



Olfactory sensitivity and threat-sensitive responses to alarm cue in an invasive fish

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Received: 28 February 2023 / Accepted: 16 May 2023 / Published online: 26 May 2023
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Abstract Establishing how invasive animals perceive and manage the threat of predation improves our understanding of why certain species rapidly invade unfamiliar environs where others do not. The threat-sensitive response hypothesis predicts that prey that modulate their antipredator behavior in response to the perceived intensity of the threat will have a selective advantage. We investigated the behavioral and olfactory antipredator responses of migrating invasive sea lamprey to varying concentrations of a conspecific alarm cue in the laboratory and field.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10530-023-03092-6>.

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We hypothesized the odor indicates the presence and location of predation risk on river shorelines, allowing sea lamprey to either alter their migratory path and/or movement timing to reduce exposure to the predator. Migratory sea lampreys exhibited threat-sensitivity in space-use both in the laboratory and nature by increasingly avoiding areas as the concentration of alarm cue increased when safe space was available (areas without the alarm cue). The intensity of the avoidance at higher concentrations was greater in the field. Electrophysiological recordings from the olfactory organ confirmed a concomitant increase in signal output to the olfactory bulb of the brain when exposed to increased alarm cue concentrations. However, increasing the alarm cue concentration did not alter the animal's motivation to move upstream in either avoidable risk or unavoidable risk scenarios. Exposure to alarm cue affected decisions regarding how to move, but not whether to move. Knowledge of how the animal uses the cue in nature reveals ways to use the cue to manipulate movement and achieve conservation goals.

Keywords Behavioral plasticity · Chemical ecology · Movement ecology · *Petromyzon marinus*

Introduction

A defining characteristic of an invasive species is its capacity to succeed in new places. Because many

introduced species are prey for all or a portion of their lives, the ability to manage risk posed by unfamiliar predators promotes invasion success (Saul et al. 2013; Penk et al. 2017). In fact, naiveté to native predators is an oft-cited mechanism underlying biotic resistance to invasion (deRiviera et al. 2005; Carlsson et al. 2009; Carthey and Banks 2014). Thus, considerable insight into invasion biology and the creation of effective conservation practices may be gained from mechanistic examination of the antipredator strategies of successful invaders (Saul and Jeschke 2015; Gregor et al. 2020).

Animals frequently assess predation risk by monitoring public information (Danchin et al. 2004; Guttal and Couzin 2011). Public information can prove more useful than prior personal experiences when the identity of the predator or the magnitude of risk is uncertain, as during the initial stages of invasion (Dall et al. 2005; Webster and Laland 2008; Mathot et al. 2012; Brown et al. 2013). In aquatic environments, public information often takes the form of semiochemicals—molecules conveyed by water that contain information regarding opportunities or perils that strongly influence decision making. Aquatic organisms attend to a rich array of chemical information emitted from both predators and prey to evaluate the immediacy of predation risk (Wisenden 2000; Burnard et al. 2008). Perhaps best understood are the damage-released alarm cues, substances emitted from injured prey tissue during predator attack that induce antipredator responses in closely related taxa including freezing, flight, shelter use, avoidance of the area, increased vigilance, and neophobia (Chivers and Smith 1998; Ferrari et al. 2009; Wisenden 2015).

Reliance on alarm cues to detect predation risk may be particularly useful to newly introduced species that do not enjoy eco-evolutionary overlap with native predators, but do with native prey. For example, ‘eavesdropping’ on the alarm cues emitted from native prey may provide an early warning system, alerting newly arrived invaders (Magrath et al. 2015). There is substantial evidence that aquatic prey detect and respond to alarm cues emitted from closely related heterospecifics (e.g. confamilial taxa), with the intensity of the response often diminishing with increased phylogenetic distance between releaser and receiver (Hazlett and McLay 2005; Dalesman et al. 2007; Schoeppner and Relyea 2009; Pecor et al. 2010; Mitchell et al. 2012; Ituarte et al. 2022). Further,

when familiar alarm cues are paired with unfamiliar predator odors, evidence suggests prey may acquire olfactory recognition of the predator (Batabyal et al. 2014; Gonzalo et al. 2007; Mitchell et al. 2011; Leduc et al. 2007). Alarm cues therefore unite innate and acquired predator recognition, and reliance on alarm cues to assess predation risk may improve the likelihood of survival in the early stages of biological invasion.

Invasive prey will acquire a further fitness advantage when they vary the amount of time and energy invested in antipredator behavior in proportion to predation risk (Helfman 1989; Mathis and Vincent 2000; Mirza and Chivers 2003; Ferrari et al. 2009; Roux et al. 2014). Accordingly, a relatively high concentration of alarm cue may indicate an acute threat, whereas low concentrations may indicate more distant threats, in time or space, that merit less attention (Chivers and Smith 1998; Fraker 2008; Ferrari et al. 2009; Wisenden et al. 2010; Roux et al. 2014). Alternatively, antipredator responses may be expressed in non-graded patterns where the animal never (threat-insensitive) or always (threat-hypersensitive) avoids apparent risk (Helfman and Winkleman 1997; Brown et al. 2006). Non-graded responses to alarm cue are likely detrimental to the establishment of invading species. Ignoring cues that reliably signal risk will result in greater mortality, whereas hypersensitivity may result in reduced foraging success, negatively affecting growth, survival, and reproductive performance (Brown et al. 2006; Carthey and Blumstein 2018).

In this study, we investigated olfactory sensitivity and behavioral threat sensitivity to a conspecific alarm cue in the sea lamprey (*Petromyzon marinus*), a diadromous fish with a complex life history that successfully invaded the Laurentian Great Lakes of North America from the Atlantic Ocean. Our subjects were sub-adult (maturing) fish actively migrating into rivers to spawn, the penultimate event in a sea lamprey’s life. The sea lamprey relies extensively on attractive conspecific cues to select migratory routes into and through rivers, and cross-reactivity of these odors with those produced by native lampreys is thought to have facilitated rapid invasion and establishment (Teeter 1980; Sorensen et al. 2005; Wagner et al. 2006, 2009; Vreize et al. 2010; Meckley et al. 2012). The sea lamprey also utilizes an alarm cue that is likely a chemical mixture

to detect and respond to predation events (Wagner et al. 2011; Bals and Wagner 2012; Imre et al. 2014; Mensch et al. 2022). Great Lakes sea lamprey react to alarm cues produced by confamilials native to the basin, but not to those from teleost fishes (Bals and Wagner 2012; Hume and Wagner 2018). In a series of laboratory and field experiments, we investigated the following hypotheses:

1. Avoidance of the alarm cue increases in response to increased cue concentration.
 - a. We first examined this hypothesis in a two-choice laboratory assay, predicting the time spent on the side of the raceway activated with alarm cue decreases with increasing cue concentration.
 - b. We next conducted two field experiments where either half or the entire width of a small river channel was activated with the alarm cue, monitoring the upstream movements of migrating sea lamprey as they passed through the activated reach. We predicted increased avoidance of the alarm cue in space (half-channel activation only) and altered timing of upstream movement (either faster or slower as risk minimization tactics (minimize time vs maximize caution) in both experiments), as a function of the cue concentration.
2. Threat-sensitive responses to olfactory cues require the ability to perceive the intensity (concentration) of the odorants that compose the cue. In fishes, an intracellular transduction cascade resulting in depolarization of the olfactory sensory neuron (OSN) occurs when its receptor binds a complementary odorant. The signal then propagates to the olfactory bulb, and the perception of signal strength is partly a function of the number of OSNs simultaneously binding the target odorant(s). We therefore hypothesized the mechanism of threat-sensitivity is related to the strength of the signal sent to the olfactory bulb from OSNs that bind alarm cue components. We predicted an exponential dose–response relationship between alarm cue dilution and electrical potential recorded at the surface of the olfactory

epithelium as revealed by electro-olfactogram (EOG) recording.

Materials and methods

Test subjects

Experiments were conducted with wild actively migrating sea lamprey collected from four tributaries draining to Lakes Huron (Cheboygan, Ocqueoc and St. Mary's Rivers, Michigan, USA) and Michigan (Manistee River, Michigan, USA) from May–June in 2012, 2014, and 2020. The specimens were captured by the U.S. Fish and Wildlife Service in traps affixed to dams as part of the annual population assessment. Each subject was transported to the Hammond Bay Biological Station (Millersburg, Michigan, USA) and placed into 1000 L holding tanks receiving a continuous flow of fresh water from Lake Huron (100% exchange every 2 h). Sea lamprey used in the electro-olfactogram (EOG) study were subsequently transported to Michigan State University Research Containment Facility (East Lansing, Michigan, USA), and held in similar conditions (flow-through tanks, temperature 7–9 °C). All subjects were monitored for a minimum of 48 h prior to experimental use to ensure normal behavior, and each specimen was physically examined for epidermal damage. Only undamaged and active specimens were used in the experiments, and each individual was used once in a single trial. No food was provided during holding as sea lamprey cease feeding prior to the onset of the migration. Use of sea lamprey and all experimental procedures were approved by the Michigan State University Institutional Animal Care and Use Committee via Animal Use Forms Nos. 02/11-027-00, 01/14-007-00, and 2/18-025-00.

Alarm cue preparation

Alarm cue stock solutions were prepared from two sources: whole sea lamprey carcass (all experiments) or sea lamprey skin (Experiment #1 only). The whole carcass cue was collected via Soxhlet extraction per the general methods of Bals and Wagner (2012). Whole carcasses were extracted singly in a 1 L Soxhlet apparatus (Experiment #1) or in groups of nine (Experiments #2–4) in a 10 L Soxhlet apparatus,

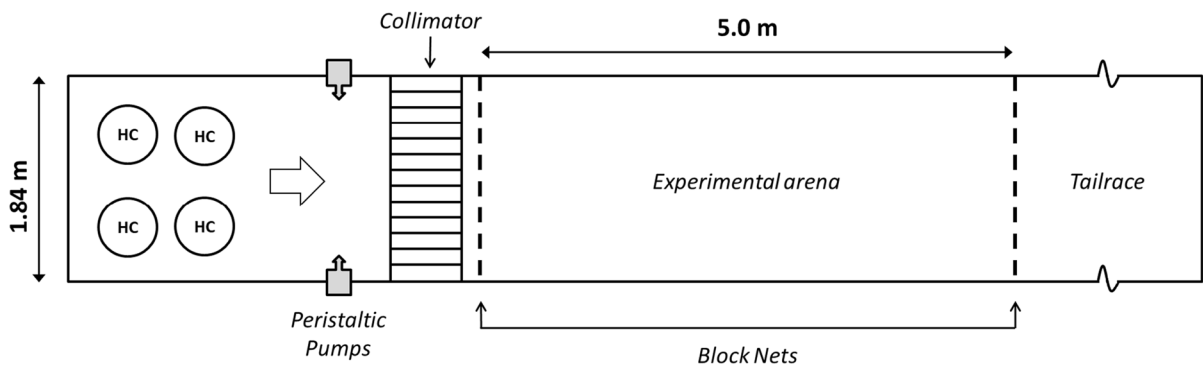


Fig. 1 Schematic of the laboratory raceway, water flowed left to right (large arrow). The experimental reach was enclosed with block nets. Subjects were held in the upstream section in holding cages (HC) until release into the experimental arena. Stimulus odors were introduced with a peristaltic pump from

equipped with hemispherical mantels that heated the solvent to 75–80 °C (Ace Glass, Inc., Vineland, NJ). Prior to performing the extractions, a solvent (50:50 w/w solution of 200 proof ethyl alcohol and deionized water) was prepared and the apparatus was loaded with adult sea lamprey carcass(es) that had been euthanized via cervical dislocation with a razor blade and rinsed for 30 min in 150 ml of solvent prior to placement into the extractor body (the rinsate was retained). The extractor was allowed to cycle three times (approximately 6 h). After cooling to room temperature, both the extract and the initial rinsate were combined, vacuum-filtered (Whatman cellulose paper filter 8–12 μm), and stored at $-20\text{ }^{\circ}\text{C}$ until use. The final carcass extracts had concentrations of 0.117 g tissue ml^{-1} solvent (Experiment #1), 0.235 g tissue ml^{-1} solvent (Experiments #2–4). For Experiment #1, the cue contained in sea lamprey skin was collected from a single adult male (skin weight 40.4 g) and a single adult female (skin weight 31.9 g). Each specimen was euthanized via cervical dislocation with a razor blade. An incision was made around the circumference of the animal posterior to the final gill opening. The skin was peeled from the carcass in a single piece and rinsed in 1 L of deionized water to remove any blood. Each skin was ground with a mortar and pestle for 15 min in 100 ml of solvent (50:50 w/w solution of 200 proof ethyl alcohol and deionized water). Each skin extract was combined with 900 ml of deionized water to reach a final volume of 1000 ml and vacuum-filtered (Whatman cellulose

paper filter 8–12 μm). The individual skin extracts were combined, separated into 200 ml aliquots and stored at $-20\text{ }^{\circ}\text{C}$ until use. The final skin extract had a concentration of 0.036 g skin ml^{-1} solvent.

Threat-sensitive responses in a laboratory raceway (Experiment #1)

Apparatus

Behavioral responses to the alarm cue were examined in a two-choice assay conducted in two identical laboratory raceways (Fig. 1). The raceways were rectangular concrete troughs, 1.84 m wide by 20 m long, divided by nets into an upstream holding section (7.5 m long), an experimental Sect. (5.0 m long), and a tailrace (7.5 m long). Each raceway received a continuous flow of water pumped directly from Lake Huron at a rate of $0.01\text{ m}^3\text{ s}^{-1}$. Water temperature ranged from 7.1 to 9.3 °C over the course of the experiments. A natural day-night schedule was maintained by dimming the lights in the room at 19:00 and leaving a large window and the end of the raceway uncovered while natural light diminished. After 90 min, the window was covered to render complete darkness. The bottom of each experimental section was lined with white fiberglass and marked with gridlines to facilitate detection of lampreys against the background. During trials, infrared light arrays mounted directly over each experimental section illuminated the animals. Lamprey movements were

recorded with a single IR-sensitive video camera mounted over the center of the experimental section. Prior to introducing the test odor into the raceway, we mixed each dilution of the alarm cue or solvent control (see below) in a 500 ml Erlenmeyer flask, adding water from the raceway to bring the final volume to 400 ml. The mixture was continuously stirred with a 2 cm magnetic stir bar during each trial, and was introduced into one half of each raceway at a rate of 15 ml min⁻¹ via laboratory-grade peristaltic pumps (MasterFlex model 7533-20). To ensure no cross-contamination of odors, a separate set of pump-head and delivery tubing was used for each stimulus odor.

Experimental protocol

To ascertain whether sea lamprey exhibited threat-sensitive responses to increasing alarm cue concentration, we tested behavioral responses (avoidance) to whole carcass extract or skin extract in separate experiments. Four dilutions (= treatments) representing 0.01, 0.1, 1.0, and 10.0 µl L⁻¹ of alarm cue extract to receiving water (by volume) were completed for skin or whole carcass odors. During the control trials, the extraction solvent was pumped into the raceway at a concentration of 10 µl L⁻¹. Ten replicate trials were completed for each alarm cue dilution and the control. On a given night, groups of ten male sea lamprey were stocked into each of the holding sections at 15:00 to acclimate the animals to the raceway. Males were chosen because (a) male and female sexually immature sea lamprey do not differ in their response to the extracted predator cue, and (b) the response does not attenuate in males at the onset of maturation, but does in females (Bals and Wagner 2012). The first trial each night began at 22:00. A single trial lasted for 30 min and consisted of a 10-min pre-stimulus period, when one group (ten subjects) was moved into the experimental reach and allowed to swim freely, and a 20-min stimulus period when we introduced the odor into one half of the raceway. We observed individual movements in an adjacent room on video monitors and recorded their activity onto digital media. To analyze animal distributions, we recorded the position of each subject every 30 s after the start of a trial by replaying the video and assigning each subject to the stimulus or non-stimulus side of the raceway based on the position of its head. We analyzed spatial distribution during the final ten minutes

of the stimulus period to obtain a mean proportion of animals on the stimulus side of the raceway for each trial. The pre-stimulus period was used as an acclimation period and the first ten minutes of the stimulus period was not analyzed to provide time for the distribution of the subjects to stabilize after introduction of the stimulus odor (typically less than 5 min, Wagner et al. 2011).

Data analysis

To determine if the tissue source of the alarm cue or dilution affected the spatial distribution of migratory-phase sea lamprey, a two-way analysis of variance (ANOVA) was performed with the proportion of animals on the stimulus side of the raceway as the response and alarm cue dilution and odor source (skin or whole carcass) as fixed factors in the model. Prior to analysis the proportion data were logit transformed and passed a normality test (Shapiro–Wilk, $W=0.98$, $P=0.25$). Because odor source was not a significant factor in the model (see below), subsequent one-way ANOVAs were performed to determine the effect of odor concentration. Pairwise post-hoc Tukey's HSD tests ($\alpha=0.05$) were performed to assign statistically significant differences in repellence across all pairs of concentration. All statistical analyses were performed using STATA (ver. 14.1, StataCorp LP).

Threat-sensitive responses in a natural stream (Experiments #2 and #3)

Study site

Responses of migrating sea lamprey to the alarm cue were observed in the Ocqueoc River, a tributary to Lake Huron in Michigan, USA (45°24'37.52" N, 84°2'54.05"W) from 31 May to 20 June 2012 (Experiment #2) and 28 May to 7 July 2014 (Experiment #3). The experimental reach was located upstream of a barrier used to limit the distribution of the invasive sea lamprey population in Lake Huron; thus, we were only permitted to release females to prevent reproduction above the barrier. An 8 m section of the stream was divided into two equal-width channels with a temporary barrier (Fig. 2). Subjects were acclimated to stream conditions in holding cages placed at the lower end of the experimental reach. Individual movement through the experimental reach

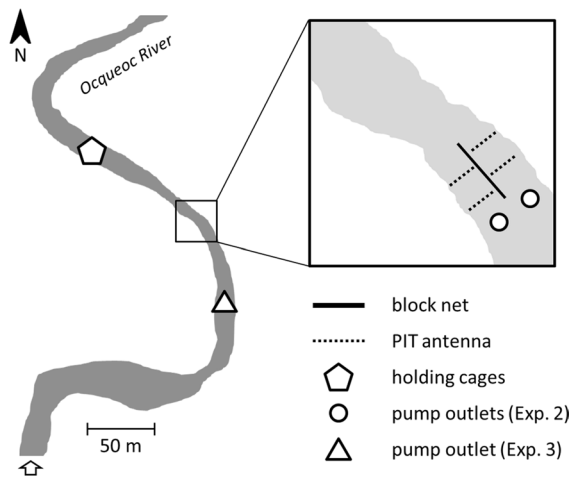


Fig. 2 Schematic of the field site for Experiments #2 and #3. Sea lamprey were released from two holding cages 100 m downstream of the detection area. A set of four PIT antennas tracked lampreys as they swam through the detection area which was separated longitudinally into two equal-width channels by an 8 m block net. The alarm cue extract was pumped one of the three locations, depending on the experiment (see text for details.)

was monitored by placing four fixed PIT antennas (two per side) 100 m upstream of the holding cages, to monitor direction of movement and which side of the channel each animal selected. Subjects released into the stream were internally tagged with a passive-integrated transponder (PIT) tag (32 mm, Oregon RFID, Oregon, USA) by making a 3 mm lateral incision along the lower abdomen below the first dorsal fin. After inserting the tag, the incision was sealed with tissue adhesive (VetBond™ tissue adhesive, 3 M Company). Temperature in the stream ranged from 12 to 24 °C in 2012 (Experiment #2) and 14 to 22 °C in 2014 (Experiment #3). Prior to each trial, discharge at the site was estimated with a flow meter (Marsh-McBirney Flow Mate 2000 or Hach H950, Hach Company, Frederick, MD, USA) using the velocity-area method (Gore 2006) and ranged from 1.1 to 2.4 m³ s⁻¹ in 2012 and in 1.1 to 2.2 m³ s⁻¹ in 2014.

Experiment #2: Half-channel activation

During the half-channel trials, the alarm cue was pumped into the stream along one streambank at four increasing dilutions to achieve the following fully mixed concentrations at the holding cages: 0 (solvent

control) 0.1, 0.2, and 1.0 µL L⁻¹. The chosen dilutions represented the range of demonstrated behavioral reactivity in the lab. Twenty hours prior to the start of a trial (approximately 02:00) 40 PIT tagged female subjects were placed into two holding cages (20 per cage) at the downstream end of the study site to acclimatize the subjects to stream conditions. Each trial lasted 3 h. At the start of a trial, the required amount of extracted alarm cue was mixed with river water in a 20 L Nalgene carboy to reach a final volume of 14 L. The alarm cue mixture was pumped into half of the channel at a fixed rate of 75 ml min⁻¹ using a laboratory grade peristaltic pump (MasterFlex model 7533-20) beginning 15 min after sunset. An equal volume solution of extraction solvent and river water was pumped into the opposing half of the channel. The sides of the channel receiving the stimulus and control odors were alternated across replicates (N=4) within each treatment such that each treatment received an equal number of trials (2) with the stimulus applied on the right and left sides of the channel. The holding cages were opened 15 min after odor pumping began and the subjects were allowed to exit volitionally. PIT antenna crossings were recorded onto a PDA (Meazure, model MEZ1000) attached to a four-antenna multiplexing PIT reader (Oregon RFID) for three hours. Data were downloaded from the reader at the conclusion of each trial. Sixteen trials were completed (four per treatment) using 640 individual subjects.

We examined three attributes of upstream movement: (1) the migration rate, defined as the proportion of subjects moving upstream and detected at any PIT antenna; (2) swim-up time, estimated as the time from cage-opening to first detection at a PIT antenna for each individual that moved upstream; and, (3) channel selection, defined as the proportion choosing the alarm cue activated half-channel as determined by first passage through the right- or left-side PIT array for each trial. We tested the predictions that migration rate would decrease and individuals would increasingly avoid the activated side of the channel (response variables) with separate three-way analysis of variance (ANOVA) tests with alarm cue concentration (factor), water temperature (continuous), and river discharge (continuous) as fixed effects in the model. Prior to analysis, the proportion data were arcsine (square root) transformed and tested for normality via Shapiro–Wilk’s test. The transformed values for

migration rate ($W=0.95$, $P=0.51$) and channel selection ($W=0.91$, $P=0.13$) met the normality assumption for ANOVA. Post-hoc pairwise Tukey's HSD tests ($\alpha=0.05$) were performed on significant ANOVAs to determine significant differences between alarm cue concentrations. Swim-up times were highly left-skewed and failed normality tests after applying standard transformations. Consequently, we tested the prediction that swim-up time would become increasingly later as alarm cue concentration increased with a non-parametric Kruskal–Wallis equality-of-populations rank test, followed by a post-hoc pairwise rank-means test to detect differences among cue concentrations using adjusted significance levels. Because the Kruskal–Wallis test is one-way, effects of water temperature and discharge covariates could not be accounted for.

Experiment #3: Full-channel activation

In Experiment #3, we tested whether migrating female sea lamprey would exhibit threat-sensitive movement tendencies when the full channel was activated with the alarm cue. Specifically, whether (a) the tendency to move upstream (migration rate) would diminish and/or (b) become later (swim-up time) with increasingly higher alarm cue concentration. Experimental procedures mirrored those of Experiment #2 with exception of the alarm cue introduction. During the trials, the alarm cue was pumped into the center of the river 120 m upstream of the PIT array, the distance necessary to ensure an even bank-to-bank distribution of the odor at the PIT array based on a preliminary dye study. Sixteen trials were completed (four replicates per treatment) using 640 individual subjects. We tested the predictions that migration rate would decrease and individual swim-up times would slow with three-way analysis of variance (ANOVA) tests with alarm cue concentration (factor), and water temperature (continuous) and river discharge (continuous) included as fixed effects. Prior to analysis, the proportion data were arcsine (square root) transformed and tested for normality via Shapiro–Wilk's test. The transformed values for migration rate ($W=0.97$, $P=0.86$) met the normality assumption for ANOVA. Post-hoc pairwise Tukey's HSD tests ($\alpha=0.05$) were performed on the migration rate ANOVA to detect any significant differences between alarm cue dilutions. As with Experiment #2,

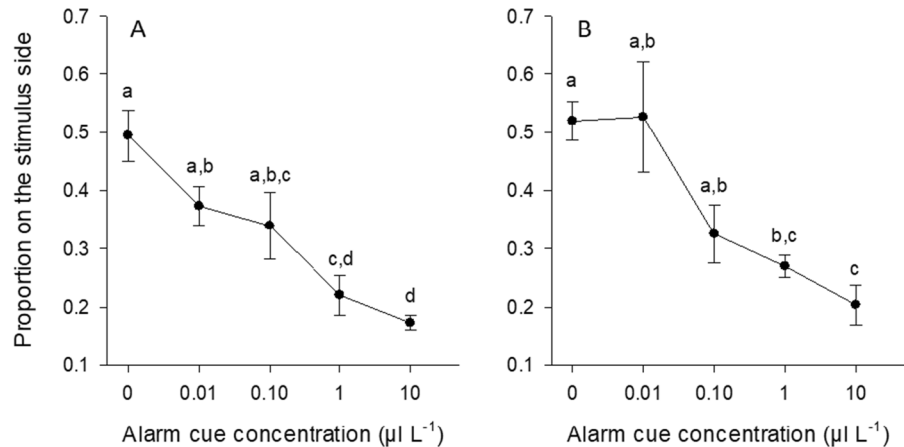
swim-up times were highly left-skewed and failed normality tests after applying standard transformations. Consequently, we tested the prediction that swim-up time would become increasingly later with another non-parametric Kruskal–Wallis equality-of-populations rank test, followed by a post-hoc pairwise test to detect significant differences among cue concentrations. In both field experiments, all statistical analyses were performed with STATA ver. 14.1 (StataCorp LP). The post-hoc comparisons after the Kruskal–Wallis tests were performed in STATA using the KWALLIS2 module developed by H.M. Caci (ver.1.1).

Olfactory organ sensitivity to alarm cue (Experiment #4)

EOG setup and recordings followed previously established procedures (Scott et al. 2019) to record the adult sea lamprey olfactory organ sensitivity to sea lamprey alarm cue. Briefly, a migratory-phase adult sea lamprey was anesthetized with 3-aminobenzoic acid ethyl ester (100 mg L^{-1} , MS222, Sigma-Aldrich) and immobilized with an injection of gallamine triethiodide (30 mg kg^{-1} of body weight, Sigma-Aldrich). Gills were continuously irrigated with aerated, anesthetic water (50 mg L^{-1} MS222) throughout the experiment. The olfactory lamellae were surgically exposed by removing the skin on the surface of the olfactory capsule. The recording electrode was placed on the surface of the olfactory epithelium and the reference electrode on the external skin near the naris. The differential EOG response magnitude induced by exposure to the stimulus was recorded using glass capillary borosilicate electrodes filled with 0.4% agar in 0.9% saline and connected to solid state electrodes with Ag/AgCl pellets (model ESP-M15N, Warner Instruments LLC) in 3 M potassium chloride. Electrical signals were amplified (NeuroLog system model NL102, Digitimer Ltd., Hertfordshire, England, UK), filtered (low-pass 60 Hz, model NL125, Digitimer Ltd.), digitized (Molecular Devices LLC, Digidata 1440A, Sunnyvale, CA, USA), and recorded on a computer running AxoScope 10.4 software (Molecular Devices LLC).

On the day of experiment, an aliquot of whole sea lamprey carcass alarm cue was diluted with filtered water to yield 6 concentrations: 0.001, 0.01, 0.1, 1, 10, and $100 \mu\text{L L}^{-1}$. A 10^{-3} M stock solution of L -arginine

Fig. 3 Mean proportion (± 1 se) of sea lamprey observed swimming on the side of the raceway activated with the alarm cue collected from **a** whole carcass or **b** skin ($0 \mu\text{L}^{-1}$)=control, solvent only). Means marked with different letters were significantly different in post-hoc Tukey's pairwise comparisons. Note the alarm cue concentrations are plotted on a log scale. $N=10$ for each odor



in deionized water was prepared, stored at $4\text{ }^{\circ}\text{C}$, and diluted with filtered water to yield a 10^{-5} M solution. At the start of the recording session, a 10^{-5} M L-arginine solution was introduced to the olfactory epithelium for 4 s, and the response was recorded to correct for variations in olfactory sensitivity among individuals. The olfactory epithelium was flushed with filtered water for 2 min, the blank control was introduced, and the response was recorded. Next, the alarm cue starting at $0.001\text{--}100\ \mu\text{L}^{-1}$ was applied in \log_{10} increments, recorded, and flushed. The response to the blank control and 10^{-5} M L-arginine standard were measured throughout each recording session. The EOG response magnitudes were measured in mV. The normalized EOG response was calculated as normalized EOG amplitude = $(R_t - R_b) - (R_a - R_b)$, where R_t is the response magnitude to the test stimulus (alarm cue), R_b is the response magnitude to the blank, and R_a is the response magnitude to 10^{-5} M L-arginine. Responses were recorded on 7 migratory-phase adult sea lamprey (4 males, 3 females). The olfactory detection threshold of alarm cue, defined as the lowest concentration in which the alarm cue elicited a larger response than the blank, was evaluated with a paired one tailed t -test with a Bonferroni adjustment ($\alpha=0.025$).

Results

Experiment #1: Threat sensitivity in the laboratory

Migratory-phase sea lamprey responded to increasing concentration of alarm cue by reducing the

amount of time spent on the side of the raceway activated by the cue (two-way ANOVA overall model, $F_{5,99}=13.25$, $P<0.0001$; alarm cue concentration effect, $F_{4,99}=16.03$, $P<0.0001$). However, the source of the cue (skin vs. whole carcass) had no effect on the pattern of response to cue dilution (two-way ANOVA, odor source effect, $F_{1,99}=2.13$, $P=0.15$). The pattern of increased avoidance in response to concentrated alarm cue was confirmed in individual one-way ANOVAs for both skin-derived ($F_{4,50}=7.55$, $P<0.0001$) and carcass-derived ($F_{4,50}=10.67$, $P<0.0001$) cues (Fig. 3). In both cases, sea lamprey were strongly repelled at the higher concentrations vs. the control (1 and $10\ \mu\text{L}^{-1}$ vs. 0, see Fig. 3 for full Tukey's HSD results). At these higher concentrations, we observed sea lamprey quickly turning away from the odor plume and proceeding to the lower end of the experimental section of the raceway in an apparent attempt to flee downstream. Once they encountered the block net they began to move back and forth across the net, apparently seeking a clear path of escape. No individuals were observed attempting escape in the upstream direction.

Experiment #2: Threat-sensitivity in a natural stream (half-channel activation)

The proportion of animals observed swimming upstream during the 3 h trials was high (mean ± 1 se, 0.86 ± 0.03 , Fig. 4a), and consistent with previous experiments in this river in the absence of alarm cue addition (e.g., Wagner et al. 2009). The addition of the alarm cue to one-half of the channel at any concentration (vs. control) had no effect on the

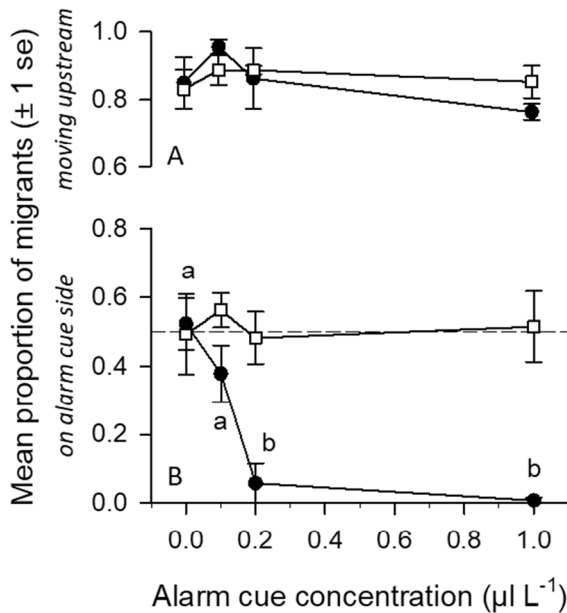


Fig. 4 **a** Mean proportion (± 1 se) of sea lamprey that swam upstream and were detected at the PIT array. Filled circles = Experiment #2 (half-channel), white boxes = Experiment #3 (full-channel). **b** The proportion of upstream swimming lampreys that were detected on the side of the channel activated with the alarm cue (Exp. #2) and the proportion on the side of the channel activated with extraction solvent (Exp. #3). The dashed line indicates the null expectation of equal proportion on either side of the channel. Different letters indicate statistically significant different values per Tukey's HSD (Exp. #3). All other comparisons were non-significant

proportion of animals swimming upstream (ANOVA, overall model $F_{5, 16} = 2.13$, $P = 0.14$; alarm cue concentration effect $F_{3, 16} = 1.14$, $P = 0.38$; Fig. 4a). Likewise, neither temperature ($F_{1, 16} = 1.14$, $P = 0.38$) nor discharge ($F_{1, 16} = 1.14$, $P = 0.38$) elicited observable effects. However, presence of the cue had a strong effect on which side of the channel sea lamprey chose when swimming upstream (ANOVA, overall model $F_{5, 16} = 21.75$, $P < 0.0001$; Fig. 4b). Neither water temperature ($F_{1, 16} = 0.00$, $P = 0.98$) nor river discharge ($F_{1, 16} = 0.56$, $P = 0.47$) affected channel selection. The alarm cue had a strong repellent effect ($F_{5, 16} = 29.65$, $P < 0.001$), causing migrants to move upstream along the side of the channel opposite to the point of odor introduction. Post-hoc Tukey's HSD tests revealed an effect of alarm cue concentration on repellence, distinguishing the two higher concentrations (0.2 and 1.0 μL^{-1}) from the lowest concentration (0.1 μL^{-1}) and the solvent control. At the high

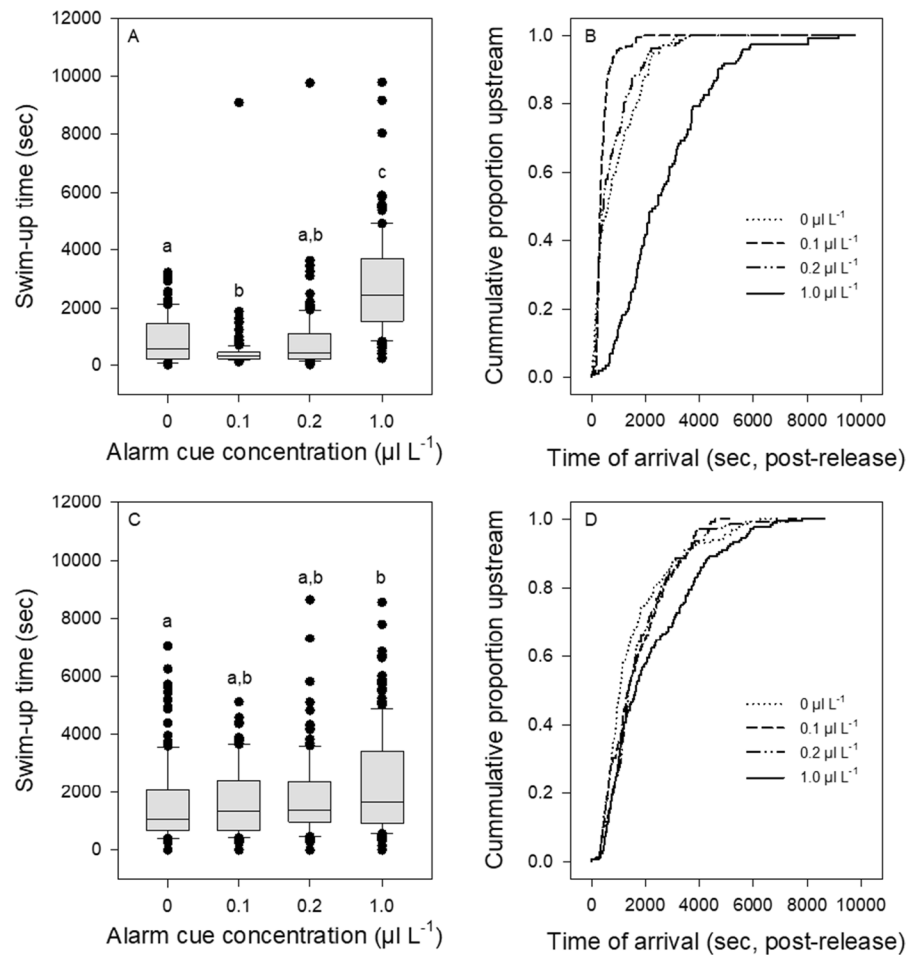
concentrations, 93.8% (0.2 μL^{-1} , 122 of 130 subjects) and 99.2% (1.0 μL^{-1} , 121 of 122 subjects) of the migrants avoided the alarm cue while continuing to move upstream.

Swim-up time differed across alarm cue concentrations (Kruskal–Wallis, chi-square = 202.03, $P < 0.0001$; Fig. 5a). The post-hoc rank-means test revealed quick movement upstream during control trials and when experiencing low alarm cue concentrations of 0.1 and 0.2 μL^{-1} , with evidence for significantly faster upstream movement at 0.1 μL^{-1} (Table 1). At the highest alarm cue concentration (1.0 μL^{-1}), upstream movement was significantly slower than all other concentrations. Median time of arrival at the highest concentration (1.0 μL^{-1}) was 40.92 min post-release, a decrease of 305–676% vs. other concentrations. Visual inspection of the rank series within each concentration (the basis of the Kruskal–Wallis test) suggests two patterns of interest (Fig. 5b). First, there was little distinction in time of arrival across the three lower concentrations for the first 50% of animals that moved upstream, with somewhat later arriving individuals more common during the control and 0.2 μL^{-1} treatments. Second, at the highest concentration of alarm cue, animals arrived consistently later (i.e., the earliest arriving individuals at high cue concentration were arriving later than the earliest individuals at low concentrations or when the cue was absent), suggesting the highest concentration affected all individuals exposed. Similar to the response in the lab, at the highest odor concentration several subjects per trial were observed exiting the holding cage and quickly moving downstream in an apparent attempt to avoid the area. These animals later returned upstream.

Experiment #3: Threat-sensitivity in a natural stream (full channel activation)

As in the half-channel experiment, the presence of the alarm cue at any concentration (vs. control) had no effect on the number of animals moving upstream (ANOVA, overall model $F_{5, 16} = 1.06$, $P = 0.44$; alarm cue concentration effect $F_{3, 16} = 0.44$, $P = 0.72$; Fig. 4a). Neither water temperature ($F_{1, 16} = 3.20$, $P = 0.10$) nor discharge ($F_{1, 16} = 0.01$, $P = 0.92$) elicited observable effects on the tendency to move upstream. In contrast to the half-channel treatments, there was no tendency to move on a preferred side of

Fig. 5 **a, c** Box-plots of the individual swim-up times (elapsed time of arrival at the PIT antennas) during the field experiments for each cue concentration ($0 \mu\text{L}^{-1}$ = solvent control) (A = half-channel activation, C = full channel activation). Different letters indicate statistically different times per post-hoc pairwise tests performed on rank data. **b, d** Cumulative proportion of animals detected at the PIT antennas as a function of time since release for each alarm cue concentration (B = half-channel activation, D = full channel activation). Only individuals that swam up to the PIT antennas are included to illustrate time-of-arrival differences



the channel when the alarm cue odor was distributed bank-to-bank (ANOVA, overall model $F_{5, 16}=0.39$, $P=0.84$; alarm cue concentration effect $F_{3, 16}=0.49$, $P=0.73$; Fig. 4b). Differences in the swim-up time across alarm cue concentrations were observed (Kruskal–Wallis, chi-square = 15.97, $P < 0.001$; Fig. 5a). Post-hoc tests revealed a significantly later time of arrival only for the highest concentration ($1.0 \mu\text{L}^{-1}$) versus control (Table 1). Median time of arrival at the highest concentration ($1.0 \mu\text{L}^{-1}$) was 27.25 min post-release, an increase of 119–154% versus other concentrations. At the control and low concentrations, times of arrival appeared later during the full-channel experiment (2014) versus the half-channel experiment (2012). There were differences in both water temperature (warmer in 2012: $^{\circ}\text{C}$, mean ± 1 se, 20.20 ± 0.85 in 2012, 17.54 ± 0.37 in 2014) and discharge (wetter in 2012: $\text{m}^3 \text{s}^{-1}$, 1.90 ± 0.075 in 2012, 1.31 ± 0.075 in 2014) across years that may have be

associated with the apparent difference (two-tailed t-tests for unequal variance: temperature, $t = -2.87$, $P < 0.001$, $df = 21$; discharge, $t = -5.54$, $P < 0.01$, $df = 30$).

Experiment #4: Olfactory organ sensitivity to alarm cue

Sea lamprey alarm cue elicited concentration-dependent electrophysiological responses in adult sea lamprey olfactory epithelia (Fig. 6). Exposure to increasing concentration of alarm cue induced increasingly larger magnitude olfactory responses. The olfactory organ was sensitive to alarm cue even at low concentrations given the detection threshold was determined to be less than $0.001 \mu\text{L}^{-1}$ (one-tailed paired t-test, $t = -3.0278$, $P = 0.0116$, $df = 6$).

Discussion

Establishing how invasive animals perceive and manage the threat of predation in nature is important to understanding how invaders rapidly establish and spread in unfamiliar environs. More broadly, it lends greater understanding to how perceived predation risk drives the evolution of movement strategies in prey. The results of this study illustrate how an invasive fish that does not exhibit natal homing or breeding site philopatry, and cannot defer reproduction to a later year, manages exposure to predation risk by modulating behavioral responses to an alarm cue during the spawning migration. The data suggest that migrating sea lamprey exhibit pronounced threat-sensitivity in space use, increasing their avoidance of areas activated with a conspecific alarm cue as the cue concentration increases. Although increasing the concentration of the alarm cue did not similarly affect the decision to move upstream in nature, sea lamprey did exhibit stronger avoidance of the alarm cue (vs. the laboratory trials), highlighting the importance of ecological and experimental contexts, and spatial scale, in the interpretation of antipredator behavior.

Threat-sensitive movement paths

Migratory sea lamprey exhibited threat-sensitive spatial avoidance of the chemical alarm cue both in lab and field environments. In the lab, extracts from skin and whole carcass elicited similar patterns in the proportional response. Though Bals and Wagner (2012) demonstrated the cue's presence in multiple tissues in sea lamprey, these data suggest the cue may be principally aggregated in the skin as has been demonstrated for other fishes (Chivers et al. 2007). In the field, the spatial avoidance response at high concentrations was stronger than in the lab, despite the fact that the fully-mixed concentration experienced at the holding cages in the natural setting was half that of the lab.

In nature, the antipredator response clearly entailed increasingly avoiding the area of the channel activated with the cue as concentration increased, in this case a simulated area of predation associated with one river bank. We suspect the animal's movement was guided by persistent spatial information in cue concentration. Specifically, the coupled longitudinal and lateral gradients in cue concentration likely allowed the animal to select upstream routes that

ensure reduced proximity to the threat. When seeking an odor's source, aquatic organisms frequently employ odor-gated rheotaxis, whereby the animal uses the edges of the odor plume to constrain lateral movement to stay within the plume, coupled with rheotaxis to move up-current to the source (Debose and Nevitt 2008). Sea lamprey are thought to use odor-gated rheotaxis when seeking attractive odors, including pheromones (Johnson et al. 2012). However, unlike many odor-tracking animals, sea lamprey have a single opening to the olfactory apparatus (i.e., a single sensor) and must move to assess spatial distributions in odor fields. Input from a single sensor can modulate effective tracking of odor plumes, as demonstrated in the nudibranch mollusk *Tritonia diomedea*, though that animal moves slowly relative to fishes and may be able to detect the chemical gradient via temporal averaging of the rhinophore's activity (McCullagh et al. 2014). When confronted with aversive odor plumes, many organisms move in opposition to the odor plume (negative chemotaxis) or flow. Such movements have been observed in *Drosophila* that dynamically anti-track turbulent aversive plumes (Wasserman et al. 2012; Gao et al. 2013). In the present study, it is unlikely that sea lamprey employed an aversive chemotaxis or rheotaxis in nature, as downstream movements were rare and short-lived, and increasing the concentration of the cue did not appear to alter the animal's decision to move upstream (though timing was affected, see below). It is also unlikely the animal maintained contact with the edge of the plume as it moved upstream, as that would ensure a close encounter with the source of the odor, and possibly the predator. Rather, it appears the animal may have moved to the opposite side of the channel, though we could not assess whether the movement path took it fully to the opposite bank. In rivers, sea lamprey regularly move in close contact with the substrate, and in proximity to the riverbank (Holbrook et al. 2015). Such movement paths may reduce the energetic cost of swimming, as the highest velocities in streams are near the surface and center of the channel, and provide easy access to stones for attachment during resting phases, as well as daytime refuge.

Table 1 Results of the post-hoc Rank-Means (RM) test of the times of arrival at the PIT antennas during each field experiment

Half-channel experiment				Full-channel experiment			
Conc	RM diff	Crit. value	Unadj. <i>P</i>	Conc	RM diff	Crit. value	Unadj. <i>P</i>
0 vs. 0.1	62.03	48.73	< 0.001	0 vs. 0.1	32.4	53.56	0.055
0 vs. 0.2	13.34	50.67	0.244	0 vs. 0.2	46.47	53.56	0.011
0 vs. 1.0	197.46	52.04	< 0.001	0 vs. 1.0	76.98	51.74	< 0.001
0.1 vs. 0.2	48.69	49.12	0.004	0.1 vs. 0.2	14.07	52.47	0.24
0.1 vs. 1.0	259.49	50.54	< 0.001	0.1 vs. 1.0	44.58	50.61	0.01
0.2 vs. 1.0	210.8	52.4	< 0.001	0.2 vs. 1.0	30.51	50.61	0.056

Conc. = concentrations being compared ($\mu\text{L L}^{-1}$); RM Diff. = the reported RM difference; Crit. Value = the critical RM value for that comparison. The unadjusted *P* value is reported. *P* values in bold indicate significantly different rank-means after adjustment for multiple comparisons

Threat-insensitive movement propensity

Whether the odor plume was laterally distinct or dispersed in the river channel, increasing the concentration of the alarm cue did not reduce the number of animals that chose to move upstream. The decision to continue upstream movement in the presence of a predation risk cue may be reasonable given the natural context of nocturnal migration in rivers. Damage-released alarm cues are released principally during predator attack (including handling and consumption). Later, during defecation, the cue may be released from the digestive system of the predator as a diet-released alarm cue (Chivers and Mirza 2001; Mirza and Chivers 2003). Regardless of route, once liberated the odorant(s) become dilute via mixing while flowing downstream, during which biochemical breakdown may occur (Sih et al. 2000; Peacor 2006; Wisenden et al. 2009). The result is a potentially short-lived signal that pulses downstream, eventually decoupling from the location of the attack. Several important implications arise from this circumstance. First, dilute cues may indicate distant risk, in space and/or time. In the absence of lateral gradients in cue strength, sea lamprey displayed weak behavioral responses to low concentrations in both lab and field, a circumstance recorded in other animal systems, including insects (Roux et al. 2014), amphibians (Buskirk and Arioli 2002; Fraker 2008), reptiles (Cisterne et al. 2014), and gastropods (Rochette et al. 1997). It is important to note, however, that it is problematic to judge what constitutes an ecologically relevant 'weak' signal as we are aware of no studies that have attempted to quantify the amount

of alarm cue released from a single predator attack, and the chemical nature of fish alarm cues remains largely unknown. The active time of chemical alarm cues appears generally limited, with half-lives ranging from 0.2 to 14.4 h when not coupled with predator odor (van Buskirk et al. 2014). Wisenden et al. (2009) suggested a short half-life observed for some fish alarm cues implicates proteins as the molecular class. The sea lamprey cue may prove longer-lived; the process of Soxhlet extraction is destructive, heating the tissue to $>80\text{ }^{\circ}\text{C}$ for several hours in the present study, precluding the use of a protein. Despite that extraction process, the cue was abundant in the extract.

Although the decision to swim upstream was ultimately unaffected by alarm cue concentration, there was evidence for later arrival at the highest alarm cue concentration. During the high-concentration trials, we noticed a tendency in some animals to immediately exit the holding cage and move rapidly downstream, akin to the response of the laboratory animals when they encountered the plume when moving across the raceway. We suspect this may have been an artefact of suddenly exposing them to the odor while confined during the acclimation period, which may have simulated the timing of a predator attack. At the highest concentration, the caged animal may have perceived the attack to be nearby, if responses are driven by graduated activity in the olfactory receptor (Lastein et al. 2008). The difference in timing was less in the full-channel experiment, which occurred two years later and during cooler, drier conditions. However, there may be strong selective pressure to respond to the cue by continuing upstream

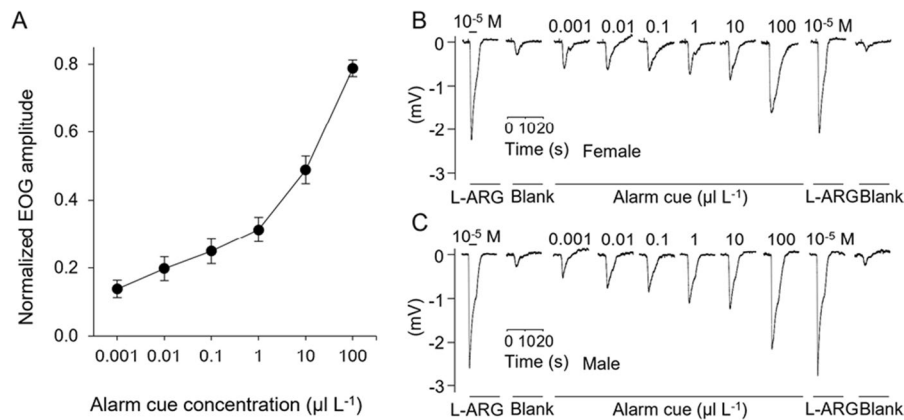


Fig. 6 Sea lamprey alarm cue elicited olfactory responses measured with electro-olfactogram (EOG). **a** Mean normalized EOG amplitude (± 1 se) of adult sea lamprey exposed to alarm cue ($n=7$). The response amplitude was blank-corrected (vehicle) and normalized to the response amplitude of 10^{-5} M L-arginine (L-ARG). Note the alarm cue corrections are plotted

on a log scale. Representative electro-olfactogram traces of a **b** female and **c** male sea lamprey olfactory epithelia exposed to 10^{-5} M L-ARG and increasing concentrations of alarm cue. The bar above the L-ARG trace (Left) represents the 4 s duration of stimuli exposure

movement, especially at higher concentrations and in the presence of a lateral gradient in cue strength. By progressing upstream immediately, the animal may be more likely to accurately detect the location in which the attacks are occurring (i.e., downstream displacement of odor plume's head has not occurred), and the predator may be engaged with the current prey item, reducing the likelihood of attack. Waiting to move upstream until the odor is no longer detectable reduces the information content of the system. Moving upstream quickly may also preserve the animal's ability to perform a chemical 'predator inspection'. Several fishes have demonstrated the ability to learn the odors of novel predators when the predator's odor is combined with a conspecific alarm cue (Chivers and Smith 1998; Lönnstedt et al. 2012). If lamprey similarly can learn the identity of predators active in the stream through which they are migrating, they will be able to assess risk in the absence of the alarm cue when moving further upstream. It is important to note this animal is nocturnal. Nocturnal activity likely reduces both the predator field (number of species) and predation intensity in riverine systems, as many piscivorous fishes are active feeders only during daylight hours or crepuscular periods (Helfman 1986), though there are notable exceptions (e.g., catfishes, Pohlmann et al. 2001; Boulêtreau et al. 2020). Further, the timing of sea lamprey movements in rivers is responsive to water temperature, generally increasing

activity on nights experiencing warmer temperatures (Applegate 1950; Binder and MacDonald 2008). Sea lamprey swimming efficiency and burst-swimming intensities are improved at the warmer temperatures in the range observed in this study (Beamish 1974). The animal may modulate its movement (day to day) in partial response to the ability to burst-swim away from predator cues when suddenly encountered.

During the field experiments, sea lamprey were navigating through a semiochemical landscape typical of areas low in the spawning watershed. Larval odor was present and dispersed (bank-to-bank), indicating the presence of suitable spawning habitat upstream, and likely stimulating upstream movement towards that location. Over the two-year period of the experiments it is probable the quantity of odor produced by the larvae increased with somatic growth, and perhaps additional recruitment (Sorensen and Vrieze 2003), leading to a more concentrated odor during the second experiment. However, we observed no apparent effects of the putatively greater larval odor on either upstream movement, or the effect of alarm cue application on upstream movement. Unlike the alarm cue, migrating sea lamprey do not exhibit strong responses to lateral differences in larval odor intensity by moving through waters with the greatest concentration (Wagner et al. 2009). They do, however, respond strongly to lateral gradients in odor detectability (presence/absence: Bjerselius

et al. 2000; Wagner et al. 2006, 2009; Neeson et al. 2011), but only when the complete mixture of odorants is present (Meckley et al. 2012). This is reasonable, as the alarm cue is associated with a discrete point in space, whereas the larval odor is associated with a dispersed larval population. What might we expect migrants to do when confronted with a collision of spatially explicit risk and opportunity information? Specifically, at the confluence of two streams where larval habitation is confined to one tributary, and where predation on migrants is limited to that same stream, causing the odors to overlap completely. Here, selection of the tributary likely to support offspring and contain potential mates is also the risky choice. We might expect a change in risk-management currency to occur. Luhring et al. (2016) recently demonstrated that when a whole river is activated with the alarm cue, sea lamprey increase their ground speed while continuing to move upstream. In effect, when the odor was not spatially avoidable in the river, the animals changed from a spatial currency (movement path) to a temporal one (movement speed) to minimize their exposure to predation risk. Because the channel dimensions (depth, distance to shore) are typically smaller high in the watershed, the animal's sense of its vulnerability should be greater, perhaps leading to behavior that is more cautious.

Olfactory sensitivity to alarm cue

The sea lamprey olfactory system does indeed have the ability to perceive the intensity (concentration) of the odorants that compose the alarm cue. Using electro-olfactogram recordings, we observed an exponential relationship between alarm cue concentration and electrophysiological response, which is a phenomenon that has been previously documented in sea lamprey for attractant pheromones (e.g., Siefkes and Li 2004; Li et al. 2018). The EOG recordings confirmed the olfactory epithelium was sensitive to the alarm cue concentrations used in laboratory and field behavioral experiments ($0.1\text{--}1.0\ \mu\text{l L}^{-1}$). The olfactory response detection threshold to alarm cue was two orders of magnitude lower than the lowest concentration applied in behavioral experiments, suggesting that the compounds composing the alarm cue are potent odorants. The normalized EOG responses of migratory-phase males and females exposed to conspecific alarm cue were comparable; however,

this does not preclude the possibility that behavioral responses to alarm cue may differ between sexes or life stages due to differences in signal integration and processing in the brain. As Bals and Wagner (2012) observed, female sea lamprey cease responding to the alarm cue after maturation, whereas males continue to avoid the odor in laboratory settings. EOGs measure the activity in the peripheral olfactory organ after odor stimulation, but they do not provide information about the central processing of the chemosensory cue that mediate behavioral responses. Both sexes conceivably benefit from the ability to detect predation risk via alarm cue and subsequently modulate their behavioral responses.

The neurobiological and sensory mechanisms that regulate the expression of threat-sensitive alarm responses in fishes remain largely unresolved, though evidence is available from a few cases (Døving and Lastein 2009; Wisenden 2015). Olfactory sensory neurons in teleost fishes exhibit three polymorphisms (Hamdani and Døving 2007), an arrangement mirrored in lampreys (Laframboise et al. 2007). Ciliated olfactory sensory neurons have been associated with the alarm response in the olfactory epithelium of fathead minnows (*Pimephales promelas*) and yellow perch (*Perca flavescens*) (Dew et al. 2014), and Crucian carp (*Carassius carassius*, Hamdani and Døving 2003). In carp, the conspecific alarm odor induces activity in the medial region of the olfactory bulb in proportion to the concentration of the extracted skin odor (Hamdani and Døving 2003), with diminishing activity in response to skin odors from more distant heterospecifics (Lastein et al. 2008). Reduced behavioral response to the alarm cue of heterospecifics is frequently observed, suggesting ciliated olfactory neurons are specifically tuned to the odor of conspecifics, but overlap in the set of odorants occurs in related species, at least at the family level (Brown et al. 2010; Júnior et al. 2010; Lastein et al. 2012; Mirza and Chivers 2001; Mitchell et al. 2012). This pattern also occurs in sea lamprey (Hume and Wagner 2018). Thus, mixtures of odorants have been implicated as the explanation for the differential intensity of conspecific vs. heterospecific responses (Mensch et al. 2022). Because experiments typically use extracted odors, we do not yet know if threat-sensitivity arises from reduced activity in the sensory neurons for all of the molecules in the mixture (the concentration effect), or if the animal detects a

reduced set of odorants at low concentration due to one or more of the molecules being diluted to below the detection threshold (the detection effect). In the latter case, the subordinate compounds in the mixture must either be produced at a lower rate, break down at a more rapid rate, or have fewer receptors on sensory neurons that complement the particular compound. Interestingly, differing detection thresholds across the compounds in a mixture would also allow the animal to estimate its proximity to the threat in time or space by comparing the relative activity across sensory neurons tuned to different compounds vs. their absolute activities.

Implications for the sea lamprey invasion of the Great Lakes

Sea lamprey rapidly and extensively invaded the upper Great Lakes after barriers to dispersal were removed (Eshenroder 2014; Hansen et al. 2016). Two features of their response to alarm cue may have contributed to their remarkable success. First, four species of lamprey native to the Great Lakes basin release alarm cues that elicit avoidance responses from sea lamprey (Hume and Wagner 2018). Second, Luhring et al. (2016) observed increased migration into a river when it was activated with sea lamprey alarm cue, postulating it indicated that other migrants (potential mates) were also selecting that watershed. Given how effectively sea lamprey both perceived relative risk via alarm cue, and navigated around the source of that risk (present study), alarm cues from native lamprey may have facilitated both predator avoidance and the location of suitable spawning habitat during the establishment phase of the invasion. The regularity of these behavioral responses may also contribute to the creation of effective behavioral manipulation practices to achieve control. Recent field studies demonstrate that application of alarm cue to one half of a river channel, simulating shoreline attacks, will effectively guide migrating sea lamprey toward traps placed in association with the opposite bank (Hume et al. 2015, 2020a). A similar manipulation is being developed to create a selective fish passage device that would prevent sea lamprey from moving through fishways, facilitating reconnection of Great Lakes streams to the lakes (Hume et al. 2020b; Zielinski et al. 2020). Each of these manipulations are more likely to be successful when performed during

the spawning migration, as the final stages of sexual maturation eliminates the alarm response in female sea lamprey (Bals and Wagner 2012).

Conclusion

Establishing how invasive animals perceive and manage the threat of predation in nature is important to understanding why certain species rapidly establish and spread in unfamiliar environs where others do not. More broadly, it lends greater understanding to how perceived predation risk drives the evolution of movement strategies in prey. During the sea lamprey's terminal reproductive migration, it appears the alarm cue strongly affects decisions regarding how to move, but not decisions regarding whether to move, and these decisions are in direct response to varying signal output from the olfactory organ. Through the lens of foraging-vigilance trade-offs, movement in response to encounter with an alarm cue has generally been associated with risky behavior in fishes, as the predator may become alerted to the prey's presence and position, increasing the likelihood of attack. However, flowing water imposes a useful spatial structure on odor plumes, and migrating animals may use this structure to discern and avoid relatively risky areas through sensory and behavioral mechanisms typically associated with navigation and cognition. Thus, the utility of movement in the presence of chemically perceived predation risk is a function of both the task (migration vs. foraging) and the circumstance (spatial scale, fluid dynamics). Ultimately, we must begin to experimentally manipulate combinations of odors, and cues received on other sensory modalities, to map how animals evaluate both the presence of risk (via cues), and vulnerability to that risk (aka safety, perhaps related to spatial arrangements of information, habitat, and social elements), in light of internal drivers of risk tolerance (e.g., residual energy stores). Such studies will afford us better opportunities to investigate and model the impact of perceived predation risk on the evolution of decision-making strategies in the complex natural circumstances that are typically excised from laboratory investigations. Sea lamprey may prove a particularly useful model for such studies.

Acknowledgements Access to laboratory raceways and test subjects was generously provided by the Hammond Bay Biological Station (U.S. Geological Survey) and the U.S. Fish and Wildlife Service. We are thankful for laboratory and field assistance provided by Skye Fissette, Carrie Baker (née Kozel), Thomas Luhring, Trevor Meckley, Amanda Pomranke, Sarah Ptasznik, and Nick Snowgold.

Author contributions All authors contributed to study design and completion. The manuscript was principally compiled and written by CMW with significant contributions and revisions from each co-author. CMW designed Experiments #1–3, with contributions from JDB and GFB. JDB executed and analyzed Experiments #1–2. GFB executed and analyzed Experiment #3. AMS and MEF designed, analyzed, and interpreted the electro-olfactogram study (Experiment #4).

Funding Financial support for the work reported here was provided by the Great Lakes Fishery Commission Sea Lamprey Research Program.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Data availability All analyses are reproducible with the data provided in “Appendix 1” in the supplementary materials.

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