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A deathly odor suggests a new sustainable tool for controlling a costly invasive species

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Abstract: Here we confirm a long-standing anecdotal observation; the sea lamprey (*Petromyzon marinus*) actively avoids the odor emitted by decaying conspecifics. We extracted the semiochemical mixture produced by the putrefying carcasses of sea lampreys via Soxhlet extraction in ethanol and exposed groups of 10 migratory-phase lampreys to either the putrefaction extract ($N = 8$) or an ethanol control ($N = 8$) in a laboratory raceway. Sea lampreys rapidly avoided the putrefaction odor while exhibiting no response to the ethanol control. This response was elicited with a diluted mixture (1:373 000) and was maintained for 40 min (the duration of exposure), after which the lampreys quickly returned to their nominal distribution. The ease with which this odor is obtained, and the rapid and consistent behavioral response, suggests the substance will prove useful as a repellent in the sea lamprey control program carried out in the Laurentian Great Lakes.

Résumé : Nous confirmons ici une observation anecdotique de longue date, voulant que la grande lamproie marine (*Petromyzon marinus*) évite de manière active l'odeur émise par les cadavres en décomposition des lamproies de même espèce. Nous avons extrait le mélange sémiochimique produit par des carcasses en putréfaction de grandes lamproies marines par extraction Soxhlet en éthanol; nous avons ensuite exposé des groupes de 10 lamproies en phase migratrice à soit l'extrait de putréfaction ($N = 8$), soit à un témoin d'éthanol ($N = 8$) dans un canal de nage en laboratoire. Les grandes lamproies marines évitent rapidement l'odeur de putréfaction, alors qu'elles n'ont aucune réaction au témoin d'éthanol. Cette réaction est provoquée par un mélange dilué (1:373 000) et se maintient pendant 40 min (durée de l'exposition), après quoi les lamproies retrouvent rapidement leur répartition normale. La facilité avec laquelle on peut obtenir cette odeur et la réaction comportementale constante qu'elle provoque font croire que cette substance s'avérera utile comme répulsif dans le programme de lutte contre la grande lamproie marine en cours dans les Grands Lacs laurentiens.

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Introduction

When an organism's lifetime fitness is bound to a single reproductive episode, the ability to locate a habitat that is favorable for the acquisition of mates and subsequent deposition of offspring becomes a vital enterprise. The sea lamprey, *Petromyzon marinus*, a devastating invasive species in the Laurentian Great Lakes, relies on the odor emitted by overlapping generations of larvae to navigate into streams with suitable spawning and rearing habitat (Sorensen and Hoye 2007). Upon arrival, another odor emitted by mature males lures females onto nests to complete spawning (Johnson et al. 2009). These pheromones have proven to be powerful attractants when applied to stream water, and considerable effort has been expended to identify novel ways to exploit this communication system to achieve pest management purposes (Johnson et al. 2009; Wagner et al. 2009). To date, those efforts have been fully focused on the manipulation of attraction.

The sea lamprey reproductive migration is also notable in that it is nocturnal. This tendency is consistent with the avoidance of risk and reasonable given that a migrant is required to enter increasingly narrow and shallow waterways. Rich arrays of olfactory alarm cues are used by aquatic organism to assess risk (Ferrari et al. 2010). Among these are odors emitted by dead and decaying conspecifics (so-called necromones; Yao et al. 2009). Recognition and avoidance of the death odor may commonly serve to reduce risks associated with predators or sources of contagion in aquatic environments (Brown 2003). For a migrating sea lamprey, this odor may also indicate the cessation of spawning, particularly in the absence of the reproductive pheromone.

Based largely on anecdotal information, the existence of a novel alarm cue, possibly a necromone, has recently been speculated for the sea lamprey (Imre et al. 2010). If elucidated, a lamprey alarm cue could prove useful in pest management that employs behavioral manipulation via chemical stimuli. Here, we report a laboratory experiment that demonstrates the sea lamprey is chemically aware of, and actively

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avoids, the odor emitted by decaying conspecifics. We discuss the implications of this finding for the rapid development of novel pest management practices based on the simultaneous use of attractants and repellents.

Materials and methods

Experimental subjects

We obtained adult migratory-phase sea lampreys from the US Fish and Wildlife Service, who trapped the animals as part of the their annual control program. All subjects came from two tributaries to Lake Huron, the Ocqueoc and Cheboygan rivers in northern Michigan, and were captured during May 2010. After capture, we placed the lampreys into flow-through tanks receiving Lake Huron water (5–10 °C depending on date with 100% exchange every 2 h) at the nearby US Geological Survey Hammond Bay Biological Station (Millersburg, Michigan). Prior to use, we selected lampreys in visibly good condition and moved them into pretrial holding tanks receiving the same Lake Huron water as the experimental raceway. Use of sea lampreys was approved by the Michigan State University Institutional Animal Care & Use Committee (AUF No. 02/10-020-00).

Collection of the semiochemical mixture

We extracted the putative alarm odor from individual lamprey carcasses at four time points postmortem (0, 24, 48, and 120 h) using a 1 L 71/60 Soxhlet apparatus with a six-bulb, water-cooled Allihn condenser and a 3 L solvent reservoir heated with a hemispherical mantle. Aerobic decay of each carcass took place under laboratory conditions in a 1 L high-density polyethylene bottle at room temperature. Prior to extraction we washed the carcasses with 100 mL of solvent (50% *w*/*w* solution of 190 proof ethyl alcohol and deionized water) and set aside the rinsate. We then mounted a single decayed carcass onto a glass extraction thimble and added 800 mL of the solvent solution. We maintained the solvent temperature in the reservoir at 65–80 °C and cycled the extractor three times per carcass. At the conclusion of the extraction we allowed the solvent to cool to room temperature before combining it with the rinsate to form the time-specific putrefaction extract. Because we did not know when during aerobic decay the compound(s) responsible for the behavioral response are produced or released, we formed the final test mixture by combining 500 mL from each of the four time-specific extracts (0, 24, 48, and 120 h), hereafter referred to as the putrefaction extract.

Behavioral assay

To determine whether exposure to the putrefaction extract repels sea lampreys, we examined space use by 16 groups of 10 migratory-phase adult sea lampreys (160 individuals total, each used once) in a 5.00 m × 1.85 m section of laboratory raceway receiving a continuous discharge of 680 L·min^{-1} at a depth of 20 cm (Fig. 1). We examined the lampreys' movements in response to two treatments: (*i*) the composited mixture of time-specific putrefaction extracts (described above) and (*ii*) an ethanol control. We completed eight replicates of each treatment. All trials took place 1 h after sunset.

Four hours prior to the start of a trial, we placed a new group of 10 migratory-phase adult sea lampreys (five male, five female) into a holding cage in the downstream portion of the raceway to allow for acclimation to test conditions. Each trial was 100 min in duration and comprised three periods: (*i*) a 20 min pretrial observation period, where lampreys were released from the cage and allowed to swim freely; (*ii*) a 40 min stimulus period, where an odor was pumped into one side of the channel; and (*iii*) a 40 min post-trial observation period that began after stimulus introduction was ceased. We mixed 40 mL of a stimulus (ethanol or putrefaction extract) with 400 mL of raceway water and pumped the mixture at a rate of 10 mL·min^{-1} into one side of the channel using a laboratory peristaltic pump. We alternated the side of the channel receiving the stimulus across replicates within each treatment (four left-side trials and four right-side trials for each treatment).

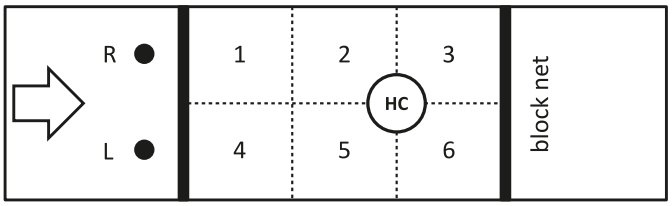
Data collection and analysis

We illuminated the raceway with infrared lights and recorded lamprey movements with an overhead night-vision video camera. At the conclusion of the experiment we reviewed the video from each trial and assigned each lamprey a position on a six-square grid every 30 s based on the location of the animal's head (Fig. 1). From these data we calculated the proportion of lampreys on the stimulus and nonstimulus side of the raceway during each period (prestimulus, stimulus, and poststimulus). We used a general linear model (GLM; SYSTAT v.12, Systat Software Inc., Chicago, Illinois) to ascertain whether differences in the proportion of lampreys on the scented side of the channel (dependent variable) vs. the unscented side were a function of treatment (ethanol or putrefaction extract), time period (prestimulus, stimulus, or poststimulus), or the side of the channel receiving the scent (right or left). All two-way interactions were included. Because we predicted (*i*) strong avoidance of the putrefaction extract during the stimulus period, (*ii*) no change in distribution in response to exposure to the ethanol control, and (*iii*) no effect of the side of the channel receiving the scent, we expected significant main effects of treatment and time period and a significant interaction between the two. To ascertain whether the differences detected in the GLM were due to avoidance during the stimulus period, we performed an individual analysis of variance (ANOVA) for each treatment, testing for differences between time periods with Tukey's honestly significant difference test (HSD, $\alpha = 0.05$).

Results

As predicted, there were significant treatment ($F_{[1,38]} = 53.8$, $P < 0.001$) and time period main effects ($F_{[2,38]} = 53.0$, $P < 0.001$) and a treatment × time period interaction ($F_{[2,38]} = 46.5$, $P < 0.001$) in the GLM. The side of the tank that received the stimulus (main effect, $F_{[1,38]} = 1.1$, $P = 0.29$) and all other two-way interactions were not significant (treatment × side, $F_{[1,38]} = 3.7$, $P = 0.06$; time period × side, $F_{[2,38]} = 0.937$, $P = 0.40$). The behavioral responses of migratory phase sea lampreys in the raceway were consistent with a strong avoidance of the putrefaction extract (Fig. 2). During the ethanol control trials, the proportion of lampreys that chose the scented side of the raceway varied between 45% and 55%, with no significant preference for either side (ANOVA, $F_{[2,21]} = 0.54$, $P = 0.59$; Fig. 2*a*). Conversely, ad-

Fig. 1. Overhead view of the raceway setup for behavioral avoidance testing. Water flowed from left to right (arrow), and odors were introduced from peristaltic pumps (solid circles) on the right (R) or left (L) side of the channel placed upstream of a block net (thick vertical line). The bottom of the raceway was lined with white plexiglass and marked into six equal-sized grid squares to ascertain space use (dotted lines and numbers). At the start of a trial, subjects were released from the holding cage (HC) and recorded with an overhead video camera.



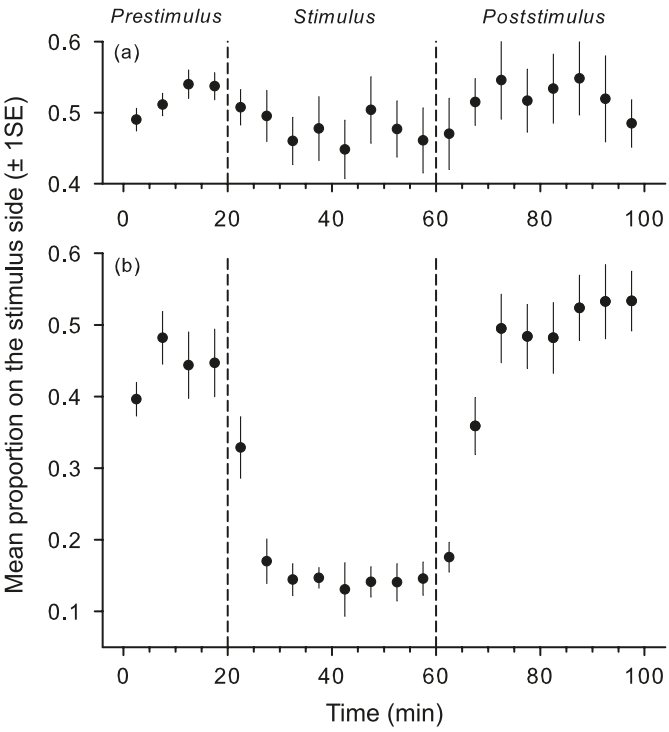
dition of the putrefaction extract quickly induced strong avoidance of the scented side during the 40 min stimulus period (ANOVA, $F_{[2,21]} = 29.6$, $P < 0.001$; Tukey's HSD, prestimulus vs. stimulus, $P < 0.001$, poststimulus vs. stimulus, $P < 0.001$; Fig. 2*b*). On average, lampreys spent 12%–15% of the time on the scented side during the stimulus period, often darting back to the unscented side and quickly swimming downstream. Lampreys exposed to the putrefaction extract returned to the nominal prestimulus distribution 6–8 min after odor introduction ceased (Tukey's HSD, prestimulus vs. poststimulus, $P = 0.99$).

Discussion

There is considerable evidence that risk-indicating olfactory cues are used by many aquatic species to assess potential fitness costs associated with space and resource use (Lima and Dill 1990; Brown 2003). Laframboise et al. (2007) recently described three distinct olfactory sensory neuron morphotypes intermingled in the sea lamprey olfactory organ. This arrangement closely parallels that of teleost fishes (Hamdani and Doving 2007), where the axons of each morphotype converge to a separate and specific region of the olfactory bulb, and three distinct connections to the brain arise, thereby mediating three fundamental behavioral processes. One pathway is tuned to reproductive pheromones, another to food odors, and the third to social cues and alarm substances. The similarities revealed in the sea lamprey olfactory architecture imply a reliance on odors for fundamental processes beyond reproduction, and the behavioral evidence reported here suggests the avoidance of harm is also mediated by olfaction in the sea lamprey.

We observed sea lampreys actively avoiding a dilute odor of decaying conspecifics (≈ 1:373 000, assuming full mixing into one half of the channel). By avoiding the odor of decaying conspecifics during the annual spawning migration, we hypothesize that sea lampreys may be alleviating one or more of three circumstances that potentially have strong negative effects on reproductive success: (*i*) entering streams where spawning has already ceased (if combined with the absence of reproductive pheromones), (*ii*) movement through areas of the watershed where terrestrial and (or) aquatic predators are killing migrating lampreys, and (*iii*) deposition of offspring in streams where high larval mortality is occurring.

Fig. 2. Mean (± 1 SE) proportion of lampreys on the scented side of the channel for ethanol control (*a*) and putrefaction extract (*b*) trials ($N = 8$). Sea lampreys actively avoided the putrefaction extract but exhibited no response to the ethanol control.



Regardless of the ultimate function, the confluence between reliance on innately acquired responses to olfactory stimuli and a geographic feature of the sea lamprey migration reveals a unique opportunity to achieve sea lamprey control with a natural repellent. This rationale was first proposed by James Miller (Michigan State University, East Lansing, Michigan) and is also mentioned in Imre et al. (2010).

The push–pull approach (Miller and Cowles 1990) involves the use of behavior-modifying chemical stimuli to manipulate the distribution of herbivorous insects and thereby reduce crop damage. Repellents "push" pests away from sites needing protection and attractants "pull" them into areas where they become vulnerable to pesticide application. As sea lampreys migrate, they transition from unconstrained movements in a three-dimensional environment (lake or ocean) to constrained movements in a network of stream channels. As noted previously, migrating sea lampreys appear to choose a river based on the emission of larval odor (Sorensen and Hoye 2007; Wagner et al. 2009) and are not preconditioned to enter the natal stream (Waldman et al. 2008). Because migrants attempting to move into spawning grounds located high in the watershed must first pass through the lower river channels, they sequentially pass bifurcations in the network. Thus, we may divert migrating lampreys away from a repellent-treated tributary and into the adjacent fork if activated with the migratory pheromone. Because successful blockage at any point low in the network protects all areas upstream of the node, a large fraction of the watershed may be blocked off with applications at a few sites, greatly limiting the amount of spawning area available and thereby the area in need of subsequent pesticide treatment. The success

of the push–pull manipulation, whether perpetrated at the river mouth or at a branch higher in the watershed, will likely rely on providing the lampreys with a simultaneous and simple choice between two options, one noxious and one attractive.

Existence of the sea lamprey alarm substance presents two substantial opportunities. Scientifically, it may represent a habitat-quality cue that mediates reproductive decision making by an ancient migratory species: a potentially novel use of a substance typically viewed as an indirect predator cue. Second, because it can be cheaply and rapidly collected, its utility in pest management may be verified prior to undertaking the costly and time-intensive process of isolation and full chemical elucidation.

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