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Research to Guide Use of Pheromones to Control Sea Lamprey

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ABSTRACT. *The Great Lakes Fishery Commission (GLFC) considers the application of sea lamprey pheromones a promising alternative-control method for its sea lamprey management program. Several components of two pheromones that regulate migration and reproduction, respectively, have been identified and synthesized. Potential utility of these pheromone compounds in lamprey management have been demonstrated in a series of field experiments. These discoveries have laid a solid foundation for development of pheromone-based management. In order to identify potential strategies that will be practical, effective, and economical, we propose a hypothesis driven approach that integrates concepts and experimental methods from several disciplines of biological science, such as neurobiology, biochemistry, and behavioral ecology to illustrate the exact function of identified compounds. In the interim, we identify the necessary steps, or issues critical to eventual implementation, to charter a pathway that leads from laboratory research to effective deployment of pheromones. Finally, we highlight a strategy that fosters collaboration among scientists across disciplines, as well as among research institutes and lamprey control agencies, to accomplish this research agenda.*

INDEX WORDS: *Pheromone, sea lamprey,* Petromyzon*, reproduction, migration, control.*

INTRODUCTION

Controlling the sea lamprey (*Petromyzon marinus)*, an introduced parasitic predator of commercially and ecologically important fishes, is essential to maintaining a healthy and sustainable ecosystem in the Great Lakes. The Great Lakes Fishery Commission (GLFC), in partnership with Fisheries and Oceans Canada (DFO) and the U.S. Fish and Wildlife Service (USFWS), carries out an integrated pest management program to control sea lampreys in the Great Lakes. The main elements of this program are stock assessments, lampricide application to kill larvae, maintaining barriers to migration, trapping of adults, and controlled release of sterilized males. The future of sea lamprey management lies in the pursuit of new and effective control tactics, including alternatives to lampricide applications.

The GLFC (2001) identified field deployment of one new alternative-control method by 2010 as an important milestone for its sea lamprey management program. They also identified the most promising method for future implementation: the application of recently identified pheromones that regulate migratory and reproductive behaviors of the sea lamprey. We prepared this review to construct a visible, transparent, and robust framework for research that supports and catalyzes the development and field deployment of pheromone-based applications. Our primary objective is to discuss the essential research issues through an analysis of current understanding of sea lamprey chemical communication and the information needs. We also seek to develop new research strategies that foster

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collaboration among scientists across disciplines, as well as among research institutes and lamprey control agencies, to accomplish these objectives.

This review contains three primary sections. The first establishes a basic understanding of pheromone communication, much of which derived in the laboratory and from numerous disciplines, ranging from molecular biology to behavioral biology and ecology. It describes both our current understanding and remaining basic research needs. The next section of the paper identifies critical questions and research needs necessary to move pheromone research "from the laboratory to a tool for control"—that is, from basic knowledge to practical implementation as a management tool. Here, we consider some broad questions that relate to the ecological context for the use of pheromones in control, and we present a set of research questions organized around three broad pathways by which pheromones might be used to the greatest effect. The final section lays out the research strategy we envision for realizing all the promises held by lamprey pheromones into an integrated pest management strategy for the Great Lakes.

FUNDAMENTAL UNDERSTANDING OF SEA LAMPREY PHEROMONES

Sea Lamprey Olfactory Biology

Biologically relevant odorants are critical for the sea lamprey to complete its complex life history. Each developmental stage—larvae, parasite, and spawning adult (Applegate 1950, Hardisty and Potter 1971)—is regulated to some extent by odors. Sedentary larval sea lampreys (ammocoetes) have sensitive and mature receptor neurons (VanDen-Bossche *et al.* 1995). A study on Pacific lampreys (*Lampetra tridentate*) implicated odorants in regulation of growth (Mallatt 1983). During the radical transformation from larvae to parasite, the olfactory system undergoes marked elaboration (VanDen-Bossche *et al.* 1997) where the sensory epithelium becomes enlarged and integrated into an olfactory apparatus that is highly sensitive to a unique repertoire of compounds (Kleerekoper 1972, Li *et al.* 1995, Li *et al.* 2002, Li and Sorensen 1997, Siefkes and Li 2004, Fine *et al.* 2004, Moore and Schleen 1980, Teeter 1980, Vrieze and Sorensen 2001). Among chordates, sea lamprey olfactory bulbs are exceptionally large relative to the brain (Stoddart 1990). Olfaction is believed to be the key modality that regulates and motivates basic behaviors in post-larval sea lampreys (Kleerekoper 1972), including migration (Bjerselius *et al.* 2000, Sorensen *et al.* 2005), mating behavior (Li *et al.* 2002, 2003a, b; Siefkes *et al.* 2003a, b; Teeter 1980), and sexual maturation (Li *et al.* unpublished data). Clearly, the sea lamprey's complex life history and reliance on identifiable odorants offer ample targets for development of control strategies that exploit a multitude of odor-induced reactions. These strategies, once developed, are likely to be effective, efficient and environmentally benign.

The natural odorants that hold the foremost promise for application in lamprey management are pheromones, or "substances that are excreted to the outside by an individual and received by a second individual of the same species in which they release a specific reaction, for example a definite behavior or developmental process" (Karlson and Luscher 1959). According to their primary function, pheromones are either considered *releasers*, which elicit immediate behavioral changes, or *primers*, which induce changes in development and physiology (Wilson and Bossert 1963). Many pheromones have both releasing and priming functions, and the actions of primers often predispose those of releasers. In principle, both types of pheromones have several features favorable for exploitation in population management. Because responses to pheromones are often instinctual, they can be expected from all conspecific individuals at the proper developmental stage (Li *et al.* 2003b). Overall, we may expect these responses to be specific and robust, rendering target animals vulnerable to manipulation with minute amounts of pheromones (cf. Siefkes *et al.* 2005, Wagner *et al.* 2006).

The driving force for research into lamprey olfaction is the development of new control methods. However, the research findings also reveal the sea lamprey's utility as an animal model for the elucidation of fundamental principles of pheromone communication and olfactory transduction in vertebrates. Its male releasing pheromone (a mating pheromone) is the only one identified among fishes (Li *et al.* 2002, Yun *et al.* 2002), and the first found to have a large active space (Li *et al.* 2003b, Siefkes *et al.* 2005). Lamprey male pheromone function differs from the "chemical spying" of most previously identified fish pheromones (Sorensen and Stacey 1999). In addition, sea lamprey is the only species with a larval migratory pheromone identified (Bjerselius *et al.* 2000, Fine *et al.* 2004, Moore and Schleen 1980, Teeter 1980, Vrieze and Sorensen 2001, Sorensen *et al.* 2005). Sea lamprey and their close relatives typically have large sensory neurons (Thornhill 1967) and a limited number of odorant receptors (Dryer 2000) that respond to a small set of specific compounds (Li 1994). This arrangement renders matching receptors to odorants somewhat easier, and should facilitate experimental inquiry into olfactory transduction mechanisms at cellular and molecular levels.

Research into pheromone communication contributes to our understanding of many other aspects of lamprey biology. The complexity of the lamprey chemical communication, encompassing several possible pheromones and feeding cues, has spurred comparative work in biochemistry and electrophysiology throughout its life history (cf. Li *et al.* 1995, Polkinghorne *et al.* 2001, VanDenBossche *et al.* 1995, Zielinski *et al.* 1995), and of other lamprey species (cf. Fine *et al.* 2004, Yun *et al.* 2003a, b). The multidimensional nature of each pheromone system has further stimulated integrative approaches that integrate different disciplines of science (cf. Li *et al.* 2002) such as reproductive endocrinology and cell and molecular biology (cf. Sower and Kawauchi 2001). These studies, in turn, provide information for understanding of pheromone function in the context of sea lamprey life history and ecology.

Information Needs for Developing Pheromone Applications

One direct and rational way to identify critical information needs is to identify issues directly pertinent to the control application. However, leading candidate applications are only beginning to emerge from the long list of potential strategies proposed over the last two decades (Li *et al.* 2003a, Sorensen and Vrieze 2003, Teeter 1980, Twohey *et al.* 2003a). As our understanding of the mechanisms that mediate lamprey pheromone communication progresses, we need to simultaneously develop cost-effective approaches to producing pheromones at a large scale.

First, we believe we should generate the basic biological information essential to elucidate the lamprey pheromone system. Each of the strategies proposed so far relies on the premise that the biosynthesis, release, and reaction to pheromones can be mimicked, altered, or disrupted. Thus, any application that exploits a pheromone will only be as good as our knowledge of the mechanisms that regulate that particular function. In the long term, the most efficient and practical control strategies will emerge from a thorough understanding of all facets of lamprey pheromones. Herein, we focus on those aspects of the sea lamprey pheromone system that determine the specificity and robustness of behavior, and that predict the potential utilities and constraints on candidate application approaches.

Studies of animal behavior often address four basic questions, respectively related to ultimate function (“why”), proximate causation (“how”), phylogeny (lineage), and ontogeny (development). Although variations on this theme are common, the general utility of this approach is well established. These questions constitute a framework that enables a logical, inclusive, and structured approach to identifying critical information needs. The “why” question is often addressed in the context of life history and ecology; its answers shed light on the most probable applications and the potential constraints confronting each. The “how” question is most often addressed at the organism, cellular, and molecular scales. It requires an integration of methods from numerous disciplines, ranging from molecular biology to chemistry to behavioral biology. Knowing “how” provides insight into which control tactics are likely to be useful. Lineage questions address the species-specificity of the pheromone system, and potentially inform the optimization of pheromone component blends in recipient streams with differing lamprey assemblages. It also provides information useful for registration by predicting unintended impacts to native species. Finally, the ontogeny question provides information on the timing and versatility of application methods.

A comprehensive answer to each question can only be attained by understanding how a pheromone message flows from the *emitter* through *signal* molecules to the *receiver*. Typically, internal and external stimuli act upon emitter animals (or tissues) to transmit a signal in coded form through water to a receiver. The receiver registers the message via the olfactory organ, interprets it according to the context, and provides a particular “meaning” which provokes a physiological or behavioral response.

Emitters

Because most pest-control applications employ man-made copies of the pheromone to induce or disrupt behaviors, emitters often receive less attention than the two subsequent components. However, understanding this component contributes to an overall understanding of ultimate function, conceivably leading to novel strategies to eliminate or strengthen message flow in the communication channel. The exact developmental stage and/or physiological state during which the sea lamprey actively synthesizes and releases each pheromone need to be identified. Mechanistic studies should focus on the biochemical pathways of biosynthesis, the cellular and molecular mechanisms facilitating pheromone release, and the regulation thereof, in particular by endocrine factors. Potential contextual stimuli that initiate and maintain the pheromone synthesis and release should be screened extensively.

Message Molecules

The identification of message molecules often predisposes intensive and efficient studies on the reactions of the receiver. Hence, it is central to understanding the mechanistic processes of any particular pheromone system and usually the initial focus of research on newly discovered pheromones. Desirable information includes the chemical structures of all components, the optimal and working range of ratios among all mixture components, mechanisms that govern the rate, timing, and duration of release, and the range of concentrations in nature. The stability of each component molecule should also be examined. If one component is more susceptible to chemical, physical or enzymatic modifications in water, then the ratio of the components, and thus the message, may change substantially over time and distance.

Pheromone molecules carry a message through a "noisy" channel, a multitude of chemicals which at times can vary substantially across habitats. How do sea lampreys optimize the "signal-to-noise" ratio to ensure their pheromones carry unequivocal messages? Most animals maximize the signal-to-noise ratios through efficient biosyntheses, unique molecular structures, and co-evolution of a physiological filter in the receiver organ. According to information theory, "redundancy" is the predominant means by which error-free information can be transmitted in a noisy channel. In pheromones, this could be achieved by multiple components if each of them is detected by a separate receptor. Actual examples for the second strategy are limited but do exist. Knowing which mechanism(s) evolved in the sea lamprey to ensure efficient signal reception, and whether it is possible and practical to exploit these mechanisms for control, is essential.

Receivers

Receivers are typically the ultimate target of pheromone-based pest management. Their reactions to a pheromone signal are mediated through a complex cascade of sensory input, signal processing in the central nervous system, motor or endocrine output (or both), and associated changes in gene expression. The cascade is augmented and modified by an extensive array of internal and external factors, an understanding of which leads to predicting the versatility, utility, constraints, and probable strategies for management.

Virtually every element of this communication system given its relevant contextual stimuli offers a potential target for control. Specifically,

- the exact developmental stage and physiological state at which the receiver lampreys detect and respond to a pheromone must be determined in order to choose proper targets for subsequent study and control;
- physiological and environmental factors that augment the specific reactions must be identified and fully characterized;
- neurobiology of the olfactory organ needs to be examined to determine the detection limit for specific pheromones, response dynamics, habituations, specificity to each pheromone component and typical noisy chemicals, possible physiological filters for noise, overall mechanisms for optimizing the detection of conspecific pheromones, and the environmental, physiological and developmental factors that modulate its responsiveness;
- how each pheromone component is processed should be determined, including presentation and integration in the olfactory bulbs, the subsequent pathways for olfactory information processing and modification, mechanisms causing motor output or endocrine changes, and central discrimination of the pheromone signal;
- plasticity in reactions due to learning and habituation, and its impact on effectiveness of control methods, should be determined;
- at the organismal level, the behavioral or physiological reactions (or both) to each component and their mixtures should be fully characterized.

Current Understanding of Sea Lamprey Pheromone Communication

Our current understanding of pheromone communication in the sea lamprey has accrued principally through research supported by the Great Lakes Fishery Commission. Following, we highlight the results and conclusions about the two pheromones that have received intensive studies: the larval migratory pheromone and the male pheromone.

Migratory Pheromone

The migratory pheromone and its potential applications in lamprey management have been reviewed recently (Sorensen *et al.* 2003, Sorensen and Vrieze 2003). Migratory adult sea lampreys rely on odorants released by the larvae to locate spawning streams. Across the Great Lakes basin, these adults do not select their natal streams for reproduction (Bergstedt and Seelye 1995), but do prefer streams that contain higher densities of larval lampreys (Moore and Schleen 1980). In the laboratory, migratory adults placed in mazes are attracted to the odor of larvae (Bjerselius *et al.* 2000, Teeter 1980), and to water collected from streams that possess larvae (Vrieze and Sorensen 2001). Further, the attractiveness of the water collected from streams without larvae can be enhanced by the addition of larval washings (Vrieze and Sorensen 2001).

Several lines of evidence indicate that petromyzonol sulfate (PS; Fig. 1; Haselwood and Tokes 1969) is only one component of the migratory pheromone. This molecule stimulates the olfactory epithelium of migratory adults (Li *et al.* 1995, Li and Sorensen 1997), attracts adults in a laboratory maze (Bjerselius *et al.* 2000), and is released in sufficient quantities to function as a component of the migratory pheromone (Polkinghorne *et al.* 2001). PS was less attractive than the entire signal released by larvae suggesting there were more components to the migratory pheromone (Sorensen *et al.* 2003). Recently, two novel compounds have been discovered as additional components of the sea lamprey migratory pheromone (Sorensen *et al.* 2005). Petromyzonamine disulfate (PADS; Fig. 1) is the major component of the migratory pheromone being biologically more active than PS and attracts sea lampreys at a concentration of 10^{-13}M (Sorensen *et al.* 2005). Petromyzosterol disulfate (PSDS; Fig. 1) was discovered as a minor component of the migratory pheromone being attractive at concentrations of 10^{-11}M (Sorensen *et al.* 2005). The behavioral effect of another larval lamprey bile acid, allocholic acid (ACA; Haslewood and Tokes 1969), has not been observed. Fine *et al.* (2004) discovered that lampreys of the family Petromyzontidae may produce a common migratory pheromone. If so, the distribution of native lampreys may bear greatly on the development of an effective migratory pheromone-based application and needs to be examined.

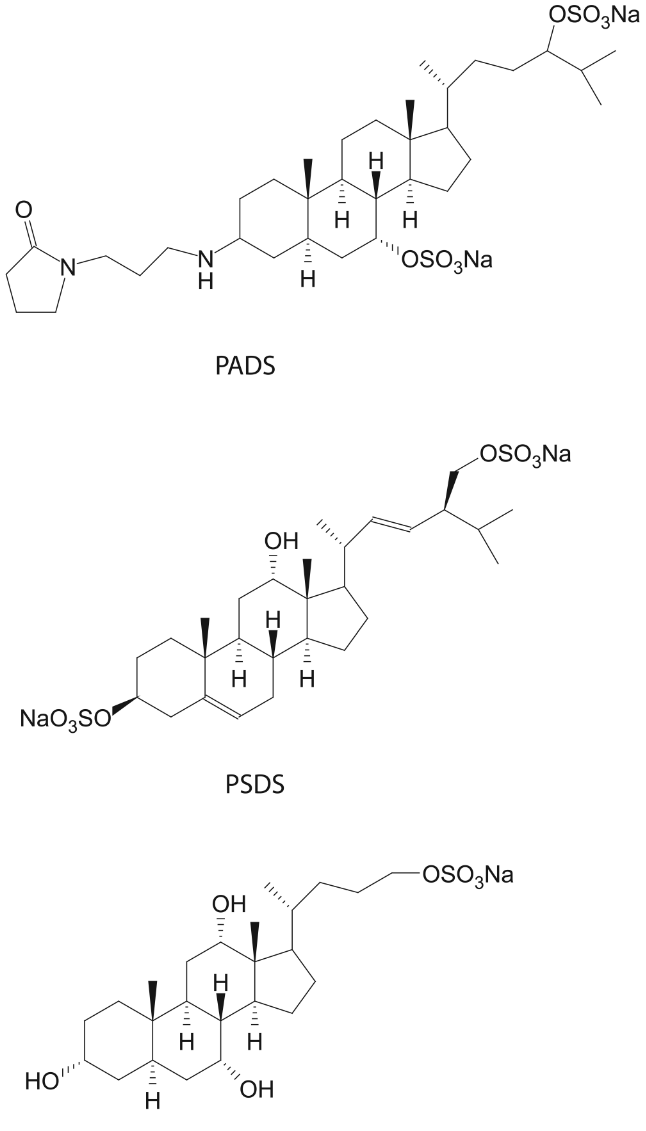


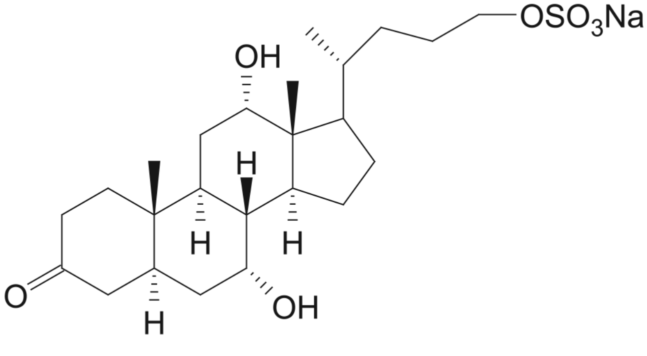
FIG. 1. The chemical structures of three components of the migratory pheromone as identified by Sorensen* et al. *(2005).

Male Pheromone

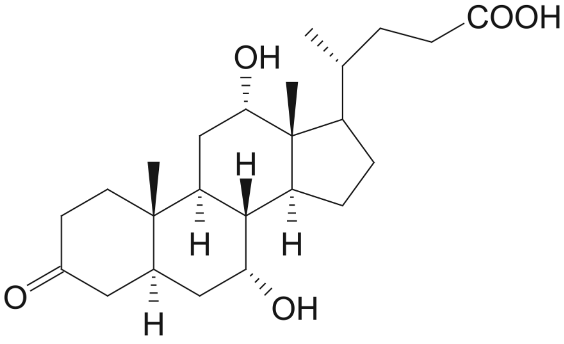
In a two-choice maze mature females tend to stay longer in the arm conditioned with mature males (Teeter 1980). In fact, it is the ovulated females, and not the males or pre-ovulatory females, that prefer water conditioned with spermiating males (Li et al 2002, Siefkes *et al.* 2003b). During these trials ovulated females also exhibited dramatic increases in search behavior. At a natural spawning site (Ocqueoc River, MI), ovulated females tagged with radio transmitters swam upstream for 65 m to reach the exact site where spermiating males were held (Li *et al.* 2002). It appears likely this pheromone guides ovulated females to nests and stimulates their participation in further nest construction and spawning. Traps baited with spermiating males (Johnson *et al.* 2005) or washings from spermiating males (Johnson *et al.* 2006) capture a large proportion of ovulated females whereas unbaited traps do not capture females. Further, traps baited with multiple males are more attractive than traps baited with single males (Wagner *et al.* 2006).

The structures of two potential components of the male pheromone have been identified as 3-keto petromyzonol sulfate (3KPZS; Fig. 2; Li *et al.* 2002) and 3-keto allocholic acid (3KACA; Fig. 2; Yun *et al.* 2002). 3KPZS differs from PS by its 3-keto, as apposed to a 3α-hydroxy, group (Haslewood and Tokes *et al.* 1969, Li *et al.* 2002). These two bile acids are highly stimulatory for the female olfactory organs (Siefkes and Li 2004). 3kPZS induces preference and search behaviors in ovulatory females in laboratory mazes (Li *et al.* 2002, Siefkes *et al.* 2003a), and is released in large quantities (Yun *et al.* 2002) through specialized cells in the gills (Siefkes *et al.* 2003a, b). In a natural stream, a synthetic copy of 3kPZS at 10^{-12} molar guided ovulated females to the exact location where 3kPZS was introduced (Siefkes *et al.* 2005). 3kPZS appears to be the main component of the male pheromone; the potential effects of 3kACA on female behavior have not been quantified. Whether these two compounds are released by any native fishes of the Great Lakes has also not been determined.

The migratory and male pheromones play different but sequential roles in an exquisite signaling system that guides lacustrine adults into suitable spawning streams, and then mature females to mature males to complete the act of spawning. Clearly, requirements for timing and precision are different in these two systems. The migratory pheromone needs to last through the season when adults enter the spawning ground and move upstream. The male pheromone, in contrast, needs to guide ovulating females to the exact nest where males are spermiating, which is particularly important to sea lampreys as they die soon after completing sexual maturation.



3kPZS



3kACA

FIG. 2. The chemical structures of two components of the male pheromone as identified by Li* et al. *2002 and Yun* et al. *2002b.

The biosynthesis and release of each pheromone appears to be well suited for their respective functions. A component of the migratory pheromone, petromyzonol sulfate (PS), is released by all larvae throughout the year (Polkinghorne *et al.* 2001). Rate of release is enhanced by feeding activity which peaks in the spring as adults enter spawning streams. In contrast, the male pheromone is not released until the male becomes reproductively available for spawning (i.e., at spermiation) (Siefkes *et al.* 2003a, b). The physiological and environmental factors controlling the onset and intensity of its release have not been clearly defined. Nevertheless, the precise synchronization of its release with the onset of spermiation suggests each is regulated by a common mechanism. Spermiation is closely regulated by the hypothalamus-pituitary gland-gonad axis (reviewed by Sower 1998), and pheromone release may be similarly regulated. The identity of the hormone (or hormones) that regulates biosynthesis of the male pheromone is not known.

One potential additional pheromone, although less intensively studied, also holds promise for developing into control methods. A *female pheromone* has been implicated in two-choice mazes where spermiating males (but not pre-spermiating males or females) spent more time in the compartment conditioned with ovulating females, but not in that conditioned with immature females (Li *et al.* 2002, Siefkes *et al.* 2005, Teeter 1980). Unlike the male pheromone, the proposed female pheromone does not induce an increase in search behavior of its receiver in either the laboratory or field (Li *et al.* 2002, Siefkes *et al.* 2005). Notably, male lampreys typically precede females and initiate nest construction (Applegate 1950, Manion and Hanson 1980). Therefore, spermiating males could benefit from a pheromone that stimulates the females to search for males (Li *et al.* 2002), whereas females may benefit from a pheromone that promotes forming and maintaining of spawning pairs (Teeter 1980).

Finally, pheromones may play a role in regulating larval distribution and the onset of metamorphosis. Larval sea lampreys have mature olfactory receptor neurons (VanDenBossche *et al.* 1995) that respond at least to some of the same odorants as adults (Zielinski *et al.* 1995, Li unpublished). Although the role of odors in the lives of larval sea lampreys is not known, excreted metabolites may be involved in inhibiting growth of other larvae (Mallatt 1983, Rodrigeuz-Munoz *et al.* 2003) and could also play a role in triggering metamorphosis.

Research Needs for the Biology and Chemistry of Lamprey Pheromones

Although our understanding of lamprey pheromones has progressed along several fronts, the unknown still exceeds the known. For the migratory and the male pheromone, we have an understanding of the complexity of their signal molecules, their potency and specificity for the olfactory epithelium, and the basic characteristics of induced behaviors. The regulation of behavioral responses, the neurobiological mechanisms that generate motor output in response to sensory input of pheromones, and regulation and biochemical pathways for pheromone synthesis remain elusive. Here, we highlight critical research needs emphasizing knowledge gaps that prevent timely development of control methods, hinder insight into the full application potential of these pheromones, and impede an initial assessment of other demonstrated pheromones.

1. Identify and characterize the variables that regulate biosynthesis of pheromones, in particular the hormones that augment the synthetic pathways of 3KPZS and 3KACA. The anticipated information will be useful for alteration of pheromone release.
2. Develop inexpensive chemical syntheses of, or other means to produce, pheromones identified in the sea lamprey at large scales and sufficient levels of purity. Synthesized compounds enable 1) direct examination of the role of each component in natural habitats, and 2) the large scale field experiments necessary to develop effective implementation protocols.
3. Elucidate the function of each identified pheromone component as well as how mixtures within the range of ratios expressed in natural pheromones operate. Results from laboratory experiments should be validated in field trials; these data will be useful in developing end-use products for eventual EPA registration.
4. Develop accurate, sensitive, and timely methods for measuring concentrations of mating and migratory pheromone components from field samples. In order to achieve effective pheromone applications, we will need to know how much pheromone to add, and for how long to add it. Competing natural sources of pheromone will be a significant impediment to successful implementation. With these methods we may 1) estimate the concentration and variation of pheromone components in lamprey habitat, 2) characterize their dispersal and fate under different environmental conditions, and 3) relate these findings to the local density of larvae and spawners.

5. Determine the physiological factors and contextual stimuli that modify lamprey responses to pheromones. This task is daunting but the resultant information will contribute to optimizing the design of application protocols.
6. Determine the impacts of physiological adaptation and habituation on the plasticity of lamprey responses to pheromones. Whether applications that require prolonged introduction of pheromones will be effective is largely determined by the plasticity of individual responses.
7. Characterize the central nervous system pathways, signal processing apparatus, and motor or endocrine outputs in response to pheromones. Determine if sex and development stage-specific regions in the brain govern where pheromone constituents converge. Identify hormones and neuromodulators that augment the neural pathway for pheromone sensory input and output during spawning and migration, and the suppression thereof. These data will 1) complement the currently available results on neurobiological responses of the peripheral sensory organs, and 2) illustrate the mechanisms mediating impacts of contextual factors on the strength of pheromone responses.
8. Determine the fitness "value" of individual pheromones in the sea lamprey. In this context, the value is difficult to define but could be roughly estimated by determining the impact on reproduction if a particular pheromone system is impeded. Nonetheless, the information, be it vague, could potentially indicate the long-term effectiveness and full potential of pheromone based controls.

MOVING FROM THE LABORATORY TO A TOOL FOR CONTROL

Background

For pheromones to become a practical tool for sea lamprey control, fundamental research aimed at elucidating the nature and functions of lamprey pheromones must be complemented by research that evaluates potential control tactics. Here, we address critical questions and research needs to move pheromone research "from the laboratory to the field"—that is, from basic knowledge to practical implementation. We first consider some broad questions that relate to this topic—the ecological context for use of pheromones in control. Then, we present a set of research questions organized around three broad pathways by which pheromones might be used to effect control. The questions are summarized in Table 1.

Field trials to assess the efficacy of using pheromones to control lampreys will require access to large quantities of either naturally produced or synthesized pheromones. Logistical constraints are likely to severely limit the supply of naturally produced pheromone, especially for program-scale implementation of a strategy. This reveals the critical importance of research on the characterization and synthesis of sea lamprey pheromones, a topic already addressed in the laboratory section of this paper. For pheromones to become a practical tool for sea lamprey control, the structure of key pheromone components must be identified, and economically viable methods of synthesis need to be developed. Finally, pheromone products must be approved by regulatory agencies in the U.S. and Canada before their application. Regulatory issues are not a subject of this review.

Recruitment Dynamics

The pheromone strategies currently envisioned are aimed at reducing sea lamprey reproductive success (Twohey *et al.* 2003a). The effectiveness of actions that reduce reproductive success will depend on sea lamprey recruitment dynamics, because the recruitment that results from the sea lamprey that are able to spawn will determine the effect of the action on production of parasitic lamprey. Both density-dependent (compensatory) changes in hatching, growth, and mortality of larvae and density-independent recruitment variation (Jones *et al.* 2003) can lead to greater than desirable recruitment despite efforts to reduce reproductive success. Thus, an integrated pest management strategy for sea lamprey must effectively reduce reproduction to levels where density-dependent and density-independent compensatory effects are negligible. Effective integrated pest management typically relies on a variety of highly effective tools that multiple vulnerabilities in the life history of the pest (Knipling 1979, Sawyer 1980). This implies an aggressive strategy, integrating pheromone use with other actions that also aim to reduce reproduction.

Four outcomes that were developed to guide implementation and evaluation of the sterile-male-

TABLE 1. Summary of research needs for sea lamprey pheromones.

Biology and Chemistry of Lamprey Pheromones

1. What variables regulate biosynthesis of pheromones?
2. What are the most efficient and inexpensive procedures to synthesize or produce sea lamprey pheromones at large scales and sufficient levels of purity?
3. What is the function of each identified pheromone component and the effects of their mixtures in eliciting behavioral responses?
4. What is an accurate, sensitive, and convenient method for measuring pheromone components for determination of purity and rapid field assessment of concentration in streams?
5. What are the physiological factors and contextual stimuli that augment and modify responsiveness of lamprey to pheromones?
6. What are the effects of physiological adaptation and habituation elements in plasticity of lamprey responses to pheromones?
7. What is the pathway in the central nervous system for sensory input of pheromones, subsequent signal processing, and motor or endocrine output?
8. Are there hormones and neuromodulators that augment the neural pathway for pheromone sensory input and output during spawning and migration, and the suppression thereof?

Redistribution Strategy

9. How do sea lampreys search for pheromone sources in lake and streams?
10. How does the lamprey's physiological condition affect the lamprey's response to pheromones?
11. How well can sea lamprey distinguish gradients/different sources?
12. How does the attractiveness of sea lamprey pheromones compare to other odorants?
13. How can we alter the distribution of lamprey during their spawning migration?
15. How do environmental variables affect exposure to the animal, dose to the animal, and the proportion of animals exposed; and the effect of pheromones on redistribution?
16. Once lampreys have been successfully redistributed in a watershed, how can this redistribution be maintained?
17. Can male and female lamprey be differentially redistributed with pheromones?
18. Are there environmentally benign compounds that repel lampreys from a stream or tributary?

Trapping Strategy

19. How does exposure to pheromones affect lamprey behavior?
20. What proportion of sea lampreys that encounter a detectable level of pheromone will search for its source (a trap)?
21. What trap designs are most effective in combination with pheromone baits?
22. Do native species of larval lampreys produce pheromones that are equally attractive as those produced by sea lamprey larvae?
23. How does the natural background concentration of pheromones affect attractiveness of introduced pheromones?
24. Can lamprey be attracted into traps or tributaries in the presence of pheromones from natural sources?
25. What concentrations of pheromone are effective in attracting sea lampreys to traps?
26. What environmental cues might enhance or reduce pheromonally mediated attraction behaviors?

Mating Disruption Strategy

27. Do sea lamprey behavioral responses to mating pheromones become less robust after prolonged exposure?
28. Can a synthetic or natural antagonist to sea lamprey pheromones be produced?
29. How is lamprey behavior affected by high concentrations of pheromone?
30. How do different components of each pheromone affect lamprey, and how do they interact?
31. Can pheromones be used to alter the natural timing of maturation?
32. What level of disruption is needed for the technique to effectively reduce sea lamprey populations?
33. Can male pheromone production be up-regulated or enhanced in individual animals to achieve a control strategy?

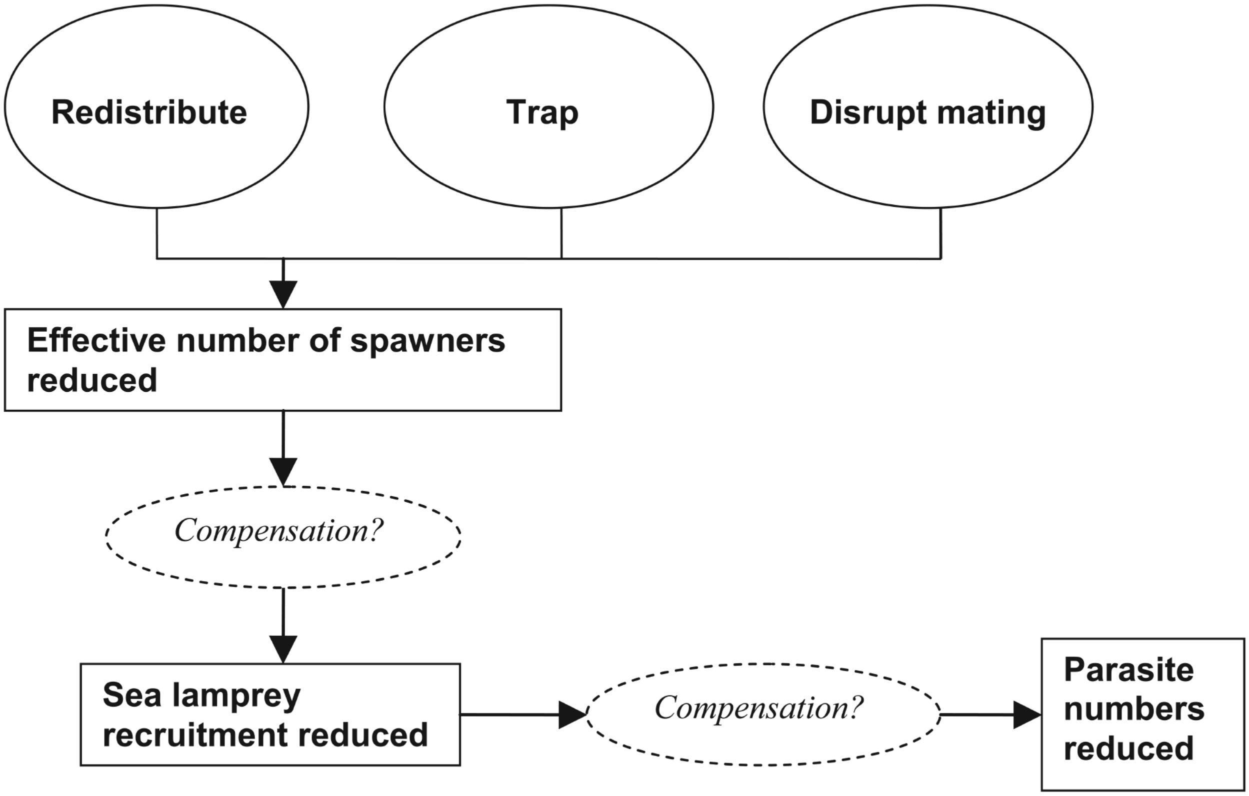


FIG. 3. Conceptual framework of pheromone control strategies based on redistribution.

release technique (from Twohey *et al.* 2003b), apply equally well to pheromone use:

1. The abundance of burrowed larvae in each year class (after leaving the nest) is reduced in individual streams.
2. Reductions in abundance of larvae persist through the larval life stage and result in reductions in the number of metamorphosing sea lampreys in individual streams.
3. The number of parasitic-phase sea lampreys in the lake is reduced.
4. Damage to fish in the lake is reduced.

Each of these outcomes is subject to the phenomenon of compensation discussed above. Research to increase our understanding of the population dynamics of larval and juvenile sea lamprey, particularly as it relates to compensatory mechanisms, is key to understanding the potential of pheromone-based control. However, the remainder of this discussion will focus on the specific use of pheromones to disrupt reproduction in sea lampreys.

Pathways to Control Strategies

The current understanding of pheromone communication suggests that a research focus on reducing the effective number of mature reproducing sea lampreys and enhancing existing control efforts (e.g., trapping and the release of sterilized males) (Twohey *et al.* 2003b) may very well lead to new management measures. Several pheromone-based tactics have been proposed for further investigation (Li *et al.* 2003a, Sorensen *et al.* 2003, Twohey *et al.* 2003a). A conceptual framework for using pheromones to reduce the number of reproducing lampreys is presented in Figure 3.

Three pathways to control are envisioned: 1) *Redistribution*—lure mature sea lampreys to habitats where they can not reproduce or where they can be effectively removed or killed. 2) *Trap*—lure sea lampreys into traps baited with pheromone. 3) *Mating disruption*—strategies to confuse and confound the reproductive cues that lampreys rely upon. Whereas these categories are useful to organize our thinking about key research questions, we note that many of the proposed questions will apply to more than one category.

Research Needs for Control

Redistribution Strategy

Pheromones have the potential to draw sea lampreys into streams or parts of streams advantageous for control (Sorensen *et al.* 2003, Twohey *et al.* 2003a, Wagner *et al.* 2006). Lampreys could be attracted into a tributary 1) with poor attributes for egg or larval survival, 2) that is scheduled for a lampricide treatment, or 3) where effective traps can be located. Reproduction might also be reduced in adjacent tributaries, delaying the need for future lampricide treatments. This approach could be enhanced by eliminating sources of migratory pheromone or by the application of a repellent. Currently, no environmentally benign repellents are known. Additionally, it might be possible to lure male and female sea lampreys to separate streams or areas of a stream, thereby preventing mating.

Important research questions related to the potential for redistribution are:

- How do sea lampreys find and subsequently behave in detection of pheromones in lakes and streams?
  a. How do sea lampreys find and select streams?
  b. When during their life history do they respond to pheromones?
  c. When during the spawning migration do sea lampreys cease to follow pheromone plumes, and how does their physiological condition affect this response?
  d. What cues other than pheromones guide them to their spawning destination?
  e. What behaviors do sea lamprey exhibit when searching for pheromones in lakes and rivers?
- How does the attractiveness of sea lamprey pheromone compare to other sources?
  a. What is the threshold concentration that attracts sea lampreys into a stream?
  b. What is the effect of background, or competing sources of pheromone?
  c. What is the dose-response relationship to pheromones?
  d. What other variables affect the attractiveness of pheromones?
  e. What distance of stream or lake can be effectively activated by pheromones?
- How can we alter the distribution of lamprey during their spawning migration?
  a. Within a watershed (among tributaries)?
  b. Among tributaries to the Great Lakes?
  c. What proportion of the population is affected, or what proportion of activated animals can be lured?
  d. Can recruitment of sea lampreys be reduced or eliminated by eliminating all larvae from a stream, including native species?
- How do environmental variables affect lamprey responses to pheromones?
  a. How do flow/current, temperature, shoreline configuration, and turbulence influence the amount and area of exposure to the pheromone?
  b. How do proximity of stream mouths, number and distribution of spawning areas and rearing areas, and lamprey distribution in lake affect movement of lampreys?
- Once lampreys have been successfully redistributed in a watershed, how can this redistribution be maintained?
  a. Through prolonged exposure to the pheromone stimuli?
  b. Will redistribution be maintained after the stimuli are no longer present?
- Can male and female lampreys be differentially attracted with pheromones?
- Are there environmentally benign compounds that will repel lampreys from a stream or tributary?

Trapping Strategy

Trapping with pheromones or other attractants has been used to combat insect pests dating back perhaps to ancient Rome (Lanier 1990). More recent successes include the spruce bark beetle (*Ips typographus L.*), fruit flies (*Tephritidae sp.*), cotton boll weevil (*Anthonomus grandis*), ambrosia beetles (*Gnathotrichus sulcatus, G. retusus and Trypodendron lineatum*), the European elm bark beetle (*Scolytus multistriatus*), and several species of Lepidoptera. The five operating principles for the attraction-annihilation strategy in insects (Lanier 1990) are, 1) optimal synthetic lures are usually identical to the natural lure, 2) effectiveness is increased as the lure is increased relative to the size of the population, 3) the reduction in damage is proportional to the reduction of the population that is in the noxious life stage, but is less than that if the life stage removed is not that which causes injury, 4) among polygamous species, lures that attract females will have greater impact on reproduction than attractants that affect only males, and 5) increasing release rate of the lure will attract a larger proportion of the population over a wider area, but the proportion of the population in the affected area that will be trapped will decrease. Attraction to traps has proven most effective when the area treated is small and the population density of insects is low. This is largely true when the goal is to suppress populations within a limited space (e.g., a corn field) to reduce damage rates. Population suppression on a wider-scale is more problematic.

Control agents in the U.S. and Canada currently operate a network of lamprey traps in about 60 Great Lakes tributaries to assess the adult population (Mullett *et al.* 2003). About 20 of these sites are used to harvest males for the sterile-male-release technique (Twohey *et al.* 2003b). Current trapping technology is not considered sufficiently effective to be used independently for control. Its use is currently limited to an integrated approach in the St. Marys River where a low density of spawning sea lampreys, release of sterile males, and periodic spot treatments with lampricide are used to control the population (Schleen *et al.* 2003, Twohey *et al.* 2003b). Conventional sea lamprey traps are located at barriers to upstream migration and are most effective with a water flow that attracts lampreys into them (Schuldt and Heinrich 1982). Though trapping is difficult or ineffective at sites without these characteristics, it could be improved with pheromones.

Lampreys are captured on their spawning migrations prior to full sexual maturity, ostensibly attracted to streams by the migratory pheromone and other environmental cues such as temperature and stream discharge. Once lampreys mature and spawning commences, few lampreys are captured in conventional traps. Recently, however, Johnson *et al.* (2005) and Wagner *et al.* (2006) demonstrated that ovulating females could be lured into non-conventional trapping locations using spermiated males as bait.

The migratory and male releasing pheromones have great potential to increase capture of lampreys by increasing effectiveness of traps at existing locations, making new sites practical for trapping, and extending the period during which trapping is effective (by making animals of differing maturity vulnerable). In addition to the direct reduction of reproductive success that would result, other potential benefits could include increased supply of males for SMRT and improved assessment of adult populations.

Important research questions related to luring sea lampreys into traps are:

- How does exposure to pheromones affect lamprey behavior?
    a. At different times during migration and spawning
    b. In ways that influence their vulnerability to traps
    c. With regard to locating a "point source" of pheromone?
    d. In response to the size or the area of a stream or lake that can be activated by pheromone?
    e. During approach to a source of male pheromone or migratory pheromone?
    f. With regard to the likelihood a lamprey that encounters a detectable pheromone plume will follow it to the source and enter a trap?
- What trap designs are most effective when combined with pheromone baits?
    a. Can pheromones be used with conventional traps to increase rates of capture of lamprey?
    b. Can migrating sea lampreys be captured in traps independent of barriers?
    c. What are the movement tendencies between river entry and encounter with a barrier?
    d. Are there alternative trapping methods that are better suited for use with pheromones?
- Do native species of larval lampreys produce pheromones that are equally attractive as those produced by sea lamprey larvae?
- Does the natural background concentration of pheromone affect attractiveness of introduced pheromones?
- What concentrations of pheromone are most effective at attracting sea lampreys into traps?
- What environmental cues might enhance or reduce pheromonally-mediated attraction behaviors?

Mating Disruption Strategy

Just as pheromones provide cues to assist sea lampreys in successful reproduction, these cues could be used to confuse and confound mating communication. Several tactics for using pheromones to disrupt insect mating have been proposed, tested, and implemented with some success (Carde 1990, Carde and Minks 1995). These approaches are based on generating sensory adaptation or habituation, promoting competition between the natural pheromone and a synthesized disruptor, camouflaging natural pheromone plumes, creating an imbalance in sensory inputs, or by releasing antagonists (Carde 1990). Each of these tactics has the potential to be applied in sea lamprey management and could reduce reproduction in treated rivers, particularly if integrated with other control activities. Experimentally, female sea lampreys retain full responsiveness to male washings up to 90 minutes following exposure (Li *et al.* 2003a, b; J. Teeter, personal communication, Monell Chemical Sciences Center, Philadelphia, PA). This should enhance the effectiveness of pheromones in trapping, but also could mean that sensory adaptation or habituation may be unlikely. Disrupting maturation may be possible through use of mating pheromones. Recent evidence suggests that the male pheromone play a role in stimulating maturity in conspecifics (M. Siefkes, personal communication, U.S. Fish and Wildlife Service Marquette Biological Station).

Pheromones have also been proposed by several investigators as a means to enhance effectiveness of sterile-male releases. Knipling (1979) theorized that release of sterile insects with "super" attraction will reduce mate finding in low density populations. He examined the effectiveness of pheromone-enhanced males and females with models, and suggested that the benefits of using both sexes together might exceed model predictions. Kaspi and Yuval (2000) found that the male Mediterranean fruit fly, *Ceratitis capitata*, when fed protein and sugar was significantly more likely to emit pheromone, and more likely to copulate than males fed only sugar. McCoy and Wright (1990) used selective breeding to increase the total pheromone produced by individual male boll weevils (*Anthonomus grandis*), rendering sterilized males more attractive than wild males. Others have proposed or discussed methods to enhance the attractiveness of sterile sea lampreys with pheromones. Li *et al.* (2003a, b) reported investigating the feasibility of inducing sterilized males to synthesize and release the male releasing pheromone at higher concentrations, for longer periods of time, or both. This technology also could enhance the attractiveness of male lampreys used as bait in traps. In another approach, Klassen *et al.* (2005) attached synthetic male mating pheromone emitters to sterilized males and enhanced their mating competitiveness; the attachment of male mating pheromone emitters to sterilized females also was effective in disrupting mate finding by wild females.

Important research questions relating to mating disruption are:

- Do sea lamprey behavioral responses to mating pheromones become less sensitive after prolonged exposure? And for how long?
- Can a synthetic or natural antagonist to sea lamprey pheromones be produced?
- How is lamprey behavior affected by high concentrations of pheromone?
  a. Will high concentrations of pheromone suppress behavioral responses?
  b. How much pheromone would be needed to maintain levels high enough and for a sufficiently long period to achieve goals?
  c. How do differing pheromone background concentrations affect this response?
- How do different components of each pheromone affect lamprey, and how do they interact?
- Can pheromones be used to alter the natural timing of maturation?
- What level of disruption is needed for the technique to effectively reduce sea lamprey populations?
- Can the male pheromone production be upregulated or enhanced in individual animals to achieve a control strategy?
  a. Will males that release more male pheromone have greater success in finding mates and mate with more females than wild males?
  b. What is the optimum release rate of pheromone from a single male to enhance mating success?
  c. How does density of spawning males and background of male pheromone affect competitiveness of males with enhanced pheromone production?
  d. Is there a formulation of pheromone that could be attached to an adult sea lamprey and be dispensed at an effective concentration over time?

RESEARCH STRATEGY

The GLFC seeks to deploy a pheromone-based control method by 2010. This vision has placed us at the forefront of developing pheromone-based controls for a vertebrate pest. Our pheromone research promises not only to revolutionize the integrated management of sea lampreys, but also may set a precedent in the application of bio-pesticides to control aquatic nuisance species. This pheromone-based method aims at suppressing the reproduction of sea lampreys in a stream or streams so as to improve the effectiveness of the overall program. That is, the application of pheromones will reduce the amount of lampricides required, the frequency of stream treatments, and/or suppress sea lampreys to lower levels in one or more of the Great Lakes. Meeting this management objective will require a concerted and collaborative effort from all sectors of lamprey research and management community. Consequently, the role of research must be explicitly identified. Application driven research was central to the discovery of two pheromones that are potent modifiers of lamprey behaviors, and will drive them to the threshold of field application by 2010. Over the long term, the GLFC recognizes that research is the essential first step to realizing all the promises held by lamprey pheromones in integrated sea lamprey management.

The challenges are clear. First, pheromone research is inherently interdisciplinary. As multifaceted communication systems, pheromones function in the context of an extensive array of environmental and physiological factors. To effectively explore the many facets of lamprey pheromone communication, our research must be holistic and integrated. The exchange of ideas, information, and techniques among researchers will be promoted through intensive workshops and the development of teams of scientists with complementary backgrounds. A second challenge is that sea lamprey adults are readily available for only 2 months each year, constraining our progress toward the 2010 milestone. Clearly, we need to pursue our research goal on several fronts simultaneously. Finally, given our short-term and long-term research needs, and the limited resources available, we must make the correct investment choices. The male releasing pheromone and the migratory pheromone remain the most promising candidates for development. However, we need to exploit olfaction-mediated reactions to the fullest potential, a goal that requires knowledge of all channels of lamprey pheromone communication. Eventually, the most effective strategy may be one that integrates applications of several pheromones to target all possible behaviors or physiology throughout the lamprey's life history.

A hallmark of the GLFC sea lamprey research program has been the integration of control agencies in evaluating research ideas, providing logistical support, and often participating directly in research projects. By fostering a synergy among researchers and control agencies, novel ideas and innovative approaches often come to light. The large scale of field studies to investigate the effectiveness of application strategies will require the involvement of the assessment and control staffs of the U.S. Fish & Wildlife Service and the Canadian Department of Fisheries and Oceans. Through this integrative approach, we hope to ensure continuity in important lines of research, mediate collaborative links between control agencies and research institutes, and when needed initiate field oriented research projects.

The GLFC has spearheaded the concept and research for pheromone application in control of sea lamprey, a vertebrate pest. The sea lamprey is becoming a model for pheromone communication and biomedical research. Outreach to, and coordination with, the scientific community is critical to the development of pheromones as an alternative control method. This paper provides the key questions and processes that potential researchers can use to develop relevant proposals, and will be used as a guiding document in GLFC-sponsored task forces, workshops, and workgroups.

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REFERENCES

Applegate, V.C. 1950. *Natural history of the sea lamprey* (Petromyzon marinus) *in Michigan.* Special Scientific Report Fisheries 61, U. S. Fish and Wildlife Service.

Bergstedt, R., and Seelye, J. 1995. Evidence for lack of homing by sea lampreys. *Trans. Am. Fish. Soc.* 124:235–239.

Bjerselius, R., Li, W., Teeter, J.H., Seelye, J.G., Johnsen, P.B., Maniak, P.J., Grant, G.C., Polkinghorne, C.N., and Sorenson, P.W. 2000. Direct behavioural evidence that unique bile acids released by larval sea lamprey (*Petromyzon marinus*) function as a migratory pheromone. *Can. J. Fish. Aquat. Sci.* 57: 557–569.

Carde, R.T. 1990. Principles of mating disruption. In *Behavior-modifying chemicals for pest management: applications of pheromones and other attractants.* R.L. Ridgway, R.M. Silverstein, and M.N. Inscoe, eds., New York: Marcel Dekker.

______, and Minks, A.K. 1995. Control of moth pests by mating disruption: successes and constraints. *Annu. Rev. Entomol.* 40:559–585.

Dryer, L. 2000. Evolution of odorant receptors. *Bioessays* 22:803–810.

Fine, J.M., Vrieze, L.A., and Sorensen, P.W. 2004. Evidence that petromyzontid lampreys employ a common migratory pheromone that is partially comprised of bile acids. *J. Chem. Eco.* 30:2091–2110.

Great Lakes Fishery Commission. 2001. *Strategic Vision of the Great Lakes Fishery Commission for the First Decade of the New Millennium.* Ann Arbor, MI, USA.

Hardisty, M.W., and Potter, I.C. 1971. *The Biology of Lampreys.* New York: Academic Press.

Haslewood, G.A., and Tokes, L. 1969. Comparative studies of bile salts—bile salts of lamprey *Petromyzon marinus* L. *Biochem. J.* 114:179.

Johnson, N.S., Siefkes, M.J., and Li, W. 2005. Capture of ovulating female sea lampreys in traps baited with spermiating male sea lampreys. *N. Am. J. Fish. Manaeg.* 25:67–72.

______, Luehring, M.A., Siefkes, M.J., and Li, W. 2006. Mating pheromone reception nand induced behavior in ovulating female sea lampreys. *N. Am. J. Fish. Manage.* 26:88–96.

Jones, M.L., Bergstedt, R.A., Twohey, M.B., Fodale, M.F., Cuddy, D.W., and Slade, J.W. 2003. Compensatory mechanisms in Great Lakes sea lamprey populations. *J. Great Lakes Res.* 29 (Suppl. 1):113–129.

Karlson, P., and Luscher, M. 1959. "Pheromones": a new term for a class of biologically active substances. *Nature* 183:55–56.

Kaspi, R., and Yuval, B. 2000. Post-teneral protein feeding improves sexual competitiveness but reduces longevity of mass-reared sterile male mediterranean fruit flies (Diptera: Tephritidae). *Ann. Entomol. Soc. Am.* 4:949–955.

Klassen, W., Adams, J.V., and Twohey, M.B. 2005. Modeling the suppression of sea lamprey populations by use of the male sex pheromone. *J. Great Lakes Res.* 31:166–173.

Kleerekoper, H. 1972. The sense organs. In *The Biology of Lampreys,* vol. 2, M.W. Hardisty and I.C. Potter, eds. New York: Academic Press.

Knipling, E.F. 1979. *The basic principles of insect population suppression and management.* U.S. Dep. Agric. ESA Agriculture Handbook No. 512.

Lanier, G.N. 1990. Principles of attraction annihilation: mass trapping and other means. In *Behavior modifying chemicals for insect pest management: applications of pheromones and other attractants.* R.L. Ridgway, R.M. Silverstein, and M.N. Inscoe, eds., pp 25–45. New York: Marcel Dekker.

Li., W. 1994. The olfactory biology of adult sea lamprey (*Petromyzon marinus*). Ph.D. thesis, University of Minnesota, St. Paul. MN.

______, and Sorensen, P.W. 1997. Highly independent olfactory receptor sites for naturally occurring bile acids in the sea lamprey, *Petromyzon marinus*. *J. Comp. Physiol.* A, 180:429–438.

______, Sorensen, P.W., and Gallaher, D.D. 1995. The olfactory system of migratory adult sea lamprey (*Petromyzon marinus*) is specifically and acutely sensitive to unique bile acids released by conspecific larvae. *J. Gen. Physiol.* 105:569–587.

______, Scott, A.P., Siefkes, M.J., Yan, H.G., Liu, Q., Yun, S.S., and Gage, D.A. 2002. Bile acid secreted by mate sea lamprey that acts as a sex pheromone. *Science* 296:138–141.

______, Scott, A.P., Siefkes, M.J., Yun, S.S., and Zielinski, B. 2003a. A male pheromone in the sea lamprey (*Petromyzon marinus*): an overview. *Fish Physiol. Biochem.* 28:259–262.

______, Scott, A.P., Siefkes, M.J., and Teeter J.H. 2003b. Sex pheromone communication in the sea lamprey: Implications for integrated management. *J. Great Lakes Res.* 29:85–94.

Mallatt, J. 1983. Laboratory growth of larval lampreys (*Lampetra (Entosphenus) tridentata* Richardson) at different food concentrations and animal densities. *J. Fish Biol.* 22:293–301.

Manion, P.J., and Hanson, L.H. 1980. Spawning behavior and fecundity of lampreys from the upper three Great Lakes. *Can. J. Fish. Aquat. Sci.* 37:1635–1640.

McCoy, J.R., and Wright, J.E. 1990. Selective breeding for increased pheromone production in the boll weevil (Coleoptera: Curculionidae). *J. Econ. Entomol.* 83:610–613.

Moore, H.H., and Schleen, I.P. 1980. Changes in spawning runs of sea lamprey (*Petromyzon marinus*) in selected streams of lake superior after chemical control. *Can. J. Fish. Aquat. Sci.* 37:1851–1960.

Mullett, K.M., Heinrich, J.W., Young, R.J., Henson, M.P., McDonald, R.B., and Fodale, M.F. 2003. Discharge regression model to estimate abundance of spawning-phase sea lampreys (*Petromyzon marinus*) in the Great Lakes. *J. Great Lakes Res.* 29 (Suppl. 1):240–252.

Polkinghorne, C.N., Olson, J.M., Gallaher, D.G., and Sorensen, P.W. 2001. Larval sea lamprey release two unique bile acids to the water at a rate sufficient to produce detectable riverine pheromone plumes. *Fish Physiol. Biochem.* 24:15–30.

Rodriguez-Munoz, R., Nicieza, A.G., and Brana, F. 2003. Density-dependent growth of sea lamprey larvae: evidence for chemical interference. *Funct. Ecol.* 17:403–408.

Sawyer, A.J. 1980. Prospects for integrated pest management of the sea lamprey (*Petromyzon marinus*). *Can. J. Fish. Aquat. Sci.* 37:2081–2092.

Schleen, L.P., Christie, G.C., Heinrich, J.W., Bergstedt, R.A., Young, R.J., Morse, T.J, Lavis, D.S., Bills, T.D., Johnson, J., and Ebner, M.P. 2003. Control of sea lampreys in the St. Marys River: A case study in integrated and coordinated fisheries management. *J. Great Lakes Res.* 29 (Suppl. 1):677–693.

Schuldt, R.J., and Heinrich, J. W. 1982. Portable trap for collecting adult sea lampreys. *Prog. Fish-Cult.* 44:220–221.

Siefkes, M.J., and Li, W. 2004. Electrophysiological evidence for detection and discrimination of pheromonal bile acids by the olfactory epithelium of female sea lampreys (*Petromyzon marinus*). *J. Comp. Physiol.* A 190:193–199.

______, Winterstein, S., and Li, W. 2005. 3-keto petromyzonol sulfate specifically attracts ovulating female sea lamprey (*Petromyzon marinus*). *Anim. Behav.* 70:1037–1045.

______, Bergstedt, R.A., Twohey, M.B., and Li, W. 2003a. Chemosterilization of male sea lampreys (*Petromyzon marinus*) does not affect sex pheromone release. *Can. J. Fish. Aquat. Sci.* 60:23–31.

______, Scott, A.P., Zielinski, B., Yun, S.S., and Li, W. 2003b. Male sea lampreys, *Petromyzon marinus* L., excrete a sex pheromone from gill epithelia. *Biol. Reprod.* 69:125–132.

Sorensen, P.W., and Stacey, N.E. 1999. Evolution and specialization of fish hormonal pheromones. In *Advances in Chemical Signals in Vertebrates*, R.E. Johnston, D. Muller-Schwarze, and P.W. Sorensen, eds. New York: Kluwer Academic, Plenum Publishers.

______, and Vrieze, L.A. 2003. Recent progress understanding the chemical ecology and potential application of the sea lamprey migratory pheromone. *J. Great Lakes Res.* 29 (Suppl. 1):66–84.

______, Vrieze, L.A., and Fine, J.M. 2003. A multicomponent migratory pheromone in the sea lamprey. *Fish Physiol. Biochem.* 28:253–257.

______, Fine, J.M., Dvornikoves, V., Jeffrey, C.S., Shao, F., Wang, J., Vrieze, L.A., Anderson, K.R., and Hoye, T.R. 2005. Mixture of new sulfated steroids function as a migratory pheromone in the sea lamprey. *Nature Chem. Biol.* 1:324–328.

Sower, S.A. 1998. Brain and pituitary hormones of lampreys, recent findings and their evolutionary significance. *Am. Zool.* 38:15–38.

______, and Kawauchi, H. 2001. Update: brain and pituitary hormones of lampreys. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 129:291–302.

Stoddart, D.M. 1990. *The scented ape: the biology and culture human odor.* Cambridge: Cambridge University Press.

Teeter, J. 1980. Pheromone communication in sea lampreys (*Petromyzon marinus*): implications for population management. *Can. J. Fish. Aquat. Sci.* 37: 2123–2132.

Thornhill, R.A. 1967. The ultrastructure of the olfactory epithelium of the lamprey *Lampetra fluviatilis*. *J. Cell Sci.* 2:591–602.

Twohey, M.B., Sorensen, P.W., and Li, W. 2003a. Possible applications of pheromones in an integrated program of sea lamprey management. *J. Great Lakes Res.* 29 (Suppl. 1):794–800.

______, Heinrich, J.W., Seelye, J.G., Bergstedt, R.A., Fredricks, K.T., Kaye, C.A., Scholefield, R.J., McDonald, R.B., and Christie, G.C. 2003b. The sterile male release technique in Great Lakes sea lamprey management. *J. Great Lakes Res.* 29 (Suppl. 1):410–423.

VanDenBossche, J., Seelye, J.G., and Zielinski, B.S. 1995. The morphology of the olfactory epithelium in larval, juvenile and upstream migrant stages of the sea lamprey, *Petromyzon marinus*. *Brain Behav. Evol.* 45:19–24.

______, Youson, J.H., Pohlman, D., Wong, E., and Zielinski, B.S. 1997. Metamorphosis of the olfactory organ of the sea lamprey (*Petromyzon marinus* L): morphological changes and morphometric analysis. *J. Morphol.* 231:41–52.

Vrieze, L.A., and Sorensen P.W. 2001. Laboratory assessment of the role of a larval pheromone and natural stream odor in spawning stream localization by migratory sea lamprey (*Petromyzon marinus*). *Can. J. Fish. Aquat. Sci.* 58:2374–2385.

Wagner, C.M., Jones, M.L., Twohey, M.B., and Sorensen, P.W. 2006. A field test verifies that pheromones can be useful in sea lamprey (*Petromyzon marinus*) control in the Great Lakes. *Can. J. Fish. Aquat. Sci.* 63:475–479.

Wilson, E.O., and Bossert, W.H. 1963. Chemical communication among animals. *Recent Prog. Horm. Res.* 19:673–716.

Yun, S.S., Scott, A.P., Siefkes, M.J., and Li, W. 2002. Development and application of an ELISA for a sex pheromone released by the male sea lamprey *(Petromyzon marinus L.). Gen. Comp. Endocrinol.* 129:163–170.

______, Scott, A.P., Bayer, J.M., Seelye, J.G., Close, D.A., and Li, W. 2003a. HPLC and ELISA analyses of larval bile acids from Pacific and western brook lampreys. *Steroids* 68:515–523.

______, Scott, A.P., and Li, W. 2003b. Pheromones of the male sea lamprey, *Petromyzon marinus* L.: structural studies on a new compound, 3-keto allocholic acid, and 3-keto petromyzonol sulfate. *Steroids* 68:297–304.

Zielinski, B.S., Israel, S., and Mastellotto, M. 1995. Behavioural responses of prolarval and larval sea lamprey (*Petromyzon marinus*) to L-arginine. *Chemical Senses* 20(6):343–343.

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