond Bay Biological Station, 11188 Ray Road, Millersburg, MI 49759, U.S.A*; njohnson@usgs.gov

[5] *Great Lakes Fishery Commission, 2100 Commonwealth Boulevard, Suite 100, Ann Arbor, MI 48105, U.S.A*; msiefkes@glfc.org

[†]Corresponding author: jhume@msu.edu, 517-353-0731

## ABSTRACT

The sea lamprey *Petromyzon marinus* is an invasive pest in the Laurentian Great Lakes basin, threatening the persistence of important commercial and recreational fisheries. There is substantial interest in developing effective trapping practices *via* the application of behavior-modifying semiochemicals (odors). Here we report on the effectiveness of utilizing repellent and attractant odors in a push-pull configuration, commonly employed to tackle invertebrate pests, to improve trapping efficacy at permanent barriers to sea lamprey migration. When a half-stream channel was activated by a naturally derived repellent odor (a putative alarm cue), we found that sea lamprey located a trap entrance significantly faster than when no odor was present as a result of their redistribution within the stream. The presence of a partial sex pheromone, acting as an attractant within the trap, was not found to further decrease the time to when sea lamprey located a trap entrance, relative to when the alarm cue alone was applied. Neither the application of alarm cue singly, or alarm cue and partial sex pheromone in combination, was found to improve the numbers of sea lamprey captured in the trap *vs*. when no odor was present - likely because nominal capture rate during control trials was unusually high during the study period. Behavioural guidance using these odors has the potential to both improve control of invasive non-native sea lamprey in the Great Lakes as well as improving the efficiency of fish passage devices used in the restoration of threatened lamprey species elsewhere.

Keywords: cue; invasive; lamprey; management

# INTRODUCTION

The Laurentian Great Lakes are highly susceptible to the establishment of invasive species and, of the approximately 180 non-native species currently recognized in this region, the sea lamprey *Petromyzon marinus* represents the most pernicious threat to fisheries. Despite suppressing sea lamprey to *c*. 10% of their historical peak abundance, the attack rate on economically valuable fishes remains unacceptably high, threatening the sustainability of commercial and recreational fisheries that generate $7 billion USD of economic activity each year (Christie & Goddard 2003). The United States (US) and Canadian governments, whose borders encircle the Great Lakes, jointly expend more than $20 million USD *per annum* to execute an integrated pest management program to suppress sea lamprey population size in the Great Lakes, primarily by killing larvae with targeted pesticides applied to rivers (Applegate et al 1961; Howell et al 1964; McDonald & Kolar 2007).

Control of non-native sea lamprey is also partly achieved through the maintenance of dams that limit the access of adults to suitable spawning habitat. Federal agencies currently operate a network of 77 sea lamprey traps in association with dams in 72 streams across the Great Lakes. The majority of this trapping effort entails the construction and deployment of both permanent and seasonal traps (Heinrich et al 2007). Permanent traps are concrete or steel boxes in fixed locations, often at one or both ends of a dam face, that capture all fishes capable of passing through the entrance. A few are incorporated into trap-and-sort fishways; devices that comprise a series of chambers with openings that progressively decrease in size to passively sort captured fishes based on body size. Few streams without barriers contain traps, and those that do employ nets or temporary trap-and-weir combinations that suffer from trap efficiencies of less than 10% (defined as the proportion of the spawning population in a given river removed by the traps) (Heinrich et al 2007). In all cases, the traps are passive (i.e., unbaited), capturing only those lamprey that choose to enter the device.

The poor performance of sea lamprey traps has been tolerated as captures are used solely for the purpose of indexing basin-wide adult abundance. Nevertheless, there is substantial interest in developing an effective trapping scheme to begin to fish-down the sea lamprey population through adult removal (GLFC 2011). The US Fish and Wildlife Service (USFWS) currently monitor 960 dams in the Great Lakes basin that block sea lamprey movement. Of these, more than 200 have conditions conducive to trapping (J. Barber, Unites States Fish & Wildlife Service, *pers. comm.* 2014). Expansion of the trapping programme into these streams will only occur if effective traps and trapping scenarios are developed. Sea lamprey traps are thought to underperform for two reasons. Firstly, encounter rates with traps are often low (Bravener & McLaughlin 2013), and secondly, once a trap is encountered, the rate of entrance by sea lamprey is also low (Johnson et al 2013). The key to improving the performance of these traps may lie in a better understanding of the factors that regulate lamprey movements in the vicinity of the trap, and attempting to manipulate those movements to increase capture success.

A number of physical modifications have been attempted to improve performance (e.g., the addition of attractant lighting), but with limited or equivocal success (Purvis et al 1985; McLaughlin et al 2007; Stamplecoskie et al 2012). More recently the focus has shifted to the use of semiochemicals – odor molecules released by animals into their environment and used to either directly communicate with another individual (pheromones) or to broadcast information about the environment (cues). As a result of their nocturnal habits, sea lamprey appear to rely extensively on such odors to successfully forage (Kleerekoper & Mogensen 1963), migrate (Vrieze et al 2010; Meckley et al 2014) and reproduce (Li et al 2002). In recent years three odors have been recognized and partially identified, including two attractants and one repellent. The attractants include the odor of stream-resident larvae, which act to guide migrants into streams with suitable rearing habitat (Sorensen et al 2005; Wagner et al 2009; Vrieze et al 2010), and a male sex pheromone that attracts sexually mature females to nests containing mature males (Li et al 2002; Luehring et al 2011; Johnson et al 2012). Sea lamprey also produce a repellent; a putative alarm cue that is released from the body upon injury or death, labeling areas of high risk along the migratory route to spawning grounds (Bals & Wagner 2012). Such risk may be interpreted as having arisen from predation and/or larval habitat degradation (Wagner et al 2011; Imre et al 2014).

Reliance on contrasting chemical information to guide natural migratory movements presents the opportunity to utilize ‘push-pull’ tactics to achieve pest management (Miller & Cowles 1990). Sea lamprey migrating to spawning grounds tend to be pulled to the side of a river channel where the presence of attractant pheromones reveals either suitable spawning habitat (Wagner et al 2006, 2009) or potential mates (Johnson et al 2009). Johnson *et al.* (2013) recently demonstrated that emission of a single component of the male sex pheromone, 3-keto-petromyzonol sulfate (3kPZS, Li et al 2002) will increase the likelihood that a migrating, sexually-immature sea lamprey enters a trap. However, whether pheromone-baiting proves effective in management will rely on firstly identifying and synthesizing the full pheromone (Luehring et al 2011; Meckley et al 2012), and secondly maximizing the proportion of migrants that encounter a baited trap. The putative alarm cue, functioning as a repellent, may achieve this maximization by generating an odor-mediated ‘exclusion zone’, thus pushing migrants to one side of a channel where a ‘target zone’ may be located (e.g., a trap) (Imre et al 2010; Wagner et al 2011; Johnson et al 2015).

This study was designed to test the utility of applying sea lamprey odors in a push-pull configuration to a river containing a single barrier-integrated trap in order to improve trap performance. We hypothesized that applying an extract containing the putative alarm cue (hereafter referred to as "alarm cue") to one-half of a river channel opposite a trap location will create an 'exclusion zone', thus increasing the likelihood an upstream migrating sea lamprey will encounter that trap by driving them to the area downstream of the trap (our 'target zone'). And that emitting a synthesized partial pheromone (3kPZS) through the trap entrance will increase the likelihood a sea lamprey will subsequently enter the trap once encountered. The experimental design included three treatments: a control, where no odors were added; push only, where extract containing the alarm cue was added; and push-pull, where both extract containing the alarm cue and the partial pheromone were added. Given the limited time to test these applications we chose not to include the pull-only treatment, as this was extensively tested as reported in Johnson *et al*. (2013). We predicted the following responses: (1) odor application will reduce the proportion of sea lamprey migrating through the 'exclusion zone' compared to nights without odor application; (2) the proportion of sea lamprey captured by the trap will be higher on nights with odor application, compared to nights when no odors are applied; and (3) the time taken to encounter the trap will be reduced on nights when odors are applied compared to nights with no odor application. For all tests we compared push only with push-pull applications to discern whether any observed improvements in trap performance were attributable to the presence of 3kPZS.

## MATERIALS & METHODS

*Study location*

The experiment was conducted on the lower reach of Carp Lake River, a tributary of Lake Michigan located near Mackinac City, Michigan USA (45° 44' 56.45" N, 84° 49' 45.90" W) (Fig. 1). This site was equipped with a sea lamprey barrier that incorporates a fixed, seasonally operated trap. The trap itself is located on the west bank of the river *c.* 500 m upstream of the confluence with Lake Michigan, affixed to the downstream side of a sheet-pile dam. River water is directed through the trap entrance to stimulate vigorous upstream swimming in those lamprey searching for a way around the barrier. The river receives a reliable run of sea lamprey each year (mean 914, range 11 – 3110, years 1984 – 2013), and average trap efficiency at this site is 46% (range 23 – 82, years 1996 - 2008) based on mark-recapture estimates.

*Capture & preparation of experimental lamprey*

The use of sea lamprey was approved by the Michigan State University (MSU) Institutional Animal Care & Use Committee (IACUC) *per* animal use permit 01/14-007-00. Immature sea lamprey were captured in barrier-integrated traps during their annual spawning migration into the Cheboygan, Manistique and Ocqueoc Rivers, northern tributaries of Lakes Huron and Michigan, by staff of the USFWS and maintained at the US Geological Survey's (USGS) Hammond Bay Biological Station (HBBS) near Millersburg, Michigan USA. Prior to use, sea lamprey were segregated by sex and held in 1000 L capacity flow-through tanks supplied with Lake Huron water at ambient temperatures ranging from 5 – 10 °C.

A total of 816 (408 male, 408 female) sea lamprey were selected for inclusion in field trials. Prior to inclusion in trials, sea lamprey were weighed (mean 233.8 g ± 54.4 S.D., range 65 – 585), measured (mean $T_L$ 483.5 cm ± 37.2 S.D., range 344 – 595) and implanted with a uniquely-encoded 32 mm half-duplex passive integrated transponder (PIT tag, Oregon RFID Inc., Portland, Oregon USA). PIT tags were inserted into the abdomen *via* a 5 mm incision and wounds were sealed using Vetbond Tissue Adhesive (n-butyl cyanoacrylate, 3M, Minnesota USA). Lamprey were allowed to recover for a minimum of 24 hours in 200 L holding tanks prior to release into the stream.

*Preparation of chemical cues*

Alarm cue was extracted following the procedure detailed in Bals & Wagner (2012). The repellent was derived using three 2.08 m Soxhlet apparatus' equipped with water-cooled Allihn condensers and 12 L solvent reservoirs heated with hemispherical mantles to 75 – 80 °C. Carcasses of adult male and female sea lamprey (9 per batch in a single extractor, mean mass per batch = 2361 g) were extracted with a 50:50 solution of 200 proof ethyl alcohol and deionized water for a minimum of three cycles. Extract was then rotovaporated under vacuum at 35°C to remove ethanol to produce *c*. 5.2 L of putative alarm cue *per* batch and stored at -20 °C until use. 3kPZS was synthesized in salt form (ammonium salt dihydrate) by Bridge Organics Co. (Kalamazoo, Michigan USA) with a purity of > 95% and stored at -80 °C until required. Stock solutions (10 mg $mL^{-1}$) of the synthesized form of the pheromone were created in a 50/50 *w/w* mixture of methanol and distilled water.

*Field trials*

Field trials were conducted between 26 May and 28 June 2014, consistent with the natural extent of the sea lamprey spawning migration in northern Lake Michigan. This included 10 nights when no odor was applied (control), 10 nights when alarm cue was applied to the stream (push), and 10 nights when alarm cue + 3kPZS was applied to the stream (push-pull) (Fig. 2). The order of treatment nights were randomized in three-day blocks to ensure an even spread across the 30 day trial period. However, odor application side could not be altered as the trap was affixed to the barrier. On each trial day 24 tagged sea lamprey were selected (12 male, 12 female) from holding tanks at HBBS and released, following acclimation to river temperature, at a point 350 m downstream of the barrier on Carp Lake River. Sea lamprey release occurred at 16:00 each day, and individuals were observed rapidly moving into cover. Lamprey movements were monitored by an array of four PIT antennas wired to a single multiplexer (Oregon RFID Inc.) that recorded the date, time and identification number of each tagged lamprey swimming over an antenna (Fig. 1).

A single antenna (A1) spanning the stream width 50 m below the barrier was used to detect those lamprey moving upstream towards the barrier from the release site. Two antennas, each spanning half the stream width were positioned (relative to facing upstream) on the left (A2) and right side (A3) of the channel 10 m further upstream of A1, to detect the position of individual sea lamprey as they approached the barrier [i.e., within the exclusion (left) or target (right) zones]. The final antenna (A4) was positioned on the trap entrance, to detect when tagged lamprey encountered the trap itself. The read range of this antenna was *c.* two body lengths, resulting in sea lamprey detection within and out-with the trap entrance. As a consequence it was not possible to determine the probability that a tagged individual entered the trap following trap encounter from PIT data alone, an important factor in determining whether 3kPZS was increasing the likelihood of trap entrance (see Johnson et al 2013).

Approximately 4 hours prior to the start of a trial stream discharge ($m^3$ $sec^{-1}$) was measured at the mid-point of the site using the USGS mid-section method (Gore, 1996) with a Doppler flow meter (Flo-Mate Model 2000, Marsh-McBirney). On nights when odors were applied discharge estimation was used to calculate the volume of alarm cue necessary to ensure a concentration of 1 ppm when fully mixed into one half of the stream discharge. The alarm cue extract was mixed with a volume of river water, collected upstream of the test area, to bring the total volume of the mixture to 9 L. The final mixture was pumped into the river immediately upstream of the barrier, at the extreme edge of the half-channel receiving alarm cue at a rate of 60 mL $h^{-1}$ for four hours (21:00 – 01:00), by a laboratory-grade peristaltic pump (Masterflex 7553-70, Cole-Parmer) powered by a 12-volt battery. The pumping location was determined after several dye releases (Rhodamine WT) confirmed where one-half of the channel would be activated downstream of the barrier.

During trials when 3kPZS was applied, it was pumped directly into the trap for 4 hours (21:00 - 01:00) using a battery operated peristaltic pump (Admiral Reef Dosing Pump, Norwich, Connecticut USA). The rate of pheromone application (2.5 mg $h^{-1}$, equivalent to the emission from three to five spermiating males, Yun et al 2012; *pers. comm.* C. Brant, Michigan State University 2015) is an amount that is highly attractive to sexually mature females, and may represent an upper behavioral threshold in the attraction of female sea lamprey in circumstances with no competing pheromone source (Wagner et al 2006). Application of 3kPZS to Carp Lake River was approved by the State of Michigan and the United States Environmental Protection

Agency (USEPA) (Permit no. 75437-EUP-3) as required by the Federal Insecticide, Fungicide, and Rodenticide Act (P.L. 75-717).

*Data Treatment*

Only lamprey detected during the hours of treatment application (21:00 – 01:00) on the night of their release were used in data analysis, because individuals not recaptured during their first night would have been exposed to a different set of experimental conditions. Only first detections of individual tagged lamprey on each antenna were used in data analyses. Of those tagged individuals that were detected on A1, we recorded the proportion of those animals that subsequently moved through the target zone (hereafter referred to as percent in target zone) on their first passage. We also recorded the proportion of tagged lamprey that were recaptured in the trap following detection on A1 on the first night of their release only. Transit time was recorded as the difference in time between detections on A1 and A4 during the hours of odor application (21:00 – 01:00).

*Statistical Analyses*

To test the effect of treatment (no odor, alarm cue, alarm cue + 3kPZS) on the percent of sea lamprey moving through the exclusion zone, we used treatment as a main effect with discharge, and river temperature as covariates in a generalized linear model (GLM). To test the effect of treatment on the percent of lamprey recaptured we used treatment as a main effect with discharge, river temperature and number of untagged wild sea lamprey captured in the trap that night as covariates. To test for the effects of treatment on individual transit times between A1 and A4, we used treatment as a main effect with discharge, river temperature, sex and body length as covariates in a GLM. All data met the assumptions of normality and heteroscedasticity. No covariates were significant, thus all were subsequently dropped from analyses. For models with a significant effect of treatment we used Tukey's HSD for post hoc means separation. All analyses were carried out in SPSS (V. 22, IBM Corp., New York USA).

## RESULTS

*Prediction 1* – Treatment affected the percent of sea lamprey moving through the exclusion zone on the night of their release ($F_{[2, 28]} = 5.172$, $P = 0.013$). A lower percent of sea lamprey moved through the exclusion zone on alarm cue (mean 20.42 ± 3.5 S.E., $P = 0.027$) and alarm cue + 3kPZS application nights (mean 19.91 ± 1.9 S.E., $P = 0.026$) compared to nights without odor application (mean 37.08 ± 6.1 S.E.) (Fig. 3). There was no added effect of 3kPZS on the percent of sea lamprey moving through the exclusion zone (alarm cue *vs*. alarm cue + 3kPZS, $P = 0.996$).

*Prediction 2* – There was no distinguishable effect of treatment on recapture rates. The average number of tagged lamprey that moved upstream and were detected on the first night of their release was high (control 20.1, range 13 – 23; alarm cue 19.7, range 16 – 22; alarm cue + 3kPZS 20.9, range 18 – 23). Thus, recapture rates were high across all treatments (control 91.4% ± 2.9 S.E.; alarm cue 96.6% ± 1.5 S.E.; alarm cue + 3kPZS 92.9% ± 2.5 S.E.). In addition, sex ratios (male to female) of recaptured sea lamprey did not deviate substantially from 1:1 across treatments (control 1.02:1, alarm cue 1.09:1, alarm cue + 3kPZS 1.22:1).

*Prediction 3* – Treatment affected individual transit times on the night of release ($F_{[2, 363]}$ = 11.619, $P < 0.001$; Fig. 4). Sea lamprey located the trap 54.1% faster during alarm cue applications ($P < 0.001$) (mean 14.7 min ± 1.54 S.E., range 1 – 86.1) and 53.5% faster during alarm cue + 3kPZS applications ($P < 0.001$) (mean 14.5 min ± 1.8 S.E., range 1 – 122), compared to nights without odor application (mean 27.1 min ± 2.9 S.E., range 2.4 – 153). There was no added effect of 3kPZS on transit time (alarm cue *vs*. alarm cue + 3kPZS, $P = 0.998$).

## DISCUSSION

Here we report a field test of the putative sea lamprey alarm cue in a management scenario for the first time. These results are consistent with previous laboratory findings that have demonstrated an unequivocally strong, negative reaction to the odor of dead conspecifics, which is likely to mitigate risk exposure in actively migrating sea lamprey (Wagner et al 2011; Bals & Wagner 2012). Our results confirm two of our predictions, in that the application of the putative sea lamprey alarm cue to a river containing a barrier-integrated trap redistributed migrants away from the alarm cue (Prediction 1) such that they arrived at the trap entrance in half the time (*vs*. control, Prediction 3). As sea lamprey in this study located the entrance to the trap significantly faster on nights when odors were applied to the stream, this would indicate that there was a reduction in search time prior to encountering the trap. However, these manipulations of behaviour did not result in a significant increase in trap captures. In 2014 sea lamprey were substantially more likely to be captured than the historical average on control nights (91% this study *vs*. 46%, years 1996 – 2008). The relatively small mean width (8.3 m, range = 5 – 11.5 m) and discharge (1.1 $m^3 s^{-1}$ ± 0.53 S. D.) of Carp Lake River during the trial period could have created conditions whereby migrants were likely to encounter the trap several times over the course of a night (more so than is typical), helping explain why capture rate was so far above average.

Despite the lack of support for increased capture rates on nights when odors were applied (*vs*. control), these findings generally affirm the framework presented by Bravener & McLaughlin (2013) – conditions that create a high probability of multiple encounters with a trap will increase the likelihood of eventual capture. We suspect guiding migrants into the vicinity of the trap with odors will have a greater impact on trap success in larger rivers where the animal may spend considerable time away from the trap location as it seeks a passage around a barrier. Furthermore, larger rivers may contain a lower density of adult sea lamprey relative to larval habitat. Dawson and Jones (2009) surmised that large sea lamprey recruitment events did not occur when adult female density was less than 0.2 females *per* 100 $m^2$ of preferred larval habitat. Therefore, trapping adult sea lamprey for control in the Great Lakes may be most successful when deploying odors in large streams where adult sea lamprey density is already low.

The addition of a synthesized partial sex pheromone (3kPZS), applied in conjunction with the alarm cue in a push-pull configuration, did not reduce the time sexually-immature male and female sea lamprey took to encounter the trap *vs*. alarm cue alone, nor increase trap capture success. Because we were unable to detect when an individual in this study first entered the trap, it remains unclear in what way, if at all, 3kPZS affected trap entrance. This pheromone component increases upstream movement in sexually mature females and enables them to locate trap entrances in environments lacking odor competition (Johnson et al 2009). Although 3kPZS alone can act to increase encounter rate with a trap entrance in controlled environments, its ability to hold mature female sea lamprey near traps is quite limited *vs* the full pheromone

(Luehring et al 2011). For example, Johnson *et al*. (2013) found that the application of 3kPZS to barrier-integrated traps only marginally increased catches of both mature and immature migrants (*c*. 8%). However, recent analyses indicate that 3kPZS was most effective at capturing sea lamprey in wide streams (> 25 m) where more than 8 mg 3kPZS hour$^{-1}$ was applied, and in streams where adult sea lamprey density was low (Johnson *et al*. *in review*). Thus, as Carp Lake River is 8 m wide at the trap site, 2.5 mg 3kPZS hour$^{-1}$ was applied, and sexually immature sea lamprey were targeted, the apparent lack of a response to 3kPZS may not be entirely unexpected in the study given these new data and the circumstances in which it was applied. Employing 3kPZS as an attractant odor under different circumstances (e.g., wider streams and at higher application rates), while targeting mature sea lamprey in streams with lower adult density may reveal those management scenarios where it could be more complementary to the alarm cue. In this study tagged males and females were recaptured in approximately equal ratios, despite the presence of 3kPZS. This is unsurprising however as tagged lamprey in this study were not yet sexually mature.

Conceivably, larval odors represent a better option for motivating vigorous upstream swimming near a trap entrance, and its application through a trap will increase capture success (Wagner et al 2006). However, the full chemical composition of this odor remains uncertain. Although Sorensen *et al*. (2005) identified three compounds (petromyzonamine disulfate, petromyzosterol disulfate and petromyzonol sulfate) as being the full migratory pheromone; a field test found that only whole larval odor elicited a behavioural response by migratory sea lamprey within a river (Meckley et al 2012). Discovery of the full chemical makeup of the larval odor could provide a superior ‘pull’ *vs*. 3kPZS when targeting sexually-immature migrants. We suggest re-examining the push-pull configuration as a sea lamprey pest control strategy once the full migratory pheromone mixture has been identified and can be synthesized.

Despite achieving a high rate of recapture when applying the push and push-pull configurations (*c.* 90%), similar proportions of sea lamprey were also recaptured on nights when no odors were present, thus weakening our ability to distinguish any significant effect of semiochemical application on trap efficacy. The natural variation in trap success at this site (22 – 85%) broadly corresponds with what we saw during control nights in this study. Therefore, the ability to detect any effect resulting from odor application may have been precluded by environmental factors during the experiment making baseline conditions unusually conducive to effective trapping. One potential explanation for a high capture rate on control nights is that individual lamprey were encountering the trap multiple times throughout the night, as the presence of the barrier prevented them from bypassing the trap entrance, and instead their natural behaviour in searching for a way around the barrier led to their eventual capture (Bravener & McLaughlin 2013). In hindsight, removing the trap immediately following the period when odors were applied (01:00) may have given a truer representation of capture efficacy between control and treatment nights, given that when odors were applied sea lamprey located the trap earlier in the evening. However, as these odors were applied during the peak period of diel activity in migratory sea lamprey (e.g., Binder & McDonald 2008) this action may have had a negligible impact on capture rates.

That sea lamprey in this study quickly located a barrier-integrated trap entrance and large proportions were subsequently captured, is an indication that a similar methodology could be employed as an effective management strategy in other systems. These findings corroborate, and add to, a burgeoning number of field experiments that indicate sea lamprey control in the Great

Lakes could be improved by behavioural manipulation *via* the application of odors to their environment (Johnson et al 2009; Wagner et al 2009; Luehring et al 2011; Johnson et al 2013; Meckley et al 2014). However, these same behavioural manipulations could also protect sea lamprey in their native range. Around the north Atlantic coast the sea lamprey is a species of conservation concern, particularly in regards to its gastronomic importance in some regions (Beaulaton et al 2008). In Iberia, for example, sea lamprey are subject to extensive levels of poaching (Andrade et al 2007) in part because they struggle to surmount in-stream barriers (Almeida et al 2002). Anadromous sea lamprey employ multiple bursts of high-intensity swimming to pass areas of high flow and are therefore subjected to fatigue when forced to employ successive high-energy bursts (Quintella et al 2004). Thus, where sea lamprey are faced with multiple in-stream barriers during their migration our data strongly suggests that minimizing the time required to get them to a passage device may increase passage success (by individuals) and overall passage rates (by populations). One of the greatest factors contributing to the decline of native sea lamprey populations is the extensive network of anthropogenic barriers preventing them from reaching spawning grounds (Maitland et al. 2014). Yet evidence suggests that by improving access to a greater extent of spawning habitat, typically prevented by in-stream barriers, sea lamprey will rapidly recolonize habitat previously unavailable to them (Hogg et al 2013; Lasne et al 2014).

More broadly, lamprey species across the globe are threatened by their inability to pass man-made barriers (Moser et al 2002; Russon and Kemp 2011; Yamazaki et al 2011; Stewart & Baker 2012), despite great variability in passage requirements (e.g., Laine et al 1998; Reinhardt et al 2008; Mesa et al 2010). The presence of a putative alarm cue has been indicated in the *Ichthyomyzon* genus as well as *Petromyzon* (Bals & Wagner 2012), and petromyzonol sulfate –

the larval precursor of 3kPZS (Brant et al 2013) – has been identified in at least four other genera (*Entosphenus, Ichthyomyzon, Lampetra* and *Lethenteron*) (Yun 2012). Should members of these genera prove responsive to odors in a manner similar to sea lamprey in the Great Lakes, then behavioural manipulation *via* the application of semiochemicals could significantly improve the efficacy of lamprey passage devices.

One uncertainty related to these findings remains to be considered; the applicability of utilizing odors to increase trapping efficacy in a system with a free-standing trap (i.e., one that does not incorporate a barrier). However, given that we demonstrated that sea lamprey can be pushed towards an area containing a trap, and be induced to locate that trap quickly, it is anticipated that catch rates would be significantly higher in such systems compared to those that contain a free-standing trap without the use of odors. In a remarkable twist of fate the sea lamprey finds itself both the subject of intensive control measures as well as large-scale conservation efforts across North America and Europe. In the same way that their inability to pass barriers in their native range can inform control strategies in the Great Lakes, so too should the application of odors to improve trapping success in the Great Lakes be used to assist in lamprey passage at dams and weirs where they require protection.

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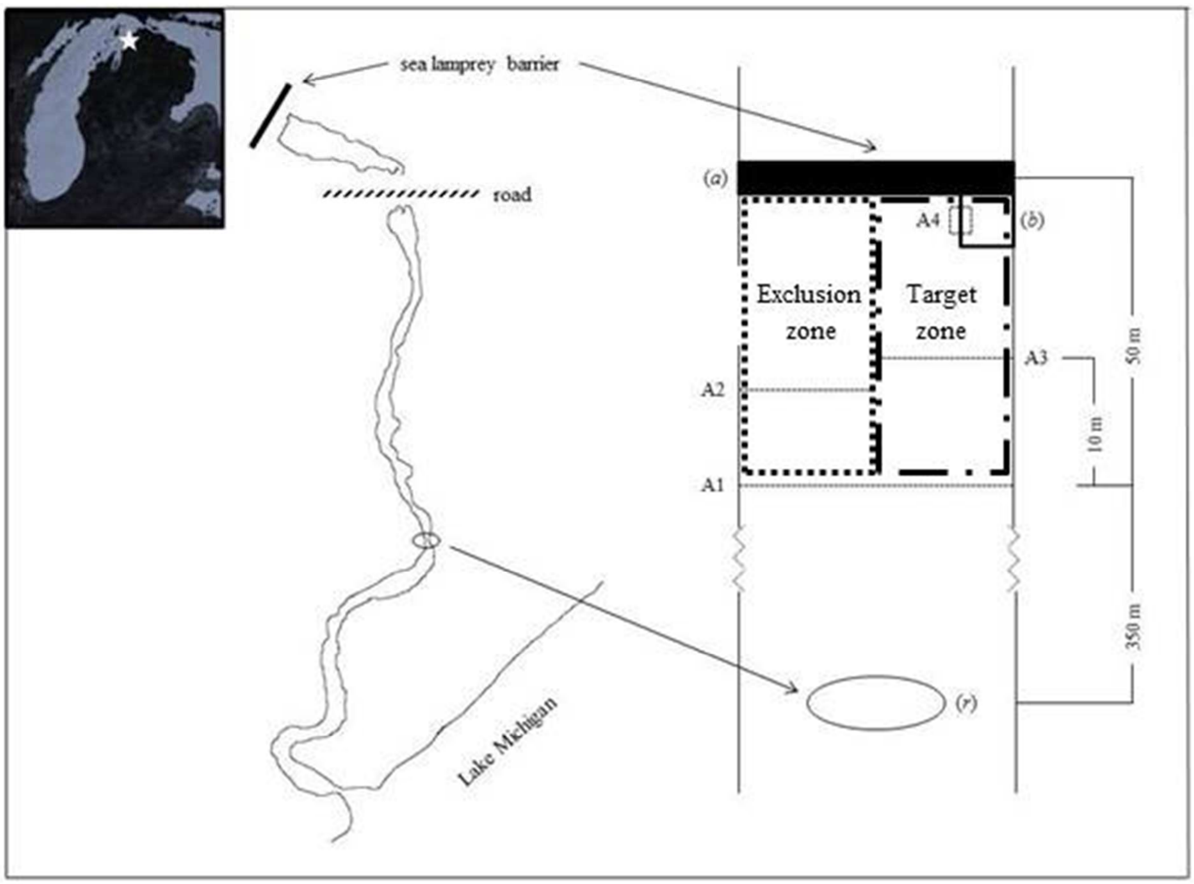
FIGURES

Figure 1



536 Figure 2

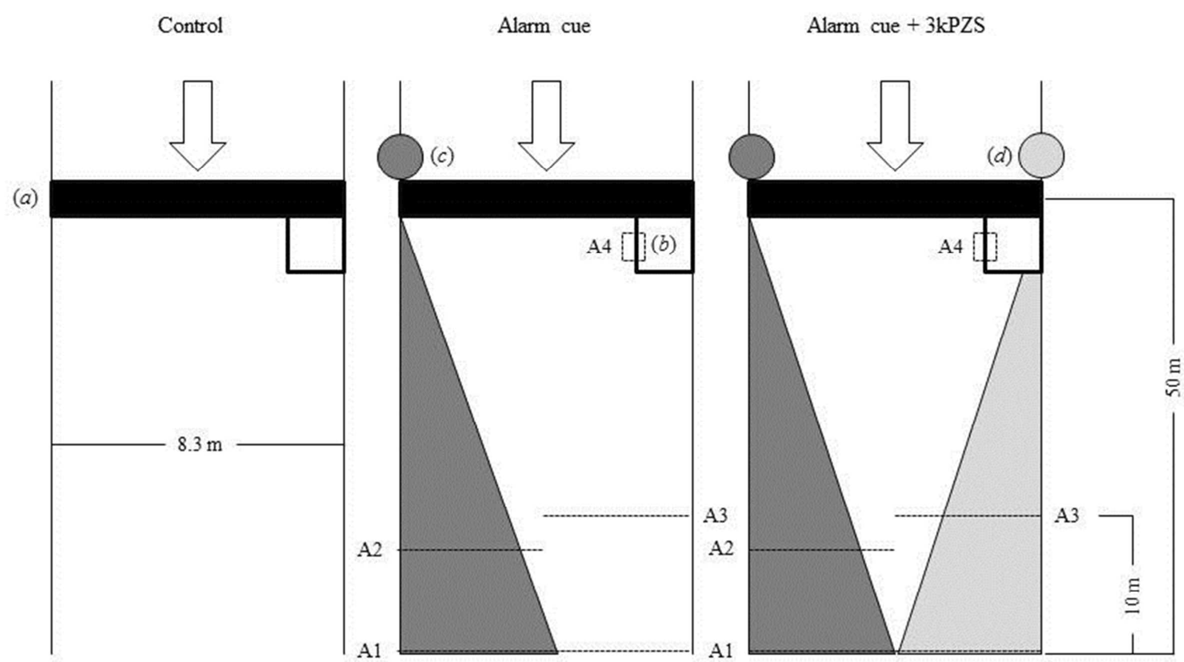
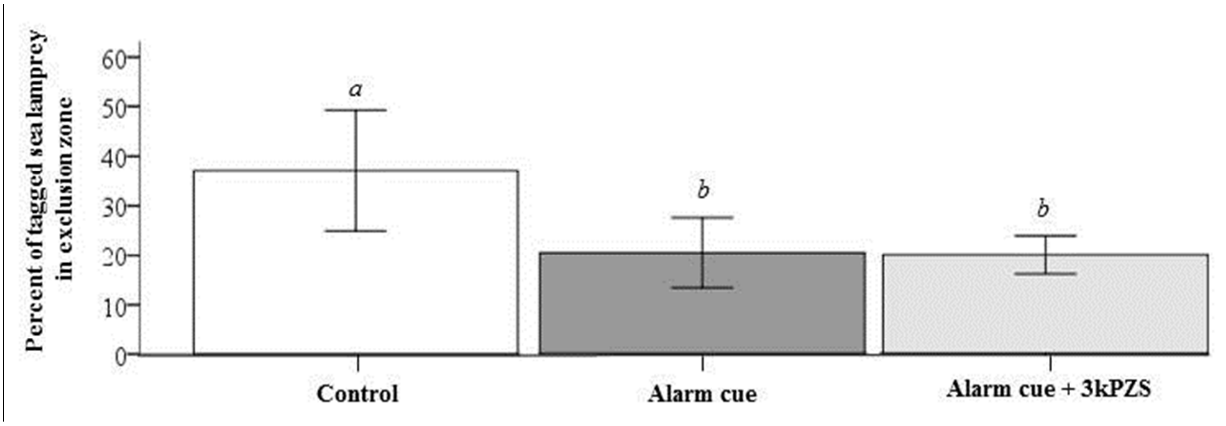
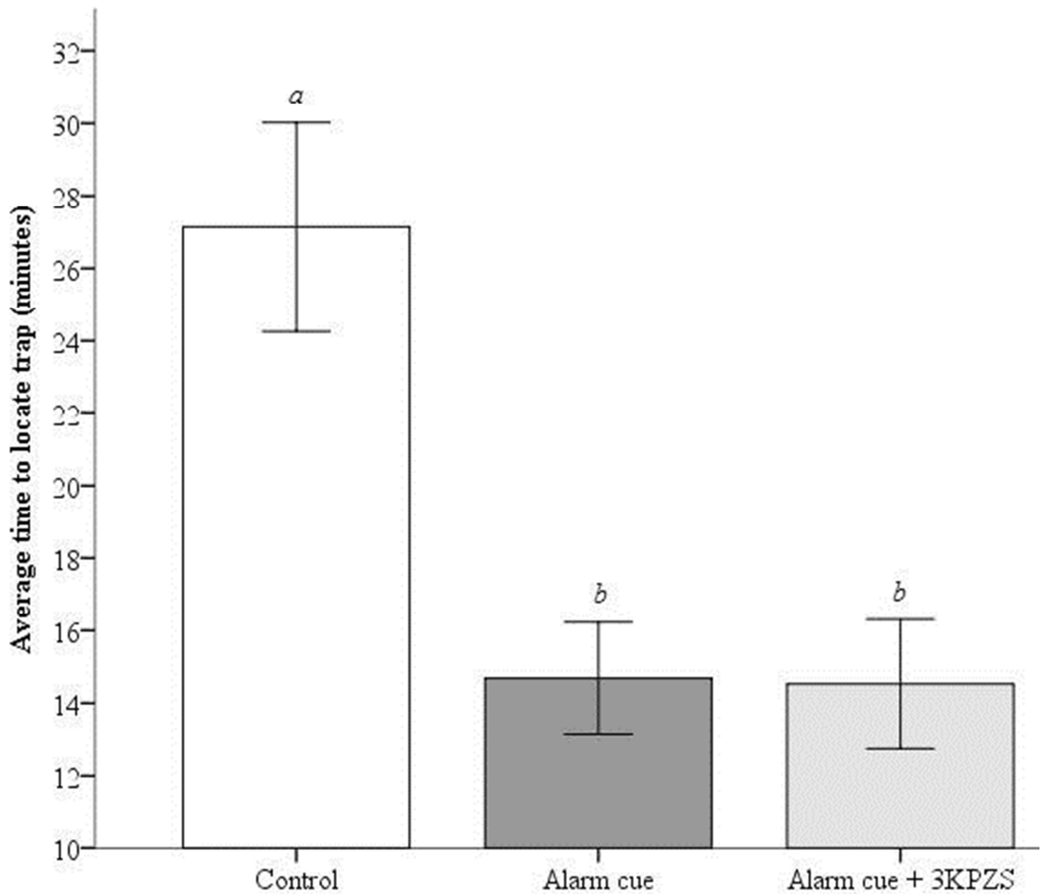

Control
Alarm cue
Alarm cue + 3kPZS
(a)
(b)
(c)
(d)
A4
A4
8.3 m
50 m
A3
A3
A2
A2
A1
A1
10 m

Figure 3


Percent of tagged sea lamprey in exclusion zone
60
50
40
30
20
10
0
a
b
b
Control
Alarm cue
Alarm cue + 3kPZS

538 Figure 4


Average time to locate trap (minutes)
32
30
28
26
24
22
20
18
16
14
12
10
a
b
b
Control
Alarm cue
Alarm cue + 3KPZS

## FIGURE CAPTIONS

Fig. 1. Location of study site. Carp Lake River is situated in northern Michigan, U.S.A. and is an affluent of Lake Michigan. There are 464 stream meters from the mouth to a sheet pile dam, which acts as a sea lamprey barrier. Relative position (not to scale) of PIT antennas used to detect tagged sea lamprey movement and important study features are noted, where: (*a*) is a sea lamprey barrier, (*b*) is a barrier-integrated trap used to collect sea lamprey, and (*r*) is the release point for tagged sea lamprey, located 350 m downstream of (*a*). A1 - 4 reflect positions of PIT antennas in the stream. The relative positions of the ‘exclusion’ (···) and ‘target zones’ (-·-·-·) referred to in the text are also indicated.

Fig. 2. Treatment regimens used to test predictions in Carp Lake River. Relative position (not to scale) of PIT antennas used to detect tagged sea lamprey movement and important study features are noted, where (*a*) is a sea lamprey barrier, (*b*) is a barrier-integrated trap used to collect sea lamprey, (*c*) is a pump used to apply alarm cue to the left side of the stream channel, and (*d*) is a pump used to apply 3kPZS to the right side of the stream channel. On control nights no odor was applied.

Fig. 3. Percent of sea lamprey detected within the odor-mediated exclusion zone in Carp Lake River between the hours of treatment application (21:00 - 01:00). Alarm cue was applied to the exclusion zone (left) and 3kPZS was applied to the target zone (right). On nights when odors were applied, fewer sea lamprey were detected within the exclusion zone, compared to nights when no odors were applied. Differences between *a* and *b* were significant at the 0.05 level.

Fig. 4. Transit time (mean ± 2 S.E.) of tagged sea lamprey between antennas A1 and A4 after the onset of treatment application (21:00). On nights when odors were applied sea lamprey were detected at the trap entrance faster compared to nights when no odor was applied. Differences between *a* and *b* were significant at the 0.05 level.