



Fatty-acid derivative acts as a sea lamprey migratory pheromone

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Olfactory cues provide critical information for spatial orientation of fish, especially in the context of anadromous migrations. Born in freshwater, juveniles of anadromous fish descend to the ocean where they grow into adults before migrating back into freshwater to spawn. The reproductive migrants, therefore, are under selective pressures to locate streams optimal for offspring survival. Many anadromous fish use olfactory cues to orient toward suitable streams. However, no behaviorally active compounds have been identified as migratory cues. Extensive studies have shown that the migratory adult sea lampreys (*Petromyzon marinus*), a jawless fish, track a pheromone emitted by their stream-dwelling larvae, and, consequently, enter streams with abundant larvae. We fractionated extracts of larval sea lamprey washings with guidance from a bioassay that measures in-stream migratory behaviors of adults and identified four dihydroxylated tetrahydrofuran fatty acids, of which (+)-(2*S*,3*S*,5*R*)-tetrahydro-3-hydroxy-5-[(1*R*)-1-hydroxyhexyl]-2-furanoctanoic acid was shown as a migratory pheromone. The chemical structure was elucidated by spectroscopies and confirmed by chemical synthesis and X-ray crystallography. The four fatty acids were isomer-specific and enantiomer-specific in their olfactory and behavioral activities. A synthetic copy of the identified pheromone was a potent stimulant of the adult olfactory epithelium, and, at 5×10^{-13} M, replicated the extracts of larval washings in biasing adults into a tributary stream. Our results reveal a pheromone that bridges two distinct life stages and guides orientation over a large space that spans two different habitats. The identified molecule may be useful for control of the sea lamprey.

chemical ecology | anadromous migration | olfaction | animal behavior | Agnatha

Fish and birds use olfactory cues for spatial orientation in fluid mediums over a wide range of scales (1). A well-known example is the olfactory input that provides indispensable information for anadromous fish to orient toward suitable spawning streams during their spawning migration, which has been suspected for centuries and demonstrated repeatedly in a wide range of species over the last seven decades (2, 3). Two types of olfactory cues have been posited (and proven) to guide stream selection during upstream movement of anadromous adults (3). In several salmon species, odorants originating from natal streams have been shown to guide home stream search over hundreds of kilometers (2). In the sea lamprey, pheromones emitted by the larvae guide migration of adults toward spawning grounds (3, 4). Along the Atlantic coast, sea lamprey historically ascended up to 850 km to reach spawning grounds in large rivers in Europe (5). However, no compounds have been definitively identified as a natural olfactory cue for fish migration, which hinders further elucidation of olfaction-based orientation and navigation of fish over large spatial scales on the order of tens to hundreds of kilometers.

The sea lamprey is a model species in the quest to identify a migratory pheromone because preponderant evidence indicates larval chemicals heavily influence selection of spawning streams by migratory adults (6). This jawless fish develops through distinct larval, juvenile, and adult stages (*SI Appendix, Fig. S1*). The

larvae spend 3–15 y in freshwater streams before metamorphosing into juveniles that migrate to the Atlantic Ocean or a Laurentian Great Lake (Lake Superior, Michigan, Huron, Erie, or Ontario) and parasitize on large fish for ~1.5–2.5 y. Finally, the adults migrate into streams to reproduce in the spring (7). Adult migrants are highly selective of streams to enter; in the Great Lakes basin, they use only 8% of the roughly 5,000 tributary streams as spawning habitat (8, 9). Further, sea lampreys do not home to their natal streams in the Laurentian Great Lakes (10) or in the Atlantic Ocean (11). Rather, they selectively enter streams with high densities of lamprey larvae (9) by tracking larval odors (9, 12). Once in spawning streams, larval odors stimulate upstream swimming and induce preference in migratory but not in sexually mature adults (13). Evidently, there is a larval pheromone that guides adult sea lampreys in their migration to spawning grounds.

A major impetus for identifying the migratory pheromone in sea lampreys is its potential application in population management. After invading the Laurentian Great Lakes, the sea lamprey populations thrived, causing catastrophic damage to the fisheries and the ecosystem (14). Sea lamprey predation has remained a primary cause for the mortality of large-sized fishes in the Laurentian Great Lakes, even after decades of lampricide application that has held sea lamprey populations in check (15). Ironically, the sea lamprey populations are imperiled in its native

Significance

Anadromous fishes are those that migrate from the ocean into freshwater to reproduce and need to orient toward a suitable spawning stream or risk leaving no offspring. These migrants are known to use olfactory cues to guide stream selection, but the cues' identities are not known. Incidentally, it is well-known that stream-dwelling larval sea lampreys emit a pheromone that guides conspecific adults ascending freshwater streams. We identified this pheromone as a fatty-acid derivative that attracts migratory adults into the baited channel in a natural spawning stream. These results illustrate an olfactory mechanism whereby lampreys reliably identify and orient toward a proven spawning ground over a large spatial scale and implicate a potential strategy for sea lamprey control.

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range and even considered threatened in several European countries (15). The migratory pheromone, once identified, is potentially useful for both control and conservation of sea lamprey populations (6, 15).

Previous studies identified several larval bile acids that attracted migratory adult sea lampreys in laboratory mazes but not in natural spawning streams (8, 12, 16). Two bile acids, petromyzonamine disulfate (PADS) and petromyzosterol disulfate (PSDS), isolated from extracts of larval washings, induced preference behaviors in a maze (17, 18), but this preference was not replicated in an identical maze (19). In two separate lamprey spawning streams, combinations of PADS and PSDS did not induce migratory behavior in migratory adults, while extracts of washings of the larvae did (20, 21). Furthermore, PADS and PSDS together did not increase the likelihood of river entry by the migrants (22). Hence, washings of larval sea lampreys must contain compounds other than PADS and PSDS that induce migratory behaviors and guide stream selection of conspecific adults (18).

In this study, we sought to identify the larval pheromone that aids stream selection of migratory adult sea lampreys. Pheromones are anonymous chemical signals that elicit stereotyped reactions in conspecifics (23, 24). We reasoned that a bioassay carried out in a natural spawning system to track migratory behaviors over a large spatial scale would be imperative to guide the fractionation for the active compound(s). With this approach, we isolated and identified four dihydroxylated tetrahydrofuran fatty acids from extracts of larval sea lamprey washings and found that one of the compounds replicated the activity of the crude extracts in biasing the migratory adults into a treatment channel in a spawning stream. We conclude that this compound

[(+)-(2*S*,3*S*,5*R*)-tetrahydro-3-hydroxy-5-[(1*R*)-1-hydroxyhexyl]-2-furanooctanoic acid (25)] is a component of the migratory pheromone of the sea lamprey and suggest that it be called (+)-petromyric acid A [(+)-PMA].

Results

Bioassay-Guided Fractionation Traced the Migratory Pheromone Activity to a Single Fraction. We developed a robust behavioral assay that determined the odor preferences of migratory adult sea lampreys in a natural stream. Migratory adult sea lampreys exposed to odors of conspecific larvae exhibit a robust preference behavior after swimming upstream to a confluence in spawning streams (13, 20). To track this behavior, we used a large-scale in-stream bioassay that allowed migratory adults to move upstream for about 200 m and then enter one of two similar channels (20, 26) (Fig. 1*A*). Previous studies conducted in this same system showed that a 200-m stretch ensures the treatment odorant is adequately mixed at our target stream concentration by the time it reaches the downstream point where test subjects are released (27). All tests were carried out at night because migratory adults are nocturnal (28, 29). In May 2009, we further validated this bioassay by testing larval washing extracts (LE) over a wide range of concentrations (*SI Appendix, Table S1*). We found that 87% of the test subjects that swam upstream for 200 m subsequently entered the channel treated with LE (with PADS reaching 5×10^{-14} M in the test stream), and 13% entered the vehicle (methanol) control channel ($P < 0.001$; *SI Appendix, Table S1*). We chose LE with PADS reaching 5×10^{-14} M in the test stream as the positive control in subsequent bioassays. As an

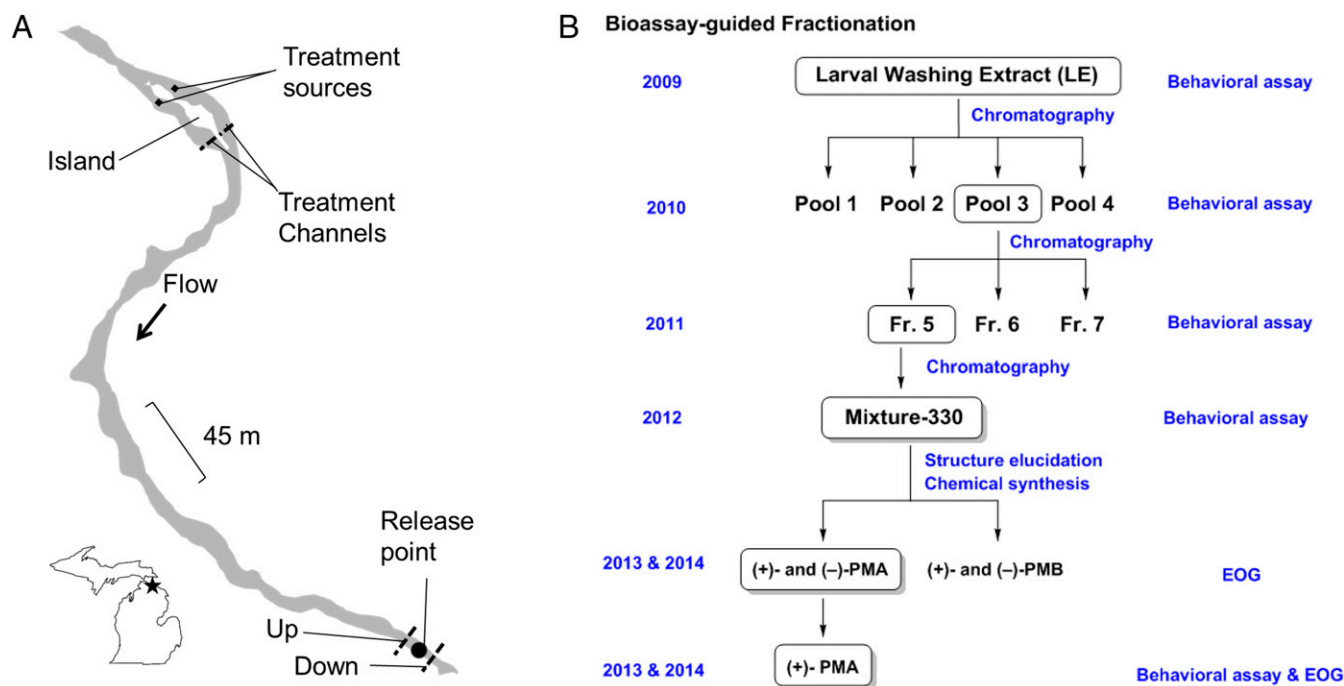


Fig. 1. Schematic of the field site in a 250-m-long section of the Upper Ocqueoc River, Millersburg, MI, used for behavioral assays that guided the stepwise fractionations for the active compound. (*A*) The river is shown in gray shade. The downstream animal release point is shown, along with the two passive integrated transponder (PIT) antennas (shown in dashed lines) used to monitor the response variable "Upstream movement" (no. of subjects moving upstream 200 m to the confluence of the two subchannels after release) in Table 1 and *SI Appendix, Table S1*. The upper 45 m of the section is naturally bifurcated by an Island. The response variable "Selection of treatment channel" [no. of subjects that moved upstream and entered either the treatment channel or blank (vehicle) channel; Table 1 and *SI Appendix, Table S1*] was monitored by the respective treatment channel PIT antennas. The treatment sources were 1 m² areas, where odorants were administered into the stream. Only female sea lampreys were used because this site is located above a sea lamprey control weir. (*Inset*) Map of the state of Michigan on which the star indicates where the test stream is located. (*B*) Stepwise fractionation and subsequent identification of the pheromone component, guided by the behavioral assays or EOG. Fr., fraction. Boxed fractions were the active fractions that replicated the activity of LE in inducing a bias in entrance to the treatment channel or highly stimulatory for the olfactory epithelium (or both); the year when the test was carried out is indicated on the left, and the type of assay is indicated on the right.

additional blank control, we also introduced the vehicle stimulant to both channels and found the number of test subjects entering the two channels were not different (*SI Appendix, Table S1*). Consistent with previous studies (13, 22), these results confirmed LE contains at least one compound that biases migrants in stream selection.

We used this in-stream bioassay to guide fractionation of LE in search for the migratory pheromone. LE, the crude extract shown to be behaviorally active, was fractionated through three iterations to obtain a pure material (>95%) that induces preference behavior in migratory adults (Fig. 1*B*). In the first iteration, LE was chromatographed on silica gel and eluted with gradient chloroform and methanol, resulting in nine fractions, which were grouped into four pools (pool 1: fractions 1 and 2; pool 2: fractions 3 and 4; pool 3: fractions 5–7; and pool 4: fractions 8 and 9). These pools were assayed for behavioral activities in May 2010. The combination of all four pools biased migrants into the treatment channel ($P = 0.004$; Fig. 1*B* and *SI Appendix, Table S1*), replicating the activity of the LE ($P = 0.032$; *SI Appendix, Table S1*). Tested individually, pool 3 differed from the vehicle ($P = 0.009$) and replicated the combined pools 1–4 in activity, whereas pools 1, 2, or 4 were not different from the vehicle control ($P > 0.10$, for each of the three pools; *SI Appendix, Table S1*). Pool 3 did not contain PADS or PSDS (18), while pool 4 did (*SI Appendix, Fig. S2*).

In the second iteration, we determined the active fraction(s) in pool 3 by testing fractions 5, 6, and 7 individually for their behavioral activities in 2011. Only fraction 5 induced the migratory behavior ($P = 0.033$; Fig. 1*B* and *SI Appendix, Table S1*).

In the third iteration, we further analyzed fraction 5 to obtain compounds that induce preference behaviors in the migratory adults. Mass spectrometric analyses of fraction 5 showed the presence of one (or more) unknown compounds with an m/z of 329 amu (negative ion ESI, *SI Appendix, Fig. S3*) as well as two known compounds—namely, petromyzonin (m/z 307) (30) and petromyoxols (m/z 273) (31). The material with nominal mass of 330 was isolated through multiple, successive chromatographic purifications as a colorless oil in the amount of 2.4 mg. We called the material at this stage of purification “mixture-330.” When mixture-330 was field tested at a final concentration of 5×10^{-13} M (based on its nominal molecular mass of 300) in 2012, it replicated the activity of LE in inducing a migratory behavior of the adult subjects (Fig. 1*B* and *SI Appendix, Table S1*). We therefore focused on elucidating the structures of compounds in mixture-330.

Mixture-330 Comprised Four Related Fatty-Acid Derivatives. Mass spectrometry and spectroscopy indicated four fatty-acid derivatives in mixture-330. Reverse-phase HPLC-MS showed a single peak with a nominal mass of 330 amu (*SI Appendix, Fig. S3*), suggesting a molecular formula of $C_{18}H_{34}O_5$ for the component(s) present in mixture-330. Many of the carbon resonances were doubled, indicating the presence of two diastereomerically or constitutionally isomeric compounds that contained a fatty-acid-like backbone as well as oxygenated methine protons. A sample of mixture-330 was converted to its methyl ester, persilylated, and subjected to GC-MS analysis (*SI Appendix, Supplementary Text*). This clearly indicated the presence of two distinct compounds, each having a nominal mass of 488 amu. This mass gain indicated that each of the two diastereomers or constitutional isomers was a methylated, bis-TMS compound, arising therefore from a diol derivative of a carboxylic (fatty) acid (i.e., from a trihydroxy-containing compound). Here, we name these components as petromyric acid A and petromyric acid B (PMA and PMB, respectively) to acknowledge their relationship to both *Petromyzon* and a fatty acid.

Further inspection of the NMR data, including the 2D COSY and HMBC spectra, revealed connectivity that pointed to the presence of a dihydroxylated tetrahydrofuran subunit in each of the two compounds. By comparing the chemical shifts of each of these with known structures containing such subunits (32), we

hypothesized the presence of a dihydroxytetrahydrofuran moiety like that shown in structures PMA and PMB (Fig. 2) as well as in the petromyoxols (31). From the fragmentation patterns in the electron impact GC-MS experiments (32, 33), we deduced the location of the ether ring and its flanking hydroxyl group, which allowed us to assign the differing constitutions of PMA vs. PMB, the two principal components in mixture-330. More detailed discussion of the analyses summarized here can be found in the *SI Appendix, Supplementary Text*.

To determine whether PMA and PMB were present in mixture-330 in racemic or enantiomerically enriched form, each of the four stereoisomers shown in Fig. 2 was synthesized (*SI Appendix, Supplementary Text*). The absolute configuration of each of the synthetic stereoisomers was established by Mosher ester analysis and shown to be as indicated in Fig. 2 for each of the (+)- and (–)-antipodes of the constitutional isomers PMA and PMB. These four isomers proved to be resolvable on a chiral HPLC column (*SI Appendix, Supplementary Text*). The natural sample, when subjected to this analysis, showed the compounds to be present in the following amounts (in order of elution): (+)-PMB, 14.4%; (+)-PMA, 18.8%; (–)-PMB, 28.6%; and (–)-PMA, 38.2%. This 57:43 ratio of (enantiomerically enriched) PMA:PMB was consistent with the relative intensities of several pairs of resonances in the ^{13}C NMR spectrum of this mixture of constitutional isomers (*SI Appendix, Supplementary Text*).

Compounds (+)-PMA and (–)-PMA Stimulated the Olfactory Epithelium.

Synthetic samples of both (+)-PMA and (–)-PMA induced strong responses in the olfactory epithelia of migratory adults, with a threshold of detection at or below 10^{-11} M for each and concentration–response relationships expected for odorants (Fig. 3*A–C*). In contrast, neither (+)-PMB nor (–)-PMB elicited a concentration–response relationship typical of an odorant–receptor interaction (Fig. 3*A*). To further characterize possible interactions between (+)-PMA and (–)-PMA on olfactory epithelium, we established cross-adaptation between the two compounds as measured by electro-olfactogram (EOG) responses. The experiments, in which the olfactory epithelium was subjected to a prolonged perfusion of (i.e., preadaptation to) one compound and, at the same time, the response to a second compound was measured, indicated that (+)-PMA and (–)-PMA suppressed EOG responses of each other (*SI Appendix, Fig. S14*). Further, we cross-adapted (+)-PMA and (–)-PMA over a wide range of concentrations for each compound and found that (+)-PMA was more effective in stimulating olfactory receptor neurons than (–)-PMA (Fig. 3*D* and *E*).

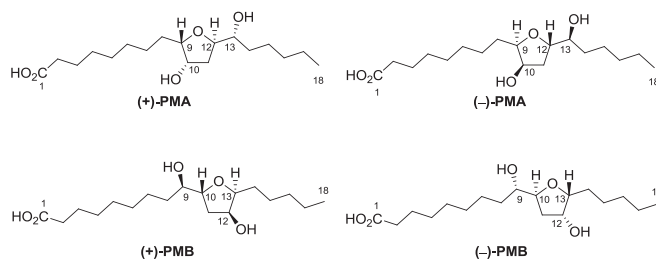


Fig. 2. Structures of (+)-PMA, (–)-PMA, (+)-PMB, and (–)-PMB. The enantiomeric composition of PMA and PMB in mixture-330 was assessed by HPLC using a Chiralpak AD-H column; each of the two constitutional isomers was judged to be a mixture of the (+)- and (–)-antipodes. The absolute configuration of each compound is as follows: (+)-PMA 9*S*,10*S*,12*R*,13*R*; (–)-PMA 9*R*,10*R*,12*S*,13*S*; (+)-PMB 9*R*,10*R*,12*S*,13*S*; and (–)-PMB 9*S*,10*S*,12*R*,13*R*. For convenience and clarity, the skeleton numbering used here (and in *SI Appendix*) is based on the fatty-acid chain from which the PMAs are derived and does not follow the numbering of the systematic nomenclature assigned by Chemical Abstracts [e.g., (+)-(2*S*,3*S*,5*R*)-tetrahydro-3-hydroxy-5-[(1*R*)-1-hydroxyhexyl]-2-furanoctanoic acid for (+)-PMA].

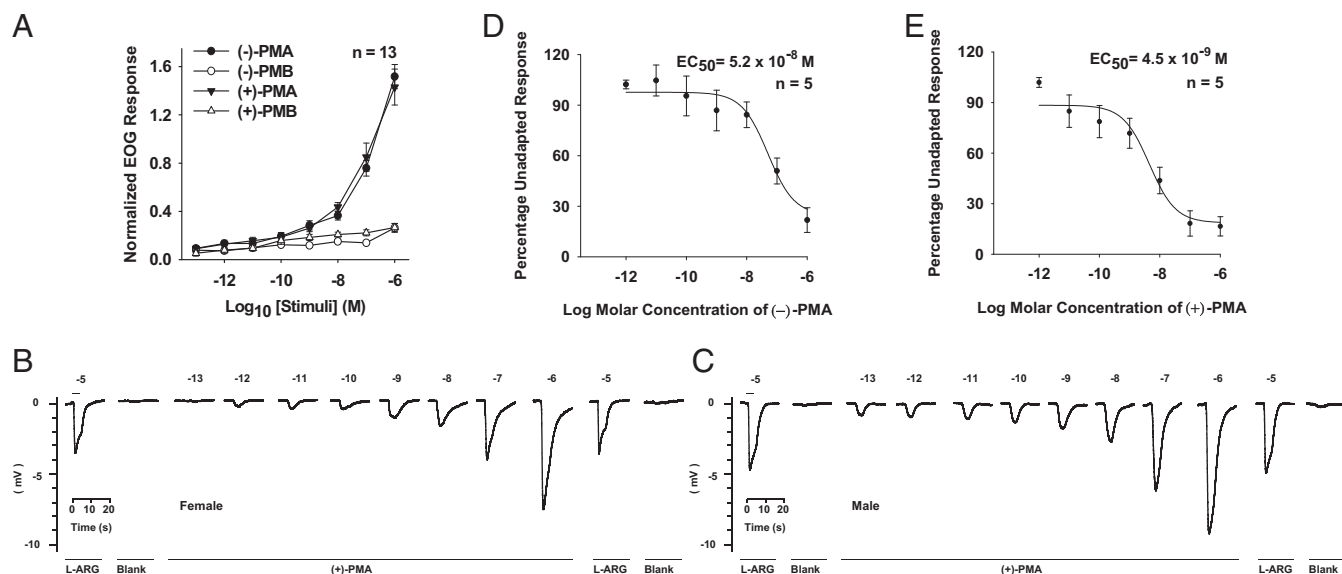


Fig. 3. Responses of adult sea lamprey olfactory epithelia, as measured by EOG recording, to synthetic samples of the dihydroxylated tetrahydrofuran fatty acids identified from larval sea lamprey. (A) Semilogarithmic plots of concentration–response relationships of each of four synthesized stereoisomers (see below) that compose mixture-330 ($n = 13$; six females and seven males), later characterized as the enantiomers of petromyric acid A and B. (B) EOG traces of a female migratory adult olfactory epithelium exposed to (+)-PMA at concentrations between 10^{-14} and 10^{-6} M. Blank, vehicle solution; L-ARG, L-arginine. The number above each trace represents the logarithmic value of the molar concentration of each stimulant. The bar above the L-ARG trace on the left represents the duration of odorant treatment. (C) EOG traces of a male migratory adult olfactory epithelium exposed to (+)-PMA. Displacement curve of EOG response for synthetic samples of the stereoisomers (–)-PMA against (+)-PMA (D) and of (+)-PMA against (–)-PMA (E). Curve fitting and EC_{50} calculations were performed using SigmaPlot (the binding ligand module; $n = 5$). All “normalized EOG responses” were blank corrected and normalized to the amplitude of the responses to 10^{-5} M L-ARG. Percentage of unadapted responses were the normalized EOG responses to an isomer after the olfactory epithelia were exposed to the other isomer divided by the normalized EOG responses to an isomer before the exposure and expressed as percentage.

Specifically, 50% of the EOG response magnitude of (+)-PMA at 10^{-7} M was suppressed by (–)-PMA at 5.8×10^{-8} M (EC_{50}), whereas 50% of the EOG response magnitude of (–)-PMA at 10^{-7} M was suppressed by (+)-PMA at 4.1×10^{-9} M, a lower concentration than for the inverse.

Compound (+)-PMA Induced Migratory Behavior. In our final step to identify the compound that replicates LE in inducing channel preference, we examined the behavioral effects of synthetic (+)-PMA and (–)-PMA across two migratory seasons (2013 and 2014). As expected (from the 2009 observations; *SI Appendix, Table S1*), LE biased a higher percentage of the test subjects into the treatment channel than the vehicle (Table 1, $P < 0.001$). Compound (+)-PMA ($P < 0.001$) did so as well when applied to reach a final in-stream concentration of 5×10^{-13} M. In contrast, compound (–)-PMA, when also applied at 5×10^{-13} M, was not attractive over the vehicle control ($P = 0.869$). In an additional experiment, subjects showed no preference for LE vs. (+)-PMA when each stream channel contained one of the two at equivalent concentrations ($P = 0.389$). Finally, animals were biased to favor LE vs. (–)-PMA ($P = 0.007$).

We deduced that the concentration of (+)-PMA in sea lamprey spawning streams are within the range that induces the migratory behavior in adult sea lampreys, by comparing ratios of compounds released by the larvae. A previous study estimated that a larval bile acid, petromyzonol sulfate (PZS; ref. 34), is present at concentrations between 1.3×10^{-13} and 1.43×10^{-12} M (average: 7.5×10^{-13} M) in six Lake Huron streams that support sea lamprey migration and reproduction (35). We analyzed extracts of larval washings and found the extracts contained (+)-PMA and PZS at a ratio of $\sim 3:1$ (*SI Appendix, Table S5*). We extrapolated that sea lamprey spawning streams contain (+)-PMA at a range of $\sim 3.9 \times 10^{-13}$ and 4.3×10^{-12} M. These estimates provide further support that (+)-PMA is a component of the sea lamprey migratory pheromone.

Discussion

In this study, we identified (+)-PMA as a migratory pheromone using accepted protocols of proof for identification of novel pheromones, as set forth by Butenandt et al. (36) and more recently summarized by Wyatt (37). We first established that LE, extracts of larval washings, biased the migratory sea lampreys toward the treatment tributary channel, as predicted from previous observations (20). We then proceeded with studies aimed at identifying the chemical(s) that replicate this activity. We fractionated the LE and tracked pheromone activity, in sequential steps, to pool 3, fraction 5, and mixture-330. Mixture-330 was subsequently shown to comprise four isomeric oxidized stearic acids containing a dihydroxylated tetrahydrofuran embedded in the C_{18} fatty-acid chain. We elucidated the relative and absolute configurations of each of the four compounds (two pairs of enantiomers) through spectroscopic and chromatographic analyses and correlation with chemically synthesized samples of each. Single crystal X-ray diffraction analysis of an analog of one of the synthetic compounds confirmed both its constitution and relative configuration. Furthermore, we demonstrated that (synthetic samples of) each of (+)-PMA and (–)-PMA stimulates the olfactory epithelium with high levels of specificity and potency and that (+)-PMA replicated the LE in biasing migratory adults into the treatment channel.

The observation that female adult sea lampreys are attracted to (+)-PMA implicates a strategy for adult sea lampreys to navigate toward spawning grounds over large spatial scales. The adults migrate into only a fraction of streams for spawning (8, 9). Once in a stream, they ascend up to 100 km to reach spawning grounds in the Laurentian Great Lakes. In their native range, the distance traveled by sea lamprey adults from the mouth of estuary to the final spawning ground varies between 20 km (southwest England) and 850 km (historical range in Rhine River, Europe) (5, 38). Although the distance migrated may vary

Table 1. Behavioral responses of migratory female sea lampreys to larval washing extracts and to each enantiomer of synthesized petromyric acid A

Treatment or measurement	No. of trials	Subjects released	Upstream movement (<i>n</i>)	Selection of treatment channel (<i>n</i>)
Treatment				
Vehicle	36	709	77% (546) A	47% (259) A
LE	15	300	87% (262) B	61% (161) B
(+)-PMA	11	219	79% (174) A	66% (114) B
(-)-PMA	12	240	68% (164) C	48% (79) A
Measurement				
χ^2	—	—	34.50	27.00
df	—	—	3	3
<i>P</i> value	—	—	<0.001	<0.001

Trials were conducted over the 2013 and 2014 migratory season in the Upper Ocqueoc River, Millersburg, MI (as shown in Fig. 1A). "Subjects released" indicates the total number of female sea lampreys released for each treatment (test subjects were released in groups of 20 for each trial). "Upstream movement" indicates the number of subjects moving 200 m upstream to the confluence of the two subchannels for each treatment. "Selection of treatment channel" indicates the number of subjects that moved upstream to the confluence and entered the subchannel containing indicated "Treatment." Treatments included the following: Vehicle (a blank control where 50% MeOH solution was applied at the same volume as the treatment odorant solutions to both subchannels simultaneously); LE (positive control larval wash water extract applied to one tributary channel at 5×10^{-14} M benchmark PADS, see *SI Appendix, Table S1a*, and vehicle solution applied to the adjacent channel); (+)-PMA [(+)-PMA, 5×10^{-13} M; final in stream concentration assuming complete mixing with stream water] vs. vehicle; and (-)-PMA [(-)-PMA, 5×10^{-13} M] vs. vehicle. Treatment and vehicle subchannels were alternated. Each response was evaluated using a generalized linear model with a binomial distribution. Within each treatment, trials were grouped and the number of individuals in each response variable (from the total number of "Subjects released") was fitted to a binomial distribution for statistical analyses. Overall significance of the logistic regression models within each response variable is shown (χ^2). Responses that share a letter (A, B, or C) are not significantly different ($\alpha = 0.05$).

dramatically for each adult sea lamprey, they all face a series of decision points when approaching river mouths and, subsequently, confluences within a river system. At each decision point, the adults need to orient toward one of the several channels. Larval odors have been demonstrated to guide the orientation at these decision points (12, 13, 20). Our in-stream test system simulated such a decision point where extracts of larval washings induced orientation of adults toward the treatment channel.

Our behavioral assays showed that (+)-PMA was able to bias tributary channel selection at approximately 5×10^{-13} M, a concentration that was comparable to the estimated levels of (+)-PMA in river systems that attract populations of adult sea lampreys each year. Although the EOG detection threshold of (+)-PMA is at 10^{-11} M, it has been shown previously, including in the sea lamprey (26), that the lowest effective concentration of pheromones in inducing behavioral response is often one or two orders of magnitude lower than that which induces EOG responses. One possible factor that may have contributed to this discrepancy is the high concentration pockets of odorants interspersed in turbulent water flows (39), as have been well demonstrated for odorants in wind (40). In our test site, sea lampreys may have encountered pockets with odorants at concentrations much higher than 5×10^{-13} M, which is expected based on calculations assuming completely uniform odor intensity. In contrast, the odorant solutions delivered to the olfactory epithelium during EOG recording were completely mixed and likely represented uniform odorant intensity. The potency of (+)-PMA observed in behavioral assays is consistent with previous observations that larval odor still elicits behavioral responses after substantial dilutions (41). Our behavior and physiology data demonstrate that (+)-PMA is an important component of the larval odor that guides oriented movements of adult sea lampreys to reach a spawning ground.

Adult sea lamprey responses to the tetrahydrofuran diols are isomer-selective (A vs. B constitution) and enantiomer-selective. Our EOG analysis of each individual, synthetic compound showed that both enantiomers of PMA were more stimulatory for the olfactory epithelium than those of PMB. Of the PMA enantiomers,

the positive antipode appeared to have a higher potency, consistent with results of in-stream assays in which (+)-PMA induced the preference behavior, whereas (-)-PMA at a same concentration did not. The importance of stereochemical features in semi-chemicals has been extensively described in insects (42–44). Our cross-adaptation experiments indicated that (+)-PMA and (-)-PMA interacted with similar detection mechanisms; hence, further investigation is required to determine if and how ratios of the PMAs and PMBs in mixture-330 influence behavioral activity. Because (-)-PMA is present at 2–10 times the concentration of (+)-PMA, future studies should focus on testing (-)-PMA at higher concentrations and on mixtures of these two enantiomers with ratios skewed toward (-)-PMA. It remains possible that (-)-PMA is an effective component of the pheromone at natural concentration. These fatty-acid derivatives represent a molecular template for fish pheromones that differ from known fish pheromones, including bile acid derivatives, prostaglandins, sex steroids, and an amino acid (45–48). Fatty-acid analogs are pheromones in many insects (49) but have not been identified as pheromones in fish.

Fatty-acid derivatives with an embedded THF moiety are related to the acetogenin family of natural compounds. PMA and PMB are constitutionally distinct (note the inequivalent head and tail side chains) and each has four stereogenic centers. Each constitutional isomer allows the possibility of 16 stereoisomers (eight diastereomeric pairs of enantiomers). Numerous homologs of varying side chain length are possible depending on the length of the fatty-acid precursor. In our previous studies, a pair of diastereomeric, 14 carbon-containing dihydroxylated THFs, the petromyroxols (31) and *iso*-petromyroxols (50), were isolated and characterized from water that had been conditioned with sea lamprey larvae; the role of these compounds in modulating behaviors of sea lamprey has not yet been examined. Previously, fatty acids with dihydroxylated THF moieties have been isolated, and their constitution, but not configurations, elucidated by mass spectrometric analysis (32, 51). Here, we deduced the full structures of both the (+)- and (-)-enantiomers of PMA and PMB and propose a plausible biosynthetic pathway for these compounds (*SI Appendix, Supplementary Text and Fig. S15*).

Based on chemical, physiological, and behavioral evidence, we conclude that (+)-PMA functions as a component of the sea lamprey migratory pheromone. The sea lamprey is a destructive invader that has thrived in the Laurentian Great Lakes and is the subject of intensive pest control. In Europe, sea lamprey is an iconic and highly prized delicacy but its populations have declined precipitously. We suggest that (+)-PMA may be applied for both control and conservation of sea lamprey populations.

Methods

The in-stream bioassay procedure (Fig. 1) was slightly modified from those described (20, 28). Only adult females were used in field studies to avoid infesting the study site. Each animal was used only once in field tests. EOG measurements have been described (31), and details are given in *SI Appendix*. Details on extraction and fractionation (52); chiral ultra performance

liquid chromatography-MS/MS analysis (31); NMR and LC-MS analyses; procedures used to synthesize (+)-PMA, (–)-PMA, (+)-PMB, and (–)-PMB; mosher ester analysis (53, 54); and single-crystal X-ray diffraction analysis are provided in *SI Appendix*.

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