

REVIEW

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Chemical cues and pheromones in the sea lamprey (*Petromyzon marinus*)

Tyler J. Buchinger¹, Michael J. Siefkes², Barbara S. Zielinski³, Cory O. Brant¹ and Weiming Li^{1*}

Abstract

Chemical cues and pheromones guide decisions in organisms throughout the animal kingdom. The neurobiology, function, and evolution of olfaction are particularly well described in insects, and resulting concepts have driven novel approaches to pest control. However, aside from several exceptions, the olfactory biology of vertebrates remains poorly understood. One exception is the sea lamprey (*Petromyzon marinus*), which relies heavily upon olfaction during reproduction. Here, we provide a broad review of the chemical cues and pheromones used by the sea lamprey during reproduction, including overviews of the sea lamprey olfactory system, chemical cues and pheromones, and potential applications to population management. The critical role of olfaction in mediating the sea lamprey life cycle is evident by a well-developed olfactory system. Sea lamprey use chemical cues and pheromones to identify productive spawning habitat, coordinate spawning behaviors, and avoid risk. Manipulation of olfactory biology offers opportunities for management of populations in the Laurentian Great Lakes, where the sea lamprey is a destructive invader. We suggest that the sea lamprey is a broadly useful organism with which to study vertebrate olfaction because of its simple but well-developed olfactory organ, the dominant role of olfaction in guiding behaviors during reproduction, and the direct implications for vertebrate pest management.

Keywords: Chemical communication, Olfaction, Sensory biology, Integrated pest management

Background

Sensory input from conspecific odors guides decisions for organisms throughout the animal kingdom [1]. Early studies focused on insects, with the first behaviorally active conspecific odorant identified in the silkworm (bombykol; *Bombyx mori*) [2]. Since then, behaviors mediated by conspecific odors have been described in crustaceans [3], fishes [4], reptiles and amphibians [5], birds [6], and mammals [7], including hypothesized functions associated with reproduction, foraging, conspecific recognition, and predator avoidance [1]. Detection of chemicals can be integrated into the decision making processes of organisms via adaptations in receivers (chemical cues) or both receivers and signalers (pheromones) [1]. While much of our understanding of chemical communication is based upon research on insects, the olfactory biology and ecology of some vertebrates is increasingly understood. In particular, chemical communication in some fishes, including

the sea lamprey (*Petromyzon marinus*), is relatively well described [4].

The sea lamprey is a basal vertebrate with a complex life history comprised of distinct larval, juvenile, and adult stages. Larval sea lamprey burrow into stream sediment and filter feed on organic material and microorganisms. Following a larval stage of 3–5 years, sea lamprey undergo a drastic metamorphosis into the juvenile stage, migrate downstream into the Atlantic Ocean or a Laurentian Great Lake, and parasitize on large fish for approximately 1.5 years. Finally, adult sea lamprey migrate into streams during the spring, where a male will construct a nest and later be joined by one or more females, spawn intermittently for a number of days, and die [8]. Olfaction is hypothesized to influence sea lamprey behavior throughout the larval, juvenile, and adult stages [9–11], but only during the terminal adult phase has the role of conspecific odors been evaluated.

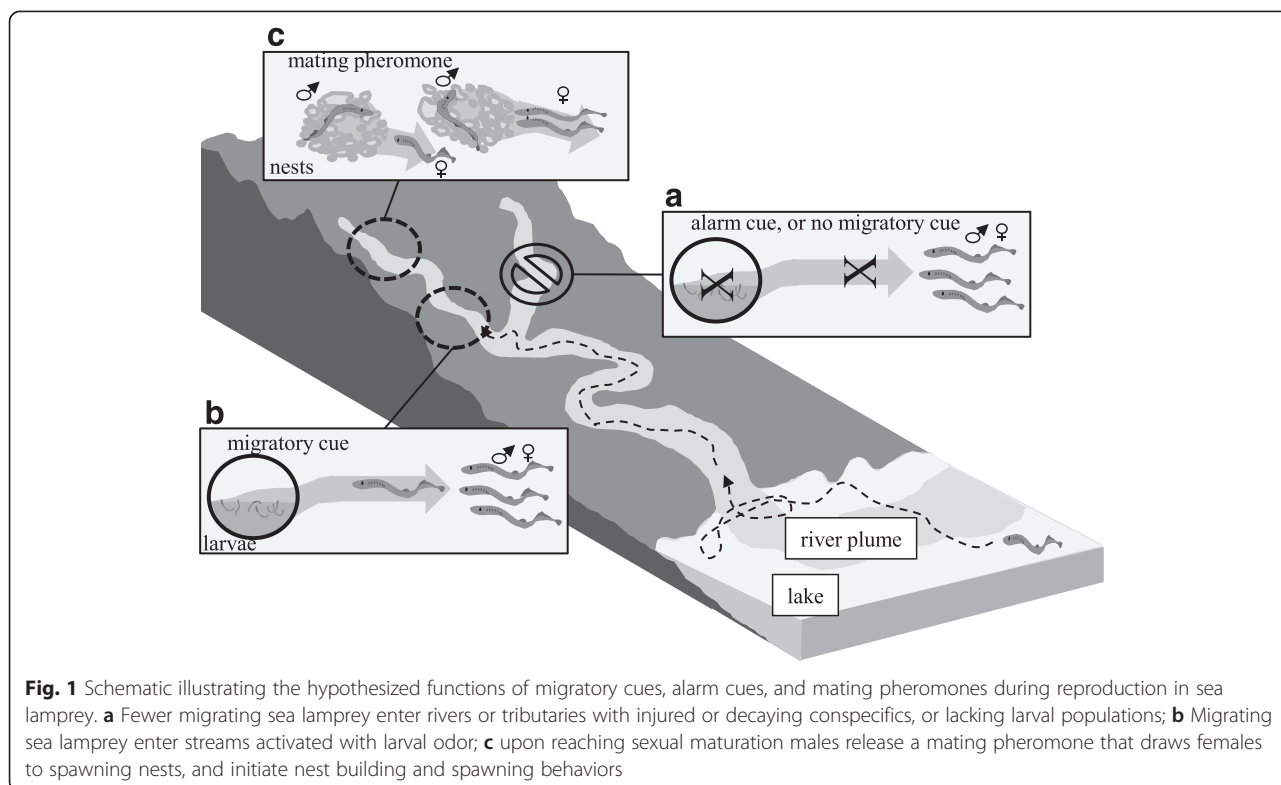
Adult sea lamprey use conspecific odors to identify suitable spawning habitat, search for mates, and avoid risk (Fig. 1) [11, 12]. Migrating adults select spawning

* Correspondence: liweim@msu.edu

¹Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI, USA

Full list of author information is available at the end of the article





tributaries based upon the odor of previous years' larvae that reside in the stream. Upon arrival at the spawning grounds, gravid females move upstream and locate spawning nests using the odor of sexually mature males [11, 13]. Alarm substances are hypothesized to guide adults away from areas where larval or adult populations have high mortality [12, 14, 15].

Here, we summarize the current understanding of the chemical cues and pheromones used by the sea lamprey during reproduction. Previous reviews of sea lamprey olfaction focus primarily on applications to fisheries management in the Laurentian Great Lakes [16–20]. Our objective is to develop a broader perspective on sea lamprey olfaction, spanning from odorants up to evolutionary patterns. We provide overviews on the neurobiology of olfaction, the ecology and evolution of chemical cues and pheromones, and potential applications to population management. We suggest that the simple but well-developed olfactory organ, dominant role of olfaction in guiding behaviors during reproduction, and direct implications for vertebrate pest management position the sea lamprey as a useful organism with which to study vertebrate olfaction.

The olfactory system

Anatomy of the olfactory apparatus

A critical role of olfaction in mediating the sea lamprey life cycle is evident by a well-developed olfactory system [21]. The large olfactory organ in sea lamprey

[22] is comprised of a peripheral olfactory organ containing both a main olfactory epithelium and tubular diverticula known as the accessory olfactory organ [23]. Early in their life cycle, prior to leaving the spawning nest, sea lamprey possess functional olfactory sensory neurons that are stimulated by conspecific odorants [9, 24]. During the metamorphosis from larvae into adults, the peripheral olfactory organ enlarges while changing from an epithelial lined tube to a nasal sac with lamellar folds [25]. The accessory olfactory organ also exhibits the formation of diverticula surrounded by blood vessels and nerve bundles [23, 25].

Olfactory sensory neurons

Olfactory sensory neurons intercept odor information using dendrites that extend into the mucus of the peripheral olfactory organ. The olfactory sensory neurons are ciliated [24, 26, 27], but exhibit distinct morphotypes similar to ciliated, microvillous, and crypt olfactory sensory neurons documented in teleost fishes [28–30]. Neuron morphotypes differ in the distance the dendrite extends into the olfactory mucus surrounding the olfactory epithelium, and may relay information from different classes of odorants (feeding, risk, reproduction) [31]. Dendrites of sensory neurons express olfactory receptors, which mark the beginning of signal transduction.

Signal transduction

Olfactory receptors on the olfactory sensory neurons bind odorants and trigger a signal transduction cascade. Receptor proteins of olfactory sensory neurons are members of the seven-transmembrane G-protein coupled receptor superfamily [32]. In the sea lamprey, chemosensory receptor genes include at least 27 olfactory receptor (OR)-type genes, 28 trace amino acid receptors (TAAR)-type and 4 vomeronasal type one (V1R)-type genes [33–35]. Signal transduction following odorant binding is not yet fully described in lamprey. On the main olfactory epithelium, the binding of an odorant by an OR likely triggers a second messenger cascade via the G-protein G_{olf} which stimulates an increase in cyclic adenosine monophosphate (cAMP), opening the cyclic nucleotide gated ion channel [36–38]. The G-proteins in the olfactory sensory neurons on the accessory olfactory organ, however, have not been identified. The signal transduction cascade leads to depolarization of the neuron and propagation of the signal to the olfactory bulb [36, 37].

Olfactory bulb

Spatially distinct regions of the olfactory bulb receive and integrate olfactory signals from the main and accessory olfactory systems (Fig. 2). Olfactory sensory neuron axons projecting from the main olfactory epithelium and the accessory olfactory organ merge into the olfactory nerve. Axons from the accessory olfactory organ project to the medial region of the olfactory bulb, while axons from olfactory sensory neurons in main olfactory epithelium extend to all other regions [27]. After entering the olfactory bulb, olfactory sensory neuron axons pass through the olfactory nerve layer and form synaptic contacts in spherical regions of neuropil known as glomeruli. Glomeruli in all regions, except the medial region, express immunoreactive G_{olf} a G protein thought to be necessary for odorant reception [38]. Within the glomeruli, axon terminals of the olfactory sensory neurons synapse with the dendritic endings of output neurons (projection neurons). Projection neurons in the medial olfactory bulb are spatially isolated from projection neurons in non-medial olfactory bulb regions and have larger cell bodies than non-medial projection neurons [39]. Lastly, projection neurons interact with interneurons and signal higher olfactory processing centers in the brain.

Projections to the brain and behavioral output

Projections from the medial olfactory bulb to higher olfactory processing centers create a direct link between olfactory input and locomotory output [40]. Odorant and electrical stimulation of the medial region of the olfactory bulb stimulates locomotion [40]. The medial region of the olfactory bulb projects to the posterior tuberculum, which is located in the ventral diencephalon and projects to the

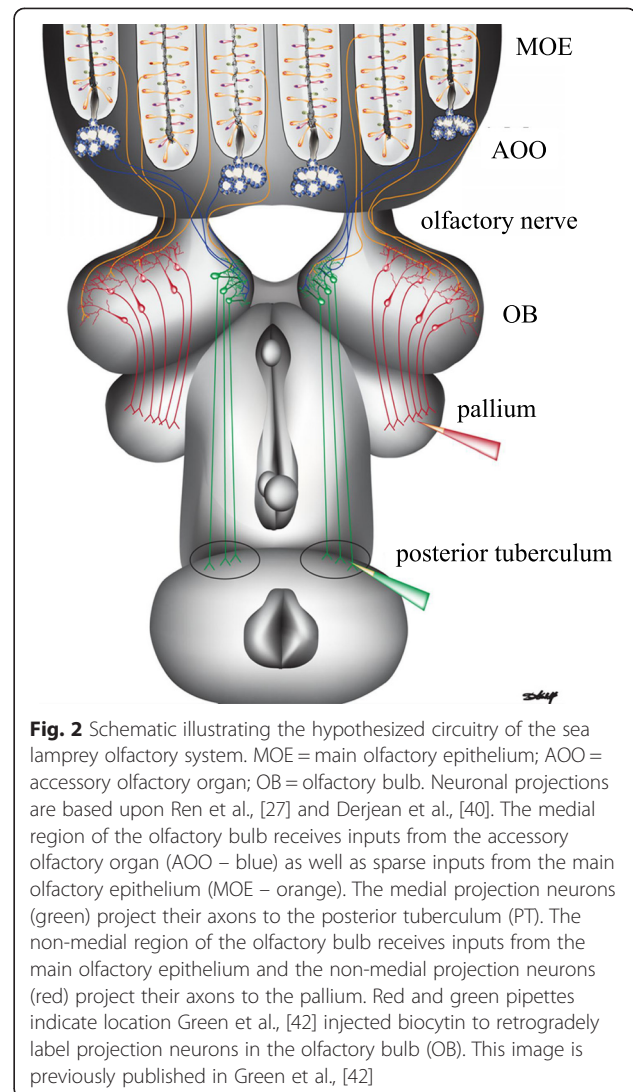


Fig. 2 Schematic illustrating the hypothesized circuitry of the sea lamprey olfactory system. MOE = main olfactory epithelium; AOO = accessory olfactory organ; OB = olfactory bulb. Neuronal projections are based upon Ren et al., [27] and Derjean et al., [40]. The medial region of the olfactory bulb receives inputs from the accessory olfactory organ (AOO – blue) as well as sparse inputs from the main olfactory epithelium (MOE – orange). The medial projection neurons (green) project their axons to the posterior tuberculum (PT). The non-medial region of the olfactory bulb receives inputs from the main olfactory epithelium and the non-medial projection neurons (red) project their axons to the pallium. Red and green pipettes indicate location Green et al., [42] injected biocytin to retrogradely label projection neurons in the olfactory bulb (OB). This image is previously published in Green et al., [42]

mesencephalic locomotor region. The mesencephalic locomotor region initiates locomotion by acting on brainstem pre-motor neurons, the reticulospinal neurons, which directly activate the locomotor networks of the spinal cord [41]. Hence, a direct pathway from a sensory neuron up to the spinal cord likely triggers odor-driven behavioral responses in sea lamprey [40].

In contrast, projections from non-medial regions may be involved in the integration of odor information. Non-medial output neurons project to several forebrain structures, including the lateral pallium. The somata of non-medial projection neurons are below the glomerular neuropil and are smaller than the somata of the medial projection neurons [39]. The receptive fields of the projection neurons in the medial and non-medial output pathways do not overlap [39]. Local field potential recordings from the non-medial olfactory bulb region have shown that the dorsal olfactory bulb territory responds

to lamprey sex pheromones and migratory pheromones while lateral olfactory bulb recordings exhibit responses to basic amino acids, and not to pheromones [42]. The hypothesized olfactory-locomotor link created by the accessory olfactory organ may be modulated by the detection and discrimination of specific odorants in the main olfactory organ.

Olfaction in lamprey compared to other vertebrates

The lamprey olfactory system exhibits many features common among vertebrates, along with several characteristics that are unique. Most organisms, including lamprey, possess similar adaptations for detecting and processing olfactory stimuli [43]. For example, the cellular and molecular mechanisms of olfaction appear to be generally shared among vertebrates, including lamprey; olfactory receptors are G protein-coupled receptors and similar transduction pathways carry olfactory signals [43]. A detailed report of the similarities and differences between the olfactory systems of lamprey and other vertebrates is outside the scope of this review, but several examples of unique features of the lamprey olfactory system should be noted. First, lamprey, along with hagfish, are unique in having a single nostril. Notably, although lamprey have a single nostril, the olfactory organ is comprised of two regions and a paired olfactory nerve. While the functional implications of having a single nostril are unclear, having two nostrils has clear adaptive significance in some fish [44]. Second, the accessory olfactory organ of lamprey appears to be a unique adaptation [35], and offers an interesting comparison to the vomeronasal organ in tetrapods. Taken together, the common and unique features of the sea lamprey olfactory system offers a useful system to answer fundamental questions of vertebrate olfaction.

Chemical cueing and pheromone communication in sea lamprey

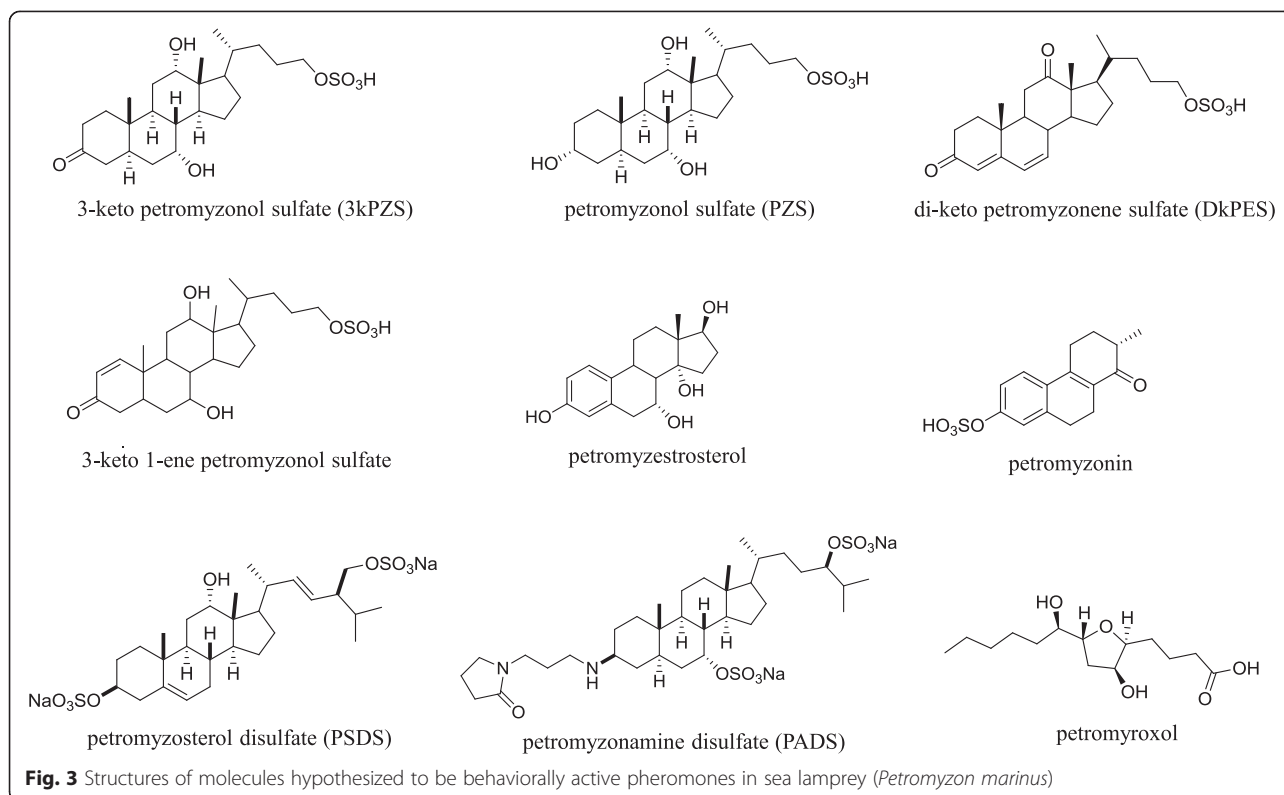
Reproductive behaviors in sea lamprey rely largely upon olfactory input [45, 46]. In contrast to many anadromous fishes (e.g. salmonids), sea lamprey do not exhibit natal homing behaviors [47, 48]. Rather, sea lamprey evaluate the suitability of a stream based on the presence of larval populations [11, 49]. Migratory sea lamprey are acutely tuned to the larval odor (*migratory cue*); putative components are detected at low concentrations [50] and larval odor elicits behavioral responses at the concentrations produced by a single larvae diluted several thousand fold [51]. Once sea lamprey arrive at the spawning grounds, final sexual maturation is partially triggered by conspecific odors [52, 53]. Upon complete sexual maturation, mate search and spawning are guided by the odors of the opposite sex [11]. Although males are attracted to the odor of females [11, 54], the odors released by males (*male*

mating pheromone) and subsequent behavioral responses in females are better understood. The male odor appears multi-functional, mediating upstream movement behaviors [55] and proximate nest construction and spawning synchronization behaviors [56]. Finally, throughout the spawning season, sea lamprey are hypothesized to evaluate risk using conspecific and heterospecific semiochemicals (*alarm cue*) [12, 14, 15].

Migratory cues and mating pheromones identities

Bile acids and derivatives are implicated as components of the sea lamprey migratory cue and male mating pheromone [19]. The olfactory epithelium of many fishes is sensitive to sex steroids, prostaglandins, amino acids, and bile acids [57]. Sex steroids and prostaglandins are commonly implicated as mating pheromones in teleosts [58]. Amino acids are likely used by anadromous salmonids during natal homing [59, 60], and as a mating pheromone in at least one species (Masu salmon, *Oncorhynchus masou*) [61]. Sea lamprey, however, only show sensitivity to a small number of amino acids and sex steroids [62]. In many species, including sea lamprey, conspecific-released bile acids evoke strong physiological responses in electro-olfactograms (EOG) [50, 63–65], thus implicating behavioral functions. High-performance liquid chromatography (HPLC) and mass spectrometry (MS) combined with EOG screening and behavioral assays have continued to amass support for bile acids and related cholesterol derivatives as components of the male mating pheromone in sea lamprey (Fig. 3) [66].

Research into the sea lamprey migratory cue provides support for the hypothesis that conspecific bile acids [50] guide spawning migrations of anadromous fishes [67]. Larvae excrete lamprey-specific bile acids [68] into the water at rates sufficient to create a detectable concentration in a river (~10 ng/h) [69, 70]. Three bile acids, petromyzonol sulfate (PZS), petromyzonamine disulfate (PADS), and petromyzosterol disulfate (PSDS) are released into the water [70], elicit strong electrophysiological responses from the olfactory epithelium [50, 65], and influence the behavior of migratory lamprey in laboratory mazes [65, 71]. While the mixture of PADS, PSDS, and PZS replicates the proximal preference elicited by larval odor in laboratory tests [65] and may influence search behavior at the junction of the lake and the river [72], the mixture does not replicate larval odor in eliciting upstream movement and stream channel preference in natural stream environments [73], suggesting crucial components of the migratory cue remain unidentified. Several additional components of larval metabolites have been identified and are potent odorants, but have not been evaluated in behavioral assays [74–76].



The first link between bile acids and reproduction was revealed by the discovery that a bile acid functions as a major component of the sea lamprey male mating pheromone [77]. The bile alcohol 3keto petromyzonol sulfate (3kPZS) is released at high rates by males (~0.5 mg/h) [78], detected with acute sensitivity and specificity [64], and elicits an attraction response in sexually mature females both in the laboratory [54, 77] and in the field [54, 55, 77]. While robust behavioral responses in large-scale field tests confirm that 3kPZS is the major component of the male pheromone [55, 79], unknown components appear to be required to match the full suite of nesting and courtship behaviors elicited by the full male odor [56]. A bile acid structurally similar to 3kPZS but lacking in the C24 sulfate, 3 keto allocholic acid (3kACA) was hypothesized to function as an additional component [64, 80, 81], but has now been resolved behaviorally inactive [56]. Notably, sea lamprey detect 3kACA with high sensitivity and specificity [64], and steroidogenesis in males is inhibited by exposure to 3kACA [53]. A 4 oxidized, unsaturated compound similar to 3kPZS elicited attraction in females [82]. Another bile acid 3,12-diketo-4,6-petromyzonene-24-sulfate (DkPES), is a potent male odorant that, when mixed with 3kPZS, increases the number of females that approach the source of 3kPZS [66]. An additional constituent of the male odor, petromyzestrosterol, elicits olfactory responses in EOG recordings but has not yet been tested in behavioral assays [66].

Sources and release

Sea lamprey possess unique mechanisms of synthesizing and excreting bile acids associated with chemical cues and pheromone. Larval sea lamprey regulate bile acids as do most vertebrates: synthesis in the liver, storage in the gall bladder, and secretion into the intestine via the bile duct. At this stage, putative migratory cue components, including the mating pheromone 3kPZS [83], are slowly released into the water via intestinal contents (~10 ng/larva/h) [69, 70, 83]. A drastic reduction both in expression of genes coding for bile acid biosynthetic enzymes in the liver [83] and in the concentration of bile acids in tissues follows the transformation of larvae into parasitic adults [69, 83]. Migratory adults likewise exhibit a down-regulation of hepatic synthesis of bile acids, but appear to regulate bile acid equilibrium through renal excretion [84]. Upon sexual maturation, males up-regulate expression of genes coding for enzymes involved in bile acid anabolism, yielding an increase in hepatic concentrations of PZS and 3kPZS [77, 78, 85]. The compounds are carried by the cardiovascular system to the gills, where PZS is hypothesized to be oxidized to 3kPZS, and released through glandular cells that develop at the final stages of maturation in males [78, 85]. Additional components of the male pheromone are likely also released by the gills [56]. The cessation of feeding and atrophy of the intestine during reproduction may favor the renal system and gills as alternative mechanisms of bile acid equilibrium.

Behavioral ecology

The migratory cue informs migrating sea lamprey regarding potential offspring success and reduces the risk of selecting poor stream habitat. Following host detachment, sea lamprey are hypothesized to identify productive offspring habitat using a series of environmental cues [73]. Adult sea lamprey search for river plumes extending into the lake or ocean and display a preference for the general odor of stream water [45, 86]. Migrating adults enter rivers and tributaries that are activated with the odor of larvae, which is directly related to potential for future offspring success [51, 86, 87]. Release of bile acids hypothesized as components of the migratory cue is linked to larval feeding [69, 70]. Although the migratory cue appear to be comprised of a mixture of multiple known and unknown components [65, 73], the functional differences between components is unknown.

The male mating pheromone mediates pre-spawning upstream migration [88] and sexual maturation in males and females [52], and spawning upstream movement [54] and a suite of spawning behaviors in females [56]. The response elicited depends upon the spatial, environmental, and physiological context. Pre-spawning upstream migration of males and females is reduced at low temperatures [89, 90], but maintained in the presence of 3kPZS [88]. Mature male odor facilitates sexual maturation of males and females [52]. Sexually mature females display strong odor-conditioned rheotaxis in response to male odor, primarily in response to 3kPZS [54–56]. Upon reaching the spawning nest, however, nest construction and gamete release in females is largely mediated by the mixture of 3kPZS, DkPES, and unknown compounds [56, 66]. The mechanisms through which pheromone mixtures operate in sea lamprey remain unknown, but specific ratios appear to be important [66].

Evolution

The migratory cue appears to be an adaptation of stream-searching adults rather than a specialized signal released by larvae (Fig. 4). Natural selection likely maintains the strong preference for larval odor, where individuals choosing to spawn in streams with clear evidence of historical success realize higher fitness relative to individuals that chose streams at random or undertake high-cost and less effective evaluation of stream habitat via direct assessment [87]. Larvae presumably receive no direct benefit by releasing an attractive odor, thus attraction to larval odor is likely an adaptation of migratory adults [91]. The hypothesis that larval odor represents a cue rather than a signal is supported by the apparent non-specificity of release and response across lampreys [91–96].

The male mating pheromone is likely the result of a more complex evolutionary history. Many fish pheromone systems, including the sea lamprey migratory pheromone

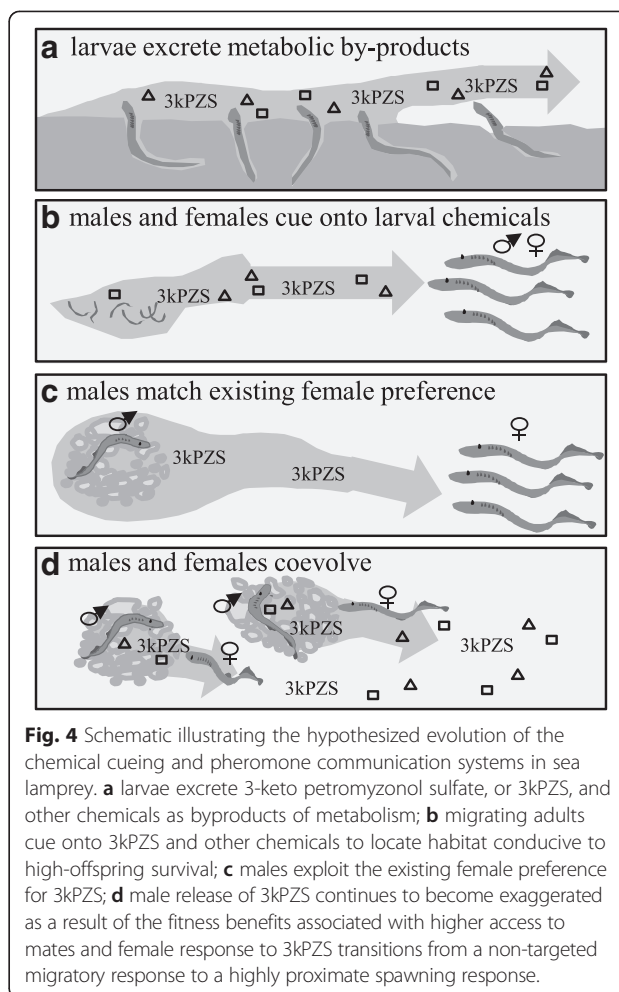


Fig. 4 Schematic illustrating the hypothesized evolution of the chemical cueing and pheromone communication systems in sea lamprey. **a** larvae excrete 3-keto petromyzonol sulfate, or 3kPZS, and other chemicals as byproducts of metabolism; **b** migrating adults cue onto 3kPZS and other chemicals to locate habitat conducive to high-offspring survival; **c** males exploit the existing female preference for 3kPZS; **d** male release of 3kPZS continues to become exaggerated as a result of the fitness benefits associated with higher access to mates and female preference for 3kPZS transitions from a non-targeted migratory response to a highly proximate spawning response.

[91], appear to represent behavioral adaptations of the receiving fish [91, 97]. Evidence that the silver lamprey (*Ichthyomyzon unicuspis*) uses larval 3kPZS as a migratory cue rather than a male-released mating pheromone suggests female preference for 3kPZS may have originated as an adaptation of receiving fish [96]. The development of glandular cells involved in 3kPZS release [78] and the extremely high rate at which 3kPZS is released [77], however, suggest that male adaptation drove a transition of 3kPZS into a mating signal. Adding further complexity to the mating pheromone is the role of multiple components influencing multiple behaviors [56]. While 3kPZS as a mating pheromone may have evolved through male manipulation of an existing female preference [96], the evolutionary processes driving male release and female preference for the remaining components of the male odor remain unknown.

Alarm cues

Identity

Pursuit of the identities of sea lamprey alarm cues is a recent endeavor, and, as such, the chemical structures remain unknown. In fact, despite many years of research

on alarm cues in fish, only two alarm odorants have been identified; hypoxanthine-3-N-oxide, an alarm cue in various teleosts [98, 99], and glycosaminoglycan chondroitin, a recently discovered alarm cue in zebrafish (*Danio rerio*) [100]. Although the identity of the sea lamprey alarm cue is uncharacterized, the odor, or part of the odor and is stable past 96 h of aerobic decay [14]. Notably, commercial 2-phenylethylamine (PEA-HCL), a hypothesized predator cue used by rodents [101], elicits an anti-predator response in sea lamprey in the laboratory [15, 102]. Whether the chemically-mediated risk assessment in sea lamprey shows parallels to teleost and other fishes remains unknown.

Sources and release

Sea lamprey alarm cues originate from conspecific tissues and bodily fluids of predators [14, 15, 102]. Damaged and decayed tissues from larval and adult conspecifics elicit alarm responses [14]. Consistent with much of the literature on fish alarm cues, damaged skin elicits a stronger aversion response compared to whole skin [14]. In contrast to skin-released alarm cues of many fishes [103], the sea lamprey alarm cue appears to be distributed throughout the skin, organ tissue, and muscle [14]. The hypothesized predator cue PEA is released via urine of carnivorous mammals [101] and other unknown predator cues may be released via saliva [102].

Behavioral ecology

Alarm cues used by sea lamprey could indicate 1) a regional end of the spawning, 2) low offspring survival, or 3) risky spawning habitat [12]. Alarm cues are emitted by both larvae and adults [14], indicating the role of conspecific alarm cues likely spans across the proposed ecological functions. Sea lamprey are semelparous and die following a single reproductive season. Hence, the scent of dead lamprey may indicate the end of the reproductive season in a tributary. Alternatively, alarm cue could indicate low survival of larvae or adults due to poor quality habitat or high predation. Additional functions of alarm cues outside of reproduction are supported by observations of possible alarm responses to damaged conspecific tissue in larval sea lamprey [104]. Larvae also show olfactory sensitivity to odors of non-damaged conspecifics [9]. Whether responses to non-damaged or damaged conspecific odors are ecologically relevant, perhaps influencing settlement behavior, or developmental precursors to the responses during the adult phases remains unknown.

Evolution

Alarm cues, including sea those used by sea lamprey, are hypothesized to be the result of receiver specializations [103]. Natural selection likely favors an aversion to alarm

cues in parallel to the attraction to larval odor, resulting in a multi-faceted mechanism to evaluate spawning habitat and optimize success during the single reproductive event. Notably, sea lamprey also exhibit alarm responses to alarm cue collected from closely related silver lamprey [14], and distantly related white sucker (*Catostomus commersonii*), but not Amazon sailfin catfish (*Pterygoplichthys pardalis*) [15]. Reproductive migrations of sea lamprey, silver lamprey, and white suckers overlap temporally and spatially, hence aversion to alarm cues of heterospecifics is ecologically relevant. However, whether the behaviorally active chemicals are shared across species, or if sea lamprey have evolved to use different compounds released by heterospecific fishes remains unknown.

Population management

Manipulation of sea lamprey olfactory biology offers opportunities for management of invasive populations in the Laurentian Great Lakes [11]. Based largely upon pheromone control of insects [105], integration of olfactory stimulants into sea lamprey control has been proposed in the forms of trapping, redistribution, disruption, and monitoring [16–19, 106]. However, only trapping has been evaluated in management scale tests [79, 107].

Baiting traps with conspecific odors increases the efficacy of sea lamprey traps [108, 109]. Field experiments in environments lacking background pheromones demonstrate that traps baited with the natural migratory cue and male mating pheromone, and synthesized 3kPZS catch more sea lamprey than unbaited traps [46, 55, 79, 107–110]. However, only 3kPZS has been tested in management-scale experiments, and only in the context of augmenting the existing trapping effort with pheromone as bait [79]. Traps baited with 3kPZS caught more sea lamprey than unbaited traps, and trapping efficiencies averaged about 10 % higher during years when 3kPZS was applied as bait [79]. The modest increase in trapping efficiency is unlikely large enough to justify wide-spread use of 3kPZS as a control measure unless application can be further optimized to improve effectiveness and reduce cost [111]. Notably, the natural, whole odor of males catches a higher proportion of sea lamprey compared to 3kPZS alone [107]. A recent evaluation of the push-pull method using alarm cue to activate one side of a stream and 3kPZS as bait for a trap on the other side of the stream failed to increase the number of sea lamprey caught in traps, although alarm cue did decrease the time taken for individuals to locate a trap [112]. Identification of all components of the male mating pheromone combined with refined trapping methods is needed to further developing odor-baited traps as a control tool.

Methods other than trapping, such as redistribution, disruption, and monitoring [16–19, 106], remain largely unexplored. Field experiments in pristine, odor-controlled environments indicate the migratory cue and male mating pheromone can be used for redistribution [55, 87] and spawning disruption [55]. Redistribution via a combination of conspecific attractants and alarm cues could be especially useful, but has not been evaluated. Quantification of 3kPZS in streams may also offer a cost-effective method to determine the presence and size of sea lamprey populations [113, 114]. Additional alternatives including antagonists [19] and integrating odor manipulation with electrical guidance [115], may too be useful, but have not been explored. Clearly, more research is needed to further develop olfactory cues as tools for sea lamprey control.

Utility of the sea lamprey model

The sea lamprey presents a simple and unique model for studying olfactory communication in vertebrates. The opportunity for insight into the biology of early vertebrates is matched only by the hagfish. However, the basic biology of sea lamprey is better understood as a result of better accessibility and decades of research associated with pest control programs in the Laurentian Great Lakes. The robust understanding of basic sea lamprey biology combined with the continued elucidation of chemical cues and pheromones, and recent advances, such as the sequencing of the genome [116], allows for novel research avenues.

A simple but well-developed olfactory system makes sea lamprey well-poised for elucidating the path from odorant detection to behavioral output. Sea lamprey detect a limited range of odorants; bile acids, a few amines and sex steroids, and L-arginine [50, 62]. The repertoire of chemosensory receptor genes is correspondingly small, consisting of only three families and an estimated 59 intact genes [34]. Ciliated sensory cells exhibit short, medium, and tall morphotypes that may be precursors to crypt, microvilous, and ciliated sensory cells documented in teleosts [30]. Despite being simple, the sea lamprey olfactory system is well-developed. The distinct accessory olfactory organ with sensory neurons that project to specific regions of the olfactory bulb allows an interesting comparison to the vomeronasal organ of higher vertebrates [27, 35]. The medial olfactory bulb, where the sensory neurons in the accessory organ project axons, forms a direct connection with brain structures that drive locomotion [40]. Strikingly, lamprey have a larger proportion of brain dedicated to processing olfactory information than any other vertebrate examined [22].

Well-adapted mechanisms of habitat and mate assessment using input from multiple olfactory stimuli and environmental cues make sea lamprey a useful organism for

studying the evolution and behavioral ecology of multi-model sensory integration and complex signals. During reproduction, sea lamprey make behavioral decisions based upon the water temperature [89, 90], time of day [117], abiotic odor of streams [51], alarm cues [12, 15], multi-component conspecific cues [65, 87] and mating pheromones [17, 55, 56, 66], as well as interactions among variables [88] and the physiological state of the receiver [54]. Furthermore, sea lamprey spawn in lek-like aggregations, where males construct and aggressively defend nests [8], and signal to females with a complex pheromone mixture, setting the stage for studies on the poorly understood role of pheromones in mate choice, the evolution of exaggerated male signals [96], and the function and evolution of multi-component pheromones [56].

Augmenting sea lamprey management with insights from olfactory communication provides a rare example of sensory-integrated control in vertebrates. Manipulation of olfactory systems is a widely used as tools to control pest insect populations [105]. Extension of olfactory integrated control of insects to invasive vertebrates is conceptually sound [16–19, 106, 118], however, after decades of research into fish olfaction, olfactory communication is not integrated into control of any invasive fish. Developing olfactory-integrated management is a challenging and costly endeavor [118], but offers a suite of potentially robust and environmentally benign tools. Olfactory-guided behaviors are not unique to sea lamprey, and insights gained while developing olfactory-integrated control of sea lamprey can be extended to other species of concern throughout the world. Furthermore, the sea lamprey model offers the opportunity to optimize olfactory-integrated control methods without the confounding interactions of other sensory modalities. For example, most organisms, including sea lamprey, incorporate information from several sensory modalities while making reproductive decisions. However, sensory-guided behaviors in sea lamprey are clearly biased towards olfaction [45, 46]. Similar to the insect model used as a conceptual foundation for sea lamprey olfaction research, the sea lamprey model can function as a model for more complex vertebrates. Likewise, the technologies and methods developed for studying sea lamprey olfaction provide a foundation that can be used to expedite future research into olfaction in other organisms that are invasive or in decline in the Laurentian Great Lakes and throughout the world.

Conclusion

The sea lamprey is a basal vertebrate with an increasingly well-characterized olfactory communication system. We suggest that the olfactory biology of the sea lamprey can be used to inform future research on olfactory systems of other species, as the understanding of the lamprey

olfactory biology has been informed by detailed descriptions of olfaction in other organisms. In particular, the simple but well-developed olfactory organ, critical functions of several reproductive chemical cues and pheromones, and potential for population control make studies on sea lamprey olfaction broadly interesting. Current research on sea lamprey olfaction focuses largely on implications for population management [16–20, 106, 118]. However, further research into sea lamprey olfaction, spanning across neurobiology, characterization of chemical cues and pheromones, and ecology and evolution, offers opportunity for a uniquely integrated understanding of chemical communication in a vertebrate.

Abbreviations

3kPZS: 3keto petromyzonal sulfate; DkPES: Diketo petromyzonene sulfate; PZS: Petromyzonol sulfate; PADS: Petromyzonamine disulfate; PSDS: Petromyzosterol disulfate; 2-phenylethylamine: PEA HCL; EOG: Electro-olfactogram; HPLC: High-performance liquid chromatography; MS: Mass spectrometry; cAMP: Cyclic adenosine monophosphate; OR: Odor receptor; V1R: Vomeronasal type 1 receptors; TAAR: Trace amino acid receptors.

Competing interests

The authors declare no competing interests.

Authors' contributions

TB participated in all aspects of developing and drafting the manuscript. MJS drafted the sections related to management applications. BSZ drafted the sections related to olfactory biology. CB designed and created the figures and contributed to editing the entire manuscript. WL conceived the manuscript and contributed to editing the entire manuscript. All authors read, corrected, and approved the final manuscript.

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Author details

¹Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI, USA. ²Great Lakes Fishery Commission, Ann Arbor, MI 48105, USA. ³Department of Biological Sciences, University of Windsor, Windsor, ON, Canada.

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