GREAT LAKES FISHERY COMMISSION

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Luring Lampreys: Assessing the Feasibility of Using Odorants to Control Sea Lamprey in the Great Lakes

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LURING LAMPREYS: ASSESSING THE FEASIBILITY OF USING ODORANTS TO CONTROL SEA LAMPREY IN THE GREAT LAKES

(Workshop sponsored by Great Lakes Fishery Commission)

Wilder Forest Nature Center
Marine-on-Croix, Minnesota
October 29-31, 1993

(Report to Great Lakes Fishery Commission)

By

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ABSTRACT

The third in a series of workshops designed to develop new approaches and ideas for controlling sea lamprey, (*Petromyzon marinus*), populations in the Great Lakes took place at the Wilder Forest Nature Center, Marine-on-Croix, Minnesota on October 29-31, 1993. The purpose of the workshop was to assess the feasibility of using odorants in an integrated sea lamprey control program. Personnel from the Great Lakes Fishery Commission and its control agents met with experts who either had knowledge of the use of odorants in controlling other organisms or were knowledgeable in fish chemoreception. Sea lamprey personnel described the current control program as well as other control methods that are being considered for use in an integrated control program. Experts in chemoreception science described how odorants are used to control terrestrial vertebrates and insect pests and studies concerning fish and sea lamprey chemoreception were described. After the presentations were made, potential ways for using different types of odorant cues (aggregants, feeding cues, repellents, migrational cues, and sex pheromones) for controlling sea lamprey populations were suggested and the research that will be necessary to evaluate the cues was described.

Group discussions identified migratory and reproductive pheromones as those cues with the greatest promise for use in lamprey control. A third topic area thought to warrant investigation was the possibility that chemical cues might influence the movement patterns of ammocoetes; however, an almost complete lack of data on this topic makes it a little less promising. Current investigations of the possibility that migratory adult lamprey select spawning streams using bile acids produced by larval sea lamprey as a cue appeared extremely promising and it was recommended that emphasis be placed on understanding the behavioral actions of these compounds, increasing our understanding of whether responsiveness to them is seasonal or life stage-dependent, and determining their species specificity. Progress understanding sex pheromone function will necessitate basic studies of the behavioral endocrinology of this species but are probably worth conducting because the sexual cues are likely to be species-specific and could be used to compliment migratory attractants and the sterile male program. The successful application of pheromonal disruptants in combination with sterile males in the pink bollworm program was impressive and highly encouraging for it demonstrates that large scale application of pheromones to control pests is technically feasible, affordable, and socially acceptable. In conclusion, the consensus of the experts was that the use of pheromones to control lamprey is definitely feasible and should be actively pursued because many basic research questions still remain.

GENERAL INTRODUCTION

Selectively toxic chemicals have been used to control sea lampreys (Petromyzon marinus) in the Great Lakes for over 30 years. Since 1970, the Great Lakes Fishery Commission (GLFC) and its control agents have been searching for other control methods that will reduce the almost total reliance on these chemicals to control lamprey populations (Smith and Tibbles 1980). Barrier dams and the sterile-male-release technique have been added to the control arsenal since that time and the GLFC is continuing to search for other control methods that will be useful in an integrated pest management program (IPM).

Recently the GLFC has sponsored a series of workshops that are designed to develop fresh approaches and ideas for controlling sea lampreys. In the first workshop, experts on sex determination and differentiation in mammals, amphibians, birds and fishes met with sea lamprey personnel to discuss the latest information and techniques available in their field (Sower and Hanson 1992). In the second workshop, pest control experts from the U.S. Department of Agriculture and the Ontario Ministry of Natural Resources met with personnel from the GLFC and the sea lamprey control program to discuss and evaluate the current sea lamprey control program and to examine other potential control options (Seelye and Hanson 1992). A third workshop was held on October 29-31, 1993 at the Wilder Forest Nature Center, Marine-on-Croix, Minnesota to assess the feasibility of using odorants to control sea lampreys in the Great Lakes. The results of this workshop are summarized in this report. Personnel from the GLFC and its control agents met with experts in chemoreception science who had knowledge of the use of odorants in controlling other organisms. The participants are listed in Appendix A. Sea lamprey personnel described the current control program as well as other control methods that are being considered in an integrated control program. The experts then made presentations describing their research. After all presentations were made, the participants compiled a list of the ways odorants might be used in an integrated sea lamprey control program.

Organizing the present proceedings has proven difficult because of the volume and diverse nature of the written and oral materials involved. The order of presentations in this manuscript follows the order of their presentation at the workshop. However, prior to the workshop all invited speakers were also asked to compile a statement on their background, a list of questions, topics, and ideas which they felt merited discussion at the workshop, a list of readings, and either an abstract and/or manuscript describing a research area relevant to the workshop. With few exceptions, speakers complied with this request. Additionally, at the meeting, Mr. Lee Hanson took notes on each presentation and discussion. Kathy Jones kindly transcribed these notes and put them on a word processor. Compiling this oral and written material has proven to be a formidable task and in the interest of keeping the manuscript to a reasonable length, extensive editing has been performed. The list of suggested readings were dropped and the personal background statements shortened. Readers interested in obtaining this information can contact the presenters directly. Their addresses and telephone numbers are found in the appendix. Talk summaries were kept to a minimal size and only included (following the background of each speaker) if immediately relevant and not covered in the abstract or manuscript. An edited version of either the abstract or manuscript presented by the presenter is given after the background information. Some liberty was taken by Dr. Sorensen in editing these. If an individual submitted both an abstract and manuscript, only the latter was included. Several workshop participants had specific suggestions for lamprey research and we have placed these into separate sections called 'Ideas'. The last section (VII) of the workshop proceedings summarizes the main concerns and ideas of the five groups formed on the last day of the workshop to evaluate the potential utility of larval aggregants, feeding cues, repellents, migrational cues, and sex pheromones in lamprey control. These topics had been identified earlier by Dr. Sorensen and the group as a whole as those odorants which the GLFC should consider most seriously in their search for odorants useful to IPM.

Literature Cited

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I. INTRODUCTIONS AND THE SEA LAMPREY PROBLEM

I.1. Introduction to the Workshop (Peter W. Sorensen--Organizer).

Background--I have been interested in fish behavior and sensory systems since my days as an undergraduate at Bates College in Maine where I earned a B.A. After taking a year off to work in the aquaculture industry, I returned to school to pursue a Ph.D. in Biological Oceanography at the University of Rhode Island. My doctoral research characterized the role of olfaction in the migration of larval American eels from the Atlantic Ocean into freshwater streams. Briefly, this research demonstrated that the chemical cue used by larval eels is innately recognized by the olfactory system. These studies led to my postdoctoral studies of sex pheromone function in goldfish at the University of Alberta under the guidance of Norm Stacey. Norm and I discovered that hormonal metabolites function as sex pheromones in the goldfish and that these compounds are potent modifiers of reproductive behavior and physiology. Although I am still actively involved with this topic, Norm Stacey will be solely responsible for describing these findings. Most recently, I have returned to my old topic of migratory cues -- this time in the sea lamprey and with the assistance of my Ph.D. student Weiming Li and Dan Gallaher, a nutritionist who specializes in bile acids. Together we have extensively characterized the olfactory sensitivity of the lamprey and discovered that migratory adult sea lamprey detect unique bile acids which are released by larval lamprey and, we believe, may function as a migratory cue. This cue appears to have potential for application as an attractant in lamprey control. Weiming will present these exciting findings which have sparked this workshop.

A note on the history of this workshop--Sea lamprey invaded the Great Lakes from the Atlantic Ocean at the turn of the century and within 40 years the fisheries industry of the Great Lakes had collapsed; each one of these primitive animals can destroy up to 20 kg of fish during its year-long parasitic phase. Presently, lamprey populations are kept more or less in check by a 15 million dollar program which applies larval toxicants to lamprey spawning streams. Rising costs and public concern now demand that a new solution(s) to lamprey control be found. The idea that odorants can be used to manage populations of sea lamprey in the Great Lakes dates back at least 20 years (see Teeter 1980 and abstract for this workshop) and is appealing because the lamprey has an extremely well-developed olfactory organ. Thanks to Weiming's hard work using electrophysiological recording, we now have a good understanding of what migrating adult lamprey detect and with what sensitivity and specificity. Consequently, we are now ready to consider behavioral testing of these cues but because large scale manipulation of wild fish using odorous cues has not been attempted before, we are unsure of how to proceed -- hence this workshop. However, although our research has focused on migrating adults, this workshop should consider all nature of chemosensory cues to manipulate all nature of lamprey (i.e. stream-resident larvae, lake-resident parasitic animals, and spawning adults).

<u>A few questions I would like this workshop to address</u>:

1) Based on your many years of experience using pheromones and other odorous cues to control and monitor insect pests, what types of cue (sex pheromones, food odors, etc.) work and what don't? Why? Also, what can fish olfactory biologists learn from the experiences gained using odors to control and monitor terrestrial animals and birds?
2) How potent are the behavioral actions of odorants likely to be in lamprey? Is it realistic to think that we can use odorants to make lamprey do things (go places) that they normally wouldn't? Should we think of odorants as behavioral modifiers or directing factors?
3) How likely is it that the individual odorants which we have identified will have behavioral activity on their own? Rather, should we expect them to be part of a more complex mixture? (These questions relate to expectations of species-specificity.)
4) Should we expect lamprey pheromones to be species-specific? And, need an odor cue be species-specific to be useful?
5) How important is it to match odor usage/importance with the specific life stage of the animal?
6) What can one deduce from electro-olfactogram recording alone?
7) Over what distances are odorants likely to be useful as attractants/repellents in aquatic environments?
8) What practical considerations should be taken into consideration when applying odors to the environment on a large (or small) scale?

    a) Should odors be administered in pulsatile or continuous fashion?
    b) What is the importance of mixtures (relates to #3)?
    c) How much odor needs to be administered relative to the animal's detection threshold?
    d) Is it important to match odor administration with other environmental cues such as temperature, flow, and time-of-day (relates to #2)?
    e) Need natural odors be licensed if they are to be applied to the environment?

9) If we had the technology to measure concentrations of lamprey pheromones easily, would this be a useful tool for assessing population densities?

10) Might pheromonal traps also be useful in monitoring lamprey abundance in remote locations (almost half the present lamprey control budget is for population assessment)?

11) What research needs to be done to answer the most salient questions?

I.2. A Word from our Sponsor (Mike Millar for Gavin Christie).

On behalf of the Great Lakes Fishery Commission, I would like to welcome you to this weekend's workshop. This session is the third in a series of workshops the Commission has sponsored to bring together experts like you to help us focus our research efforts on the development and refinement of alternatives to lampricides.

The Commission is committed to reducing its reliance on lampricides to control sea lamprey in the Great Lakes. To meet this commitment, we are carrying out a program of research into alternative methods. As in all areas of our program, the funding from the two contributing governments for this research is not at the level we really need. But we do have an active program of both solicited external and internal program research into ways to reduce lampricide use. The exciting results of Peter's work are an example of this research initiative. To get the most out of this research program and to move us as quickly as possible to the real effective alternatives which we can use in the field we need your help. We need to refine our list of research needs and priorities so that we can get the right work done.

As Peter has clearly stated in his "objective" statement in the agenda, the purpose of this session is to get your input on what research should be done to help us understand how odorants affect lamprey behavior and how these effects might be used to control sea lamprey populations. With the help of the control program experts who have come to the session, we hope you will learn about how the program of sea lamprey management works now and about our current understanding of sea lamprey behavior and ecology. This knowledge along with your specific understanding of chemical cues will hopefully stimulate an integration of what we know now and some new ideas about what we should find out in the future.

If my reading of the abstracts you have provided is at all complete, this group represents just the right mix to come up with these new ideas. So with that, and a hearty thanks to Peter for his efforts putting this session together, I repeat my welcome and thank you all for coming. I am looking forward to the session ahead.

I.3. Introduction to Sea Lamprey Biology (F.W.H. Beamish).

Summary of talk--Dr. Beamish described the general biology of the sea lamprey. Slides on the various life history stages were shown (spawning, larval, metamorphosing, and parasitic phases). Information on growth rates, sex ratios, sex determination, methods of ageing larvae, changes that occur during metamorphosis, parasitic feeding behavior and fecundity of lampreys was presented.

I.4. Impact of the Sea Lamprey (Petromyzon marinus) on the Fisheries of the Great Lakes (George Spangler).

Background--George Spangler is currently a Professor of Fisheries in the Department of Fisheries and Wildlife at the University of Minnesota. His primary research interests have been in the population dynamics of fishes of large lakes. As a research scientist for the Ontario Ministry of Natural Resources, Dr. Spangler studied the impact of lampreys on whitefish and splake hybrids in Lake Huron. Dr. Spangler has served on the Board of Technical Experts for the Great Lakes Fishery Commission.

Abstract--Since the establishment of sea lamprey populations in the Great Lakes upstream of Niagara Falls, the community of larger species of endemic fishes has declined irregularly. The lake trout and burbot which formerly served as top-level piscivores in the lakes were eliminated or severely reduced in abundance until sea lamprey populations were brought under control through aggressive management. Repercussions from the absence of predators reverberated throughout the fish community resulting in somewhat different species composition and prey fish density in each of the lakes. Attempts to artificially support a community of piscivores poorly adapted to Great Lakes conditions have been experiencing increasing costs and increasingly uncertain returns. It is clear that the introduction of Pacific salmon to the Great Lakes will not restore the productive capacity of the fishery to a level comparable to that of the pre-lamprey past. The dominance of the sea lamprey effect is apparent when examining the progress of rehabilitation of the fish communities in the upper lakes. The only significant restoration of fishery productivity in the face of lamprey predation has occurred in northern Lakes Huron and Michigan, and in Lake Superior, and then only under the circumstances of very stringent controls on sea lamprey abundance. Rejuvenation of these fisheries remains dependent upon only two species, lake trout and lake whitefish, both known to be exceedingly vulnerable to sea lamprey predation. Just as it is becoming apparent that lampreys must be further reduced in abundance to effect continuing rehabilitation of the fishery, so too has it become obvious that existing control methods are not sufficiently cost-effective or environmentally benign to be allowed to continue indefinitely. Alternatives to the current control methods are urgently needed if we are to avoid the loss of the hard-won rehabilitation that has occurred over the past two decades.

I.5. Video and Informal Discussion.

The GLFC video entitled "Great Lakes Invaders--the Sea Lamprey Battle Continues" was shown. Afterwards, a lengthy informal discussion on lamprey spawning behavior and the sterile-male-release technique took place.

II. SEA LAMPREY CONTROL TECHNIQUES

II.1. What the Great Lakes Fishery Commission is "Looking For" in a Control Strategy (F.W.H. Beamish for Gavin Christie).

The Great Lakes Fishery Commission was formed in 1955 with the general purpose of supporting the improvement and perpetuation of fishery resources and the specific purpose of carrying out a program to "eradicate or minimize" the sea lamprey in the Great Lakes. Since the inception of the lampricide control program and the application of the selective pesticide TFM the success of the control program has been astounding. The benefits of the control program in terms of successes in rehabilitation of depleted lake trout populations and the development of the current salmon and trout fisheries have been remarkable.

These successes in hand, the Commission has looked forward to what its long-term program should look like. This "vision", that I am handing out, presents the Commission's direction in three statements: 1) healthy Great Lakes ecosystems, 2) integrated management of sea lamprey, and 3) institutional/stakeholder partnerships. The Commission's view of its sea lamprey program is presented on page 21. The focus of the future program will be to: provide an integrated sea lamprey management program that supports the fish community objectives for each of the Great Lakes and that is ecologically and economically sound and socially acceptable.

The statements iterate the Commission's commitment to the principles of integrated pest management (IPM). The objectives of the overall program are determined by the value of the fish that you save by killing lamprey and by the ecological, economic, and social costs of doing the control. IPM suggests that the best way to achieve such objectives will always be with a mix of control techniques which gives you flexibility and efficiency.

The Commission has identified milestones to help it reach its objectives. The first is to define the lamprey suppression targets. If we can't eradicate lamprey, we have to define what minimize 'means' in lamprey numbers in all of the lakes. The target numbers in hand, the Commission defines the second milestone to reach these low levels of lamprey. Considerations in the process include: a) meeting all registration and environmental regulations; b)refining population assessment for lake and large river populations; and c) improving research and assessment. Our focus at this workshop is to help us reach the second milestone: to reduce reliance on lampricides to 50% of current levels by using alternate control techniques.

In this statement the Commission formalizes its commitment to reduce lampricide use and move to other methods of control. Our discussions this weekend should ultimately help us achieve this milestone. The critical feature of the Commission's plan is that all its parts be considered together. Achieving the 50% reduction must be done while meeting the overall objective of providing required control. The Commission needs effective alternatives and won't sacrifice the control we have now to meet this objective.

The use of odorants as attractants or repellents to lamprey has truly exciting possibilities to provide real improvements to all of the methods we use now and to provide for new methods not yet in our arsenal. This integrated applicability is what makes this session so important to achieving the Commission's vision for sea lamprey management in the Great Lakes.

II.2. Larval Toxicants (Larry Schleen).

Background--Since graduating from the University of Guelph in 1969 with a B.Sc. in Marine Biology, I have been employed at the Sea Lamprey Control Centre in Sault Ste. Marie, Ontario. I am presently supervisor of the Control Program (lampricide treatments and barriers) at the Centre. I have experience in the assessment program (larval and adult) but have most expertise in the lampricide treatment program, having been supervisor of one of the Centre's treatment units from 1974 to 1987.

Submitted manuscript--The lampricide treatment program has been highly successful in reducing sea lamprey numbers and has allowed the partial rehabilitation of some native fish species and the establishment of populations of several introduced salmon species. I have witnessed over the years a continual refinement in treatment techniques and extreme dedication by control personnel. The treatment program is still supported by most, but the need for alternative methods to replace or at least supplement the use of lampricides is critical.

The prospect of using natural odorants to control or manipulate populations of sea lampreys has always interested me. I co-authored a paper with Moore in 1980 entitled "Changes in spawning runs of sea lamprey in selected streams of Lake Superior after chemical control". The number of spawners captured in streams by electrical barriers the year following successful treatments, i.e., where very few residual larvae remained, was significantly less, but in streams where residual larvae were left or where lentic populations existed, adult runs were less affected. The possibility that larval sea lampreys attract adult spawners is strongly suggested. Following 'clean' treatments, the attraction of larvae or their excretions would be limited and would only slowly build up as the larval population reestablished.

Hopefully my expertise and knowledge of sea lamprey biology and distribution within the Great Lakes will contribute to discussions on the practicality of utilizing natural odorants to control sea lampreys within such a large arena. If a practical attractant were to be developed, perhaps present lampricide application personnel and many of their present application techniques would be utilized. Expertise in measuring stream discharge, conducting water chemistry tests and applying liquids to flowing systems might be highly valuable in administering an attractant to streams.

The concept of an attractant, rather than a repellent, would seem to me to be of most value. I think the attraction of spawning phase adults would be most practical as opposed to the attraction of larvae or parasitic phase animals. If spawners could be drawn into a stream or portion of a large system such as the St. Marys River, they would be susceptible to trapping or treatment. If trapped, the males could then be used in the sterile-male program. The attraction of larvae to other streams or portions of their resident stream would seem difficult and of limited value. The attraction of transformers (metamorphosing larvae) before they leave a stream would however be of considerable value.

Questions that need to be answered include:

1. How 'strong' would an adult attractant have to be to compete with the natural attractant of larvae in other tributaries while not overloading the animal's sensory abilities?
2. Do native larvae attract adult sea lampreys? Would this explain some of the original establishment and distribution of larval populations? Should this be investigated in conjunction with work on sea lamprey larvae?
3. How specific does the timing have to be to attract spawners? If the attractant lasts for a relatively short time and other factors come into play, i.e., the attraction or repulsion of other adults, in a drawn-out spawning run would you only expect to attract some of the run? How soon would you have to introduce an attractant in a stream to attract

staging adults and how long would you have to continually feed the substance into the stream?

Summary of talk--Mr. Schleen presented a brief overview and history of the use of larval toxicants to control populations of sea lampreys in the Great Lakes and described the lampricides used, their advantages and their shortfalls. He described their historical and present use patterns and attempted to predict future use problems. He stated that the applications of toxicants to flowing aquatic systems is not unique to the sea lamprey control program, but its scale must rival any similar pest control program in the world. To think that we can continue regularly to treat more than 200 of the cleanest and most productive tributaries in North America with an albeit very safe chemical is probably unrealistic. The cost and continued availability of these lampricides is another matter of concern.

II.3. Larval Toxicants-Effectiveness of the Lampricide Treatment Program (Terry Morse).

Background--I graduated from Central Michigan University in 1973 with a B.S. in Biology/Conservation. I continued at Central Michigan for 3 years (1974-76) of graduate studies in Fisheries/Aquatic Zoology. I began working for the U.S. Fish and Wildlife Service in Marquette, Michigan in 1977. I spent 14 years with the Assessment Unit of the sea lamprey control program and 4 years in my current position as supervisor of the Control Unit.

Abstract--The first sea lamprey was found above Niagara Falls in 1921. By the late 1930's sea lampreys were found in all 5 Great Lakes. By late 1950's, sea lampreys were the major cause of the collapse of lake trout, whitefish and chub populations in the Great Lakes. The first round of lampricide treatments on Lake Superior streams was completed in 1962 and resulted in an 84% reduction of the lamprey population. Under the present program, the sea lamprey population is now only 10% of pre-control level, except in northern Lake Huron. Results of recent studies on 2 Lake Superior streams show an average lampricide treatment effectiveness of at least 97%. Even at this level of control, in Lake Superior, sea lampreys still kill as many trout as are harvested in sport and commercial fisheries. The overall reduction in the lamprey population, however, has contributed to a revitalized fisheries valued at $2-4 billion annually in the Great Lakes region.

II.4. Use of Sterile-Male-Release Technique for the Control of Sea Lampreys (Jim Seelye).

Background--My college education culminated with the receipt of a PhD in limnology from Michigan State University. Training emphasis was on radioisotope cycling in aquatic systems and analytical chemistry. I worked for the Corps of Engineers in Vicksburg, Mississippi, on problems associated with the disposal of dredged materials and built a solid foundation of friends in a variety of fields across the country. Our research program involved sites from Seattle to Long Island. My frequent travel and contacts with a variety of research groups provided an education that would otherwise be difficult to obtain. Six years were spent working for the Fish and Wildlife Service on potential effects of contaminants on reproduction of lake trout. During the last 11 years, I have performed the duties of Station Chief at the Hammond Bay Biological Station. We conduct research on sea lampreys, methods to control them, and their effects on the fishery.

Abstract--In 1970, Lee Hanson at the Hammond Bay Biological Station conducted a literature search on various control methods used for pests. This review started a research program on the application of the sterile-male-release technique to sea lampreys. We needed a method to sterilize the sea lampreys without affecting their reproductive behavior. Large numbers of male sea lampreys must be available at low cost and the size of the targeted population must be known to accurately predict efficacy. To increase the potential for success, the population must be reduced by other control methods. The cost of the sterilization program must be competitive with other control techniques and reliable methods must be available to evaluate the program. We evaluated the potential of chemosterilization, immune techniques, and irradiation for sterilizing sea lampreys. Chemosterilization proved to be the most reliable technique and injection was considered the safest method to administer the chemosterilant. We are conducting the current program under the assumption the population is at a level where recruitment to the parasitic-phase is linearly related to the number of spawners. The reduction in reproduction should be proportional to the ratio of sterile males to untreated males. Release of an equal number of sterile-male sea lampreys to untreated males should result in a 50% reduction in the production of larval sea lampreys. During two field trials, this ratio was observed in an isolated population of sea lampreys in the Big Garlic River. Further studies were conducted to evaluate the best method of releasing sterile-male sea lampreys back into the lakes. Releasing the sea lampreys in individual streams was chosen as the best technique overall. The ratio of sterile to untreated males in each stream could be predicted more accurately using stream releases as

opposed to lake releases where the distribution between streams was not known. A hazardous chemical laboratory was constructed at Hammond Bay, a mechanical injector was purchased from a company in Denmark, and the program was begun in 1990. Our first solid information on the efficacy of the program will be the assessment data for larval populations in the test streams, after one generation (5-7 years). There should also be a measurable decline in the number of spawning-phase sea lampreys returning to these streams.

Ideas--Discussions about the use of various physical and chemical cues to enhance trapping of sea lamprey have taken place since the beginning of the program. Although traps have been used mostly to assess sea lamprey populations, in locations such as the Cheboygan and Brule rivers, trapping does show promise as a control technique. The only factor that separates assessment from control in this case is the efficiency of the trap. If the use of olfaction by spawning-phase sea lampreys can be understood, perhaps the efficiency of many of our traps can be increased.

Another use of olfactory cues would be to control sea lampreys by restricting their movement. This could be applied by repelling sea lampreys from good reproductive habitat or attracting them to poor habitat. Drawing the spawners into streams where good habitat for spawning exists, allowing them to spawn, and then treating the area with lampricide, dewatering the site, or modifying the temperature might all be feasible control methods.

Assessing sea lamprey populations indirectly by measuring something they produce has also been a topic of frequent conversation in the program. The presence of a specific chemical that is released proportionally to the biomass of larval sea lampreys in a stream might allow routine measurement of the production potential of this parasite.

Understanding the potential olfactory communication between individuals and the life-phases of sea lampreys could also lead to useful techniques for controlling populations. Water from high-density larval sea lamprey populations seems to influence growth in lower density populations. Identifying the cause of this decreased growth might lead to a useful management technique. Sex determination in sea lampreys might be influenced by the density of the larval population. If this is true, it could be used for our benefit.

As we discuss the potential uses of olfaction in the sea lamprey control program, we must keep one important factor in mind. The most useful techniques for control or assessment will be the ones that do not include the dispensing of any man-made chemicals. Registration of even the "safest" chemicals for use as a pesticide or drug will cost millions of dollars and years of research. If the method of introduction is by the organism, the potential success of the method increases dramatically. We do not need to eliminate the possibility of registration of a useful chemical; however, the benefit/cost ratio will eventually enter the picture and strongly influence feasibility.

II.5. The Sea Lamprey Barrier Program--History and Future Objectives (Ellie Koon).

Background--I did undergraduate and graduate work at the University of Michigan School of Natural Resources, after which I served as the Collections Manager at the University of Michigan Museums, Division of Fishes, for 5 years. I have been with the U.S. Fish and Wildlife Service Sea Lamprey Control Program for 10 years in various capacities. Since June 1993, I have been the U.S. Sea Lamprey Barrier Coordinator, a newly created position. I have a strong personal commitment to alternative control methods and a great deal of optimism that research may provide new ways to manage sea lampreys.

Ideas--Sea lamprey barriers complement the sterile male program by providing sites to trap spawning adult males for sterilization (sea lampreys are very difficult to trap without some type of barrier that concentrates them). Trapping and removal of adults may constitute an effective control method in itself in some streams. Olfaction research may lead to attractants that greatly enhance trapping efficiency by drawing lamprey into traps or even into streams with good trap sites. Attractants might be used in concert with repellents that keep lamprey out of streams that have no barriers or are difficult to treat effectively with TFM.

Abstract--Sea lamprey are most vulnerable to control during spawning migrations and as larvae resident in streams. Early control efforts focused on preventing adult spawners from entering streams. Initially (1947-1952), screen-type mechanical weirs were built. These became clogged with debris and were very difficult to maintain in flood conditions. Next (1953≈1966), electrical barriers consisting of suspended arrays of AC electrodes were installed in many streams. These caused fish mortality and were subject to flooding and power failures. Starting in 1958 adoption of the larval lampricide TFM (3-trifluoromethyl-4-nitrophenol) as the primary means of control resulted in a reduced emphasis on barriers and efforts to refine either physical or electrical barriers ceased.

In the late 1970's, the GLFC again recognized the value of barriers in sea lamprey control and promoted the construction of low-head barrier dams. Under this initiative, 39 dams were built or modified. Lack of adequate and stable Commission funding has been the major obstacle to program progress. Most low-head dams constructed during this period have functioned very well as sea lamprey barriers.

Recently, a new concept in electrical barriers using a pulsed DC gradient designed by Smith-Root, Inc., has been tested on 4 Michigan streams. Next spring in Ontario, an experimental velocity barrier will be tested which takes advantage of lampreys' poorer swimming ability compared to that of other fishes.

The GLFC has a recently-stated goal of reducing lampricide use by 50%. Other means of control that are currently available include the sterile-male-release technique (SMRT) and construction of barriers. The current GLFC barrier policy states "The Commission endorses the installation of barriers, modification of existing structures into barriers, and continued research into new barrier design and technology as part of an integrated control program. The Great Lakes Fishery Commission will develop a barrier program with concern and sensitivity to the preservation of other environmental, ecological, or recreational impacts."

The environmental impacts of sea lamprey barriers are relatively minor except for the problem of blocking passage of other fish species. New barrier designs must incorporate means of allowing fish to pass where it is deemed necessary.

II.6. An Overview of Alternative Control Strategies (Roger Bergstedt).

Background--I received a BA in philosophy from the University of Minnesota, Duluth, a BS in fisheries biology from the University of Minnesota, St. Paul, and an MS in fisheries biology from Iowa State University. Since 1975, I have been employed by the U.S. Fish and Wildlife Service. Ten years were with the Great Lakes Fishery Laboratory--two at the main laboratory in Ann Arbor in the Physiology and Contaminant Chemistry Section, and eight at the Oswego, New York, field station doing fish stock assessment on Lake Ontario. During those years I developed a strong interest in sea lampreys and their effects on fish stocks. In 1985, I transferred to the Hammond Bay Biological Station to work primarily with sea lampreys.

<u>Submitted</u> <u>Manuscript</u>--It is important to understand what is meant by alternative control methods. In Webster's Unabridged Dictionary, the most appropriate definition for "alternative" is "one of a number of things or courses offered for choice." All definitions given imply a choice <u>between</u> options. Our goal, however, is to have a number of effective methods that complement one another and reduce reliance on any individual method.

Currently, the sea lamprey control program depends almost entirely on removal of sea lampreys during their larval stage with the lampricide TFM, either alone or together with Bayer-73. The only operational alternative to TFM currently is the lowhead barrier and trap. Another alternative, sterile male release, is being used on an experimental basis. Barriers and traps did not provide effective control before the discovery of TFM and sterile male release is still experimental. At this time it is fair to say that the loss of TFM would result in the loss of effective control of sea lampreys in the Great Lakes. This is a dangerous situation for Great Lakes fisheries and one we seek to remedy. The purpose of this workshop is to identify knowledge and lines of inquiry within your disciplines that might lead either to totally new methods or to improvements in existing methods that would make them more effective.

Although the Commission has recently placed more emphasis on finding new alternatives for sea lamprey control, the Commission and its Control Agents have continually sought new methods. A paper entitled "Current and Proposed Alternative Methods for the Control of Sea Lampreys" was prepared by the Hammond Bay staff to summarize previous work on alternative control technologies, including ones that have been used, investigated, or simply hypothesized. The following is an outline of eleven ideas discussed in that paper.

1. The first efforts at control focused on blocking sea lampreys from spawning habitat. Lamprey have specific requirements for spawning and, if they can be reliably kept from suitable sites, barriers provide control. Barriers provide benefits on specific streams, but are not currently thought of as an effective control on their own. Types of barriers include mechanical weirs, electric weirs, lowhead barriers, and velocity barriers.

2. Trapping, when combined with a barrier, can remove substantial numbers of animals and their reproductive potential. Removal of mature sea lampreys will reduce the number of eggs laid, but has the most potential as an alternative technique when used with other methods such as lowhead barriers and sterile male release. An important need is for development of a technique for

effective trapping in rivers that do not have a barrier (John Heinrich, personal communication). This requires either attracting or forcing sea lampreys toward a trap, which would require an attractant or a repellent.

3. The ability to either attract or repel sea lampreys could be important in several ways. (1) Parasitic sea lampreys could be lured to traps. (2) Enhancement of trapping on streams lacking barriers could add to the effectiveness of sterile male release. (3) Spawning sea lampreys could be lured into streams that are easily treated or have poor larval habitat. (4) Sea lampreys could be repelled from "problem" streams that are difficult or costly to treat. (5) Broadcast of pheromones could disrupt spawning behavior. Because of the potential for both new methods and enhancement of other control methods, the concept of attractants and repellents is very appealing. This should be a primary area of discussion in this workshop.

4. Competitive displacement of sea lamprey by other lamprey is a theoretical possibility, but doesn't seem likely since sea lampreys colonized the Great Lakes in the face of existing native stocks. Producing large numbers of sea lamprey larvae treated to prevent metamorphosis and which would displace normal larvae has been suggested.

5. Predators do not currently provide natural control. Introduction of new predators might be possible but would meet with opposition based on concern over possible threats to other species.

6. Parasites and pathogens have been suggested, but little work has been done. Fish managers would probably be concerned about introductions of new pathogens. Participants in the last workshop, who were knowledgeable in other areas of pest control, felt this was an area where more effort should be spent.

7. Management for fish resistant to sea lamprey attacks might reduce the effects of sea lampreys. Species vary in resistance to attacks and genetic strains of lake trout seem to vary in the attack rates they sustain.

8. Sterile male release has been effective with insects and is being tested experimentally with sea lampreys. The role of olfaction and pheromone communication in mating behavior could bear on the success of sterile male release and should be discussed at this workshop.

9. Disruption of the life cycle or of reproductive behavior has obvious implication for control. Again, olfaction could be a key to progress in either area if chemical communication were important. The possible role of olfaction and natural odorants should be discussed.

10. Chemical control of growth regulation has been hypothesized. It is possible that chemical communication through olfaction might regulate growth. The possibility of identifying and using these substances should be discussed.

11. We do not rule out the possibility that new lampricides that are more desirable or that fill specific needs could be developed. However, a serious impediment to development of new lampricides is the enormous cost of registration. During the discussions in this workshop, we should strive to be realistic about the potential regulatory requirements and cost of registration for odorants.

A copy of the paper abstracted here can be obtained from either the Hammond Bay Biological Station or the GLFC Secretariat.

II.7. Introduction to Lamprey Chemoreception and the Promise of an Odorant-Based Control Strategy for Lamprey (Peter Sorensen).

Summary of talk--Because of the large size of the Great Lakes, any chemical cue that might be used to attract sea lampreys would have to be distributed over a large area. Since the olfactory system in sea lampreys is much more developed than their gustatory or visual systems, odorants may be most useful for attracting or repelling lampreys. Migratory and sexual cues would appear to have particular promise because of the relative vulnerability of the adult life stage. This will be discussed further in Li's presentation.

III. USING CHEMICAL CUES TO CONTROL TERRESTRIAL VERTEBRATES

III.1. The Potential of Pheromonal Control of Reptilian Populations (Robert T. Mason).

Background--My training is in the chemical ecology of vertebrates, specifically reptiles. I did my graduate work in Zoology at the University of Texas at Austin under David Crews. There I worked on garter snake reproduction doing both field and laboratory studies of the Canadian red-sided garter snake. I was primarily interested in how pheromones drive reproductive behavior in these animals. After finishing at Texas, I moved to the National Institutes of Health in Bethesda, Maryland where I worked for four years in a natural products laboratory learning chemistry techniques. This lab specialized in natural products and especially pheromones, mostly of insects. At NIH we were able to isolate, identify, and synthesize the garter snake sex attractiveness pheromone. This was the first, and to this day, only pheromone identified in any reptile.

Currently, I am still working on projects involving the conservation of these garter snakes in Manitoba, Canada. There, the snakes are being killed off in large numbers during spring and fall migrations across highways. We are trying to use pheromone technology involving pheromonal attractants and repellents to build what I call "chemical fences" to control the movements of the snakes. My other primary research effort is on the control of the brown tree snake on Guam and other Pacific islands. Again, we are trying to exploit the same pheromone technology to try and alter the snakes behavior. For example, one scenario involves attracting snakes into traps by means of pheromones. Once in the trap, the snakes can be exposed to viruses or fungi, leave the trap, wherein they can then spread the pathogen through the rest of the population.

Submitted manuscript--The study of chemical communication, semiochemicals (chemicals with signal function), and pheromones is recognized as a small, but critical area of reptilian social behavior. Indeed, reptiles in general, and snakes in particular are well known to be exquisitely tuned to the reception and perception of chemical cues in the environment. Chemical cues are very efficient energetically in that they are cheap to produce, they relay messages long after the producer is gone, they work in the dark and over very great distances. Squamate reptiles are interesting in this regard in that they may arguably be more sensitive to chemical stimuli than any other vertebrate.

In the constant battle with pest species, new technologies for the control of vertebrate pests have given rise to the development of several methods that utilize chemicals that modify behavior. These behavior-modifying chemicals are used to induce a variety of specific responses such as trailing, aggregation, mate selection, courtship, and repulsion. The extensive application of pesticides has not only upset the delicate biological balance of nature, but also poses a danger to human health and drastic, long-term alteration of the environment. As a result, the emerging "alternative agriculture" and a worldwide shift towards integrated pest management are bringing behavior-modifying chemicals, particularly pheromones, to the forefront of pest control (Shani, 1991). Pheromones are viewed as ecologically friendly, chemically safe, and efficient in terms of cost of production and the small quantities usually needed to produce an effect.

The prevailing approach in integrated pest management is an ecological approach to reducing pest damage by using all the available techniques to maintain pests below damaging levels (pestistasis). This strategy is based on the determination of the point at which a pest population approaches the level at which control is necessary to prevent excessive ecological damage to native fauna or excessive detrimental effects to human ecology, mainly in the form of bites.

Currently, a use of pheromones that has been gaining momentum in the fight against insect pests also shows considerable promise in application to snake pests. This growing technology is the use of pheromones as a means of monitoring pest populations, including their introduction into new, previously undisturbed environments. This is important on a smaller scale as well as on a larger scale in the introductions of snakes and lizards onto new islands in the Pacific and elsewhere.

The tropical colubrid brown tree snake, *Boiga irregularis*, is native to Australia, New Guinea, and the Solomon Islands. Sometime during or immediately after World War II, the brown tree snake became established on Guam probably due to accidental transport by aircraft and ships. During the ensuing years, its population density has increased to several thousand snakes (up to 13,000) per square mile. This snake has clearly established itself as a serious threat to native fauna and humans.

Boiga irregularis is nocturnal and arboreal. It is considered a generalist predator preferring roosting birds, nestlings and even eggs. By the late 1960's and early 1970's, bird populations on Guam had begun to decline at an alarming rate. By 1986, nine species of native forest birds were considered extinct (Savidge, 1987). The few species of birds that remain are either too large for the snake to eat or are seriously threatened. Directly related to the decline of the bird populations is the potential threat to agricultural crops and native vegetation by insect pests previously kept in check by avian predators. With the shrinking prey base caused by the mass extinctions of birds on Guam, the snake has broadened its prey base to include small mammals such as mice, rats, and shrews. Predation on the Marianas fruit bat has now caused populations of this valuable insectivore to be dangerously reduced (Wiles, 1987). The snake has even begun to seriously threaten populations of native lizards and skinks.

As the prey base continues to shrink in inhabited areas of the island, the snakes are interacting more frequently with humans. Perhaps the most disturbing and costly problem is the increasing number of power outages and damage to electrical equipment of the Guam Power Authority and Naval Public works caused by snakes climbing the power poles, presumably to stalk roosting or nesting birds (Fritts, 1988). The resulting power losses have caused aggravation to local citizens and are a serious economic burden. Since 1984 there have been over 500 power outages due to snakes with resulting damages totalling in the millions of dollars.

Finally, the snake is now regarded as a potentially hazardous problem to humans. The brown tree snake is apparently quite comfortable living in close proximity to human activity and habitation. With this increased contact, the brown tree snake is now found raiding poultry houses and backyards where domestic pets, poultry and eggs are readily encountered and consumed. The snake also enters houses and snakebites are increasing dramatically in Guam (Fritts *et al.*, 1990). Although the bites of *B. irregularis* have been described as harmless to humans (Cogger, 1975), the brown tree snake is a rear-fanged mildly venomous snake. The most serious encounters with the snakes are those involving small children. Indeed, four infants less than one year old were treated for snakebite recently on Guam (Fritts *et al.* 1990). The infants developed respiratory problems and exhibited symptoms indicative of some neurotoxins. All the infants recovered but serious injury or death could have resulted. The brown tree snake which can reach lengths of ten feet, can also be a threat if it should attempt to constrict a human infant.

This discussion will only consider three of several uses of behavior-modifying chemicals. Attraction and mating disruption have proven to be quite effective in selected examples of insect pest management (see Ridgway *et al.*, 1990). These two experimental paradigms seem to hold the most promise for application in ophidian pest management. The third, repellents, has not received as much study. I will attempt to describe current efforts by researchers investigating pheromones in the brown tree snake. Finally, a discussion of the need for future research directions will be addressed. By taking stock of where the field is today, enlightened decisions can be made to direct future efforts with the goal of effective, safe, and economical control of ophidian pest species.

Attraction and mating disruption are being focused on because they appear at this time to hold the most promise as biological control agents. They possess certain features that make them attractive candidates for further study including: behavioral responses are elicited at very low concentrations, they are highly specific, and they have potential for immediate use in pest management.

The use of pheromones as attractants and mating disruptants possesses several features that distinguish this approach from the use of chemical pesticides. Pheromones may indirectly kill snakes by luring them into traps, to toxicants or to pathogens, or they may alter normal reproductive or aggregative behavior. In some cases, such as mating behavior, pheromones will affect only one sex, in this case males. However, aggregation and trailing pheromones will affect both sexes equally. The field testing parameters including the location, number of replicates, size of the trap associated with the pheromones will vary and depend on the type of behavior being affected, the habits of the particular target species, environmental considerations, and estimated population densities of the target species.

Efficacy of the program would be defined as a lowered population density and could include quarantine maintenance, elimination of nuisance or noxious populations, or complete eradication. Efficacy must be demonstrated under field conditions. Significance of the treatment should normally be assessed by data from several experimental and control plots.

Another promising prospect for the control of ophidian pest species with pheromones is by use of a technique known as "male confusion." The basic premise is that when the environment is permeated with pheromone, the number of males locating females will be greatly reduced, and that this decrease in mating activity will result in lower population levels in subsequent generations (Birch and Haynes, 1982).

The physiological basis for mating disruption is not fully understood. Disruption could potentially result from competition between sexually attractive females and sources of synthetic pheromone. Here, a large number of sources releasing high concentrations of pheromones out compete the female. Permeation of the environment with pheromone trails and aggregation sites may lead to sensory adaptation or habituation to the pheromone. Both processes could decrease or eliminate behavioral responses to female pheromones and would decrease matings in the field.

To demonstrate mating disruption, small-scale test plots are established in which a trap baited with sexually attractive females or synthetic pheromones is surrounded by trails of synthetic pheromone. Alternatively, sexually attractive, unmated females are placed in the center of a plot to determine if males can find and mate with them. It is crucial that trails of pheromone lead to the female's location.

Historically, studies of pheromones in snakes are all extensions of earlier research conducted on a North American colubrid, the garter snake, *Thamnophis sirtalis* and the Swedish adder, *Vipera berus*. G. K. Noble (1937) was the first investigator to study the sex attraction pheromones that females use to attract males. He discovered that the source of the sex pheromones was the dorsal skin of the female and not the cloacal gland secretions as proposed by others (Baumann, 1929).

During the breeding season, male garter snakes and male adders, *Vipera berus* (Nilson, 1980; Andrén, 1982; 1986), initiate courtship behavior in response to a pheromone sequestered on the female's dorsum. Rapid tongue-flicking serves to deliver these cues to the male's vomeronasal organ. The vomeronasal organ is known to be the sole mediator of the reception and perception of pheromone cues in those snakes studied to date (Kubie *et al.*, 1978; Kubie and Halpern, 1979; Halpern and Kubie, 1980; Andrén, 1982).

That the skin is the source of sex pheromones in many snakes has been known for some time by zoo workers (Radcliffe and Murphy, 1983). Indeed this was confirmed by a series of experiments in which skin tubes from male and female garter snakes were used to induce courtship from courting males (Gillingham and Dickinson, 1980). Treatment with exogenous estrogen causes intact, reproductively inactive, and ovariectomized female *Thamnophis s. sirtalis* to become attractive to and elicit courtship from sexually active males (Crews, 1976). Thus, it appears that ovarian steroids positively affect the production and/or expression of sexual attractivity in female garter snakes. Estrogen treatment in conjunction with shedding appears to be the most effective means of eliciting pheromone production (Kubie *et al.*, 1978). In an attempt to characterize the sex pheromones of the garter snake, Garstka and Crews (1981, 1986) used plasma from estrogen-injected females and males to induce courtship from sexually active males. They originally hypothesized that the yolking protein vitellogenin or some lipidaceous subunit of vitellogenin was acting as the sex pheromone (Garstka and Crews, 1981). However, further investigations lead these authors to conclude that vitellogenin was not responsible for eliciting courtship behavior (Garstka and Crews, 1986).

The sex attraction pheromones of the red-sided garter snake, *Thamnophis sirtalis parietalis*, have now been isolated, identified and synthesized (Mason *et al.*, 1989; 1990; 1993). The pheromones, a novel series of long-chain saturated and monounsaturated methyl ketones have been shown to elicit courtship behavior from sexually active males in the field. To date, this is the only pheromone yet identified in a reptile.

The most recent work on snake pheromones has concentrated on the skin lipids as the source of the sex attractiveness pheromones. Thus, the more recent work on the brown tree snake has concentrated on the skin lipids of the female as a source for the sex attractiveness pheromones. It was hypothesized that because the garter snakes and brown tree snakes are both members of the family Colubridae, their sex pheromones might be related chemically as well. Hexane extracts of female garter snakes were fractionated and yielded one active fraction that contained a novel series of saturated and monounsaturated methyl ketones ranging in molecular weigh from 422 to 534 daltons. Using the same experimental paradigm, Murata et al. (1991) isolated a similar series of saturated and monounsaturated methyl ketones. However, in addition, the main components were a novel series of methyl ketodienes. Ketodienes have never been detected in garter snake skin lipids. Behavioral trials with brown tree snakes using ketodienes have been ambiguous. The major problem is that reliable sex behavior is difficult to induce in both male and female brown tree snakes. However, recent observations of breeding behavior of brown tree snakes in Australia may provide information of value to these investigations.

There are two other behaviors in snakes mediated by pheromones. Trailing studies have focused primarily on three behaviors: detection and location of conspecifics during the breeding season, aggregation, and migration to winter hibernacula. Only the first two are relevant to this discussion. At least 10 species in five families of snakes are known to utilize pheromone trails (see Ford, 1986; Mason, 1992 for review). Most reports of trailing in snakes concern reports of trailing during the breeding season. It is generally the case that male snakes preferentially follow female trails. Females in general tend not to trail males or females during the breeding season.

Another important behavior mediated by pheromones in snakes is aggregation. Gravid female snakes have been reported to aggregate, presumably for some benefit in gestating offspring or eggs (see review in Gillingham, 1987; Gregory *et al.*, 1987). It is not clear what proximate factors are responsible for aggregation in the field. In the laboratory, temperature, humidity, and stress have been demonstrated to be directly related to the incidence of aggregation (see Mason, 1992 for review). However, whether any or all of these are major factors operating in nature has not been tested directly.

In most investigations of aggregation in snakes, stimulus animals leave chemical cues on filter papers that can then be used as bedding under shelters. Snakes prefer to reside under shelters in laboratory conditions and probably in the field as well. In a number of experimental procedures using a variety of different species of snakes, investigators have repeatedly found that when groups of snakes are offered the chance, they choose to aggregate as opposed to remaining alone. That this tendency to aggregate is based on chemical cues left on the substrate has been demonstrated in many studies (see Mason, 1992 for review). The source of the chemical cues differs among species but the two most effective sources are skin lipids and cloacal gland secretions. Aggregation behavior has also been described in the brown tree snake. An aggregation of fourteen brown tree snakes was reported in a hollow tree on Guadalcanal (Pendleton, 1947). Experimental investigations of aggregation behavior in the brown tree snake have not been attempted.

All snakes described have paired cloacal scent glands located in the tail dorsal to the hemipenes in males and in the corresponding position in females. The glands are holocrine in nature and produce primarily lipids (Oldak, 1976). The function of these glands has been the subject of much controversy over the past century. Two of the proposed functions can be attributed to defensive aspects of ophidian behavior. The effects of the cloacal gland secretions have usually been described as repellent and/or alarming.

Some authors have attributed an alarm function to the cloacal gland secretions of disturbed snakes. Anecdotal reports frequently relate descriptions of snakes becoming very agitated and disturbed when exposed to the cloacal scent gland secretions expressed by a conspecific individual. Experimental studies have demonstrated that animals exposed to conspecific scent gland secretions react more intensely to stressful conditions than do animals that were not conditioned by the scent gland secretions (Graves and Duvall, 1988). A common response of a snake to these scent gland secretions is to flee. The current hypothesis is that since cloacal scent gland secretions are expressed under stressful conditions such as predation, it would benefit a conspecific to be able to interpret these semiochemicals as an indicator of imminent and proximate danger and leave the area at once. The exploitation of these scent gland secretions as a tool for modifying snake behavior is obvious. To date, no studies have been conducted on the repellency of brown tree snake cloacal gland secretions. Studies on the chemical constituents that comprise the cloacal gland secretions have been conducted (Mason, unpublished). Further, behavioral tests are planned in order to determine what behavioral response, if any, the brown tree snake will have to its cloacal gland secretions.

The role of pheromonal agents in integrated pest management strategies as applied to reptilian pests is still in its infancy. However, it is recognized that aspects of social behavior in the brown tree snake may be amenable to exploitation by pheromones. I have concentrated here on reviewing the research to date on four behavioral paradigms that seem to hold the most promise for use as control agents: sex behavior, trailing behavior, aggregation behavior, and alarm or repellent behavior. Much more work is needed before these pheromones can be brought to bear as one of the multifaceted methods utilized in integrated pest management strategies.

A novel strategy that has not been discussed in regard to integrated pest management of reptiles warrants inclusion here. One system that has been attempted in insect control strategies involves the use of pheromones in conjunction with naturally occurring pathogens. For example, one paradigm is to use pheromone-baited traps to attract snakes. The trap is contaminated with a virus or other pathogen and the snake allowed to escape. During aggregations or mating behavior, the pathogen is passed on to additional individuals which in turn pass it on yet again. This strategy would necessitate the investigation of potential contagious pathogens in reptiles. Published accounts of virulent pathogens affecting exclusively snakes are becoming more common as experts knowledgeable in their identification and their concomitant research have proliferated (Frye, 1981; Cooper and Jackson, 1981; Hoff *et al.*, 1984). Indeed, research into potential viral pathogens of brown tree snakes is currently underway (D. Nichols, personal communication).

One indicator that pheromones are becoming an important part of integrated pest management systems is the recent involvement of the agricultural and chemical industries. It has been suggested that the fact that pheromone success in monitoring or even controlling pest populations will reduce pesticide sales is not conducive to the involvement of industry, particularly since the quantities of pheromones needed are relatively so small and must be specifically formulated for each species (Birch and Haynes, 1982). However, as humankind becomes more reluctant to add synthetic substances of broad and lasting effectiveness to the environment, the impact of pheromone technology on industry and agriculture will continue to assume revolutionary significance. Only by integrating our knowledge of the basic biology, behavior, reproduction, and chemical ecology of ophidian pest species into an integrated pest management strategy will meaningful and significant advances be made toward controlling snake populations.

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III.2. Sulfur-containing Semiochemicals Attract Predators and Repel Prey (Russell Mason).

Background--Dr. Russ Mason is a psychologist who received his M.A. and Ph.D. degrees from Clark University. Following post-doctoral training at Brown University and the Monell Chemical Senses Center, Russ became an Assistant Member on the regular Monell staff. In 1986, he accepted a position with the Denver Wildlife Research Center. At present, Dr. Mason is a project leader for the Wildlife Research Center, and an Associate Member at Monell. He also has faculty appointments at the University of Pennsylvania (Biology) and Rutgers University (Animal Sciences).

Submitted manuscript--Regardless of vertebrate class, carnivores and omnivores are attracted by sulfurous odors and herbivores are repelled by them. This is especially true in feeding contexts. The present manuscript seeks to provide a plausible explanation for this dichotomy. As a background for the explanation, several illustrative examples are provided below.

When faced with a choice among feeding sites, Norway rats prefer locations that conspecifics are exploiting (Galef and Clark 1971, Galef and Heiber 1976). When faced with a choice among several novel foods, naive rats choose the types of novel foods that have previously been ingested by conspecifics with whom they have interacted (e.g., Strupp and Levitsky 1984). This socially mediated transfer of food preference is semiochemically mediated (Galef and Wigmore 1983, Galef and Stein 1985).

Important chemical information could be simply the smell of food. However, transmission appears to involve both the smell of the ingested diet and an endogenous (demonstrator-derived) cue (Galef et al. 1985, Galef and Stein 1985). Behavioral and gas chromatographic/mass spectroscopic experiments suggest that 1-10 ppm carbon disulfide and/or carbonyl sulfide are critical components of the endogenous signal (Bean et al. 1988, Galef et al. 1988). Carbon disulfide is as attractive to wild rats as it is to rats in the laboratory (Mason et al. 1988), and in standardized tests, carbon disulfide improves the acceptance of EPA challenge baits by wild-trapped Norway rats (Figure 1).

Carbon disulfide and other sulfurous compounds are also attractive to dogs and cats. Diallyl disulfide (garlic) is a popular flavor additive to both dog and cat foods (D. Passe, personal communication), and both diallyl disulfide and propane thiosulfinate (onion) enhance consumption by these carnivores (Mason, unpubl. obs.). Since successful coyote (Canis latrans) lure formulations contain sulfurous materials (G. Preti, unpubl. obs.), it seems likely that sulfurous compounds are attractive to these canids as well.

Anecdotal and experimental evidence support the view that sulfur-containing compounds are repellent to herbivores. Grazing ungulates such as mule deer (Odocoileus hemionus) and elk (Cervus canadensis), herbivorous rodents like mountain beaver (Aplodontia rufa), and lagomorphs like cottontailed rabbits (Sylvilagus floridanus) are repelled by hydrogen sulfide (Campbell, and Evans, 1988, Conover 1987, Conover and Kania 1987, DeVoe and Schaap 1987), an odorant that attracts coyotes (Bullard pers. commun.).

The omnivore-carnivore/herbivore dichotomy spans vertebrate classes. Among birds, there is evidence that carnivores such as turkey vultures (Cathartes aura) find food on the basis of sulfur-containing volatiles (Stager 1967, Houston 1986). Conversely, herbivorous species such as Canada geese (Branta canadensis) avoid plants like wild onion (Mason, unpubl. obs.), perhaps because these plants contain S-propyl propane thiosulfinate. Among fish, the use of sulfur-containing baits for catfish (Ictalurus nebulosus) is well-known, and sulfur-containing amino acids like cysteine and methionine are potent olfactory and taste stimuli (Caprio 1975, 1977).

One plausible explanation for the attractiveness of sulfurous odorants to carnivores and omnivores and the repellency of these same materials to herbivores is that sulfur is released during the bacterial degradation of proteins. Thus, the presence of sulfur could indicate that an animal has recently consumed meat--a fact that should attract carnivores and omnivores, but repel herbivores. It also follows that other excreta (urine, feces) should be repellent to herbivores. There is evidence consistent with this possibility. For example, the urine of a variety of arbitrarily selected predators is repellent to mountain beavers (Aplodontia rufa) when the odors are presented in the immediate vicinity of a food source (Epple et al. 1993; Figure 2).

Studies on a number of other mammals, including lagomorphs, rodents, and ungulates also document the effectiveness of predator-derived chemical cues as herbivore repellents. Feces, urine, and glandular secretions affect spacing, exploitation of food resources and damage to plants by some old world and new world Lagomorpha (Sullivan et al. 1985a, Sullivan and Crump 1984, 1986a, Robinson 1990), several species of Microtus (Stoddart 1976, 1980, 1982, Dickman and Doncaster 1984, Gorman, 1984, Sullivan and Crump 1986b, Sullivan et al. 1988a,b, 1990a, Robinson 1990), woodchucks (Marmota monax, Swihart 1991), and Norway rats (Rattus norvegicus, Vernet-Maury 1980, Vernet-Maury et al. 1984). Not surprisingly, predator odors are being evaluated as practical repellents under field conditions. Extensive laboratory and field studies by Sullivan and co-workers, testing a number of synthetic components of predator scent, resulted in a potential new rodent repellent comprised from 2 constituents of mustelid anal gland secretion (Sullivan and Crump 1986a, 1986b, Sullivan et al. 1988a, 1988b, 1990a, 1990b, Merkens et al. 1991). Both of these secretions are sulfur-containing.

Fecal material and urine from a variety of carnivores reduce feeding in ungulates, including roe deer (Capreolus capreolus), red deer (Cervus elaphus), black-tailed deer (Odocoileus hemionus columbianus), white-tailed deer (Odocoileus virginianus) and domestic sheep and cattle (Van Haaften 1963, Muller-Schwarze 1972, Melchiors and Leslie 1985, Sullivan et al. 1985b, Abbott et al. 1990, Pfister et al. 1990, Swihart et al. 1991, Weldon 1990). Very slow habituation to predator-derived chemical cues was evident in several of these studies (Sullivan and Crump 1984, Sullivan et al. 1988a, Swihart 1991).

Although the repellency of predator odors is influenced by habitat characteristics (Merkens et al. 1991), and other ecological factors (Swihart et al. 1991), there is evidence that avoidance is innate and mediated by a few key chemical cues. For example, many herbivores are repelled by chemical cues from carnivores that do not prey on them (Abbott et al. 1990, Dickman and Doncaster 1984, Gorman 1984, Muller-Schwarze 1972, Stoddart 1976, 1980, 1982, van Haaften 1963). Mountain beavers even avoid feeding from bowls scented with urine from domestic dogs. Similarly, other herbivores are repelled by chemical cues from carnivores that do not normally prey upon them.

Although data are sparse, there is evidence that fish respond to predator odors as mammalian prey do. Fathead minnows (Pimephales promelas) are repelled by the odor of northern pike (Esox lucius) (Chivers and Smith 1993). Likewise, brook trout (Salvelinus fontinalis) are repelled by the odor of Atlantic salmon (Salmo salar) (Keefe 1992). Repellency is influenced by the nature of the salmon's diet, and is significantly enhanced when salmon are eating fish (rather than invertebrates).

Semiochemicals in feeding situations are usually (if not always) sulfur-containing. In part, the repellent or attractant effectiveness of these cues is related to volatility, i.e., more volatile stimuli are more powerful cues. But volatility alone does not explain why sulfur-containing odors should be more important than other equally volatile stimuli. One explanation may be that sulfur-containing odorants are important because they reflect protein digestion. Protein digestion is an indicator of diet type. Hence, the attractiveness of sulfur compounds to carnivores and omnivores, and the repellency of these substances to herbivores. This explanation is similar to a hypothesis proposed by Albone (1984) for air-breathing vertebrates, and incorporates aspects of Bryant and Atema's (1987) explanation of the relationship between diet type and social behavior in catfish. Regardless, sulfur-containing odorants may be especially useful as vertebrate pest control agents because these odors are both attractant and repellent, depending upon the animal under consideration.

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Table 1. Results of choice tests during a diet manipulation experiment with juvenile brook trout from Angeline Brook (AB) and Beaver River (BVR).

Population	Experimental Group	Stimulus Choice	Mean Time Spent	Standard Error
AB	1	CW vs.	124.8	21.3
		ATS-M	217.8	39.2
	2	CW vs.	182.6	14.4
		ATS-G	124.8	27.8
	Control	CW vs.	129.5	21.8
		CW	113.9	22.1
BVR	1	CW vs.	181.7	16.3
		ATS-M	180.2	14.5
	2	CW vs.	252.0	18.9
		ATS-G	148.8	34.1
	Control	CW vs.	144.8	16.7
		CW	157.2	28.6

Abbreviations: CW, control water; ATS-M, Atlantic salmon fed mealworms; ATS-G, Atlantic salmon fed goldfish. From: Keefe, M. L. (1993). Chemically mediated avoidance behavior in wild brook trout, Salvelinus fontinalis: the response to familiar and unfamiliar predaceous fishes and the influence of fish diet. Can. J. Zool. 70, 288-292.

Figure Captions

Figure 1. Consumption of carbon disulfide adulterated EPA challenge bait and control bait by wild-trapped rats. (From: Mason, J. R., N. J. Bean, and B. G. Galef. 1988. Attractiveness of carbon disulfide to wild Norway rats. Proc. Vertebr. Pest Conf. 13: 95-97).

Figure 2. Mean consumption of apple paired with control odors, predator odors, or mink anal gland secretion by mountain beaver. Abbreviations: GPU = guinea pig urine, BA = butyric acid, A = dog, B = mink, C = bobcat, D = coyote. (From: Epple, G., J. R. Mason, D. L. Nolte, and D. L. Campbell. 1993. Effects of predator odors on feeding in the mountain beaver (*Aplodontia rufa*). Journal of Mammalogy 74: 715-722).

Figure 3. Mean change in total distance travelled (cm) by sham operated (control) and anosmic fathead minnows following exposure to chemical stimuli from northern pike. (From: Chivers, D. P., and R. J. F. Smith. 1993. The role of olfaction in chemosensory-based predator recognition in the fathead minnow, *Pimephales promelas*. Journal of Chemical Ecology. 19: 623-633.

Figure 1.

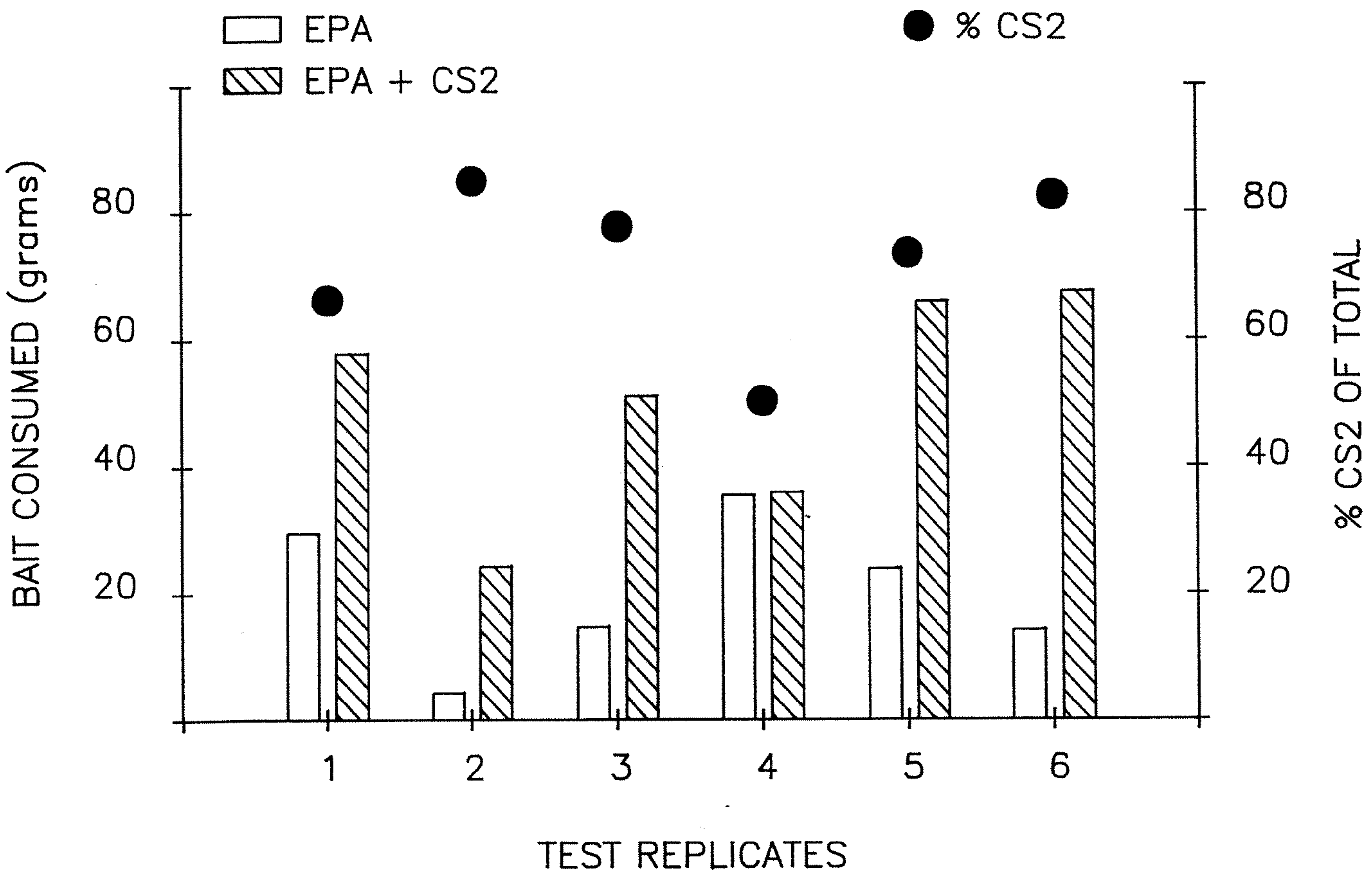

EPA
EPA + CS2
% CS2
BAIT CONSUMED (grams)
20
40
60
80
% CS2 OF TOTAL
20
40
60
80
1
2
3
4
5
6
TEST REPLICATES

Figure 2.

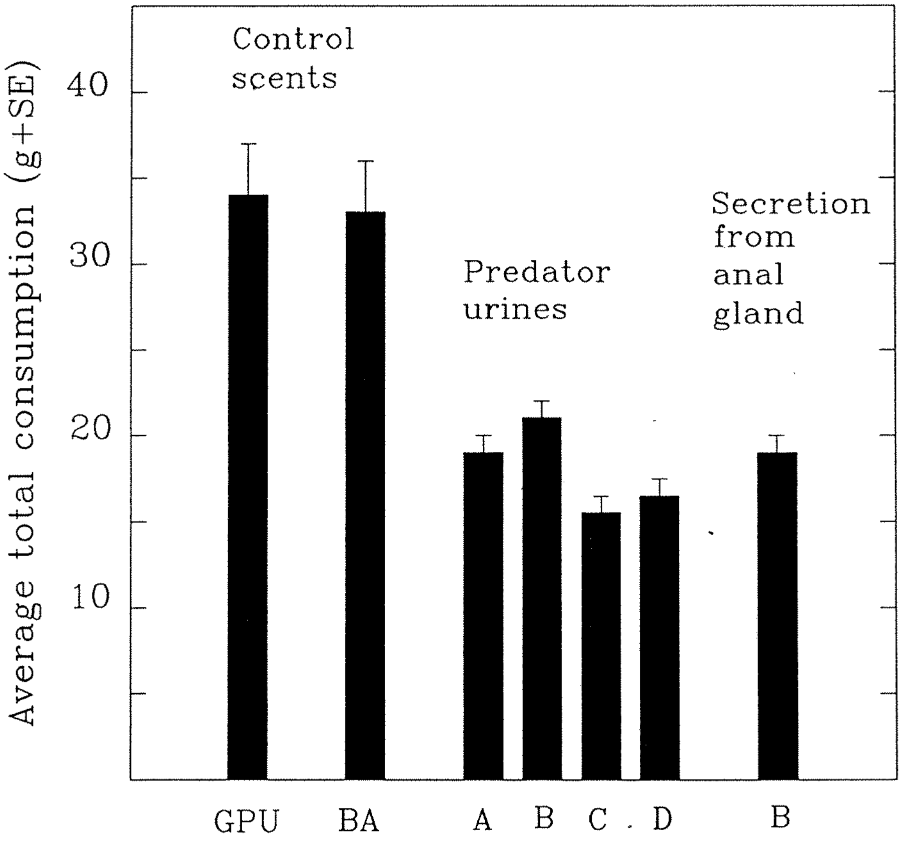
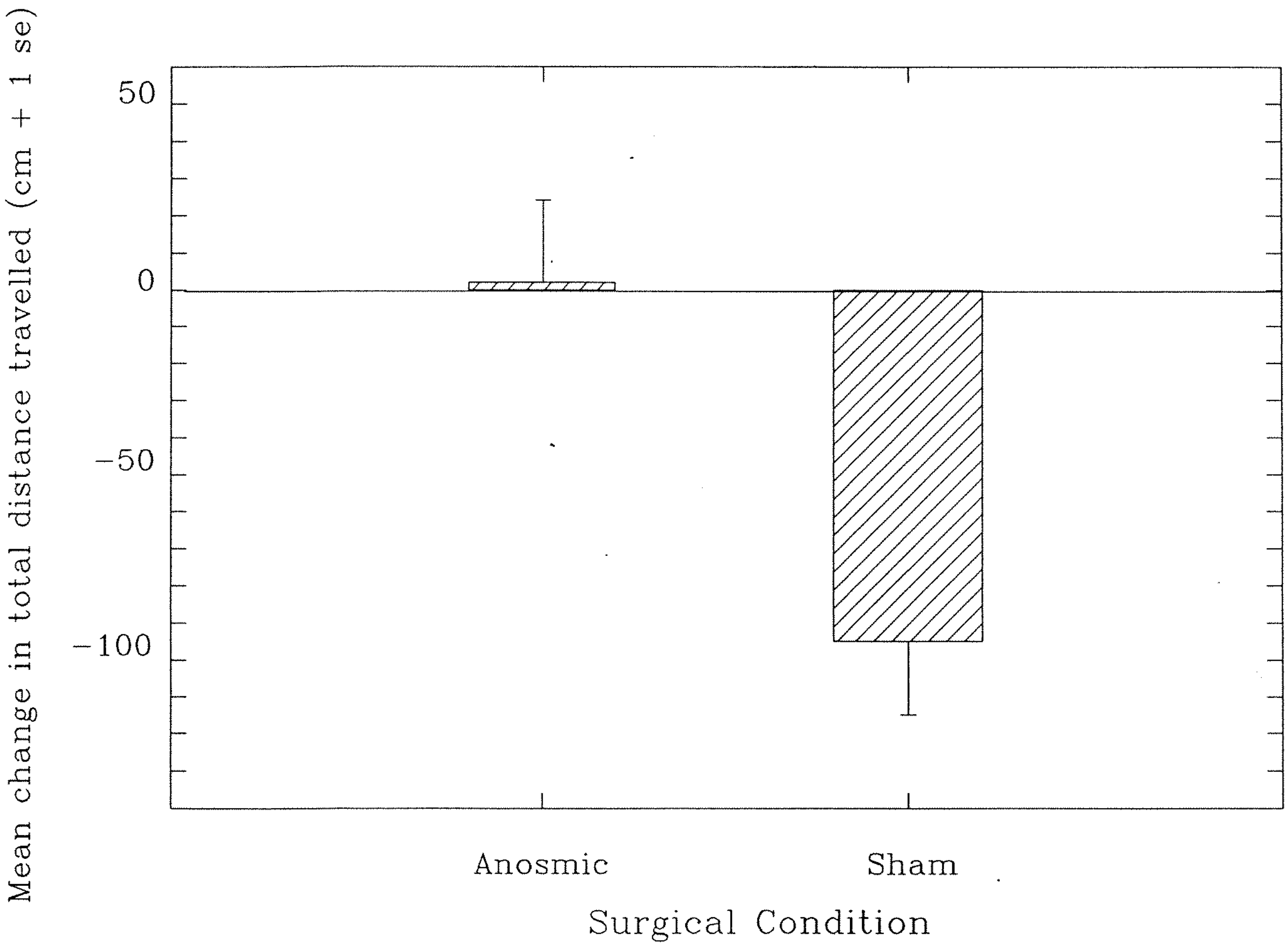

Control scents
Predator urines
Secretion from anal gland
Average total consumption (g+SE)
40
30
20
10
GPU
BA
A
B
C
D
B

Figure 3.


Mean change in total distance travelled (cm + 1 se)
50
0
−50
−100
Anosmic
Sham
Surgical Condition

IV. THE USE OF PHEROMONES AND KAIROMONES IN THE MANAGEMENT OF INSECT PESTS

IV.1. Introduction to Insect Chemoreception, Chemical Signals, Kariomones, and Control Theory (Richard Jones).

Background--Dr. Jones, Dean of the College of Agriculture at the University of Minnesota, is administratively responsible for the 12 departments of the College as well as the six agricultural experiment stations throughout the state. Dr. Jones, a faculty member in the Department of Entomology since 1977, was named head of the Department of Entomology in 1984 and for six years directed the teaching and research mission of that department prior to his selection as Dean of the College of Agriculture. Dr. Jones served on a three person team to survey and evaluate the use of biological control for stem boring insects in the People's Republic of China in 1980. From 1985-1992, he served as advisor to a PL 480 project in Yugoslavia on the integrated pest management of corn insects. Dr. Jones has taught courses in insect physiology and in chemicals and insect behavior. He has received many state and federal grants to investigate the behavior and biological control of insects, and has authored many articles and book chapters on chemical pest management. His expertise in insect physiology and behavioral chemicals is nationally and internationally recognized.

Summary of talk--Dr. Jones presented ideas and thoughts on chemoreception in insects. Insects don't see or hear very well but their bodies are covered with chemoreceptors and they use chemicals very effectively to locate mates, food, and habitat. There are several physiological factors that determine how insects respond to chemical cues. Heat and air pressure cause different responses. A high concentration of a chemical may cause a different response than a low concentration does. Parasitoids (insects that are parasitic on other insects) use chemical cues to locate the habitat where their prey is found and to locate their host. Chemicals have been identified that increase parasitism in some parasites. Parasitoids are attracted by chemicals emitted by damaged plants and have ability to learn which is useful. While pheromones can be used for disrupting or confusing insects, their greatest use is for monitoring insect populations.

IV.2. Insect Chemosensitivity (Joseph C. Dickens).

Background--Dr. Dickens is currently Research Entomologist and Lead Scientist working on a research project entitled *Modification of Insect Behavior Though Olfactory Chemicals*. Dr.

Dickens' expertise is principally in the use of morphological and electrophysiological methods to investigate insect sensory receptors and correlation of electrophysiological responses with whole organism behavior. Dr. Dickens has been with the USDA Agriculture Research Service for 11 years during which his principle research has concerned characterization of olfactory receptor neurons for pheromones and host odors in the boll weevil and other insects including parasitoids. He has also worked on hormonal modulation of olfactory sensitivity and pheromone production in insects, olfaction in Hawaiian fruit flies, and photoreception. Following an ARS Fellowship in the Netherlands two years ago, Dr. Dickens has continued his research on the boll weevil but is currently spending more time on studies of olfaction in other insects, e.g. *Spodoptera* moths, and *Lygus* bugs. Prior to joining ARS, Dr. Dickens worked on olfaction in bark beetles in the southeastern United States and Europe, and the role of protein synthesis in the frog, *Rana pipiens*.

Submitted manuscript--Chemical signals emitted from conspecifics, food sources, or potential mating or egg-laying sites play an important role in the behavior of organisms. Both chemical and physical stimuli are detected by specialized receptor neurons that send messages to the brain of the receptive organism. In the environment of every creature there may be numerous other species that are potential emitters of chemicals. Thus the organism is confronted with the problem of distinguishing chemical signals important to its survival (Dickens, 1990a).

Insects offer a unique model for understanding basic mechanisms by which chemical signals are detected and subsequently integrated with information from other sensory modalities into a behavioral symphony for species sustenance and propagation (Bell and Cardé, 1984; Dickens and Payne, 1986). Electrical responses can be relatively easily recorded from individual receptor neurons of insect olfactory organs and intracellular responses may be recorded from higher order neurons within the brain (Masson and Mustaparta, 1990). Relevant stimuli, such as pheromones and host odors, have been identified in several instances, and the specific behaviors they elicit have been characterized. The physical nature of chemical signals detected by insects has been determined (Murlis and Jones, 1981). Understanding the mechanisms involved in the detection and coding of these signals by peripheral olfactory receptor neurons is tantamount to understanding central nervous system processing of incoming signals and resulting behavior.

Olfactory reception in insects involves several steps (Kaissling, 1986; Stengl et al., 1992; Dickens et al., 1993a): 1) adsorption of the odorant to the cuticle with subsequent diffusion to a pore in a receptive sensillum; 2) transport of odorous molecules through the receptor lymph within a sensillum by pore tubules (Kaissling, 1974) or odorant binding proteins (Vogt and Riddiford, 1986); 3) interaction of the odorous molecule with a putative membrane-bound receptor with an increase in membrane conductance to produce a receptor potential (Zufall et al., 1981); 4) rapid inactivation of the odorant by removal of the molecule from the receptor by a carrier protein (Kaissling, 1986) or enzymatic degradation (Vogt et al., 1985); 5) spread of the receptor potential to an impulse generator where firing of the affected neuron occurs with subsequent transmission of the coded message to higher order neural centers in the brain. The sum of the detector machinery in insects codes not only information about the concentration of odors in the air, but also temporal information on the frequency of encounters with adequate stimuli (Kaissling, 1986; Kaissling and Kramer, 1990). Specificity of olfactory neurons in insects is thought to be a product of putative membrane-bound receptor proteins and possibly odorant-binding proteins (Vogt et al., 1991). Specificities of the reception and inactivation processes may differ for an individual odorant (Dickens et al., 1993b).

Sensitivity and specificity in a complex chemical environment is enhanced by blends of chemical signals which may be emitted in a species-specific context, *e.g.* mixtures of a multicomponent pheromone and host plant odors (Dickens, 1990a; 1990b; 1993; Dickens et al., 1984). While one may consider detection of individual odorants, it is likely that behavior results from detection and integration of a multiplicity of odorants present as a blend in the insect's environment. Insects may respond behaviorally to a blend of pheromone components, but under natural conditions the insect encounters the pheromone in combination with plant odors and emissions of other insects. For example, the boll weevil is a narrowly-oligaphagous insect which feeds only on cotton or closely related plants. Upon locating a suitable host plant, male boll weevils feed and release a blend of four oxygenated monoterpenes in their excrement. Three of these compounds are detected by specific receptor neurons housed within sensilla on the insect's antenna (Dickens, 1990b). Activation of these three neuronal types sends a message to the brain which results in attraction of male and female boll weevils. In addition, male and female boll weevils have at least seven neuronal types which are selectively activated by specific plant odors. A blend of host plant odors is also attractive to boll weevils. Since male boll weevils must feed to produce and release the pheromone, responding weevils are exposed to a complex blend of pheromone components and host plant odors. It is this complex blend which is both synergistically and preferentially attractive to the boll weevil and other insects in context with their specific pheromones and host plants.

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Topics for discussion--Previous results show sea lampreys to be very sensitive to specific bile salts. At what concentration are these compounds present in the animals' environment? What is the temporal nature of these olfactory cues in context with behavior?

Is there any evidence for synergism of behavioral responsiveness in lamprey to the bile salts by other odorants?

Based on electro-olfactograms, sea lampreys are differentially sensitive to particular compounds. What determines this specificity?

Since mature animals may be more sensitive or at least behaviorally more responsive to odorants at different times, it seems likely that the physiological state of the organism is responsible for behavioral response to various stimuli. Thus hormones are likely involved. Although hormones probably have their effects on the central nervous system, do hormones also regulate development of olfactory receptor proteins, or have effects on odorant binding proteins or odorant degrading enzymes?

IV.3. Understanding the Behavioral Ecology of Pheromone-mediated Communication Systems for Effective Use in Management Programs (Jeremy McNeil).

Background--I received my B. Sc. in Zoology from the University of Western Ontario in 1969 and completed my Ph. D. in Entomology/Ecology at North Carolina State University (under the direction of Prof. R. L. Rabb) in 1972. I accepted a faculty position at Laval University straight out of graduate school and have remained here ever since. Initially my research was of a more applied nature, establishing IPM programs for several important agricultural pests. About 12 years ago I reoriented my research to more basic aspects of behavioral ecology, with particular emphasis on chemically-mediated mating systems of Lepidoptera as I felt this was an area that, despite the potential of semiochemicals in management programs, had been neglected. My program has become more and more interdisciplinary in nature, which accounts for ongoing collaborations with physiologists, biochemists, molecular biologists, geneticists and chemists in several different countries. I have published a couple of reviews on the behavioral ecology of Lepidopteran pheromones, and these are cited below. Two other book chapters on the physiology of migration are currently in press and should be available sometime in 1994.

Ideas: Consideration of semiochemicals in management of lamprey--Based on my limited knowledge of lamprey biology (obtained from the reprints provided to participants. e.g. Teeter 1980) it seems that the most promising area to exploit for the use of infochemicals in the management of the lamprey would be the olfactory cues used by individuals migrating towards suitable spawning sites. As there is evidence that the cue originates from the juveniles developing at the spawning sites, this possibility should be first tested under field conditions by placing enclosures containing larvae in rivers that are not known to normally attract migrants. If this is the mechanism then one would expect detectable physiological changes, associated with the onset of migration, that modulate the behavioral response to the chemical cues from suitable spawning sites. Thus, I believe a detailed eco-physiological study of the responsiveness to "larval infochemicals" by migrating and non-migrating individuals could provide valuable insight into the selection of spawning sites and facilitate use in management programs. Furthermore, given that migration appears to be initiated by sexually immature individuals, it would be interesting to determine if any infochemicals used in the location of spawning sites also influences the rate of sexual maturation. It is evident that these studies would have to be conducted in concert with those to identify the composition of any behavior-modifying chemicals that are isolated.

Submitted manuscript--In the last several decades semiochemicals, usually sex or aggregation pheromones, have been successfully deployed in a variety of control programs against a number of agricultural and forestry insect pests, as evidenced in a number of recent general publications on the subject (Jutsum and Gordon, 1989; Ridgway et al., 1990). However, not all attempts have proved successful and as Tumlinson (1988) pointed out this is due, at least in part, to incomplete chemical identifications. In addition, a better understanding of the behavioral, ecological and evolutionary perspectives of the semiochemically-mediated communication systems we wish to manage would also lead to a significant improvement in the deployment of infochemicals in pest management (McNeil, 1991, 1992).

To exemplify this point I will review the use of sex pheromones to monitor lepidopteran populations, where one of the major points for consideration is the degree to which synthetic lures can effectively compete with natural sources of the infochemicals. Obviously, a lure that does not contain all of the components will not compete effectively with receptive females. However, even with the correct blend, the degree of competition may be influenced by a variety of biotic and abiotic factors. For example, in the sunflower moth, *Homoeosoma electellum*, older females spend more time calling (the behaviour associated with the release of the sex pheromone) than younger ones. Furthermore, females in the presence of host plant pollen call significantly longer than similar-aged individuals without access to this resource (McNeil and Delisle, 1989). Thus, for the same population density, the level of competition between females and lures would vary, depending on the phenological state of the host crop and the age of the females in the population. Similarly, in the European corn borer, *Ostrinia nubilalis*, females call for a significantly longer time at high (>80%) than low (<65%) relative humidity conditions (Webster and Cardé, 1982; Royer and McNeil, 1991), while male responses to synthetic lures declines with an increase in relative humidity (Royer and McNeil, 1993). Therefore, on any given night, traps may be markedly more effective if humidity conditions are low rather than high. Changes in temperature conditions may also influence the level of competition, not only through the timing and duration of female calling behaviour (see McNeil, 1991 for references) but also through modification of the responsiveness of males to different pheromone blends (Linn et al., 1988) as well as the time window during which males are physically capable of flying toward a pheromone source.

In some species migration is an important life history strategy and this may have a marked effect on the effective use of pheromone traps. The true armyworm, *Pseudaletia unipuncta*, cannot overwinter in northern United States and Canada, yet each year immigration results in the establishment of northern populations. Studies on the pheromonal ecology of this species found that the sexual maturation of both sexes is delayed by short day, low temperature conditions and lead to the hypothesis that there are northward spring and southward fall migrations in this species (McNeil 1987), in response to cues indicating predictable habitat deterioration: in the south the onset of summer when temperatures are too high for optimal development and in the north the onset of winter conditions that are lethal to all stages in the life cycle. Additional studies on the underlying physiology associated with both the production of, and response to, the sex pheromone support the idea of seasonal migration (Cusson et al. 1993). Consequently, as immature males do not respond to pheromone sources, pheromone traps are of little use in monitoring the density of migrant populations, even when very high concentration lures are used. However, while the effectiveness of pheromone traps may be limited under certain conditions when dealing with migrant Lepidoptera, an understanding of the behavioral ecology and physiology of the pheromone communication system may provide valuable insight into migratory strategies that could ameliorate management practices.

A rather different case of how migration may influence the efficacy of pheromone traps in management programs is found with the spruce budworm, *Choristoneura fumiferana*. In this species migration from an unsuitable habitat is generally undertaken by mated females only. Thus, even through immigration the density of reproducing females may change by an order of magnitude at a given site, and there may be negligible increases in the density of males captured in pheromone traps. That must be considered when using pheromones.

There is considerable evidence in the literature for female mate choice and male-male competition in Lepidoptera. Thus, it is possible that the majority of males captured in pheromone traps were those rejected by females and/or were those that lost out to superior conspecific males. If true, then the data generated from trap captures would be of little use in predicting population trends. Unfortunately there is very little information available on the reproductive status of individuals captured in pheromone traps, and this is an area that I believe merits further investigation.

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IV.4. Using Pheromones to Monitor Population Densities (Ring T. Cardé).

Background--My research interests center on chemical communication in insects, emphasizing male attraction to female moths and attraction of female wasp parasitoids to their lepidopterous hosts. A common theme in our investigations of both of these systems is the flying maneuvers employed by insects to locate an upwind odor source. As well, we study the chemistry of pheromones and kairomones, the turbulent dispersion of odor plumes, the genetic control of pheromone production and response, learning in parasitoids, and the mechanisms responsible for mating disruption when formulated synthetic pheromone is dispersed on a crop. My graduate training was in insect systematics at Cornell, but a postdoctoral fellowship with Wendell Roelofs at Geneva lured me into studying how insects communicate with chemicals. I was at the Department of Entomology at Michigan State until 1982, when I became Head of the Department of Entomology at the University of Massachusetts, a position I unfortunately assumed again this September.

Submitted manuscript--The release of a pheromone by the female to attract a male appears to be a nearly generic solution to mate finding in the 120,000 known species of moths. The chemical identity of the pheromone message is known for several hundred moth species and, based on empirical field trapping trials, attractants are known for hundreds of additional moth species. The distinction between an attractant pheromone and an empirically-defined attractant is that in the former case the chemistry of the natural pheromone has been verified; most attractants undoubtedly represent a part or the entire natural pheromone. Attractant pheromones predominate mate location behaviors in many other insect groups, including beetles, termites, bees, wasps, ants and sawflies. In some groups, most notably several of the beetle families, both males and females are attracted by the same pheromone, often in combination with host-related volatiles, often to a site where both sexes may aggregate, feed and mate. Such pheromones can be viewed as causing both attraction and aggregation.

The chemistry of attractants in moths is well understood for most families (Mayer and McLaughlin, 1991). Although some species employ a single chemical, most species utilize blends of two to as many as 5 or 6 components. Most described components consist of carbon chains of even numbers (most routinely of 12, 14 or 16 carbons) with a terminal acetate, alcohol or aldehyde. Usually one or two double bonds occur at the 11, 9 or 7 position. Specificity in the communication message is achieved mainly by each species using a unique blend of compounds, sometimes by simply using combinations of geometrical and positional isomers. Often the female releases these blends in a tightly regulated ratio and, if so, the male's response in turn may only occur when this blend is accurately mimicked by a synthetic mixture.

The behavioral reaction of a flying male moth to a pheromone is to move upwind, provided the concentration or flux of pheromone is above threshold and, based on data from a small but likely diagnostic number of species, distributed patchily in the wind. Such a patchy occurrence of course is generated by the pheromone plume being subject to turbulent diffusion. The process of attraction thus involves optomotor anemotaxis, in which the polarization of upwind displacement is due to the insect sensing its movement relative to its visual surroundings. Attraction in the field certainly occurs over distances of tens to hundreds of meters, although there is some doubt about communication over distances of kilometers.

The potential of using a sex pheromone to lure insects into a trap and employing the resulting counts to estimate population levels long predates the availability of synthetic lures. But today the ready availability of lures for many of our most injurious species has spawned a moderate industry to supply traps and lures to researchers and to pest management advisors. The uses of these sampling systems fall into three distinct categories:

1. *Survey and delimitation.* In 1992 over 400,000 traps were deployed to find gypsy moth and Asian-strain gypsy moths in North America. The grid system can be set at 4 or more traps per square mile, following an initial catch in the previous year. The pattern of catch then allows a program of insecticide treatment to be directed only at areas harboring infestations. Considerable efforts have been undertaken to calibrate trap catch by experiments using released, marked gypsy moth males (Elkinton and Cardé, 1980). Generally, it is thought that most males do not travel over a kilometer before capture, and that attraction occurs in the range of 100 meters (Elkinton et al., 1987).
2. *Initializing phenological models.* The first emergence of a given species cannot always be predicted reliably with models based on temperature. Using the first appearance of the oriental fruit moth in pheromone-baited traps as a "biofix," it is feasible to provide useful models of seasonal development (Baker et al. 1980).
3. *Direct correlations of catch and population density.* The value of such information is obvious. An inexpensive and sensitive sampling device that allows managers to base their decisions on the abundance of a pest or its potential for damage has universal appeal. Pheromone-baited traps often provide just this sort of information, as detailed in this workshop by Staten's description of area-wide management programs using pheromone-baited traps for the pink bollworm in cotton. However, as population density rises, the value of trap catch as a predictive tool seems to diminish. The reasons for this vary with species (McNeil, 1991). They may be entirely artifactual, as occurs when a sticky trap becomes paved with insects, such that the efficiency of trap catch is diminished. Other examples of non-linear relationships between trap catch and population density can have strictly behavioral and ecological causes. Unfavorable weather can suppress trap catch but not necessarily mating and hence population levels. Competition between native females and pheromone traps also may provide a misleading picture. Early in a flight males typically predominate, while the reverse can occur toward the end of emergence. Males present at the beginning of a flight may be most apt to be caught in traps and those at the end of the flight may be least likely to be captured. This phenomenon may simply be due to some

males spending an evening mating, so that they are most unlikely to be caught in a trap until the next night. In some species the ratio of males to females can vary widely, certainly an undesirable characteristic for the use of pheromone traps in monitoring density. The behavior of individual males may be affected by many environmental and experiential factors. The extent to which such ecological and behavioral vagaries modify trap catch varies widely with a given pest species, as does, to a degree, the tolerance of managers for errors in estimation.

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IV.5. Using Sex Pheromones to Disrupt Insect Population Development Through Mating Disruption (Robert Staten, L. Antilla, and O. El Lissy).

Background--Robert (Bob) Staten started work in Entomology in New Mexico with the express goal of purchasing a "hot rod Ford" at the age of 15. He has never left that field. Upon completion of a Ph.D. from University of California, Riverside, in 1970, a career in what is now APHIS started. It began with the Sterile Insect Project in the Coachella Valley. That project was short-lived, and Dr. Staten transferred to Phoenix, where a one man Methods station was started. During the ensuing years, almost all personal research and development activities have been in two main areas:

a. Using pheromones for survey and disruption.
b. In sterile insect technology.

Virtually all efforts have been placed in fostering these two technologies into Bio-Rational Management Systems, with an ultimate goal of eradication. The one-man station has become a western regional based center with responsibility for grasshopper IPM, biocontrol of whitefly, and pink bollworm program support. The Center has six professional employees and a small cadre of seasonal and permanent support personnel. Bob is the Center Director.

Submitted manuscript--There are numerous review articles covering the theory and use of phermonal mating disruption. Participants are referred particularly to Cardé (1990). This presentation will, to a limited degree, cover the more pragmatic development and its relative success using a cotton pest, the pink bollworm, *Pectinophora gossypiella,* Saunders, as a cause célèbre.

Gossyplure was the first EPA-labeled pheromone system for control of an insect. The actual pheromone was identified in 1973 in a combined effort lead by the University of California at Riverside (Hummel et al, 1973). The first commercially available pheromone system utilized repeated applications of low dosage pheromone (.75 - 1.6 g. AI/acre) in discrete point sources ranging from 3,000 to 8,000 fibers (NoMate™) or flakes (Disrupt) per acre. Relatively early in their development, insecticides were mixed with the adhesives which held these discrete sources to leaves to take advantage of an "attract and kill" concept (Conlee and Staten Patent number 4,671,010). Resulting mortality improvement is unclear.

In the irrigated cotton systems where the pink bollworm (PBW) is present, fiber formulation is still used, along with several sprayable formulations. The key disadvantage of the fiber and flake is the need for special equipment for distribution and a matched adhesive to keep them attached to plants. Sprayable systems do not produce discrete point sources, but appear to elicit extensive searching behavior on leaf surfaces by male pink bollworm moths. These formulations are being applied largely with conventional spray technology, but require higher levels of active ingredients per acre (2.5 - 5 grams AI per acre).

In all these systems, management of a pest population is much more demanding than in those using conventional insecticides. Timing is extremely important. No low-rate formulation has enough activity to block mating for more than 8 - 14 days. The actual time frame for which disruption is effective is limited and controlled by temperature and climatic conditions. Monitoring of pest populations and behavior modification through pheromone traps is extremely critical. Use of pheromones, particularly with these systems, is management intensive from a pest control advisor's perspective. The intent is not to kill the insect, but to keep a male from finding a female and mating with it. Thus, the higher the density of a pest population, the more likely is an encounter of a female with a male. The systems, from all field evidence, are inversely density-dependent. For this reason, the most successful usage of these materials is against the spring emerging moth populations from diapausing larvae and the first spring generation of moths. Overwintering larvae are subject to major mortality, and are operating at somewhat of an energy an/or nutritional deficit. The next generation is found only in flower buds before the plant fruiting cycle is developed. This generation is also usually at low population densities. Unfortunately, growers have been reluctant to expend resources when populations are not at high and damaging levels. Low rate systems were used in an early area-wide program in the Imperial Valley in 1982.

The most important lesson learned by this author was that for an area-wide program to win with soft technology, it had to be a great deal better than the conventional insecticides before it would have long term successes. Baker, et al. (1990), offers an excellent review of technology in low rate systems and some of the technology that evolved from this period. Our laboratory, and frequently the adjacent ARS lab, the Western Cotton Research Center, were involved in advances after this time. A review of the 1982 program and some of these advances can be found in Baker, et al. (1990). The most significant change in PBW disruption came about with the advent of a PB Rope first tested by Flint et al, (1985). This system is a high rate system.

The PB Rope is a radical departure from all other disruption systems used for this insect in several respects:

1. It uses one treatment with a much higher level of AI (30 grams per acre).
2. Only 400 "dispensers" per acre are hand placed in the field.

3. Effects on the population are present at least 50-60 days.
4. Cost of the individual treatment is perceived as extremely high.
5. It is, from our observations, more efficacious against higher level populations.
6. There is only one critical decision period. The application must be when cotton is small and just before the first hostable flower buds are present.

This system was tested in "small" field plots in Arizona in 1984 first by Flint, and placed immediately in large scale field tests in 1985 (Staten et al, 1987). During the mid-1980's, pesticide usage in the Imperial Valley was extensive. All growers were using a full season cotton growing system, which had maximum host potential for pink bollworm. Population pressure was extreme, and suppression was critical. Within this environment, the Rope was successful. This success was best documented in Table I from Staten et al, 1987.

Table I. Effects of PBW-Rope Treatment on Boll Damage and Insecticide Usage in the Imperial and Mexicali Valleys in 1985

Treatment	Larvae/100 bolls		No. insecticide treatments
	Aug	Sept	
	Imperial Valley[a]		
Conventional insecticide	0.85	0.88	11.4 a
Conventional pheromone	0.90	2.1	10.4 a
PBW-ROPE pheromone	0.32	0.39	6.6 b
	Mexicali Valley[b]		
Conventional insecticide	1.72 a	1.55 b	4.9 a
PBW-ROPE pheromone	0.7 b	0.72 b	2.9 b

[a] There were seven PBW-ROPE-treated fields, eight conventional insecticide-treated fields, and eight conventional pheromone-treated fields (NoMate PBW or Disrupt). Means in same column having no letters in common are significantly different according to ANOVA followed by Duncan's multiple range test ($P = 0.01$).

[b] There were 16 PBW-ROPE-treated fields and 14 conventional insecticide-treated fields. Means in same column having no letters in common are significantly different according to Student's *t test* ($P = 0.01$).

Within this test, each plot was a full field. Although the Imperial Valley of California is separated from the Mexicali Valley by only a fence, the economic structure of cotton growing is completely different. The Rope was successful in both systems, in that conventional pesticides were eliminated in an amount at least equivalent in value to the cost of the Rope. Population suppression was as good or better than full use of pesticide.

After this first year of replicated field testing, the Phoenix Methods lab initiated an area-wide test in a small semi-isolated area of Southern California, the Coachella Valley. In this area, cotton was and had always been of secondary importance to dates, grapes, citrus, and vegetables. Its growing season was virtually identical to the adjacent Imperial Valley, approximately 30 miles to the south and east. Separation was by a brackish water lake, the Salton Sea. Records were obtained on pesticide use in all fields before we began to work (1985) in the valley in 1986. Our project started as a pheromone management trial in 1986. In 1987-1989, we integrated sterile insect releases into this system. As in all our large scale field tests, at any time a pest control advisor deemed it appropriate, a developing larval population was treated with insecticides. The key to this and all other pheromone strategies for pink bollworm is to hold populations <u>below</u> a level which is a normal economic threshold for conventional insecticides. Table II shows the treatment strategies and the required conventional pesticide usage before we started our project in 1985 and in each subsequent year of the area-wide trial.

Most importantly in this table, conventional insecticides were reduced drastically from a grower system in which conventional treatments started at 7.27 per field in 1985 to no treatments in 1988. Even in 1986 and 1987, the fields requiring treatments were usually those which were closest to the Imperial Valley. Treatments also began after moths were collected in traps placed in the 30 miles of desert between the Imperial Valley and the Coachella Valley. The pink bollworm moth becomes more mobile as the season progresses, with dispersal flights occurring as cotton is terminated.

Table II. Control strategies and conventional insecticide use patterns for premanagement and area-wide management systems in the Coachella Valley.

Treatment system	Fields treated with pheromone	Mean No. of conventional insecticides	No. of fields treated with insecticides	Cumulative conventional insecticide treatments
1985 insecticide only (Pre-trial data)		7.27	56/57	414
1986 pheromone and insecticides	31/31	1.8	17/31	56
1987 sterile insects, pheromone and insecticides	17/21	1.03	7/27	28
1988 sterile insects and pheromone	4/31	0	0/31	0
1989 sterile insects, pheromone and insecticides	3/23	1.9	18/23	44

In 1989, the adjacent Imperial Valley terminated cotton growth a full month earlier than the Coachella Valley. Within a few days of the start of defoliation in the Imperial Valley, massive moth flights were found in the desert between the two valleys. Migration appeared to be a key factor in 1989 as opposed to 1988. A critical key to just how low this population was and an idea of the structure of the population is found in boll infestation data in Table III.

Table III. Summary of boll data from the Coachella Valley pink bollworm management study, showing total larvae per sample and number of bolls sampled.

	1986		1987		1988		1989	
Sample week	No. of bolls sampled	Total larvae	No. of bolls sampled	Total larvae	No. of bolls sampled	Total larvae	No. of bolls sampled	Total larvae
1	50	3			370	0		
2	500	3	500	0	726	0	560	0
3	960	2	1700	0	1200	0	720	0
4	1180	0.9	2400	7	1600	0	960	0
5	1520	1	2400	33	2000	0	1520	0
6	1520	3	2500	34	2160	0	1600	1
7	1600	1	2700	1.1	2320	0	1760	0
8	1520	1	2700	6	2480	5	1564	18
9	1600	1	2700	5	2480	5	1860	39
10	1760	43	1350	25	2480	2	1960	39
11	2200	138	1350	41	2480	0	1240	47

In all fields, population levels were the lowest of any area in the Colorado River Basin. Until late 1989, the populations appeared to be on a constant downward cycle. This observation has proven to be particularly important and has again been verified in Parker, AZ. The most important aspects of the Coachella trial are that it dealt with multiple fields showing a significant ability of this pheromone system to affect a large area. The trial represented the beginning of a new approach (i.e., the integration of sterile insect and pheromone systems for area-wide management). In 1994 this approach will be pushed into a much larger project in the Imperial Valley. It will now be interesting to balance and refine the two technologies based on costs. Pheromone technology is now in an area-wide trial in Parker, Arizona, as the key ingredient in a management system. Knowledge of its use has been advanced significantly in this area of Arizona from 1990 through 1993.

In late 1989, key Arizona Cotton Research and Protection Council personnel began to consider pink bollworm. They were completing a very successful bollweevil eradication program and were looking for a new challenge. In preliminary planning meetings, a strategy for management with pheromones on an area wide basis was developed using what we learned from our previous projects. With limited resources, this grower organization elected to set up a match funded program in which the cotton grower organization statewide would initially provide one half the cost of a program management and treatment material costs. As the project has succeeded, the local grower group has taken an increasing share of the costs. By 1994, all but supervisory costs will be paid by the local grower community. The USDA provided technical assistance and minimum vehicle assistance. The Parker area in the north central area of the Desert Colorado River cotton growing area of Arizona was chosen because it was of significant size at 27,000 acres. Pink bollworm was a major problem and pesticide usage was considerable and extensive.

The objectives of the Parker Project were relatively simple in 1990 and have become more stringent with each year. They were: a) to maintain pink bollworm at low enough levels to not require grower treatments in more than 10 percent of the fields before August 15, 1990; b) to maintain populations at levels below this threshold in 1992 until September; c) to completely control all populations throughout the growing season without the need for grower action in 1993. The treatment strategy used by the program was not exclusively pheromonal, but one in which all treatments used pheromones. When critical, a dual application of pheromones and insecticide could be used. This was, however, the "last" choice and, after 1990, not scheduled. The entire management scheme is and was cumbersome and will not be reported here in detail. Here, the basic treatments used and the total acreages treated with these systems are shown in table IV.

Table IV. The Parker Valley Pink bollworm Program Treatment strategies and Acres Treated from 1991 through 1993.

	Acres Treated			
Treatment System	1990	1991	1992	1993
PBW Rope (High Rate System)	11,826	12,524	9,545	6,808
PBW Fiber "Single"	30,478	31,192	61,538	40,974
PBW Fiber "Dual" (concurrent with insecticide overspray)	32,965	27,388	24,978	2,690
Straight Insecticide	0	841	6,066	0

Although it is beyond the scope of this paper to give all treatment rationales, the PB Rope was applied generally in the earliest cotton so long as the stand and plant height at 6 leaf sluge was uniform enough for good Rope distribution. It was also applied selectively to fields with highest spring trap capture. During the initial years of the program, dual application (concurrent pheromone treatments with an insecticide overspray) was used for the first treatment and at times of hazard or perceived risk. Straight insecticides were used very sparingly when, for example, a Rope treatment was thought to be in jeopardy. Results are best expressed as boll infestation data in larvae per 100 bolls. Table V gives pertinent boll infestation data for 45 evaluation fields as collected by program personnel.

Table V. Summary of Boll data from the Parker Valley Pink bollworm Management study showing total larvae per sample and number of bolls sampled 1989 through 1993.

WEEK OF	1989		1990		1991		1992	
	BOLLS SAMPLE D	LARVAE/ 100	BOLLS SAMPLE D	LARVAE/ 100	BOLLS SAMPLE D	LARVAE/ 100	BOLLS SAMPLE D	LARVAE/ 100
7/09	--	--	2,910	.3	--	--	2,147	.19
7/16	--	--	2,885	.6	3,332	.06	3,161	.09
7/23	2,680	3.6	3,123	1.4	3,378	.03	3,259	.95
7/30	2,758	7.7	3.037	2.7	3,320	.09	3,440	.61
8/06	3,107	17.9	3,167	1.5	3,591	.03	3,287	0
8/13	3.080	25.9	3,074	5.9	3,614	.03	3,461	0
8/20	3,032	30.6	3,096	14.8	3,782	.03	3,063	2.45
8/27	3,033	36.4	3,537	20.6	3,661	1.6	2,519	1.19
9/03	3,067	34,5	2,932	10.9	3,499	1.9	2,930	1.60
9/10	3,095	21.6	3,338	10.4	3,725	3.7	2,797	1.78
9/17	3,027	28.4	3,627	33.3	3,585	6.6	--	--

The most critical feature of this data is that before

The most critical feature of this data is that before pheromone treatment started in an organized program, populations were high (virtually no pheromone activity existed in 1989 on the part of the growers). In 1984, before our program, these populations were not held below an economic threshold of 5, 10, or even 15% in spite of significant insecticide treatments. Monitored fields averaged over 8 treatments during the season. In 1990, only 2 major areas of the low desert Arizona and California southwest did not have catastrophic populations of pink bollworm. Those were Parker, under area-wide pheromone uasge and the Imperial Valley, with very heavy pheromone usage following its first "extremely" short season in 1989. Populations have declined each subsequent year to the point that no detectable larval populations were found in the Parker area in 1992. No comparisons have been made with other areas of Arizona in 1991, but in 1992, program personnel made comparable surveys in the central part of Arizona while there were still larval populations available in Parker. This data is in Table VI. In a courtroom, this data would, of course, be considered circumstantial but the differences are obvious.

Table VI. Summary of Boll data from central Arizona compared to the data from the Parker Program.

	PARKER			CENTRAL ARIZONA		
DATE	BOLLS	LARVAE	%	BOLLS	LARVAE	%
7/09	2147	4	0.19	3300	37	1.12
7/16	3161	3	0.09	3400	27	0.79
7/23	3259	31	0.95	2600	59	2.27
7/30	3440	21	0.61	2700	209	2.27
8/06	3287	0	0.00	3300	321	9.73
8/13	3461	0	0.00	3400	337	9.91
8/30	3063	75	2.45	2700	248	9.19
8/27	2519	30	1.19	2800	401	14.32
9/03	2930	47	1.60	--	--	--
9/10	2797	50	1.78	--	--	--

Use of pheromones represents a major advance in Integrated Pest Management for cotton insect control programs. This is not an uncontroversial statement. Use of these systems have been managerially difficult. Experimental verification is difficult and very costly. It has long been hypothesized by workers in the field that pheromone disruption would work best on an area-wide basis because movement or the expected movement of mated females from conventionally-treated fields can and will obscure results. This is a paradigm that is particularly true if you have a limited budget and must work within a small plot format. The best information will then be obtained from behavioral tests and extrapolation. This means the assumption of risk in taking the next and subsequent steps. Judgment will be critical. All judgment will be questioned by a researcher's peers.

The next major issue for those contemplating development of pheromone type systems is that control of most organisms follows established commercial paths with vested interests. A pheromone system has major potential for changing established economic systems. Workers will again be confronted with questions and controversy.

The last difficult issue is that of cost. To date, profit and even gross sales in pheromones have not been large compared to a chemical pesticide. It is highly likely that a non-profit management system is an ideal developer and distributor of these types of systems. Cost of registration for insect pheromones has been notably low compared to the massive costs of a conventional pesticide. Unpublished estimates (C. Doane, personal communications) of $100,000 for a new registration of a new system are common. Developmental costs and capital expenditure ratios with market depth are much more critical issues within these systems. This is particularly true if these systems are capable of eradication, as evidenced by constantly declining and reduced populations. This use does not fit the classical tenets of Integrated Pest Management economic threshold on season by season losses. New tenets are required.

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V. FISH CHEMORECEPTION: THEORY AND APPLICATION

V.1. Introduction to Chemoreception in Fish (Toshiaki Hara).

Background--I am currently a Research Scientist at the Freshwater Institute, Fisheries and Oceans Canada, in Winnipeg and an Adjunct Professor of the Faculty of Graduate Studies (Zoology), University of Manitoba. I have been in this position since 1969. My research interest is in all aspects of fish chemoreception, olfaction and gustation in particular, and their environmental interactions. Recently I edited a book on Fish Chemoreception (Chapman and Hall) and contributed a chapter on Chemoreception for The Physiology of Fishes (D.H. Evans, Ed., CRC Press).

Submitted manuscript--The chemical senses are the most ancient of sensory systems, having evolved 500 million years ago. They mediate the functions most basic to the survival of the individuals and species: feeding and reproduction. In vertebrates the olfactory and gustatory systems comprise the major sensory organs for detection and identification of chemical stimuli in the environment. The common chemical sense and solitary chemosensory cells, though less understood, may provide additional chemoreceptor functions. Although olfactory and gustatory systems are distinct morphologically, functionally, and ontogenetically, the aquatic environment surrounding fish makes their chemical senses unique and consequently the distinction between the two sensory modalities is not always as clear as in terrestrial organisms.

Ultrastructural organization of the sensory epithelium is extremely consistent throughout fish species, despite considerable variations in structural arrangement, size and degree of development of the peripheral olfactory organ. The olfactory neuron reaches directly to the brain to sense the outside world. Four major classes of chemicals have been identified as specific olfactory stimuli, and their stimulatory effectiveness has been characterized in over 30 fish species: amino acids, sex steroids, bile acids/salts, and prostaglandins. Anatomical and physiological evidence suggest that olfactory signals such as those involved in reproduction (pheromones) and those used in feeding may be processed independently through two distinct subsystems, the lateral and medial olfactory systems.

The taste buds constitute the structural basis of the gustatory organ and chemical information detected by specialized epithelial cells, gustatory cells, is transmitted to the central nervous system by cranial nerves VII (facial), VIII (glossopharyngeal), and X (vagal). Their precise peripheral innervation is not clearly understood. Taste buds may occur not only in the oropharyngeal cavity, but on the whole body surface. Besides diverse sensitivities and specificities for amino acids, fish gustatory receptors detect various organic acids, nucleotides, bile salts, CO_2, and in some cases marine toxins.

Because high sensitivity of the fish gustatory system to certain chemicals seems to be widespread, it is no longer appropriate to consider olfaction to be the sensory modality for chemical detection only on the basis of its high sensitivity.

V.2. Responses of Teleost Fishes to Alarm Pheromones and to Chemical Stimuli from Predators (R.J.F. Smith).

Background--I began my research career with an M.Sc. on stickleback behavioral endocrinology with W.S. Hoar in 1964, followed by a Ph.D. on sunfish behavioral endocrinology with M.H.A. Keenleyside in 1967. I have been working with fish alarm pheromones since the early 1970's when I noticed that breeding male fathead minnows lost their alarm pheromone cells when their androgen levels were high. Recently my co-workers and I have been attempting to measure the costs and benefits of alarm signalling to senders and receivers.

Ideas-- Alarm pheromones have not been reported in lampreys but this does not mean they are absent. Careful examination of the possibility of lamprey alarm pheromones is probably worthwhile. Similarly, responses to predator stimuli should be carefully examined. Pfeiffer and Pletcher (1964) suggested that some predators are deterred by distasteful skin secretions from lamprey but that others, such as sturgeon and eels were not deterred. The search for predator stimuli should concentrate on the predator species that are most likely to feed on lampreys. Alarm pheromones and predator odors may be useful in redirecting fish from one course to another or steering them away from spawning areas and toward a trap or lure area. It is unlikely that alarm pheromones or predator stimuli will completely overcome the migratory or spawning motivation of the lampreys.

Pfeiffer, W. and T. F. Pletcher (1964). Club cells and granular cells in the skin of lamprey. J. Fish,. Res. Bd. Canada 21: 1083-1088.

Submitted manuscript--Karl von Frisch first reported fish alarm substance (Schreckstoff) in 1938. In the subsequent 55 years it has been found that this alarm-substance/fright-reaction system is typical of the Superorder Ostariophysi (Pfeiffer 1977, Smith 1992) a group that includes about 70% of the species of freshwater fishes. Minnows, suckers, catfish, characins, and loaches all belong to this superorder and typically have alarm substance. The alarm substance is produced and stored in fragile epidermal club cells (outside the dermal scales) and is only released by injury to the skin. When receivers, conspecifics or other ostariophysans, smell the alarm substance they perform typical antipredator behaviour for their species. This reduces their risk of immediate capture (Mathis and Smith 1993b). The evolutionary forces favoring production of the alarm substance by senders, although interesting, are beyond the scope of this document.

As well as the immediate antipredator response, receivers also learn about predators from the association of alarm substance with other stimuli. For example, European minnows, Phoxinus phoxinus, and fathead minnows, Pimephales promelas, learn the odours of predators if the odours are paired with alarm substance (Magurran 1989, Chivers & Smith In Press b) and the visual appearance of the predators present when alarm substance is detected (Chivers & Smith In Press a). Cyprinid fishes can transmit the recognition of predator odours to schoolmates (Chivers & Smith In Prep). Thus, detecting alarm substance may have long term consequences, improving the ability of the prey to recognize and avoid predators.

Even after its consumption, a minnow may continue to warn conspecifics (Mathis & Smith 1993a, 1993c). The alarm substance from consumed minnows can apparently leak out of pike, Esox lucius, and warn naive minnows to avoid pike and teach them to avoid pike odour in the future.

The active component of ostariophysan alarm substance may be Hypoxanthine-3(N)-oxide (Pfeiffer 1982) but there is some unpublished evidence suggesting that this compound is not as effective as natural alarm substance preparations. The response of receivers to alarm substance is apparently innate (Pfeiffer 1977) but fish can become habituated to its continuous presence and stop responding (Krause 1993). They can also modulate their response to alarm substance when predator avoidance conflicts with feeding or mating.

Injury-released alarm pheromones have been reported in several groups of non-ostariophysan fishes (Smith 1992). These appear to be analogous to the ostariophysan system rather than homologous. None have been studied in as much detail as the ostariophysan system. I am not aware of any published reports of alarm pheromones in lamprey. They lack the epidermal club cells associated with the ostariophysan alarm substance, although several of the teleost fishes that have analogous alarm pheromones lack these as well.

Chemical Stimuli from Predators--Weldon (1990) recently reviewed the responses of fishes to chemical stimuli from predators. A number of fish can recognize and avoid predators on the basis of chemicals, probably through olfaction (Chivers & Smith 1993), or the single cell chemosensory system (Kotrschal 1991). In some cases predator recognition is learned during the life of an individual (Magurran 1989, Chivers & Smith In Press a,b) but there is evidence for "constraints on learning" that facilitate learning the characteristics of natural predators. Pacific salmon halt their migration and move downstream in response to water that has contacted bear paws and human hands and in response to L-serine, a component in water rinses of mammalian skin (Idler et al. 1956, Rehnberg and Schreck 1987). As with alarm pheromones, fish can become habituated to predator stimuli and other motivations may override predator avoidance, as occurs when salmon migrate past bears that are actively fishing.

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V.3. Identity, Function and Application of Feeding Attractants (John Caprio).

Background--JOHN CAPRIO, Ph.D. (1976) Florida State University (Lloyd M. Beidler, Major Professor); Applewhite Professor, Louisiana State University; Adjunct Professor, Pennington Biomedical Research Center; past (1992-93) Executive Chairperson (president) of the Association for Chemoreception Sciences; Chairman for the 1994 Gordon Conference on the Chemical Senses. Two U.S. patents issued since 1992 and one pending on fish feed enhancers and fish baits. Fifty-six research publications since 1975 dealing with olfaction and taste in fish; over 50 abstracts presented at national/international scientific society meetings.

Submitted manuscript--Fish, like other vertebrates, possess the senses of olfaction and taste. Olfactory receptors are primary neurons whose ganglia are at the periphery and whose axons constitute the first cranial (olfactory) nerve that projects directly to the olfactory bulb within the central nervous system. Depending upon the species of fish, taste receptors may be located primarily on the lips and within the oral cavity or be spread across the body surface (e.g. catfish). Taste cells within the caudal oral cavity are innervated by the glossopharyngeal (IX) and vagal (X) cranial nerves, whereas taste cells within the rostral oral cavity, lips and external portions of the body are innervated by the facial (VII) cranial nerve.

The aqueous environment selects for physico-chemical properties of molecules that are quite different than those for olfactory stimuli in air breathers. Water soluble substances, such as amino acids, small peptides, nucleotides, bile salts, quaternary ammonium bases and certain steroidal compounds, can stimulate both olfactory and taste receptors of fishes and have been indicated as possibly important chemosensory stimuli for these animals. The vast majority of studies related to chemicals that release feeding activity have been of amino acids, with nucleotides a distant second. Electrophysiological studies over the past 20 years clearly indicated the high sensitivity of olfactory and gustatory receptors of fishes to amino acids. Electrophysiological thresholds to amino acids varied depending upon the species and chemosensory system tested, but for the more sensitive species thresholds were generally in the range of 10^{-7} to 10^{-9}M for both chemosensory systems. Although there is a similarity in the general sensitivity of fish taste and smell systems to amino acids, the specificity of the systems to particular amino acids can be quite different. In general (although exceptions do exist), the olfactory spectrum of sensitivity across a number of species of fish tested were highly similar; however, this did not appear to be the case for gustation. Although both olfactory and gustatory systems are used simultaneously by fish in their chemosensory behaviors, the amino acids that best stimulate the taste receptors of a particular species are highly correlated with the release of feeding behavior in that species.

From only a few studies on the chemical specificity of individual facial taste fibers of teleosts, it appears that most amino acid taste information is transmitted to the brain by way of only a few different "major types" of taste fibers, classified according to the similarity in their overall responses to the test stimuli. These studies indicated that the specificity of the amino acid taste fiber types can be quite different across different families of fishes. Recent studies in ictalurid catfish indicated that a mixture of the most effective amino acid for each major fiber type released the entire sequence of feeding activity from appetitive to consummatory patterns of feeding behavior. When presented to naive channel catfish, this combination of amino acids resulted in enhanced feeding activity, whereas combinations of other naturally occurring amino acids had little effect. It is important, however, to note that catfish are highly dependent upon their chemosensory systems for feeding, whereas other fishes (e.g. salmonids, bass) are more visually oriented in what releases appetitive feeding behavior. The limited data suggest that a specific combination of certain compounds that occur naturally in the aqueous environment can be somewhat selective in its action to promote feeding.

The following are suggested readings. All appear in: Fish Chemoreception. Fish and Fisheries Series 6, T.J. Hara, Ed., Chapman and Hall, London, 1992.

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Jones, K.A. Food search behaviour in fish and the use of chemical lures in commercial and sports fishing, pp. 288-320.

V.4. Identity, Function, and Application of Migratory Cues

(Toshiaki J. Hara).

Background--(Already given).

Abstract--The best known migratory cues are probably the ones involved in homing of salmonids. Salmon homing is well documented, and a number of studies have demonstrated the importance of chemical information in home-stream discrimination.

Despite considerable research however, many aspects of homing migration are still poorly understood and remain controversial. The imprinting hypothesis, i.e. learning of environmental chemical cues during a sensitive period of early development, and the pheromone hypothesis, i.e. innate responses to pheromones specific for local fish populations, are the two prevailing hypotheses.

Hasler and his students, in a series of census, tracking, and electrophysiological experiments, beautifully demonstrated that salmonids artificially imprinted to morpholine or phenethyl alcohol can be decoyed into streams scented with those chemicals. However, in my opinion, one fundamental question remains unanswered: do salmonids really smell morpholine and phenethyl alcohol?

The pheromones are suggested to be released from the skin mucus or produced in the liver and released into the environment via the bile and the feces. Laboratory experiments demonstrated that the bile and bile acids/salts are potent olfactory and gustatory stimulants for salmonids and they respond differentially to mucus emanating from different populations of the same species. However, field studies have failed to demonstrate that homing is based on the presence of members of the populations on or near the spawning grounds.

No new information on migratory cues in homing salmon is available since the publication of Hasler and Scholtz's book: 'Olfactory Imprinting and Homing in Salmon' (1983), and the following key questions still remain: 1) does chemosensitivity to some chemical stimuli such as sex pheromones change at certain life history stages? 2) does chemosensory capability change under specific stimulus conditions? and 3) does the sensitivity change daily and seasonally?

V.5. Identity, Function, and Application of Sex Pheromones (Norm Stacey).

Background--My long-term research interest has been hormone-sex behavior interaction in fish, with almost exclusive attention to goldfish. Early work in this area showed that Prostaglandin $P_{2\alpha}$ is a female hormone that turns on female spawning behavior during the few hours that ovulated eggs are in the reproductive tract. Current studies in this area involve examining steroid effects on sexually dimorphic behaviors and pheromone responses in goldfish and other fish.

Entry into the pheromone field was the serendipitous effect of two factors, a poorly designed experiment that inadvertently demonstrated the extraordinary olfactory potency of waterborne 17,20βP, and Peter Sorensen's arrival to begin his postdoctoral work. Developments in the seven years since then have given us a completely new perspective on behavior-endocrine interactions in fish reproduction.

<u>Ideas</u>: (Also see discussion in manuscript below)--The apparently widespread use of released hormones and hormonal metabolites as sex pheromones in freshwater gnathostome fish and evidence that female lamprey are attracted to waterborne testosterone raise the possibility that hormonal pheromone systems have evolved in the sea lamprey. Although a recent EOG recording study by Li and Sorensen failed to find any reproductive hormones or hormone metabolites (including testosterone) that were potent lamprey stimulants, there are several reasons for not rejecting the possibility of lamprey hormonal pheromones:

i) olfactory responses to hormonal pheromones are highly specific, increasing the possibility of false negatives when studying a species in which the nature of released hormones and metabolites is poorly understood.

ii) in Atlantic salmon, males that initially showed no response to high (μM) concentrations of 17,20βP-sulphate became extremely sensitive (pM EOG detection threshold) within minutes of exposure to female urine. These results suggest a further search for lamprey hormonal pheromones is warranted.

<u>Submitted manuscript</u>--It is reasonable to expect that the reproductive hormones and hormonal metabolites of all aquatic organisms are released to the water in quantities reflecting their rate of synthesis, and that they therefore contain information about an individual's changing reproductive status. Because such information can be of great potential usefulness to conspecifics, it has been predicted (Kittredge and Takahashi, 1972; Colombo et al., 1982) that evolution of adaptive behavioral and physiological responses to water-borne hormones and metabolites has been widespread among aquatic organisms. And where such responses can benefit the individual releasing the hormone(s), and thus act as a selective force for increased signalling efficiency, it would also be expected that the interaction would progress from chemical spying to true chemical communication (Stacey and Sorensen, 1991). In support of these theoretical predictions, studies in teleost fish over the past decade have clearly demonstrated hormonal pheromones in several species (Kitamura et al., 1993a; Sorensen and Stacey, 1990; Stacey and Sorensen, 1991; Van Den Hurk and Resink, 1992), and provided strong evidence that similar pheromonal systems are present in many others (Kitamura et al., 1993b; Sorensen, 1992; Stacey et al. 1994).

The hormonal pheromones of goldfish (<u>Carassius auratus</u>) are better understood than those of other fish, and thus serve as a useful model for illustrating both the nature and potential complexity of the phenomenon. Studies that have focused on male response to female odors indicate that the periovulatory female sequentially releases two distinct pheromones, a preovulatory steroidal pheromone, and a postovulatory pheromone consisting of F2-series prostaglandins (PGFs). Each of these pheromones induces distinct releaser (rapid behavioral) and primer (slower physiological) effects in the male.

Although the preovulatory pheromone appears to consist of a mixture of stimulatory C21 steroids and inhibitory C19 steroids (Sorensen et al., 1992), virtually all studies have focused on the function of the presumed major C21 component, the oocyte maturation-inducing steroid 17a,20β-dihydroxy-4-pregen-3-one (17,20β-P). Within 6 h of exposure to 17,20β-P, male goldfish increase milt (sperm and seminal fluid) quantity and quality (increased sperm swimming time) through a neuroendocrine reflex thought to involve decreased dopaminergic inhibition of pituitary gonadotropin (GtH) release, increased blood GtH levels, and GtH-induced increase in testicular 17,20β-P levels (Defraipont and Sorensen, 1993; Dulka et al., 1987, 1992). In addition to these primer effects, 17,20β-P induces immediate increases in social behaviors, and a later increase in competitive spawning motivation (DeFraipont and Sorensen, 1993; Sorensen et al., 1989). Although the combination of these behavioral and endocrine effects is believed to increase male fertility during the intense inter-male competition for access to spawning females, this has not been demonstrated experimentally.

Electro-olfactogram (EOG) recording from the olfactory sensory epithelium shows that although 17,20β-P is the most potent steroid for goldfish (1 pM EOG detection threshold), a variety of free and conjugated C21 steroids (all capable of stimulating GtH and milt increase), and some C19 steroids, are detected (Sorensen et al. 1990, 1992). Cross-adaptation studies indicate all steroids act on one of three receptor classes, which appear to be tuned to 17,20β-P, 17,20β-P-sulphate (17,20β-P-S), or androstendione (AD) (Sorensen et al., 1990), all known to be released by ovulatory females (Sorensen and Scott, unpub.). The biological significance of these three steroid receptor mechanisms is far from clear. However, because water-borne AD inhibits the effect of 17,20β-P on GtH and milt, it is possible that the male responds not only to an increase in 17,20β-P release by the female, but also is sensitive to the C21:C19 ratio. In addition, recent work on rainbow trout (*Oncorhynchus mykiss*; Scott, 1994) showing that 17,20β-P-S is excreted in urine, whereas free 17,20β-P is released by another route (likely the gills), suggests the female goldfish preovulatory steroid also would be compartmentalized, a reasonable prediction being urinary release of conjugated (glucuronidated and sulphated) 17,20β-P, and non-urinary release of free 17,20β-P and AD.

At ovulation, release of the preovulatory pheromone is abruptly terminated, and release of the postovulatory PGF pheromone commences. Although not well understood, the PGF pheromone appears to be synthesized when movement of ovulated oocytes into the oviduct triggers synthesis of prostaglandin F2a, which then enters the circulation and acts centrally to trigger female spawning behavior (Stacey, 1987). Ovulated and PGF-injected females are actively courted by males, apparently because they release metabolites of PGF, the most potent thought to be 15-keto-PGF2a (Sorensen and Goetz, 1993). In addition to this direct releaser effect on sex behavior, the postovulatory PGF pheromone also indirectly induces physiological effects (increase in milt volume and GtH level) by stimulating socio-sexual interactions (Sorensen et al., 1989). These primer effects of the PGF pheromone differ from similar responses to the steroid pheromone in that the milt response to the PGF pheromone is more rapid and independent of GtH increase, while the GtH increase appears not to involve dopamine withdrawal (Stacey et al., 1994).

Studies of hormonal pheromones in other teleosts have demonstrated male behavioral responses to PG released by ovulatory females (Misgurnus anguillicaudatus; Kitamura et al., 1993a), female behavioral responses to C21 steroids released by males (Clarias gariepinus; Van Den Hurk and Resink, 1992), and ovulatory and behavioral responses to presumed steroidal pheromones in Brachydanio rerio (Van Den Hurk and Resink, 1992). Although less than 50 species have been examined, and most information is from EOG recordings rather than studies of in vivo biological response, several generalities are emerging:

i) PG pheromones appear to be more widespread than other hormonal pheromones, likely because of the widespread use of PGF as a behavioral sex hormone in female fish (Stacey, 1987),

ii) active components of hormonal pheromones may be the native hormones or their metabolites,

iii) hormonal pheromones likely contain a mixture of active compounds, despite the fact that responses can be induced by single components (Sorensen et al., 1992),

iv) three basic chemical types of hormonal pheromone appear to have evolved (PG, C21, and C19), although a cyprinid responsive to C18 steroids has recently been found (Stacey, unpub.): however, a multiplicity of functional types within each class is to be expected, judging from the very different responses elicited by C21 steroids in male goldfish (Sorensen et al., 1989) and female Clarias (Van Den Hurk and Resink, 1992).

The feasibility of using hormonal pheromones as a tactic in the strategies to control sea lamprey (Petromyzon marinus) in the Great Lakes is contingent on at least four major considerations:

i) whether sea lampreys have evolved pheromonal responses to sex hormones,

ii) whether the nature of the evolved pheromonal response(s) is such that manipulations are biologically feasible under natural conditions,

iii) whether any biologically feasible manipulation is more cost-effective than alternative tactics, and

iv) whether there are negative environmental concerns about hormonal pheromone use, either because of the chemical nature of the pheromone, or because of effects it may have on species other than lamprey.

Hormonal pheromones in sea lamprey: At present, it is unknown whether sea lamprey have evolved pheromonal responses to released sex hormones. Adult sea lamprey are attracted to the odor of juveniles (Teeter, 1980), a response which may be induced by juvenile bile salts to which the olfactory receptors of mature adults are extremely sensitive (see Li et al., this workshop). Although mature males are attracted to the odor of ovulated eggs, and mature females are attracted to urogenital fluid from mature males (Teeter, 1980), there is no evidence these are responses to hormonal pheromones. Adult sea lampreys have been reported to respond to pM concentrations of testosterone (Adams et al., 1987); however, EOG studies (Li et al., this workshop) indicate that the olfactory system of sea lamprey is not sensitive to testosterone and related C19 steroids. Indeed, of a wide variety of steroids tested by Li and co-workers, sulphated C21 steroids were the most stimulatory (100 nM EOG threshold). Although this potency is lower than would be expected for a sex pheromone, the observed responses raise the distinct possibility that lamprey have evolved pheromonal response to a sulphated C21 steroid, the identity of which is yet unknown. Because olfactory receptors to hormonal pheromones can be remarkably specific (Sorensen et al., 1990), and because steroidogenesis and steroid excretion in sea lamprey have yet to be characterized, it would not be surprising if the purported sulphated C21 pheromone were a novel compound.

Biological feasibility of manipulating responses to hormonal pheromones: Based on what is known of teleost fishes, sea lamprey responses to hormonal pheromones might be of two basic types: tonic, in which continuous release by one individual attracts sexual partners (Clarias gariepinus, Van Den Hurk and Resink, 1992; goldfish, Sorensen et al., 1989); reflexive, in which exposure triggers either endocrine changes leading to milt production (goldfish, Dulka et al., 1987) or ovulation (zebrafish, Van den Hurk and Resink, 1992; goldfish, Sorensen and Stacey, 1987), or a behavioral response such as rheotactic upstream movement, apparently independent of continued exposure (Atlantic salmon, Moore, 1991). A reflexive pheromone system should be far easier to manipulate, because a bolus addition is technically simpler than a dispensing system, and because a reflexive system would be less likely to involve concentration gradients.

Economic feasibility: If sea lamprey do possess hormonal pheromones, the economic feasibility of their use in lamprey management will be determined by at least three major factors: the nature of the pheromonal response (tonic vs reflexive), detection threshold, and chemical cost. Even if a potential pheromone were highly potent (pM detection threshold), the costs of most synthetic steroids or prostaglandins combined with the geographic scale of the lamprey management problem suggest manipulating a tonic hormonal pheromone would not be economically feasible. However, a hormonal pheromone of the reflexive type would be far less expensive to administer than the tonic type, and also less likely to raise environmental concerns, because smaller amounts would be required and the potential for interfering with other species would be reduced.

Environmental concerns: Because application of hormonal pheromones to Great Lakes sea lamprey management would involve an existing natural product at concentrations not greatly in excess of those normally released, it is expected the major environmental concerns would be the potential for such application to interfere with reproductive processes of other agnathan or teleost species. Although species-specificity is often considered a feature of sex pheromones, such specificity in fact should be expected only between species which have had significant opportunity for interbreeding in their recent evolutionary history (Stacey and Sorensen, 1991). There obviously are great differences in the hormonal pheromones to which distantly related teleost species are most sensitive (Stacey et al., 1994). However, the few studies which have compared olfactory specificities of related, sympatric species find no evidence of species-specificity (Bjerselius and Olsen, 1993; Cardwell et al., 1992; Irvine and Sorensen, 1993), and common carp (Cyprinus carpio) exposed to water-borne 17,20β-P exhibit the same increases in GtH and milt seen in goldfish (Stacey et al., 1994). From this limited information, it would not be unreasonable to expect that a hormonal pheromone of sea lamprey could be detected by, and potentially interfere with the reproduction of, other lamprey species.

Although there is yet no direct evidence that sea lamprey have evolved pheromonal responses to released sex hormones, the widespread use of hormonal pheromones in freshwater teleost fishes (Stacey et al., 1984), the evidence for sex pheromones in lamprey (Teeter, 1980; Adams et al., 1987), the clear evidence of a pheromonal system involving juvenile bile steroids (Li et al., this workshop), and demonstrated EOG responsiveness to sulphated C21 steroids (Li et al., this workshop), all suggest that continued attempts to identify lamprey hormonal pheromones is clearly warranted. Although such research could take many approaches, the fact that extensive EOG screening has failed to identify a potent olfactory stimulant suggests that a better understanding of lamprey steroids and prostaglandins is required. Fruitful approaches could involve HPLC purification and bioassay of known pheromone sources (urogenital fluids and egg washes; Teeter, 1980), as has been done with Pacific herring, (Clupea harengus pallasi, Carolsfeld et al., 1992), or identification of the released metabolites of injected radiolabelled hormones, as is now being attempted in goldfish and other teleosts (Sorensen and Goetz, 1993).

Assessing the feasibility of pheromonal techniques to control Great Lakes sea lamprey also will require more basic information about physiological and behavioral aspects of lamprey reproductive biology to determine if this species is reproductively vulnerable to manipulation by chemical cues. For example, if ovulation is triggered pheromonally, and if there is a short period of postovulatory oocyte viability, there may be opportunity to reduce female fertility by using pheromones to trigger premature ovulation. As well, evidence that females are attracted to a male pheromone (Teeter, 1980) suggests that, if this pheromone could be identified and implanted in sustained release form in males being used for the sterile male program, there is potential to increase effectiveness of current sterile male techniques.

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VI. LAMPREY CHEMORECEPTION

VI.1. Initial Approaches to the Development of Odorant-Based Control Techniques for Landlocked Sea Lampreys (John. H. Teeter).

Background--J. Teeter holds a B.A. in biology from Albion College, an M.S. in zoology/marine biology from the University of Hawaii, a Ph.D. in physiology from the University of Pennsylvania, and a Postdoctoral Fellowship in cellular neuroscience from the Albert Einstein College of Medicine. During the period from 1977-1985, I conducted a combined behavioral/analytical chemistry study, in collaboration with the U.S. Fish & Wildlife Service and the Great Lakes Fishery Commission, designed to determine if pheromones are involved in sea lamprey migration and reproduction and, if so, their source and identity. Also during this period, several studies of chemosensory orientation and acuity in catfish and sharks were completed. In addition, from 1976 until the present, I have continued experiments to define the mechanisms of signal transduction in vertebrate olfactory and taste receptors. Our current work involves characterization of the cellular and molecular properties of two unique classes of ligand-gated ion channels; at least two amino acid taste receptors from catfish and channels in the membranes of vertebrate olfactory receptor cells which are activated by the stimulus-generated second messenger, inositol 1,4,5-trisphosphate.

Submitted manuscript--The potential usefulness of chemical attractants and/or repellents for landlocked sea lampreys in an integrated program of population management has been recognized for many years. A growing appreciation, in the early 1970's, of the importance of chemical signals in regulating a variety of behaviors in fishes [see, for example, 1-3], as well as anecdotal reports of behavioral interaction between migrating sea lampreys apparently mediated by chemical cues [4,5], led to speculation that intraspecific chemical signals (pheromones) might play significant roles during one or more life stages of sea lampreys. A cooperative program with the U.S. Fish & Wildlife Service and the Great Lakes Fishery Commission was initiated to identify potentially useful attractants and repellents for sea lampreys. The unique advantages of pheromones as highly selective and relatively nontoxic attractants led us to concentrate on the possibility that pheromones are involved in sea lamprey migration and reproduction. The results of our initial behavioral experiments indicated that sexually mature male and female sea lampreys release substances that elicit preference responses in the opposite sex [6]. Males avoided water in which other males had been held, while females appeared indifferent to rinses of other females. In addition, adults captured early in the spawning season, prior to the appearance of secondary sex characteristics, showed a marked, but transient, preference for water in which sea lamprey larvae had been held.

These observations were confirmed and expanded during the next several spawning seasons. The substance(s) released by males, which attracted females and was avoided by other males, was present in the urogenital fluid. Urine from males displaying secondary sex characteristics, both unspermiated and spermiated, evoked preference responses in females at concentrations as low as 6.4 μL/L. Milt elicited no observable response in females. Rendering the females anosmic by plugging the naris with latex resulted in the loss of the preference for male urine, indicating that the response was mediated by the olfactory system. The substance released by sexually mature females which elicited a preference response in males was present in urine and was also associated with ovarian fluid. However, ovulation did not appear to be a prerequisite for release of the attractant. These results indicate that both male and female sea lampreys, after reaching a critical stage of sexual maturation, release substances that signal their sex and reproductive state and probably act as attractants for the opposite sex.

Although significant progress has been made in recent years in characterizing pheromone communication in fishes [7,8], the precise functions of the apparent chemical signals in sea lampreys remain speculative. The male pheromone could function in attracting females to the nest, in pair-formation and in release of spawning behavior, as well as in dispersing males over the available nesting habitat. A "priming" role in maturation of females or ovulation is also possible. The attractant released by mature females could also serve a role in pair-formation and release of spawning behavior. The transient response of migrating adults to presumed metabolites from larval sea lampreys residing upstream could aid the adults in selecting a suitable river in which to spawn and in aggregating them prior to upstream movement [6,9].

Synthetic sea lamprey pheromones may prove useful in manipulating one or more of these behaviors to advantage. However, the actual utility of pheromones in population control can only be determined by carefully designed field trials once the active substances are identified and their precise biological functions determined.

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Discussion--Most of the ideas for using chemical signals in lamprey control have focused on sex attractants and migratory cues for adults. This is certainly reasonable; however, there may be additional, potentially useful, chemical cues that are important during other life stages. For example, Barb Zeilinski's lab has shown that ammocetes have a well-developed and presumably functional nose. Do ammocetes use chemical cues in selection of feeding areas? Do chemical signals play a role in triggering metamorphosis? For example, when larval population density gets high, does the increasing concentration of some metabolite signal to the larger individuals that it is time to transform and move downstream to feed?

The fecundity of sea lampreys is high. How successful will approaches like release of sterile males, trapping out one or the other sex using specific attractants, or disrupting spawning by broadcast application of a sex pheromone be in reducing reproductive success? Might the larval stage be the most vulnerable to chemosensory manipulation?

VI.2. Olfactory Development and Function in Larvae (Barbara Zielinski).

Background--I am presently a faculty member in the Department of Biological Sciences, University of Windsor. I have worked with the structure and function of the olfactory mucosa since my graduate studies with Dr. Toshiaki Hara at the University of Manitoba and the Freshwater Institute. My postgraduate studies with Drs. Marilyn and Thomas Getchell at Wayne State University focused on ultrastructural and pharmacological aspects of the olfactory mucosa, and with Dr. Anita Hendrickson at the University of Washington, on synaptic development of the visual cortex. My contribution to the Great Lakes Fishery Commission has been to provide information on that development of the olfactory organ in sea lampreys. This includes structural changes throughout the life cycle, and most recently, the function of the olfactory mucosa in larval ammocoetes.

Submitted manuscript--The study of the development of the olfactory system has important applications to the control of sea lampreys and to understanding their biology. We have undertaken a survey of the structure of the olfactory mucosa in a development series, from embryonic stages to upstream migrants; and have initiated physiological and behavioral studies in larval ammocoetes.

Light microscopy, as well as scanning and transmission electron microscopy were used to investigate the development of the olfactory organ, in particular, the distribution and relative number of olfactory receptor cells (ORC). In sea lampreys, only ciliated ORC have been observed (Thornhill, 1967; Vandenbossche et al., 1994). Embryos and prolarvae that were collected from nests in the St. Marys River were used to investigate the early development of the olfactory organ. The olfactory placode was present at Piavis's embryonic stage 14 (Piavis, 1971). ORC were observed shortly after hatching (stage 15), and were abundant before prolarvae abandoned their nests for downstream migration at stages 17 and 18. Asymmetric synapses with agranular vesicles that are characteristic of olfactory nerve synaptic contacts were present in the telencephalon adjacent to the olfactory nerve. These observations suggest that olfactory input is present in prolarvae, possibly for use in the selection of downstream feeding sites.

In larval ammocoetes (from the Hammond Bay Biological Station and Sault Ste. Marie), the olfactory mucosa was on the posterior and lateral walls of the nasal cavity. In 6 cm larvae, the olfactory mucosa had a surface area of 0.07 mm^2 and contained approximately 7,200 ORC. Along the surface of the olfactory mucosa, the density of ORC was relatively uniform (20±8 ORC per 100 μm. Electrophysiological experiments initiated with Dr. T.J. Hara have demonstrated that larvae respond to chemical stimulation. Electro-olfactogram responses to L-arginine were stereo specific, and there were responses to taurocholic acid.

Our study of structural changes of the olfactory organ during metamorphosis was from specimens provided by Dr. John Youson (University of Toronto). During metamorphosis, the olfactory organ expanded and underwent considerable remodeling. The percent weight of the nasal sac to the total body weight doubled from 0.26% at metamorphic stage 1 (M1) to 0.51% at M7. Between M2 and M7, the surface area of the olfactory epithelium almost doubled (0.51 mm^2 to 1.1 mm^2) as did the total number of ORC (43,248 ORC to 76,084 ORC). Major structural reorganization commenced at M3. A nasal valve formed between the nasal tube and olfactory organ, the walls of the olfactory organ folded into olfactory lamellae, the olfactory nerve expanded and diverticuli of the accessory olfactory organ became prominent in the lamina propria. Undoubtedly, these changes are associated with the transformation of sea lampreys from larval filter feeders to the parasitic phase and may be controlled by specific environmental or physiological signals.

The work of previous investigators has demonstrated that the olfactory system of post metamorphic stages responds to chemical stimulation and directs specific behaviors (e.g. Kleerekoper and Mogensen, 1963; Li and Sorensen, 1993; Teeter 1980 and this workshop). In parasitic phase lampreys and in upstream migrants the ORC were wider than in larvae, and the ORC density dropped to 9±2 and 6±2 ORC/100 μm, respectively. The olfactory mucosa of most upstream migrants that were trapped in the Ocqueoc, Cheboygan, and St. Marys rivers was structurally intact. The lamellae of some specimens did not contain olfactory epithelium, and we are currently examining serial sections from these specimens for the presence of ORC at any location in the olfactory organ. Therefore it appears that the olfactory organ contains ORC during the upstream spawning migration.

The olfactory system appears to direct chemoreception in sea lampreys throughout their life cycle. The olfactory mucosa has developed in prolarval and larval stages. It is physiologically active in larvae and may be functioning in prolarvae. These stages may use olfaction to select feeding sites and to remain located in these areas. During metamorphosis the olfactory organ undergoes extensive growth and re-organization. The importance of the olfactory system during upstream migration is supported by partial or total structural integrity of the olfactory mucosa in upstream migrants.

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VI. 3. Olfactory Sensitivity of Migratory Adult Sea Lamprey (*Petromyzon marinus*) to Bile Acids, Amino Acids, and Sex Hormones: Recent Developments and Future Directions (Weiming Li, Peter W. Sorensen and Daniel D. Gallaher).

Backgrounds

Weiming Li: Mr. Li is a Ph. D. candidate in the Department of Fisheries and Wildlife, University of Minnesota. Before coming to the United States, he received his B.A. and M.S. from Shanghai Fisheries University.

Peter Sorensen: Dr. Sorensen is a faculty member at the University of Minnesota and his interests and background are listed in the introduction.

Daniel Gallaher: Daniel Gallaher received his undergraduate degree in Biology from the University of California, Irvine, and an M.S. and Ph.D. in Nutritional Sciences from UC Davis. He was a member of the faculty in the Food and Nutrition Department for 5 years (1983-1988) prior to his present position. A major research interest of his laboratory for many years has been the effect of diet on bile acid metabolism. Their studies have focused primarily on how dietary fat and fiber affects fecal excretion, pool size, and bacterial metabolism of bile acids. Their interests in these areas relate both to the cholesterol lowering effect of dietary fiber and certain fats as well as the purported role of bile acids as promoters of colon cancer. However, as a result of their collaboration with Dr. Peter Sorensen, they have become involved in the study of lamprey bile acids and their possible role as odorants that may act as highly species-specific attractants. The laboratory has conducted measurements of the bile acid (used here as a generic term) profile in both ammocetes and adult lamprey in different tissues, as well as in waters known to contain lamprey. They have also carried out isolation of specific bile acids to support the olfaction studies being performed in Dr. Sorensen's laboratory.

Submitted manuscript--A preponderance of evidence suggests that the sea lamprey, *Petromyzon marinus*, is an olfactory animal whose complex life cycle is largely regulated by odorous cues found in its aqueous environment. Ongoing behavioral, anatomical, and electrophysiological studies increasingly suggest that lamprey feeding, migratory, and sexual behavior are regulated by natural odors. Behavioral experiments conducted with parasitic-phase lamprey have demonstrated that these animals use their olfactory sense to locate prey (Kleerekoper and Mogensen, 1963). Other experiments conducted with adults (Teeter, 1980, this volume; Li and Sorensen, unpublished results) have shown both that the washings of ammocoete larvae are strongly attractive to migrating animals and that mature males are attracted to washings of females while being repelled by washings of conspecific males. Notably, the olfactory system (sense of smell) of post-larval sea lamprey is extremely large and well developed, while the visual and gustatory systems of these animals are relatively poorly developed (Kleerekoper, 1972; Vandenbossche et al. 1994). Furthermore, our recent electrophysiological experiments (reviewed here) describe an extremely sensitive olfactory system which is specifically 'tuned' to detect odors of likely importance to feeding and migration.

The probable importance of olfaction to lamprey has lead many to wonder if natural odorants might be useful in lamprey control (Teeter, 1980, this volume; Zielinski, this volume). In fact, increasing interest in this hypothesis is the '*raison d'être*' for the present workshop proceedings. One idea is that natural odorants could be used to attract specific life stages of this pest to traps for removal or population estimation. Another idea is that if repellents and strong sex aggregants are found they might be used to disrupt the behavior of adult lamprey to prevent successful migration and/or spawning. A third idea is that if lamprey are found to employ pheromones, concentrations of these compounds could be monitored in natural waters to assess population abundance. Examples of how these ideas are presently used to control insect pests are found in these proceedings. The use of natural odorants to manipulate lamprey behavior appears to have many desirable attributes including: potency, specificity of action (perhaps even at the species level), ease of application, and innocuous nature. However, before one can use odorants either to control this species or to assess its abundance, one has to know what odorants are important to it. Unfortunately, this information is presently not available. As a first step, we have spent the past three years characterizing the olfactory sensitivity of migratory adult lamprey. Some preliminary behavioral tests have also been conducted. This paper briefly presents some of our key findings and discusses their potential relevance, thereby providing a knowledge base with which to interpret the ideas presented in this workshop proceedings.

The primary method which we have used to determine the olfactory potency of compounds was electro-olfactogram (EOG) recording. In addition, pilot tests of the behavioral actions of several potent olfactory stimuli were also conducted in a maze constructed in a Michigan stream at the time of the adult lamprey migration. All results are being analyzed as part of Weiming Li's Ph.D. dissertation and will be submitted for publication within a year; presently only abstracts have been published (Li and Sorensen, 1992; Li et al., 1993). This paper will concentrate on describing the EOG data because it has been the most productive. Briefly, the EOG measures the voltage transients across the surface of the olfactory epithelia which are induced by exposure to an odorant and believed to represent summated generator potentials (Getchell, 1974). This technique has proven reliable and convenient for determining the potency of pheromonal odors in teleost fish (Sorensen, 1990, 1992); however, it does not shed any light on the biological (behavioral) relevance of an odor. A detailed description of the protocols we employed may be found in Sorensen et al. (1990). We concentrated on characterizing the olfactory sensitivity of migratory adult sea lamprey to the four classes of odorants presently known to be detected by teleost fish: amino acids, steroid hormones and their metabolites, prostaglandins, and bile acids (Hara, 1992 this workshop). Our study is the first comprehensive characterization of the olfactory sensitivity of a primitive cartilaginous fish. The lampreys used for EOG recording were migratory adults captured in the Cheboygan River, Michigan and the St. Marys River, Ontario, during the early stages of their inland migration. Animals were tested at 11°C within 3 weeks of capture. We will describe our findings by odorant class and conclude with a brief discussion of key findings and perceived research needs.

Nearly 25 years of behavioral, biochemical, and electrophysiological studies suggest that L-amino acids are the major constituent of food odors recognized by teleost fish (Jones, 1992). Amino acids are detected by both the olfactory and gustatory systems of fish with sensitivities ranging down to the nanomolar level (see: Caprio, 1988, this volume; Hara, 1982, 1992). Although the sensitivity of teleost gustatory systems tends to be restricted to a few amino acids, the olfactory systems of teleost fish are sensitive to almost all L-amino acids. Furthermore, where studied, olfactory sensitivity has been found to be attributable to several types of receptor mechanisms which detect acidic, basic, long-chain neutral and short-chain neutral amino acids (Caprio and Byrd, 1984; Hara, 1992). Little variation in the amino acid sensitivities of different species of teleost fish has been described to date. However, behavioral assays of prey searching behavior of sea lamprey conducted by Kleerekoper and Mogensen (1963) suggested that, unlike teleost fish, the lamprey uses amines as feeding cues.

Our recent studies of the olfactory sensitivity of lamprey to amines and amino acids confirm that sea lamprey are different from teleost fish described to date. Most striking is our discovery that the olfactory sensitivity of sea lamprey to amino acids is largely restricted to L-arginine which they detect at concentrations down to 10^{-10} Molar (M). Other basic amino acids have detection thresholds ranging from 10^{-7} M to 10^{-4} M and most acidic and neutral amino acids are not detected even at concentrations as high as 10^{-4} M. Structure-activity studies and cross-adaptation experiments suggest that the high olfactory sensitivity of sea lamprey to L-arginine is attributable to a highly specialized receptor mechanism that is different from receptor mechanisms responsible for the detection of L-arginine in teleost olfactory systems which do not distinguish L-arginine from other basic amino acids (for example see: Caprio and Byrd, 1984; Sveinsson and Hara, 1990). In addition, the olfactory sensitivity of adult sea lamprey to amino acids differs from that of teleosts in that several D-amino acids are more potent than their L-isomers and appear to be detected by different receptor mechanisms. Finally, as suggested by Kleerekoper and Mogensen (1963), the lamprey olfactory system is highly sensitive to amines, with trimethyl amine having the lowest detection threshold of 10^{-9} M. Behavioral and EOG experiments suggest that the latter compound is repulsive to migrating lamprey, however.

Whether this unique pattern of sensitivity to amino acids in sea lamprey is directly related to the lamprey's parasitic feeding behavior is unknown, but it seems likely. Although our data partially support Kleerekoper's hypothesis that amines are important to this species, they strongly suggest that L-arginine plays a critical role in prey recognition. L-arginine is found in the skin mucous of the rainbow trout, Oncorhynchus mykiss, and the lake whitefish, Coregonus clupeaformis (Hara et al., 1985), both important prey of sea lamprey. Also, L-arginine is frequently a limiting nutrient in carnivorous animals. It is possible that by having an olfactory system which is largely restricted to this amino acid, lamprey are able to locate a prey effectively, for they are oblivious to the presence of other extraneous amino acids. Of course, such extreme sensitivity to a single compound suggests that prey recognition is likely to be highly instinctual and therefore open to manipulation by fisheries biologists. Additional behavioral research must now be conducted to determine if L-arginine and its analogs are in fact feeding cues, and to determine whether the olfactory system of adult and parasitic-phase sea lamprey have similar sensitivities and functions.

The study of sex pheromone identity and function has progressed rapidly since the recent discovery that circulating gonadal hormones and their metabolites commonly function as sex pheromones in teleost fish (Sorensen, 1992; Stacey, this volume). What is particularly striking about this discovery is the extreme sensitivity and specificity with which these compounds are detected and the potency of their actions. Hypothesizing that hormonal metabolites might also be sex pheromones in the lamprey, we used EOG recording to test the potency of over 60 sex steroids and prostaglandins. Of particular interest was the sensitivity of sexually mature females to testosterone which Adams et al. (1987) suggested to be a sex pheromone for lamprey both because of its apparent ability to attract migrating males and because of the presence of immunoreactive testosterone in male urine (also see Teeter, 1980, this volume). However, when tested by EOG recording, we found only five compounds to have olfactory activity at a concentration of 10^{-6} M, and testosterone was not one of them. Furthermore, none of these compounds had the extreme potency expected of a sex pheromone. Intriguingly, however, all five compounds were sulfated 19- and 21-carbon sex steroids. Additionally, cross-adaptation experiments suggested that they were detected by receptor mechanisms different from those which respond to sulfated bile acids. The olfactory sensitivity of adult lamprey to sex steroids was not affected by exposing their olfactory organs to washings of the opposite sex. Nor did ovulated females detect sex steroids better than non-ovulated females.

Although none of the sex hormones tested is potent enough to function as a sex pheromone, the fact that several sulfated reduced sex steroids have specific olfactory activity merits further study. This is particularly true because recent studies suggest that sulfation is a major metabolic pathway in fish (Sorensen et al., 1991). Also, our recent studies of lamprey behavior confirm those of Teeter (1980) which demonstrated that sexually mature lamprey are strongly attracted to washings of the opposite sex: it seems highly likely that sexually mature lamprey, which are nearly blind, use sex pheromones. Sex pheromones might be very useful in controlling adult sea lamprey and their reproductive success. A major impediment to understanding sex pheromone function and identity in lamprey is our poor understanding of lamprey reproductive endocrinology (particularly sex steroid pathways), and a lack of studies on the importance of olfaction in reproductive behavior. Studies of hormone metabolism in this species would be especially helpful if conducted in conjunction with EOG recording and behavioral assays. Stacey (this volume) makes a similar recommendation.

There are good reasons to believe that adult lamprey employ chemical cues emanating from conspecifics to locate spawning streams ('migratory pheromones'). The existence of a sea lamprey migratory pheromone is strongly suggested by the facts that adult lamprey tend to enter particular river systems to spawn, that these streams are not necessarily their natal streams (R. Bergstedt, unpublished results, this volume), that their preference for these streams decreases when larvae are removed from them (Moore and Schleen, 1980), and that washings of larvae are attractive to adults in a Y-maze (Teeter, 1980). Migratory pheromones have been postulated for many fish (Smith 1985) including Arctic charr, Salvelinus alpinus, which have been observed to stray into newly stocked streams (Nordeng, 1971) and to be attracted to conspecific washings (Selset and Døving, 1980). Døving et al. (1980) hypothesized that the charr migratory pheromone is comprised of bile acids emanating from fry because fish produce species-specific mixtures of bile acids in great quantities and these compounds are stable (Tammar, 1974).

Data have been collected both for and against Døving's hypothesis. Electrophysiological recording has established that the olfactory and gustatory systems of salmonids are extremely sensitive to several mammalian bile acids (Døving et al., 1980; Hara et al., 1985). Intestinal extracts and bile acids have also been shown to attract salmonids in laboratory tanks (Selset and Døving, 1980; Stabell, 1987). However, the behavioral and olfactory effects of naturally produced bile acids have never been tested on a fish. If the hypothesis that bile acids function as migratory cues is correct, it should be true for the lamprey because larval lamprey, which live in the streams for extended periods of time, contain large quantities of a unique bile acid, petromyzonol sulfate (3a,7a,12a-trihydroxy-5a-cholane-24-sulfate) in their gall bladders (Haslewood and Toakes, 1969). Accordingly, we have been testing whether this compound might function as a migratory pheromone for this species. Our study is the first direct test of the hypothesis that bile acids naturally produced by fish are released to the water where they have specific olfactory and behavioral actions.

Using high performance liquid chromatography (HPLC) we have now analyzed the bile acid composition of ammocoete gallbladders, intestines, holding water, and stream water. Considerable quantities of petromyzonol sulfate were found in the gallbladder of larval lamprey along with smaller quantities of both petromyzonol and its precursor, allocholic acid. The concentration of lamprey bile acids in a major ammocoete stream, the St. Marys River in Ontario, was estimated to be 5 x 10^{-10} M. Bile acid identification has been confirmed by mass spectrometry.

Using EOG recording we have extensively characterized the olfactory sensitivity of migratory adult lamprey to 36 bile acids including allocholic acid, petromyzonol, and petromyzonol sulfate which we have had synthesized. Our studies have clearly shown that the olfactory organ of migratory adults is extremely sensitive to petromyzonol sulfate and allocholic acid, both of which it detects down to concentrations of 10^{-12} M; adult lamprey entering the St. Marys River would have detected these compounds! The olfactory system of adult lamprey is also acutely and specifically sensitive to taurolithocholic acid sulfate which has a detection threshold of 10^{-12} M. Furthermore, structure-activity and cross-adaptation and mixture studies suggest that the olfactory system of migratory lamprey is specifically sensitive to these stimuli and that it is capable of distinguishing between them based on molecular structure. For instance, the configuration of 5-proton is recognized: bile acids with a 5a-H (a feature unique to lamprey bile acids) are 1,000 to 10,000 times more potent than similar bile acids with a 5b-H. The presence of axial hydroxyl groups, a sulfate ester at the 24 carbon, and a carboxyl group at the 23-carbon all appear critical to the olfactory potency of lamprey bile acids. We have not yet had the opportunity to test the behavioral activity of pure allocholic acid and petromyzonol sulfate but we have been able to confirm Teeter's (1980) findings that ammocoete washings are behaviorally attractive and we know that these animals release significant quantities of these bile acids to the water.

In conclusion, results to date support our hypothesis that unique bile acids may function as a migratory pheromone for adult sea lamprey, but conclusive experiments have yet to be performed. In particular, the behavioral actions of these compounds have yet to be demonstrated. Also, we have to determine whether petromyzonol sulfate and allocholic acid are specifically produced and released by sea lamprey or whether they are released by all species of lamprey larvae. Additionally, we do not know what controls the production and release of these compounds so it is difficult to interpret the significance of their levels measured in river waters. Nor do we know whether other species of fish (and lamprey) detect petromyzonol sulfate and allocholic acid. The possibility that olfactory and/or behavioral responsiveness to these compounds is influenced by life-stage and/or is seasonal has yet to be determined in lamprey. The latter is especially important because we need to know when and how lamprey might be using these cues in order to employ them in lamprey control.

The olfactory system of migratory adult sea lamprey is unique among the aquatic vertebrates which have been studied to date, both in terms of what it detects and what it does not detect. Its extreme sensitivity to two naturally produced bile acids and L-arginine is also quite extraordinary. These findings bode well for the possibility that these cues will be useful in lamprey control because they might permit this species to be targeted easily and precisely. The use of bile acids to trap migrating adults appears to have particular promise because of the relative ease with which these animals can be caught as they ascend streams to spawn, the potency of the bile acid cues, the fact that natural sources for these cues are available (eliminating licensing problems), and the value of capturing adults to use them for the sterile male program.

Developing the technology to measure bile acid cues also has great promise because once the mechanisms which control bile acid release are understood, monitoring the presence of these cues in natural waters could be used to assess population densities and also predict where spawning runs might occur. Another possibility raised by our findings is that unique, as yet undescribed, sulfated sex steroids might function as sex pheromones in this species. If identified, these cues would have great promise because they could be used in concert with migratory cues to precisely manipulate spawning populations of lamprey. Exactly how any of these compounds can be used in sea lamprey control is not clear both because their influence on sea lamprey behavior is not known and because the technology for treating aqueous environments with pheromones has not been developed. While the proceedings of the present workshop have been very useful in addressing the latter issue (for examples of the successful use of pheromones in controlling pests see: Cardé, Mason, McNeil, Staten, this volume), basic research on the biology of sea lamprey will be required to answer the first.

Several basic questions about the olfactory biology of lamprey have to be addressed before we can attempt to apply knowledge of it to lamprey control. Most importantly, we must develop an understanding of the role of the olfactory sense in the behavior of this species. First, we must understand how the behavior of lamprey is influenced by the compounds which we have identified by electrophysiological recording as being of special interest. Second, we must determine how essential the olfactory system is to the behavior of this species: if the olfactory sensitivities of these animals is ablated, how well they function. Both of these questions must, of course, be addressed within the context of the life history stage investigated and perhaps even time-of-year because of the dramatic behavioral and physiological changes which lamprey undergo during the course of their lives. Third, we must also develop an understanding of the production and distribution of the cues. In particular, we must learn what controls bile acid production and release in lamprey and whether the bile acids produced by sea lamprey are species specific. Determining the identities and functions of putative sex pheromones is also an important research topic. In conclusion, although many questions about lamprey olfactory biology remain, what we have already learned about lamprey olfaction and examples of existing odorant-based pest control technologies, suggest that natural odors and pheromones have considerable promise in an integrated lamprey control program.

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VII. GROUP DISCUSSIONS EVALUATING THE POTENTIAL OF USING DIFFERENT TYPES OF ODORANT CUES IN LAMPREY CONTROL

After the presentations were made, the workshop participants were divided into small discussion groups. Each group was instructed to review a particular type of odor cue and its potential for use in lamprey control. They were instructed to evaluate the likely importance of the cue to lampreys, the potential for the cue to be useful in bio-control, the state of our understanding of the cue, and key research needs which must be met in order to evaluate the cue. The cues that were evaluated included feeding cues, migrational cues, sex pheromones, repellents, and aggregants. The following suggestions or recommendations were made by the various groups:

VII.1. Larval Aggregants/Repellents (Group leader--Dr. Barbara Zielinski).

General comments:

1. If larval repellents exist (presently unknown), it might be useful to frighten the prolarvae from the nest. If they can be forced to leave the nests during the daytime they would perhaps be subject to increased predation by fish. If they can be forced to concentrate in certain areas it may be easier to kill them with TFM. However, fright compounds may cause larvae to burrow rather than emerge which would not be helpful.
2. If aggregants exist, perhaps we could control migration of larvae with water borne odorants found in areas of dense larval populations. We need to find out if larvae like to live with other larvae. We need to know what causes larvae to cluster in streams. Is it physiological, behavioral, or environmental (water conditions)? We need to know potency of bile compounds as olfactory stimuli for aggregating larvae.
3. It was noted that larvae sometimes congregate around salmon carcasses. Perhaps it would be useful to find out why. (Are chemical attractants involved?)
4. Bile acids such as ACA and PS could be applied in same manner that TFM is and should not affect other organisms if it is produced only by lamprey larvae. It will be very difficult to obtain the large quantities of PS that would be needed.
5. It might be possible to find a gene for a sea lamprey alarm substance and make transgenic trout that exude the sea lamprey alarm substance.
6. Perhaps larvae can be put into small traps to lure other larvae.

<u>Synopsis</u>: Nothing is known about any olfactory cues important to ammocoetes at present. We need to conduct physiological studies (feasible), behavioral studies (difficult), and field trials (very difficult) to characterize their actions and identify them. The most promising research direction would be to look for aggregants and the possibility that bile acids may have this function.

VII.2. <u>Feeding Cues</u> (Group Leader--Dr. John Caprio).

<u>General comments</u>:

<u>Larvae</u>:

1. Determine what causes larvae to concentrate around salmon carcasses (Could the possible aggregant be a foul odor?).
2. It might be useful to find a repellent that would drive larvae out of substrate and into areas of deeper water where they might not survive. It would be impractical to do this in all places. It would also require a large amount of chemical.
3. Bioenergetic study suggests that larvae can't move many times.

<u>Parasitic-phase adults</u>:

1. Lakes are much to large to be able to attract enough parasitic-phase lampreys with feeding cues. Cues do not disseminate well in water and lamprey would likely have difficulty locating source. No known practical way to do this!
2. Electromagnetic field influences feeding behavior only over short distances.
3. Destruction of anti-coagulant property of saliva might be useful.
4. Find odor that attracts downstream migrants as they leave a river to begin parasitic existence.
5. May be able to use sound or vibration since adults can be captured by towing a net behind a motor boat and lampreys sometimes attach to boat hulls.

<u>Spawning-phase adults</u>:

1. No feeding cues possible since spawning-phase adults do not feed.

<u>Synopsis</u>: Manipulation of feeding behavior in lamprey does not appear to have much promise. The only realistic possibility

could be larval attractants/aggregants discussed by Dr. Zielinski.

VII.3. Repellents (Group Leader--Dr. R.J.F. Smith).

General comments:

Parasitic-phase adults: The following suggestions were made on how to control or monitor the numbers of parasitic-phase lamprey in the lakes.

1. Condition hatchery-reared fish to avoid lampreys.
2. Because lampreys sometimes attach to boats, use vibration or electrical shock to trap lampreys in lake. Might be good assessment tool.
3. Use carcasses of dead fish as bait to trap lampreys.
4. Determine if antibodies to lamprey saliva can be produced in rainbow trout. Lampreys feeding on these fish may then die.
5. Put buoys out that will record lamprey attachments. Use these as population monitoring devices.

Spawning-run adults: They stated that spawning-run lampreys strong reaction to human saliva and dead lampreys (histamine?) should be studied (studies shouldn't be expensive). They cautioned however that spawning-run adults may not be a good place to look for alarm substances since there are competing stimuli as well as injured tissue (which may cause alarm responses) already present from lampreys spawning and injuring themselves.

Synopsis: Nothing is presently known about the topic although it appears to have some promise. The group felt that anecdotal stories about lampreys reactions to human saliva, dead lampreys or dead salmon may be a good starting point in searching for a repellent or alarm substance. Also, information on basic response of lampreys to things such as predators or low oxygen concentrations is needed. However, because responses to alarm stimuli are generally reduced in adult fish and can be influenced by learning, this line of research is not particularly promising.

VII.4. Migrational Cues (Group Leader--Dr. Peter Sorensen).

General comments: Lampreys migrating to spawning streams appear to be attracted to streams containing lamprey larvae. It has been hypothesized that the adults are attracted to bile acids released by the larvae. The group felt that this should be pursued further. The following comments were made:

1. While bile acids may be only part of the stimuli, it still should be pursued. Cue may be very simple (insect examples).
2. If cue is all or nothing, we would not have to be concerned about background stimuli.
3. Will need much more of the bile acids for application.
4. Need to know what life stages produce bile acids, effect of temperature on production, degradation of cue in water, and how to test it (good bioassays).
5. Need to know when and where spawning-run adults are using the cue (lake, river mouth, instream, etc.).
6. May be able to treat larvae some way to make them produce more bile and put them in traps. Need to know what controls bile acid production in lamprey.
7. Using something like lamprey bile as attractant would require large amount of bile and would be expensive. Should look at compounds with similar structure that might be much less expensive. Allocholic acid would be much cheaper than Petromyzonol sulfate. May be simpler to dump large numbers of larvae in a trap or seed rivers that won't support larvae to draw spawners to unsuitable streams. Could use nonparasitic lamprey larvae as "seed."
8. Bob Staten's experience with licensing pheromones suggests this technology should be very affordable. (See his manuscript.)
9. Need basic observations of migratory behavior of lampreys (use video equipment, fishways with windows, etc.).
10. Odorants will only be useful on very low populations of lampreys. Chemical control has perhaps reduced lamprey population below economic injury threshold. Use of pheromones and sterile-male releases have ability to reduce populations even further. Control will then become much more economical and less toxicants will be necessary.

Synopsis: Using migratory cues to manipulate lamprey behavior appears to have great promise. Although many questions remain to be answered about bile acids, this line of research seems to be very hopeful. Development of a good behavioral assay, establishing what controls lamprey bile acid production, and determining whether-or-not responsiveness is seasonal are key questions. Licensing a bile acid for use seems feasible (see Staten's manuscript) and should be pursued.

VII.5. Sex Pheromones (Group Leader--Dr. Norm Stacey).

General comments: The group felt that it would be very worthwhile to pursue a sex pheromone study. Some of their thoughts and ideas are as follows.

1. A sex pheromone should be very potent and specific for lampreys. They should be environmentally safe since they are a natural product.
2. There is a need to know if olfaction is involved. It would be useful to conduct an ablation study to determine if nest building or interaction between sexes is affected.
3. It would be helpful if we knew what causes ovulation. Perhaps premature ovulation can be induced. There is a need to speed up maturation (perhaps by change in photoperiod or temperature) so sexually mature animals can be available for study over a longer period of time.
4. Need to know release patterns of sex pheromones. They may only work for short distances in a stream and may not be useful for long distance attraction.
5. May possibly be able to make sterilized males super attractive to females with injection of sustained release pellets containing sex pheromones.
6. May be able to inject females with sustained release pellets containing prostaglandin so females will spawn for several weeks and decrease male fertility.
7. There is a need to determine steroidal pathways in lampreys.
8. A small amount of money should be enough to determine if pheromones are used and how potent they are. A similar study has recently been done to identify a sex pheromone in Pacific herring (see N.M. Sherwood in Can. J. Fish. Aquat. Sci.).

<u>Synopsis</u>: Manipulation of adult lamprey behavior with sex pheromones would compliment the sterile male project and the use of migratory attractants and as such offers great promise. Some basic studies on hormone identity and metabolism in conjunction with olfactory function are needed and should be a high priority. (See Stacey's manuscript.)

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