AN ABSTRACT OF THE THESIS OF

April G. Smith for the degree of Master of Science in Fisheries Science presented on February 7, 2012

Title: Effects of Atrazine on Olfactory-Mediated Behaviors in Pacific Lamprey (*Entosphenus tridentatus*)

Abstract approved:

Carl B. Schreck

Pacific lamprey (*Entosphenus tridentatus*) are experiencing population declines throughout their range. Xenobiotics could be an important risk factor for lamprey populations. Our goal was to establish if common herbicides, as used in forest management, could affect reproductive fitness. We determined that atrazine was a likely compound of greatest concern to lamprey populations. Using an odorant response behavioral assay we were able to demonstrate that environmentally relevant concentrations of atrazine caused a depressed response to adult lamprey holding tank effluent, likely pheromones. Atrazine also depressed their activity level; the number of times they crossed into the effluent arm after being treated with atrazine was significantly lower than controls. In addition, activity level post exposure to atrazine differed between adult life history stages, something which was not significantly different during control trials. Using an odorant detection assay, based on evaluating ventilation rate, we were able to show that environmentally relevant concentrations of atrazine caused a significant increase in ventilatory response to a repulsive odorant, a conspecific necromone. Through the detection study we also showed that lamprey,

exposed to atrazine, had a slight increase in ventilatory response to odor from adult lamprey. If we are concerned about the decline in Pacific lamprey populations, then we should logically be concerned with their exposure to atrazine in the environment.

Effects of Atrazine on Olfactory-mediated Behaviors in Pacific Lamprey (*Entosphenus tridentatus*)

by

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APPROVED:

Major Professor, representing Fisheries Science

Head of the Department of Fisheries and Wildlife

Dean of the Graduate School

I understand that my thesis will become a part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

April G. Smith, Author

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CONTRIBUTION OF AUTHORS

Dr. Carl Schreck contributed to development of experimental design for both experiments and editing of all chapters. Stan van de Wetering contributed to development of experimental design for both experiments. Dr. David Noakes contributed to editing of the conclusion of chapter 2. Dr. Alix Gitelman contributed to the experimental design and reviewing the statistical analysis for both experiments. Rob Chitwood contributed to experimental design for the odorant detection experiment.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDIX FIGURES

Figure Page

LIST OF APPENDIX TABLES

Effects of atrazine on olfactory-mediated behaviors in Pacific lamprey (*Entosphenus tridentatus*)

Chapter 1: Introduction

Impairment to the processing and integration of environmental cues can occur when a fish comes into contact with a toxic substance, including herbicides. The sub-lethal effects from such contact are apparent within multiple systems including: the respiratory, circulatory, nervous, immune, endocrine, and sensory systems, as well as behavior (Heath 1995). The subsequent physiological and behavioral changes that can occur after contact with toxic substances, can lead to altered outcomes in reproductive success, such as a fish being unable to find spawning grounds or a mate (Tierney et al. 2010) or having reduced gamete production (Tillitt et al. 2010). These altered outcomes in reproductive success, after contact with a contaminant, can ultimately lead to population declines.

Declines of Pacific lamprey (*Entosphenus tridentatus*) populations have been noted throughout Oregon for over 35 years (Moring and Lantz 1975; Close et al. 2002; Howard and Close 2005; Robinson and Bayer 2005). In recent years, several studies have found that toxicants can have negative effects on lamprey. Myllynen et al. (1997) found that river water, which is high in iron and low in pH, causes mortality of European lamprey (*Lampetra fluviatilis*) roe and larvae. Soimasuo et al. (2004) found that sediments contaminated with multiple persistent organic pollutants (POP's) are toxic to European lamprey ammocoetes. Substrate collected from the Port of Portland superfund site delays burrowing time and appears to retard growth in Pacific lamprey ammocoetes (Unrein, J.R., Oregon State University, 2011, personal communication). These studies all suggest that toxicants can have a negative effect on Pacific lamprey.

Olfaction in lamprey and its connection to ventilation

Cyclostomata, the class to which lamprey belong, are monorhinic; they have paired olfactory nerves that innervate an unpaired organ (Hardisty and Potter 1972). The water enters the single nostril, continues down the nasal tube, passing below the nasal capsule and into the blind nasopharyngeal pouch (Kleerekoper and Van Erkel 1960). At the entrance to the nasal capsule a small valve directs a thin stream of water into the nasal sac and into contact with the lamellae which are covered by a highly sensitive olfactory epithelium (Kleerekoper and Van Erkel 1960; Hardisty and Potter 1972). Once an odorant molecule comes into contact with the olfactory epithelium a cascade of events occur, called transduction, which leads to the brain interpreting the chemical signal and perceiving a scent. During expiration the branchial constrictor muscles contract, squeezing the flexible walls of the nasopharyngeal pouch, directing water back out of the nostril, and allowing water to flood out of the nasal capsule (Hardisty and Potter 1972).

The relationship between gill ventilation and smelling is inextricably linked in lamprey. With every contraction of the branchial muscles there is a corresponding flow of water through the nostril (Dawson 1905). It has been shown in rainbow darters (*Etheostoma caeruleum*) that ventilation rate increases with exposure to chemical cues (Gibson and Mathis 2006). Increased gill ventilation rate could be a means to increase oxygen consumption in preparation for flight or exploratory behavior. It also could be a cryptic mode of increasing water flow towards the head and olfactory epithelia, allowing darters to more thoroughly sample the chemical

signal without giving away position (Gibson and Mathis 2006). In lamprey, an increase in ventilation rate would result in the same benefits of increased oxygen consumption and increase water sampled by the olfactory epithelia.

Pacific lamprey life cycle and the importance of olfaction

Pacific lamprey are anadromous jawless fish distributed throughout the Pacific Ocean basin (Hardisty and Potter 1971). Ammocoetes, juvenile lamprey, live in freshwater for up to 7 years before smolting and migrating to the ocean where they can live for 6 months to 3.5 years in their parasitic phase before returning as adults to freshwater to spawn (Luzier et al. 2008). In ammocoetes the olfactory organ remains little developed from the larvae stage. During smoltification the olfactory organ quickly develops into a fully functional olfactory system (Hardisty and Potter 1972). The parasitic phase of lamprey uses olfaction to help locate prey (Kleerekoper and Mogensen 1963).

Adult lamprey actively choose spawning rivers based on olfactory cues and not based on stream recognition or any other physiological or environmental cue (Vrieze et al. 2010; Yun et al. 2011). Adults are drawn to conspecific odors; a combination of petromyzonal sulfate (a migratory pheromone) or 3-keto petromyzonal sulfate (a sex pheromone) with petromyzonamine disulfate, and petromyzosterol disulfate. Together, these odors make up the components of the migratory or spawning bouquet that is released into the water by larval and adult lamprey and attracts conspecifics (Sorensen et al. 2005; Yun et al. 2011). The olfactory response to these pheromones is greatest in the spring when lamprey first enter freshwater. The magnitude of

response is then somewhat diminished, though stable, for the better part of 12 months until the following spring when lamprey spawn and response subsides (Robinson et al. 2009).The olfactory system of lamprey is in nearly direct contact with the surrounding environment being separated by only a thin layer of mucus. This makes it exceptionally vulnerable to toxicants found in the water column (Tierney et al. 2010). The ability of a toxicant to disrupt this intricate system can occur for a multitude of reasons. The toxicant can damage the olfactory cells, mimic the odorant, or change the odorants chemistry; all of which can lead to an altered sensory perception by the lamprey. The lamprey perceiving an incorrect stimulus can react to the stimulation inappropriately, either physiologically or behaviorally (Tierney et al. 2010).

Atrazine

We are specifically interested in xenobiotics that are used in the management of forested land. Application of contaminants can be easily controlled by land managers, while more diffuse sources of pollution such as in-stream nutrient load are more difficult to manage (Harding et al. 1998). We have chosen to look at herbicides that are applied to forested land as the contaminant of interest.

A deterministic risk assessment was conducted to select the herbicide to be used in this project. We summarized active ingredients of herbicides detected in 10 water quality reports from reference sites in Oregon (Anderson et al. 1997; Garono and Brophy 1999; Garono and Brophy 2001; Gilliom et al. 2006; Carpenter et al. 2008; Agriculture 2009; ODEQ 2009; USEPA 2009). We found 68 unique herbicides detected and from this we examined the risk associated with the top ten herbicides,

ranked by the number of studies which detected the herbicide and the concentration detected compared to the US Environmental Protection Agency's (USEPA) NOAEC (no observed adverse effect concentration) for a freshwater fish. The following qualities of each herbicide were used to determine its risk to lamprey: USEPA registered use sites included forestry, lipophilicity, bioavailability, solubility, target organs, half life, water partitioning coefficient, and significant findings showing a negative effect on fish. From this risk assessment atrazine was selected as the herbicide of concern for this study (for full risk assessment see Appendix 1).

The USEPA has determined several standards for atrazine endpoints in water. The concentration considered safe in drinking water is 3 ug/l. The concentration considered lethal to rainbow trout (*Oncorhynchus mykiss*) in both lentic and lotic ecosystems after 96 hours is 5,300 ug/l. The highest concentration considered to have no effect on brook trout (*Salvelinus fontinalis*) after 44 weeks in both lentic and lotic systems is 65 ug/l and the concentration to have a low effect after 44 weeks is 120 ug/l (USEPA 2010).

From 1992 until 1999 the US Geological Survey (USGS) collected over 200 parameters of water quality for the Willamette River Basin Water Quality study (Wentz et al. 1998). Anderson et al (1997) analyzed the data set collected by Wentz et al. specifically for pesticides and found atrazine in 99% of the water column samples, with a maximum concentration of 90 ug/l We tested the nominal concentration of 100 ug/l for the odorant detection study as it is just below what has been shown to have a low effect on brook trout (USEPA 2009) and is just over the maximum

detection, 90 ug/l, that was reported for the Willamette River (Anderson et al. 1997). We tested the nominal concentration of 50 ug/l for the odorant response study as we wanted a concentration closer to what is found on average in streams and rivers of Oregon and the USA (Gilliom et al. 2006).

General atrazine toxicity studies

A study with male Atlantic salmon (*Salmo salar*) parr, which were acutely exposed to 1 ug/l of both simazine and atrazine, showed a significantly reduced olfactory response to female priming pheromones (Moore and Lower 2001). Atrazine has also been shown to have a significant effect on migratory activity and olfactory sensitivity in salmon smolts, as well as inhibiting the secretion of testosterone in the testes. This study also concluded that if a waterborne pollutant interferes with the olfactory sensitivity to relevant compounds, then spawning success could be reduced and long- term reductions in populations could occur (Moore and Lower 2001).

A study conducted with fat head minnows (*Pimephales promelas)* found both physiological and behavioral results. With exposure to 50 ug/l atrazine, they found 39% lower total egg production than controls. The decrease in egg production was attributed to reduced spawning events, and gonad abnormalities, as well as alteration of the final maturation of oocytes (Tillitt et al. 2010).

Tierney et al. (2007) found that after a 30 min exposure to 1 ug/l atrazine coho salmon parr (*O. Kisutch*) showed a loss of preferential response to the amino acid L-histidine as compared to controls. They also found that 10 ug/l atrazine significantly reduced EOG response to L- histidine. Tierney et al. postulated that a reduced EOG

response to amino acids can have survival implications since amino acids can elicit anti-predator behaviors in adult salmonid. They also concluded that atrazine can result in impaired imprinting of larval salmonids to the olfactory bouquet of a stream by reducing the amino acid elicited response, thus reducing a fish's ability to successfully return to spawn.

Research Goals and Objectives

The main goal of this research is to determine if atrazine has an effect on the ability of Pacific lamprey to migrate to spawning grounds and successfully spawn. In order to determine this we will conduct a laboratory study with the goal of determining if atrazine detrimentally alters the response of lamprey to biologically relevant odors. We predict that atrazine is negatively affecting lamprey olfaction, the dominant sense used for migration and spawning. We addressed this prediction by conducting two assays. First, we used a behavioral odorant response experiment to determine if lamprey behave differently to relevant odors when treated with atrazine compared to a control. Second, we conducted a physiological odorant detection study to determine if lamprey have an altered ventilation rate in response to biologically relevant odorants after being exposed to atrazine

Odorant response study

Behavior is a very obvious, measurable component of an animal's life cycle. An animal with altered behavior may find it more difficult to complete its life cycle. The behaviors we are most interested in are those which would result in a lamprey's

inability to migrate or spawn. Adult Pacific lamprey are known to actively move towards pheromones produced by juvenile lamprey and other adult lamprey (Bjerselius et al. 2000). Bjerselius et al. (2000) concluded that adult sea lamprey (*Petromyzon marinus*) swim up a concentration gradient of pheromones. By selecting streams with strong concentrations of pheromones produced by juveniles, the adults are actively choosing to spawn in streams which are proven to contain high quality rearing habitat for juveniles. Yun et al. (2011) determined that pheromones produced by adult Pacific lamprey cause other adult Pacific lamprey to elicit a similar behavioral response as sea lamprey.

To determine if atrazine may cause an altered response to adult lamprey effluent, likely pheromones, we conducted our study using a two choice y-maze. Lamprey were given the opportunity to freely move between arms and their behavior monitored. By detecting a difference in the amount of time or number of times that the lamprey moved into the effluent arm, as compared to the controls, we can determine if atrazine altered their behavioral response to the odorant.

Odorant detection study

Physiological responses of fish can be either adaptive or detrimental. Adaptive responses are vital to the survival of an individual over the long term, while short term or severe responses can lead to detrimental outcomes, including death (Barton 2002). Altered physiological responses due to exposures to contaminants can lead to a fish being unable to behave appropriately to an environmental cue (Tierney et al. 2010).

To investigate if atrazine may cause an inappropriate response to an environmental cue, we looked for a change in gill ventilation rate in reaction to behaviorally relevant odors. By detecting a change in ventilation rate in conjunction with an odorant in the water column we concluded that the lamprey recognized the odor and the change in ventilation rate is a behaviorally relevant response. If this change in ventilation rate is altered after exposure to atrazine we can conclude that atrazine altered the physiological response to the odorant.

References

Anderson C, Wood T, Morace J. 1997. Distribution of dissolved pesticides and other water quality constituents in small streams and their relation to land use, in the Willamette River basin, Oregon, 1996. Portland, OR.

Barton BA. 2002. Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology 42:517-525.

Bjerselius R, Li WM, Teeter JH, Seelye JG, Johnsen PB, Maniak PJ, Grant GC, Polkinghorne CN, Sorensen PW. 2000. Direct behavioral evidence that unique bile acids released by larval sea lamprey (P*etromyzon marinus*) function as a migratory pheromone. Canadian Journal of Fisheries and Aquatic Sciences 57:557-569.

Carpenter KD, Sobieszczyk S, Arnsberg AJ, Rinella FA. 2008. Pesticide occurance and distribution in the lower Clackamas River basin, Oregon 2000-2005. Reston, VA.

Close DA, Fitzpatrick MS, Li HW. 2002. The ecological and cultural importance of a species at risk of extinction, Pacific lamprey. Fisheries 27:19-25.

Dawson J. 1905. The breathing and feeding mechanism of the lampreys. Biological Bulletin 9:1-21.

Garono R, Brophy L. 1999. Rock creek (Siletz) watershed assessment final report. Corvallis, OR: Earth Designs consulting Inc. Available: http://www.earthdesign.com/rockcreek/DRAFT.PDF [accessed 22 April 2010].

Garono R, Brophy L. 2001. Midcoast watersheds council sixth field watershed assessment. Corvallis, OR: MidCoast Watersheds Council. Available: http://www.midcoastwatershedscouncil.org/Assessments/MCWC_Assessment.pdf [accessed 22 April 2010].

Gibson AK, Mathis A. 2006. Opercular beat rate for rainbow darters, *Etheostoma caeruleam*, exposed to chemical stimuli from conspecific and heterospecific. Journal of Fish Biology 69:224-232.

Gilliom RJ, Barbash JE, Crawford CG, Hamilton PA, Martin JD, Nakagaki N, Howell LH, Scott JC, Stackelberg PE, Thelin GP and others. 2006. The quality of our nation's waters- pesticides in the nation's streams and ground water, 1992-2001: US Geological Survey circular 1291. Reston, VA: USGS. 172 p.

Harding JS, Benfield EF, Bolstad PV, Helfman GS, Jones EBD. 1998. Stream biodiversity: The ghost of land use past. Proceedings of the National Academy of Sciences of the United States of America 95:14843-14847.

References (continued)

Hardisty MW, Potter IC, editors. 1971. The biology of lampreys, Volume 1. New York: Academic Press. 1-7 p.

Hardisty MW, Potter IC, editors. 1972. The biology of lampreys, Volume 2. New York: Academic Press. 393-399 p.

Heath AG. 1995. Water pollution and fish physiology. 2nd edition. Water pollution and fish physiology. 3-9, & 11-13 chpt.

Howard JK, Close DA. 2005. Pacific lamprey research and restoration project. 2003 annual report, project no. 199402600. BPA Report DOE/BP 00005455-7:1-132.

Kleerekoper H, Mogensen J. 1963. Role of olfaction in the orientation of *Petromyzon marinus* L. Response to a single amine in prey's body odor. Physiological Zoology 36:347-360.

Kleerekoper H, Van Erkel G. 1960. The olfactory apparatus of *Petromyzon marinus* L. Canadian Journal of Zoology 38:209-223.

Luzier CW, Brostrom J, Cook-Tabor C, Goodman D, Nelle RD, Ostrand KG, Schaller H, Streif B. Proceedings of the Pacific lamprey conservation initiative work session- October 28-29, 2008; 2008 October 28-29, 2008; Portland, OR. US Fish and Wildlife Service.

Moore A, Lower N. 2001. The impact of two pesticides on olfactory-mediated endocrine function in mature male atlantic salmon (S*almo salar* L.) parr. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology 129:269-276.

Moring, J.R. Lantz RL. 1975. The Alsea watershed study: effects of logging on aquatic resources of three headwater streams of the Alsea River, Oregon. Part I. Biological studies. Fish. Res. Rep. No. 9. Oregon Department of Fish and Wildlife, Corvallis, OR.

ODEQ. 2009. Watershed-based toxics monitoring program 2008 progress report: Willamette River basin. Hillsboro, OR: Oregon Department of Environmental Quality Laboratory and Environmental Assessment Division. Available: http://www.deq.state.or.us/lab/wqm/docs/10-LAB-007.pdf [accessed 13 May, 2010].

References (continued)

PURS-Oregon 2007. 2008. Pesticide Use Reporting System. Salem, OR: Oregon Department of Agriculture. Available: http://www.Oregon.gov/ODA/PEST/docs/pdf/pursreportweb2007.pdf [accessed 22 April 2010].

PURS-Oregon 2008. 2009. Pesticide Use Reporting System. Salem, OR: Oregon Department of Agriculture. Available: http://www.Oregon.gov/ODA/PEST/docs/pdf/pursreport08web.pdf [accessed 22 April 2010].

Robinson TC, Bayer JM. 2005. Upstream migration of Pacific lampreys in the John Day River, Oregon: Behavior, timing, and habitat use. Northwest Science 79:106-119.

Robinson, T. C., P. W. Sorensen, et al. (2009). "Olfactory Sensitivity of Pacific Lampreys to Lamprey Bile Acids." Transactions of the American Fisheries Society 138: 144-152.

Soimasuo M, Kervinen J, Sinkkonen S, Paasivirta J. 2004. Bioaccumulation of pops from contaminated sediment to lamprey (*Lampetra fluviatilis* L.) larva. Journal of Soils and Sediments 4:75-83.

Sorensen PW, Fine JM, Dvornikovs V, Jeffrey CS, Shao F, Wang JZ, Vrieze LA, Anderson KR, Hoye TR. 2005. Mixture of new sulfated steroids functions as a migratory pheromone in the sea lamprey. Nature Chemical Biology 1:324-328.

Tierney KB, Baldwin DH, Hara TJ, Ross PS, Scholz NL, Kennedy CJ. 2010. Olfactory toxicity in fishes. Aquatic Toxicology 96:2-26.

Tierney KB, Singh CR, Ross PS, Kennedy CJ. 2007. Relating olfactory neurotoxicity to altered olfactory-mediated behaviors in rainbow trout exposed to three currently-used pesticides. Aquatic Toxicology 81:55-64.

Tillitt DE, Papoulias DM, Whyte JJ, Richter CA. 2010. Atrazine reduces reproduction in fathead minnow (*Pimephales promelas).* Aquatic Toxicology 99:149-159.

USEPA. 2009. Aquatic life benchmarks for pesticies. Available: http://www.epa.gov/oppefed1/ecorisk_ders/aquatic_life_benchmark.htm [accessed 1 June 2010].

References (continued)

USEPA. 2010. Pesticide reregistration status. Available: http://www.epa.gov/opp00001/reregistration/status.htm [accessed 1 June 2010].

Vrieze LA, Bjerselius R, Sorensen PW. 2010. Importance of the olfactory sense to migratory sea lampreys *Petromyzon marinus* seeking riverine spawning habitat. Journal of Fish Biology 76:949-964.

Wentz DA, Bonn BA, Carpenter KD, Hinkle SR, Janet ML, Rinella FA, al. e. 1998. Water quality in the Willamette basin, Oregon 1991-1995. Report nr USGS Circular 1161. Available: http://pubs.usgs.gov/circ/circ1161/circ1161.pdf [accessed 22 April 2010].

Yun SS, Wildbill AJ, Siefkes M J, Moser ML, Dittman AH, Corbett SC, Li W, Close DA. 2011. Identification of putative migratory pheromones from Pacific lamprey (*Lampetra tridentata*). Canadian Journal of Fisheries and Aquatic Sciences 68:2194-2203.

Chapter 2. Effects of atrazine on olfactory-mediated behaviors in Pacific lamprey (*Entosphenus tridentatus*)

Abstract

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Keywords

Pacific Lamprey, Atrazine, Pheromones, Necromones, Y-maze, Respiration, Ventilation, Olfaction

Introduction

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A study with male Atlantic salmon (*Salmo salar*) parr, which were acutely exposed to 1 ug/l of both simazine and atrazine, showed a significantly reduced olfactory response to female priming pheromones (Moore and Lower 2001). Atrazine has also been shown to have a significant effect on migratory activity and olfactory sensitivity in Atlantic salmon smolts, as well as inhibiting the secretion of testosterone in the testes. This study also concluded that if a waterborne pollutant interferes with the olfactory sensitivity to relevant compounds, then spawning success could be reduced and long- term reductions in populations could occur (Moore and Lower 2001).

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Tierney et al. (2007) found that after a 30 min exposure to 1 ug/l atrazine coho salmon parr (*O. kisutch*) showed a loss of preferential behavior to the amino acid L-histidine as compared to controls. They also found that 10 ug/l atrazine significantly reduced EOG response to L- histidine. Tierney et al. postulated that a reduced EOG response to amino acids can have survival implications since amino acids can elicit anti-predator behaviors in adult salmonid. They also concluded that atrazine can result in impaired imprinting of larval salmonids to the olfactory bouquet of a stream by

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Pacific lamprey are known to actively move towards pheromones produced by juvenile and other adult Pacific lamprey (Yun et al. 2011). It has been shown that adult lamprey will swim up a gradient of pheromones to select locations to spawn (Bjerselius et al. 2000). By selecting streams with strong concentrations of pheromones produced by conspecific, adult lamprey are actively choosing to migrate into and spawn in streams which contain high quality rearing habitat for juveniles (Bjerselius et al. 2000). If atrazine interrupts the detection of behaviorally relevant odorants then lamprey could not reach productive spawning grounds, have limited ability to find other adults to spawn with, or be unable to avoid potentially hazardous

situations. If this happens to a large number of individuals within a population declines would result.

The closely related sea lamprey (*Petromyzon marinus*) is known to avoid risks associated with olfactory cues. Wagner et al (2011) showed that lamprey will actively avoid the odor of decaying conspecifics. They hypothesized that this olfactory cue can signal multiple events: the end of spawning in a stream, areas where predators are actively killing lamprey, or streams where high mortality of larval lamprey is occurring. Being able to detect and avoid areas of risk, regardless of the reason, would ultimately lead to high survival of adults and potentially their future reproductive investment.

We addressed our interest in causes for lamprey population decline by determining if atrazine is having a deleterious effect on Pacific lamprey response towards an attractive odorant, likely a spawning pheromone, as well as their ability to detect a repulsive odor, likely a conspecific necromone. The main goal of this research was to determine if atrazine alters lamprey olfactory mediated behaviors. We investigated this hypothesis by conducting two assays. First, we conducted a behavioral odorant response experiment to determine if lamprey behave differently to relevant odors when treated with atrazine versus a control. Second, we carried out a physiological odorant detection study to determine if lamprey have altered gill ventilation rate in response to biologically relevant odorants after being exposed to atrazine in comparison to a control.

Methods

Male and female adult lamprey were collected by hand from Willamette Falls in June 2010 and August 2011. They were transported to the Fish Performance and Genetics Laboratory at Oregon State University and placed into 750 liter tanks supplied with pathogen - free well water at approximately 13° C, at a density of 33-34 lamprey per tank, 100 lamprey total. Tanks contained 15 cm of gravel sized substrate (2-10cm) throughout bottom; this was covered with multiple slate rocks roughly 30 cm across or larger. Lamprey were inserted with passive integrated transponder (PIT tags and left to acclimatize to tanks for several months before trials began.

Odorants used in trials

The lamprey to be used in the odorant response and odorant detection studies were at two different adult life history stages. During the odorant response study approximately one third of the lamprey were showing characteristics of being reproductively mature. We assumed that natural shedding of pheromone into the effluent of their holding tanks was high during this time. We were able to confirm this assumption with a pilot study in which the lamprey were attracted to effluent compared to well water. During the odorant detection study lamprey were not showing spawning characteristics so a washing was employed to aid in capturing adult odor in methods similar to Bjerlious et al. (2000) and Yun et al. (2002). To obtain adult odor, we placed four adult Pacific lamprey into a static container with 8 liters of water for 3 hours. The container was maintained at a temperature of 12º C and aerated

throughout the odor collection. The lamprey were removed and the resulting water used as an attractive odorant.

To obtain necromone, a 139g adult lamprey, which died of natural causes, was placed in a container and left in a warm room overnight to allow for further decay. The carcass was then homogenized using a Waring commercial blender with 150 ml of e-pure water. The homogenate was then sonified using a Branson sonifier cell disrupter at 50w for 45 seconds. The homogenate was diluted serially using e-pure water. A concentration of $4.8x10^{-4}$ of this homogenate was placed into the mixing container during each trial. If complete mixing was obtained, then the lamprey received a concentration of $4.8x10^{-8}$ homogenized lamprey.

Life history stage

To investigate if atrazine has a different effect at different lamprey life history stages, we grouped the lamprey into three mutually exclusive subcategories by physical and behavioral characteristics. Beginning March 6, 2010 lamprey were checked every evening after twilight to see if they were "active." A lamprey was considered active if it was swimming in the water column when checked, as opposed to buried under substrate. Under substrate is their preferred habitat during the day. If a lamprey was active it, was then checked to see if it was showing spawning characteristics. Females, which were ready to spawn, had an obvious egg sack and males had a protruded pseudo-penis. Lamprey which were active and showed spawning characteristics are called ripe in the data analysis, regardless of sex. If there

were no signs of spawning characteristics and the fish were "active," they were considered a different life history stage from the ripe lamprey, possible a migrating lamprey, and are called active in the data analysis. Several lamprey never emerged at night and showed no signs of spawning characteristics. These lamprey are possibly hold - over lamprey, lamprey which will not migrate and spawn until the next year and are called non-active in the data analysis.

Selection of atrazine concentrations

The United States Environmental Protection Agency (USEPA) has determined several standards for atrazine endpoints in water. The concentration considered safe in drinking water is 3 ug/l. The concentration considered lethal to rainbow trout (*Oncorhynchus mykiss*) in both lentic and lotic ecosystems after 96 hours is 5,300 ug/l. The highest concentration considered to have no effect on brook trout (*Salvelinus fontinalis*) after 44 weeks in both lentic and lotic systems is 65 ug/l and to have a low effect after 44 weeks is 120 ug/l (USEPA 2010).

From 1992 until 1999 the US Geological Survey (USGS) collected over 200 parameters of water quality for the Willamette River Basin Water Quality study (Wentz et al. 1998). Anderson et al (1997) analyzed the data set collected by Wentz et al. specifically for pesticides and found atrazine in 99% of the water column samples, with a maximum concentration of 90 ug/l We tested the nominal concentration of 100 ug/l for the odorant detection study as it is just below what has been seen to have a low effect on brook trout (USEPA 2009) and is just over the maximum detection, 90 ug/l, that was reported for the Willamette River (Anderson et al. 1997). We tested the

nominal concentration of 50 ug/l for the odorant response study as we wanted a concentration closer to what is found on average in streams and rivers of Oregon and the USA (Gilliom et al. 2006).

Treatment for odorant response experiment

Stock atrazine solution was created by mixing 6.08 ml of the formulated product Atrazine 4L (EPA Registration Number: 1281-158), from Agri-Solutions, Inc., with 980 ml of well water. Atrazine 4L contains 40.8% of the active ingredient atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine).

To create a nominal concentration of 50 ug/l atrazine, 33 ml of stock Atrazine 4L solution was placed in 750 liters of recirculating well water. Tanks were identical to the lamprey holding tanks with the exception of the gravel - sized substrate that was not placed in the treatment tanks due to the ability of atrazine to bind to particulate matter. Two large slate rocks were placed in each tank for the lamprey as cover, slate rocks were also used as cover in their holding tanks. To prevent UV break down of atrazine, tanks were covered with Gila Privacy Control Black Window Film rated to reject 99% of UV rays by the National Fenestration Rating Council (NFRC). Lamprey were placed into the treatment tank approximately 30 minutes after evening twilight and treated for an average of 25.8 hours (range: 23.8 to 29.3 hours). Control treatment was identical except no atrazine was used and the lamprey were treated for an average of 25.2 hours (range- 23.4 to 29.7 hours). Each lamprey was tested twice, once after treatment with atrazine and once after treatment as a control, the order in

which each lamprey was tested was randomly selected and clearance time (average: 4 days, range: 2-7 days) was allowed between trials.

One liter of treatment water from two of the twenty-five trials was analyzed for atrazine by Pacific Agricultural Laboratory, Portland, OR. A modified EPA 8270D (GC-MS SIM) method was used and atrazine was detected at 56 ug/l and 43 ug/l, respectively.

Treatment for odorant detection study

To create a nominal concentration of 100 ug/l atrazine, 1.75 ml of stock Atrazine 4L solution was placed in a 190 liter Rubbermaid storage tote filled with 40 liters of recirculating well water. Tanks were covered with the same UV blocking film used in the odorant response study. Two large slate rocks were also placed in these tanks as cover. Lamprey were placed into the treatment tanks between 7:22 am and 7:58 am (7:39 am on average) and treated for an average of 23.7 hours (range- 23.2 to 25.0 hours). Control treatment set up was identical except no atrazine was used. Lamprey were placed in control tanks filled with 40 L of well water between 7:08 am and 7:44 am (7:29 am on average) and treated for an average of 23.9 hours (range- 23.7 to 24.5 hours). Each lamprey was tested twice, once after treatment with atrazine and once after treatment as a control, the order in which each lamprey was tested was randomly selected and clearance time was allowed between trials.

One liter of treatment water from two of the nine trials were analyzed for atrazine by Pacific Agricultural Laboratory, Portland, OR. Using the same method as the odorant response study atrazine was detected at 110 ug/l for both trials.

Odorant response behavioral study

In the spring of 2011, when lamprey were beginning to show spawning characteristics, the difference in response of adult Pacific lamprey to water conditioned with holding tank effluent was tested using a two-choice y-maze (Figure2. 1). Trials began approximately 30 minutes after sunset from April 30, 2011 to July 2, 2011. Two marriotte siphons were filled with either 2 liters of effluent from the adult tank or well water and remained on for the duration of the trial. The experiment was videotaped from above. Well water was flowing in each arm at 2857 ml min^{-1}, marriotte siphons were set to 10 ml min^{-1} for each arm. The arm that received the effluent was randomly selected for each trial. A 100 watt incandescent light bulb , centered 6 feet above the y-maze, was turned on every 5 minutes for 30 seconds to promote movement, starting as soon as the lamprey was placed in the maze. To provide light for filming an 8 W LED diffuse red light bulb passing light greater than 640 nm remained on throughout the experiment.

Lamprey were placed into the stem of the maze for a 15 minute acclimation period ending when the experimenter entered the room to remove the screen (Figure2. **1**). The trial began as soon as screens were removed. Tape was laid across the top of the tank to allow visualization of the lamprey entering or exiting an arm. The lamprey was considered in an arm of the maze if its anterior end had crossed the plane created by the tape. Between trials the tank was drained of all water, cleaned with 5% hydrochloric acid, rinsed with well water and drained again.

Odorant detection study

In the fall of 2011, prior to lamprey showing any spawning characterisitcs, the difference in olfactory response of individually identified adult Pacific lamprey to water conditioned with an odorant was tested using counts of the contractions of the branchial baskets. For each trial, a single lamprey were placed individually into a clear plastic tube covered with black plastic and viewed through video cameras placed adjacent to the tubes (Figure 2.2). Well water was flowing into a mixing container (turnover rate: 42 per hour) and then onto tube at a rate of 300 ml min^{-1} (turnover rate: 8 per hour). Before starting the trial the lamprey was given 2 hours to stabilize gill ventilation. Two of the same 8 W LED diffuse red light bulbs were used in this experiment to aid cameras in filming ventilation, they remained on throughout the experiment.

An odorant was placed in the mixing containers (B Figure 2.2) and contractions of the branchial basket (BPM) were counted for 1 minute segments for 20 continuous minutes post addition of the odorant. During our pilot study we found a diminished ventilatory response to subsequent presentations of the odorant (for results of pilot study see Appendix 2). Based on the pilot study findings odorants were given in a block design. During the first block the order which control well water or necromone was presented to the lamprey was randomly assigned. In the second block the order which control well water or adult odor was presented was randomly assigned. The maximum ventilation rate per minute for each trial was obtained. The range in ventilation rate, the maximum contractions per minute minus the lowest contractions

per minute which occurred before the maximum, was calculated for each trial. If the first minute was the maximum response for an odorant then the range would equal zero. Between trials the tube was drained of all water, cleaned with 5% hydrochloric acid, rinsed, left with water flowing through tube over night, and drained again at the start of the next trial. One trial was run per day starting at 7:22 am, on average, from October 3, 2011 to November 2, 2011.

Statistics

The statistical software program R (R 2011) was used in the analysis. All reported p-values (P) are from Wilcoxon signed rank tests unless otherwise stated.

Results

Odorant response behavioral study

For the odorant response experiment we were interested in how lamprey behaved. We defined behavior as moving in the y-maze during a trial. Six lamprey were not included in the analysis because they did not move in any trial.

Time spent in arms of odorant response

When given a choice between an arm with effluent versus one which contained only well water we found moderate evidence to suggest that lamprey did not spend equal amounts of time in the two arms (Figure 2.3) ($P = 0.043$). Lamprey selected the effluent scented arm, preferentially spending a median of 523 seconds more in the effluent arm than the non effluent arm (95% C.I.: 8 and 1049 seconds). This effluent

selection was inhibited by prior atrazine exposure. We did not find any evidence to suggest that lamprey spent more time in the effluent arm than the control arm after being treated with atrazine. (Figure 2.3) (P = 0.42)

Lamprey spent more time in the effluent scented arm when not pre-treated with atrazine than lamprey which had been pre-treated with atrazine. When comparing time spent by lamprey in only the effluent arm, we found moderately strong evidence to suggest lamprey which had not been exposed to atrazine spent a median 512 seconds more in the effluent arm than atrazine exposed lamprey (P= 0.034, 95% C.I. 19 to 914 seconds). We found no evidence to suggest that the time spent in the non-effluent arm pre versus post exposure to atrazine differed (Figure 2.3) (P=0.14).

Movement

When given a choice between an arm with effluent versus one which did not have effluent we found no evidence to suggest that adult Pacific lamprey crossed into the effluent arm any more than the non effluent arm both pre and post exposure to atrazine (Figure 2.4) (P= 0.53 and 0.28 respectively).

When comparing lamprey crossing into the effluent arm, we found moderate evidence to suggest that unexposed lamprey crossed into the effluent arm a median of 5 more times than exposed lamprey (Figure 2.4) (P= 0.043, 95% C.I.= 0 to 12 crossings). We found no evidence to suggest that the number of times lamprey crossed into the non-effluent arm differs pre versus post exposure to atrazine (Figure 2.4) (P=0.26)

Time spent in arms of odorant response by life history stage

Ripe lamprey post exposure to atrazine spent more time in the effluent arm than both active and non-active lamprey post exposure (rank sum test P=0.016 and 0.0022, respectively. Post exposure we found no evidence to suggest that active and non-active lamprey behaved differently (Table 2.1) (ranks sum test P= 0.16).

Ripe lamprey and active lamprey did not have significantly different behaviors pre versus post exposure to atrazine (Table 2.2) (P= 0.76 and 0.18 respectively). However, we found suggestive evidence that non-active lamprey behaved differently pre versus post exposure to atrazine (P= 0.063). We found suggestive evidence that ripe lamprey pre-treatment behaved differently than non-active lamprey post treatment (rank sum test P=0.098).

Movement in odorant response by life history stage

Ripe lamprey, post exposure to atrazine, behaved differently than both active and non-active lamprey post exposure (Table 2.2) (rank sum test P =0.019 and 0.038 respectively). We found suggestive evidence that ripe lamprey crossed into the odorant response arms differently pre versus post exposure to atrazine (P =0.099). We found no evidence to suggest that active and non-active lamprey behaved differently in the odorant response (Table 2.2) (P= 0.47 and 0.26, respectively). We found suggestive evidence that ripe lamprey pre-treatment behaved differently than non-active lamprey post treatment (rank sum test P=0.098). We found moderate evidence to suggest that ripe lamprey exposed to atrazine crossed into the arms differently than active lamprey which were not exposed atrazine (rank sum test P=0.075).

Odorant Detection Study

Range in response to necromone

The range of contractions of the branchial basket in response to a conspecific necromone did not increase significantly for untreated lamprey when compared to the range in contractions in response to water (Figure 2.5) (P=0.17). Atrazine treated lamprey had a significantly higher median range in response to necromone than water, 10.5 bpm (range= 5 bpm to 16.5 bpm) (P= 0.014). Lamprey post exposure to atrazine have a suggestion of a significantly higher median range in response to necromone, 5.5 bpm (range= 1.5bpm to 11.5 bpm) than pre exposed lamprey (P=0.076). Lamprey exposed to atrazine had a strongly significant difference in their median range of contractions, 8 bpm higher (range= 3bpm to 14.5 bpm), when compared to their response to water when they were not exposed to atrazine (P=0.0091).

Maximum response to necromone

The maximum number of contractions of the branchial basket in response to a conspecific necromone was not significantly different from their maximum response to well water (Figure 2.6) (P=0.13). After lamprey were exposed to atrazine contractions of the branchial basket in response to necromone increased moderately when compared to their response to water post exposure, with a median difference of 10 bpm (range= 1bpm to 18bpm) (P=0.043). Comparing pre - and post - exposure to atrazine the maximum number of contractions of the branchial basket in response to necromone had a moderately significant difference of 11.5 bpm (range= 2.5bpm to 21

bpm) (P=0.033). When comparing the response of an atrazine - exposed lamprey to necromone versus unexposed lamprey to well water we found strong evidence to suggest a median difference of 15bpm (range= 2.5bpm to28.5bpm) (P= 0.0028).

Contrary to Wagner (2011) we did not find a significant reaction to necromone versus well water. There are multiple possible reasons for this discrepancy. Wagner tested movement across a raceway to avoid necromone with Sea lamprey; the method of detection as well as the species tested are different. Wagner also collected the necromone by extracting the putative alarm signal; we used aliquots of homogenized whole decaying lamprey as our alarm signal. We also exposed our lamprey to a much smaller concentration of necromone than Wagner, 1.5×10^{-5} versus our $4.8 \times 10^{-8.}$ Additionally we allowed 2 hours post handling to conduct our experiment, while Wagner allowed 4 hours, our fish may have been stressed from handling to a greater degree causing a higher ventilation rate over all. We are unconcerned by the discrepancy.

Range in response to adult odor

The range in the maximum contractions of the branchial basket in response to adult odor was not different when compared to their response to water (P= 1.00). Their range in response to adult odors pre - and post - exposure to necromone was also not significantly different (P= 0.23). Exposed lamprey response to adult odors did have a suggestion of a difference in the median range of contractions of the branchial basket when compared to an unexposed lamprey response to well water with a median difference of 3.5 bpm (range=-1 to 7.5bpm) (P=0.076).

Maximum response to adult odor

The maximum number of contractions of the branchial basket in response to adult odor was not different when compared to their response to well water (P= 0.28). Their maximum response to adult odors pre and post exposure to atrazine was also not significantly different (P= 0.15). There was a suggestion of a difference in the median number of contractions of the branchial basket when comparing exposed lamprey response to adult odor and unexposed lamprey response to well water with a median difference of 9.5pbm (range= -1.5bpm to 24bpm) (P= 0.086). The results in reaction to this second odorant could be confounded by the order in which the fish received this odorant. Every fish received adult odor during the second block of odorant presentations. We know from our pilot study that lamprey show a sensory adaptation to a second presentation of odors (See Appendix 2).

Discussion

Pacific lamprey populations are declining throughout their range (Moring and Lantz 1975; Close et al. 2002; Howard and Close 2005; Robinson and Bayer 2005). Attempts at increasing lamprey populations have included closing the commercial lamprey fisheries in the 1940's (Close et al. 1995), stopping the use of lampricides on native populations (Close et al. 1995), and improving dam passage (Moser and Close 2003). Some causal mechanisms for their decline have not been adequately addressed for their effects on lamprey populations, including; loss of spawning and rearing habitats in streams resulting in recruitment failure (Moser et al. 2005), inadequate prey availability in ocean, and effects of pollutants (Close et al 2005).

A large gap in our knowledge of the causal mechanisms for lamprey population declines is water pollution (Close et al. 1995). We know water pollution is possibly a mechanism of lamprey population declines due to the research on water pollutant effects on teleosts, yet it is little studied in lamprey (Close et al. 1995). Our research is unique in that we investigated the behavioral effects of an instream toxicant on lamprey, specifically the effects on behaviors mediated by their most dominant sense: olfaction. We attempted to bridge this gap in knowledge by conducting two assays. First, a behavioral odorant response experiment to determine if lamprey behave differently to relevant odors when treated with atrazine versus a control. Second, an odorant detection study to determine if lamprey have altered gill ventilation rate in response to biologically relevant odorants after being exposed to atrazine. Our experiments showed that adult Pacific lamprey which have been exposed to environmentally realistic concentration of atrazine have altered behaviors and these behaviors vary by life history stage. Further, their affected behavior could support reduced reproductive fitness.

We were able to obtain statistically significant results with our experimental design despite low samples sizes, 25 and 9 lamprey for the two experiments respectively. Pacific lamprey are listed as vulnerable- at risk by the State of Oregon and thus large sample sizes were not possible to obtain. This suggests that larger sample sizes would only further increase the confirmation of alterations in behaviors caused by atrazine. In timberland in Oregon herbicide application tends to occur in the spring along with planting of saplings (Elefritz et al. 2006), which is also the time

when the highest concentrations of herbicides are detected in streams and river (Anderson et al. 1997). At this same time of year multiple lamprey life history stages are actively using the freshwater environment. Adult lamprey are migrating February to September (Close et al. 1995; Keefer et al. 2009; Clemens et al. 2012). Spawning occurs April to June, and eggs hatch approximately 19 days later. Both ammocoetes from previous year classes and adult holdover lamprey (adults which have been in freshwater longer than one year before spawning) are living in freshwater year round (Close et al. 1995; Clemens et al. 2010). All of these life history stages could be affected by the spring flush of herbicides into freshwater.

An elasticity model which incorporates all Pacific lamprey life history stages and the effect that atrazine has on them would be invaluable in determining the most effective time to limit lamprey's contact with atrazine in the environment. For example, finding that limiting lamprey contact with atrazine during the 3 weeks of embryo development could have positive effects on multiple life history stages by reducing exposure to adult migrants, holdover adults, spawning adults and larvae.

While this is the first report on the effects of atrazine in lamprey of which we are aware, there are a few studies on direct toxic effects of atrazine on teleosts. No direct mortality due to contact with atrazine in the environment has been documented in fishes (Rohr and McCoy 2010) However, the physiological effect of atrazine on growth and reproduction in teleosts has been well documented.

A meta-analysis conducted on greater than 125 peer reviewed published papers conducted by Rohr and McCoy (2010) found elevated fish activity, reduced anti-predator behaviors, reduced olfactory abilities, reduction of immune function, an increase in infection end points, alteration of gonadal morphology, altered spermatogenesis and altered sex hormone concentrations, after exposure to atrazine. However, Rohr and McCoy's meta-analysis was written in direct response to Solomon et al.'s (2008) critical review which found no conclusive evidence for causality of reproductive effects of atrazine on fish. This conclusion is based on inconsistencies found in effects reported and inconsistencies between studies conducted in different laboratories. In spite of Solomon et al.'s finding of inconsistencies, there is still strong inference that while atrazine may not be the most problematic causal mechanisms of Pacific lamprey population declines, any or all of the endpoints found by Rohr and McCoy could be contributing towards Pacific lamprey population declines as a result of contact with atrazine in the environment.

The response that lamprey exhibited to atrazine after exposure in our experiments is likely the result of inhibition by atrazine on transduction in the olfactory epithelium, reducing their ability to smell minute concentrations of behaviorally relevant odors. Moore and Wang (1998) found that atrazine was able to inhibit transduction within olfactory receptor cells of Atlantic salmon and effectively prevent males from detecting and responding to pheromones. Additionally Tierney et al. (2007) found that rainbow trout exposed for 30 minute to atrazine at 1 ug/l showed a decrease in electro-olfactory responses to an amino acid and preference behavior towards an

amino acid was also reduced. It is likely that lamprey, which have very similar olfactory structure to teleosts (Laframboise et al. 2007), have a similar toxic mode of action in response to exposure to atrazine.

Conclusion

The results of our study suggest that if lamprey encounter atrazine in the environment they will have altered responses to biologically relevant odors, most likely through inhibition of transduction in the olfactory epithelium. This could reduce their ability to smell minute concentrations of behaviorally relevant odors, altering olfactory-mediated behaviors such as migration and spawning. Lamprey which are migrating towards spawning grounds, or searching for other spawning individuals, may then have altered success at detecting attractants in the water column. For an animal which must obtain all of its nutrients to survive for up to 2 years before spawning, having a hypersensitive reaction or missing a chemical cue could result in the consumption of energy which would otherwise be used in gamete production. If the effect on gamete production occurs population wide, a reduction in population would result. If we are concerned about lamprey populations we must be concerned about their contact with atrazine in the environment.

References:

Anderson C, Wood T, Morace J. 1997. Distribution of dissolved pesticides and other water quality constituents in small streams and their relation to land use, in the Willamette river basin, Oregon, 1996. Portland, OR.

Bjerselius R, Li WM, Teeter JH, Seelye JG, Johnsen PB, Maniak PJ, Grant GC, Polkinghorne CN, Sorensen PW. 2000. Direct behavioral evidence that unique bile acids released by larval sea lamprey (P*etromyzon marinus*) function as a migratory pheromone. Canadian Journal of Fisheries and Aquatic Sciences 57:557-569.

Carpenter KD, Sobieszczyk S, Arnsberg AJ, Rinella FA. 2008. Pesticide occurance and distribution in the lower Clackamas river basin, Oregon 2000-2005. Reston, VA.

Clemens B, Mesa M, Magie R, Young D, Schreck C. 2012. Pre-spawning migration of adult Pacific lamprey, E*ntosphenus tridentatus*, in the Willamette River, Oregon, U.S.A. Environmental Biology of Fishes 93:245-254.

Clemens BJ, Binder TR, Docker MF, Moser ML, Sower SA. 2010. Similarities, differences, and unknowns in biology and management of three parasitic lampreys of North America. Fisheries 35:580-594.

Close D, Fitzpatrick M, Li H, Parker B, Hatch D, James G. 1995. Status report of the Pacific lamprey, *Lampetra tridentata,* in the columbia river basin. Portland, OR: U.S. Department of Energy. Available: https://pisces.bpa.gov/release/documents/documentviewer.aspx?doc=39067-1 [accessed 1 June 2010].

Close DA, Fitzpatrick MS, Li HW. 2002. The ecological and cultural importance of a species at risk of extinction, Pacific lamprey. Fisheries 27:19-25.

Dawson J. 1905. The breathing and feeding mechanism of the lampreys. Biological Bulletin 9:1-21.

Elefritz M, Atkinson M, Fitzgerald S. 2006. The care and planting of tree seedlings on your woodland. Available: http://extension.oregonstate.edu/catalog/pdf/ec/ec1504.pdf [accessed 24 January 2012].

Garono R, Brophy L. 1999. Rock creek (Siletz) watershed assessment final report. Corvallis, OR: Earth Designs consulting Inc. Available: http://www.earthdesign.com/rockcreek/DRAFT.PDF [accessed 22 April 2010].

References (continued)

Garono R, Brophy L. 2001. Midcoast watersheds council sixth field watershed assessment. Corvallis, OR: MidCoast Watersheds Council. Available: http://www.midcoastwatershedscouncil.org/Assessments/MCWC_Assessment.pdf [accessed 22 April 2010].

Gibson AK, Mathis A. 2006. Opercular beat rate for rainbow darters, *Etheostoma caeruleam*, exposed to chemical stimuli from conspecific and heterospecific. Journal of Fish Biology 69:224-232.

Gilliom RJ, Barbash JE, Crawford CG, Hamilton PA, Martin JD, Nakagaki N, Howell LH, Scott JC, Stackelberg PE, Thelin GP and others. 2006. The quality of our nation's waters- pesticides in the nation's streams and ground water, 1992-2001: US Geological Survey circular 1291. Reston, VA: USGS. 172 p.

Harding JS, Benfield EF, Bolstad PV, Helfman GS, Jones EBD. 1998. Stream biodiversity: The ghost of land use past. Proceedings of the National Academy of Sciences of the United States of America 95:14843-14847.

Heath AG. 1995. Water pollution and fish physiology. 2nd edition. Water pollution and fish physiology. 3-9, & 11-13 chpt.

Howard JK, Close DA. 2005. Pacific lamprey research and restoration project. 2003 annual report, project no. 199402600. BPA Report DOE/BP 00005455-7:1-132.

Keefer ML, Moser ML, Boggs CT, Daigle WR, Peery CA. 2009. Variability in migration timing of adult Pacific lamprey (*Lampetra* tridentata) in the columbia river, USA. Environmental Biology of Fishes 85:253-264.

Laframboise AJ, Ren X, Chang S, Dubuc R, Zielinski BS. 2007. Olfactory sensory neurons in the sea lamprey display polymorphisms. Neuroscience Letters 414:277-281.

Moore A, Lower N. 2001. The impact of two pesticides on olfactory-mediated endocrine function in mature male atlantic salmon (S*almo salar* L.) parr. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology 129:269-276.

Moore A, Waring CP. 1998. Mechanistic effects of a triazine pesticide on reproductive endocrine function in mature male atlantic salmon (*Salmo salar* L.) parr. Pesticide Biochemistry and Physiology 62:41-50.

References (continued)

Moring, J.R. Lantz RL. 1975. The Alsea watershed study: effects of logging on aquatic resources of three headwater streams of the Alsea River, Oregon. Part I. Biological studies. Fish. Res. Rep. No. 9. Oregon Department of Fish and Wildlife, Corvallis, OR.

Moser ML, Close DA. 2003. Assessing Pacific lamprey status in the Columbia River basin. Northwest Science 77:116-125.

Moser ML, Zabel RW, Burke BJ, Stuehrenberg LC, Bjornn TC, Spedicato MT, Lembo G, Marmulla G. 2005. Factors affecting adult Pacific lamprey passage rates at hydropower dams: Using "Time to event" Analysis of radiotelemetry data. Aquatic telemetry: advances and applications. Proceedings of the Fifth Conference on fish telemetry held in Europe, Ustica, Italy, 9-13 June 2003.:61-70.

Myllynen K, Ojutkangas E, Nikinmaa M. 1997. River water with high iron concentration and low ph causes mortality of lamprey roe and newly hatched larvae. Ecotoxicology and Environmental Safety 36:43-48.

ODEQ. 2009. Watershed-based toxics monitoring program 2008 progress report: Willamette River basin. Hillsboro, OR: Oregon Department of Environmental Quality Laboratory and Environmental Assessment Division. Available: http://www.deq.state.or.us/lab/wqm/docs/10-LAB-007.pdf [accessed 13 May, 2010].

PURS-Oregon 2007. 2008. Pesticide Use Reporting System. Salem, OR: Oregon Department of Agriculture. Available: http://www.Oregon.gov/ODA/PEST/docs/pdf/pursreportweb2007.pdf [accessed 22 April 2010].

R Development Core Team (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Robinson TC, Bayer JM. 2005. Upstream migration of Pacific lampreys in the John Day River, Oregon: Behavior, timing, and habitat use. Northwest Science 79:106-119.

Rohr JR, McCoy KA. 2010. A qualitative meta-analysis reveals consistent effects of atrazine on freshwater fish and amphibians. Environmental Health Perspectives 118:20-32.

References (continued)

Soimasuo M, Kervinen J, Sinkkonen S, Paasivirta J. 2004. Bioaccumulation of pops from contaminated sediment to lamprey (*Lampetra fluviatilis* L.) larva. Journal of Soils and Sediments 4:75-83.

Solomon KR, Carr JA, Du Preez LH, Giesy JP, Kendall RJ, Smith EE, Van Der Kraak GJ. 2008. Effects of atrazine on fish, amphibians, and aquatic reptiles: A critical review. Critical Reviews in Toxicology 38:721-772.

Tierney KB, Baldwin DH, Hara TJ, Ross PS, Scholz NL, Kennedy CJ. 2010. Olfactory toxicity in fishes. Aquatic Toxicology 96:2-26.

Tierney KB, Singh CR, Ross PS, Kennedy CJ. 2007. Relating olfactory neurotoxicity to altered olfactory-mediated behaviors in rainbow trout exposed to three currently-used pesticides. Aquatic Toxicology 81:55-64.

Tillitt DE, Papoulias DM, Whyte JJ, Richter CA. 2010. Atrazine reduces reproduction in fathead minnow (*Pimephales promelas).* Aquatic Toxicology 99:149-159.

USEPA. 2009. Aquatic life benchmarks for pesticies. Available: http://www.epa.gov/oppefed1/ecorisk_ders/aquatic_life_benchmark.htm [accessed 1 June 2010].

USEPA. 2010. Pesticide reregistration status. Available: http://www.epa.gov/opp00001/reregistration/status.htm [accessed 1 June 2010].

Wagner CM, Stroud EM, Meckley TD. 2011. A deathly odor suggests a new sustainable tool for controlling a costly invasive species. Canadian Journal of Fisheries and Aquatic Sciences 68:1157-1160.

Wentz DA, Bonn BA, Carpenter KD, Hinkle SR, Janet ML, Rinella FA, al. e. 1998. Water quality in the Willamette basin, Oregon 1991-1995. Report nr USGS Circular 1161. Available: http://pubs.usgs.gov/circ/circ1161/circ1161.pdf [accessed 22 April 2010].

Yun SS, Wildbill AJ, Siefkes M J, Moser ML, Dittman AH, Corbett SC, Li W, Close DA. 2011. Identification of putative migratory pheromones from Pacific lamprey (*Lampetra tridentata*). Canadian Journal of Fisheries and Aquatic Sciences 68:2194-2203.

Yun SS, Scott AP, Siefkes MJ, Li WM. 2002. Development and application of an elisa for a sex pheromone released by the male sea lamprey (P*etromyzon marinus* L.). General and Comparative Endocrinology 129:163-170.

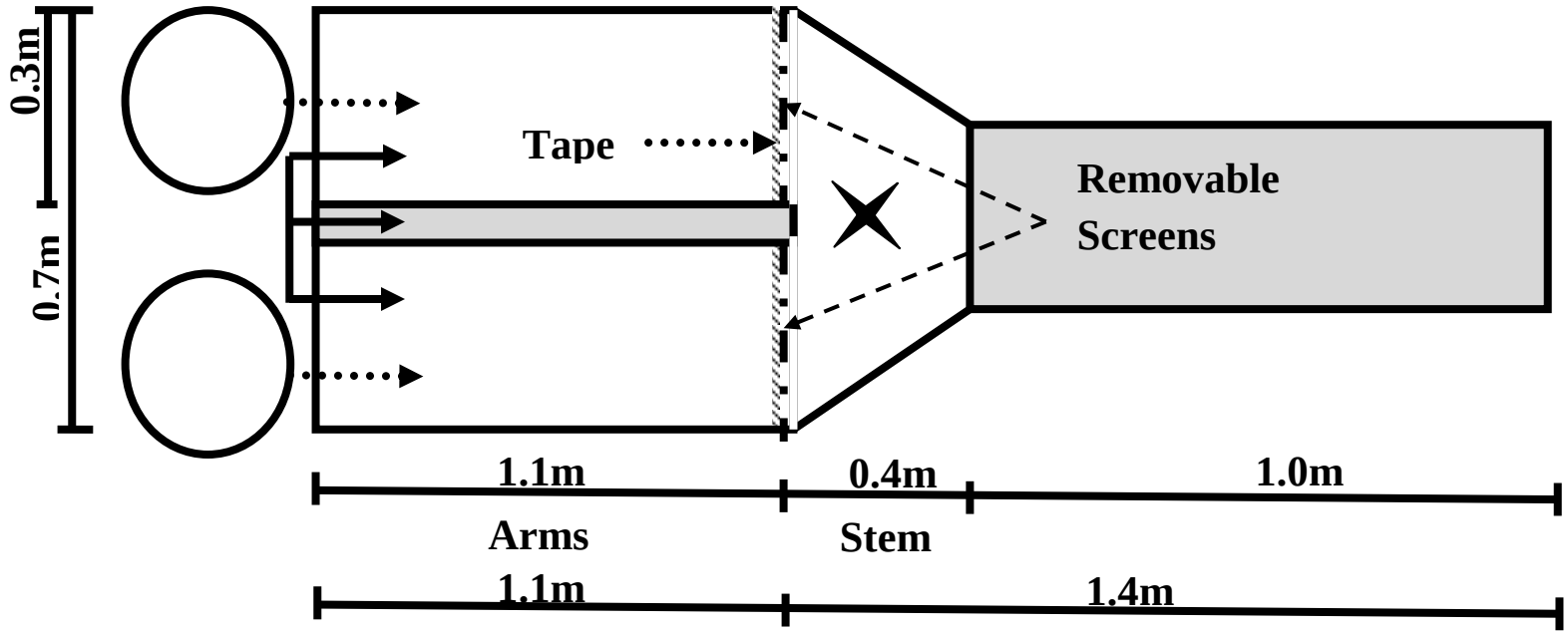

Figure2. 1. Diagram of y-maze used in odorant response study, top view, used to test attraction to effluent placed in one randomly selected arm of the maze. X is the location where lamprey were initally placed in the maze. Dash-dot lines are screens removed after 15 minutes of acclimation. Lamprey have no access to gray area during trials. Circles and dotted arrows indicate placement of marriot syphones and their flow. Solid arrows show inflow of well water into three different chambers in the maze, the center chamber acts to separate flow as they exit the arms.

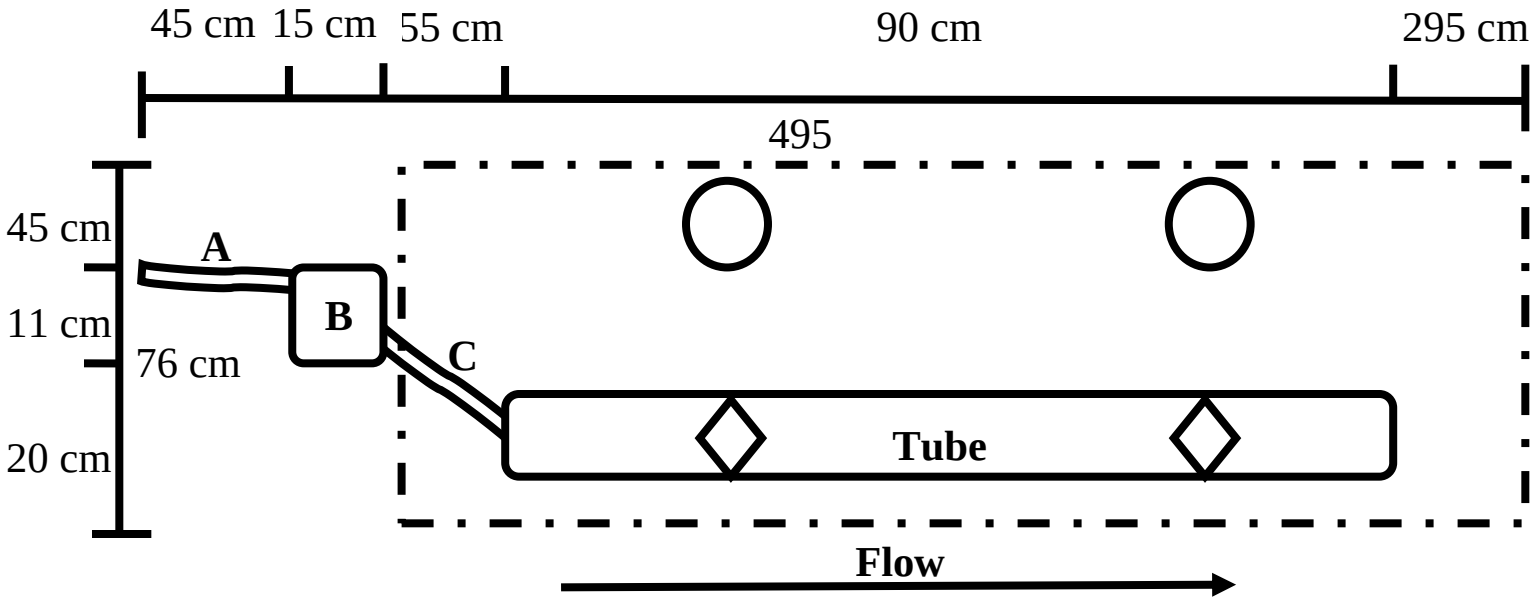

Figure 2.2. Side view of tube used to view lamprey ventilation (not drawn to scale). Lamprey were placed in clear tube and viewed through two movable cameras (diamonds). Tube was covered with black plastic to block lamprey from reacting to external stimuli (dot dash line). Well water flowed (A) into mixing container (B) where odorants were added and then into (C) tube, Circles are two 8 watt LED diffuse red light bulbs showing light greater than 640nm, out of the visual spectrum of lamprey. Lights were used to aid cameras in filming ventilation.

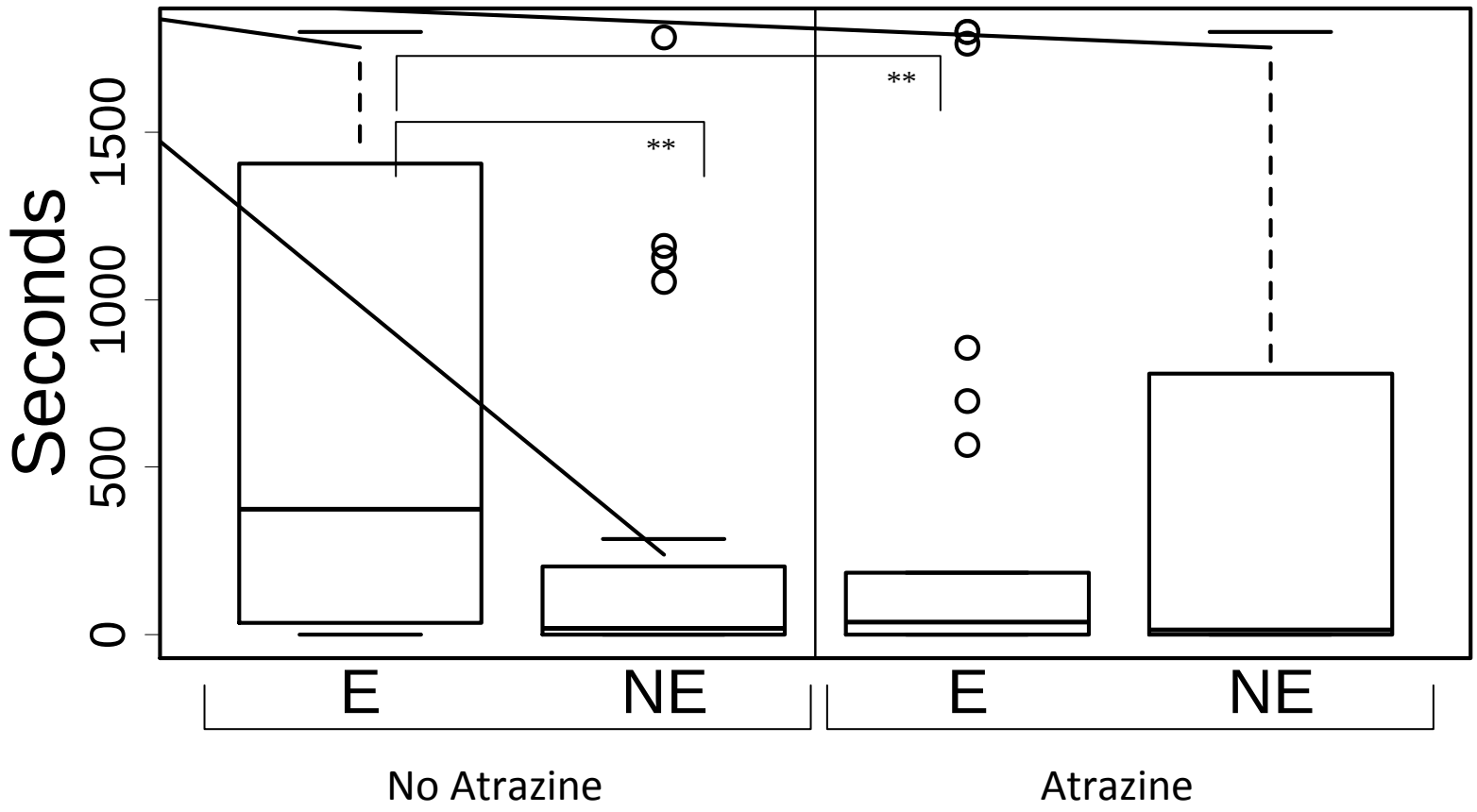

Figure 2.3. Box and whisker plots of the amount of time (1800 seconds total) lamprey spent in arm conditioned with effluent (E), water from adult lamprey holding tank, or in identical arm but uncondictioned with effluent (NE). Lamprey were tested after being treated with 50 ug/l of atrazine for 24 hours (Atrazine) or after being subjected to an identical treatment setup but without atrazine (No Atrazine). Boxes represent the interquartile range (25^{th} to 75^{th} percent). Horizontal line in the middle of the box is the median. Whisker ends indicate 1.5 time the interquartile range. Outliers are represented as empty cicles beyond whiskers. Brackets indicate significant comparisons. ** $P < 0.05$

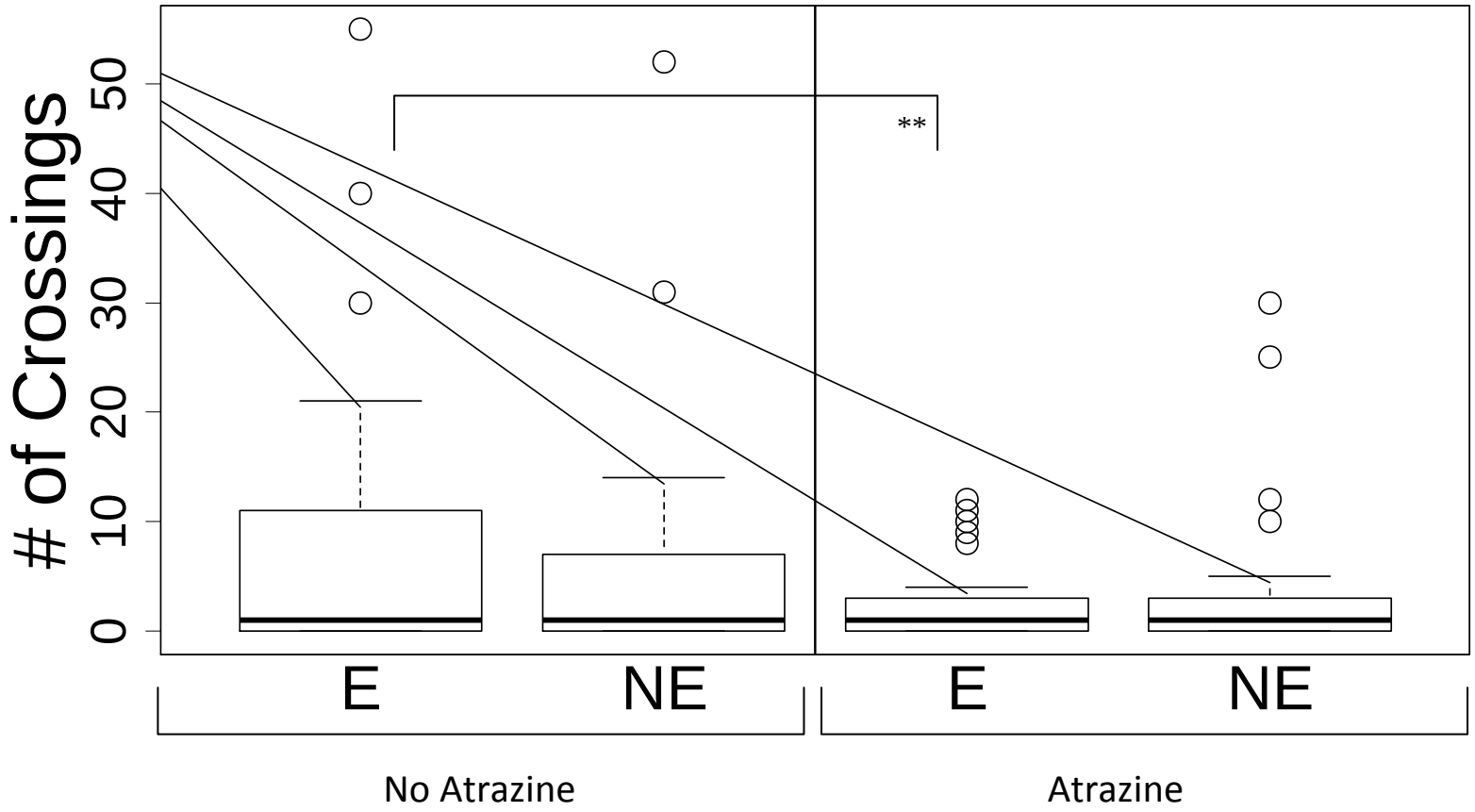

Figure 2.4. Box and whisker plots of the number of times adult lamprey crossed into arm conditioned with effluent (E) (water from adult lamprey holding tanks) or in identical arm but uncondictioned with effluent (NE). Lamprey were tested after being treated with 50 ug/l of atrazine for24 hours (Atrazine) or after being subjected to an identical treatedment setup but without atrazine (No Atrazine). Boxes represent the interquartile range (25^{th} to 75^{th} percent). Horizontal line in the middle of the box is the median. Whisker ends indicate 1.5 time the interquartile range. Outliers are represented as empty circles beyond. Brackets indicate a significant difference. ** $P < 0.05$

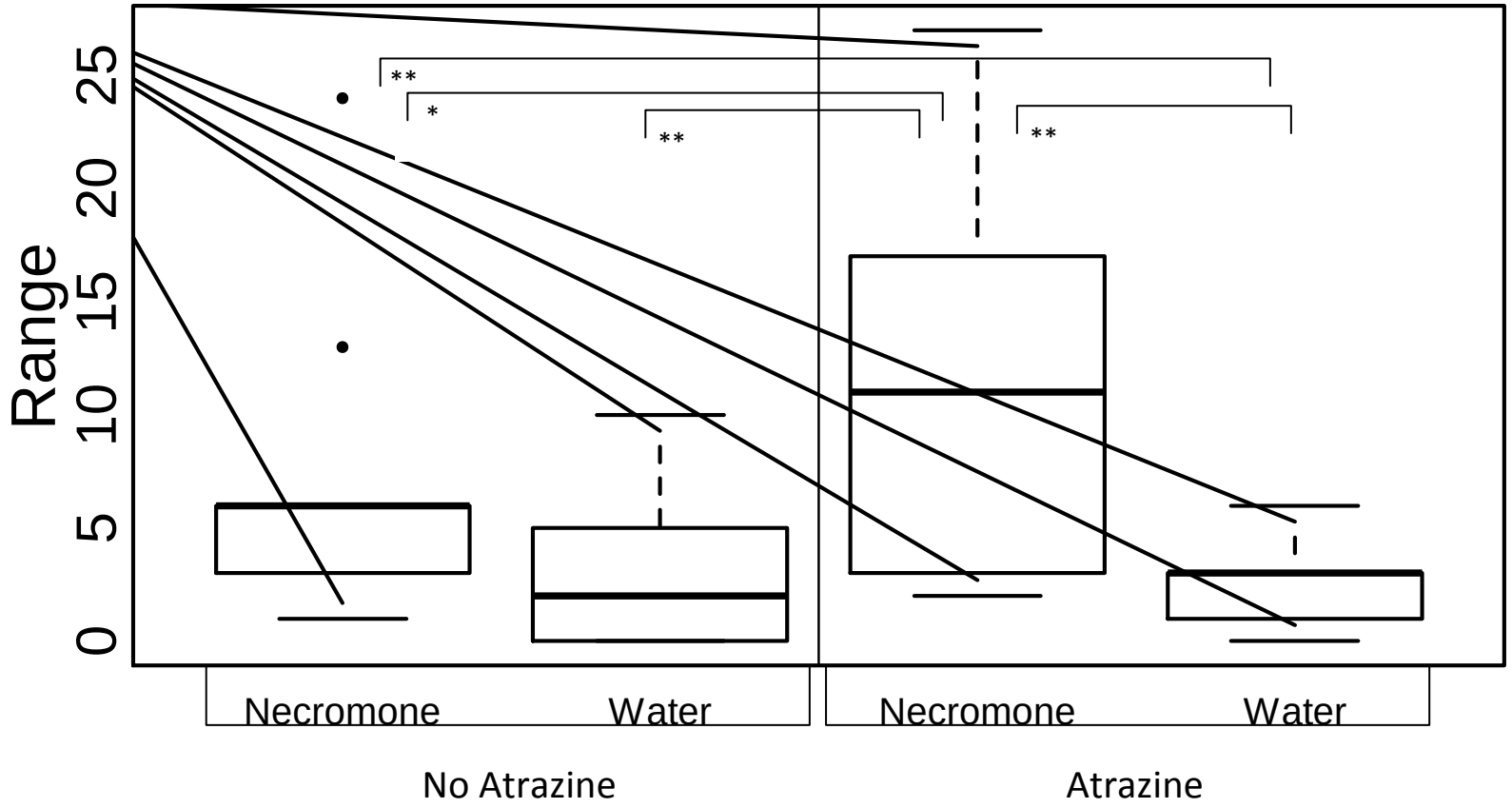

Figure 2.5. Box and whisker plots of the range in ventilation of adult lamprey in reaction to a necromone (a homogenized lamprey that died of natural causes) or well water. Lamprey were tested after being treated with 100 ug/l of atrazine for 24 hours (Atrazine) or after being subjected to an identical treatment setup but without atrazine (No Atrazine). Boxes represent the interquartile range (25^{th} to 75^{th} percent). Horizontal line in the middle of the box is the median. Whisker ends indicate 1.5 time the interquartile range. Outliers are represented as cicles beyond. Brackets indicate a significant difference. **P <0.05, * P<0.1

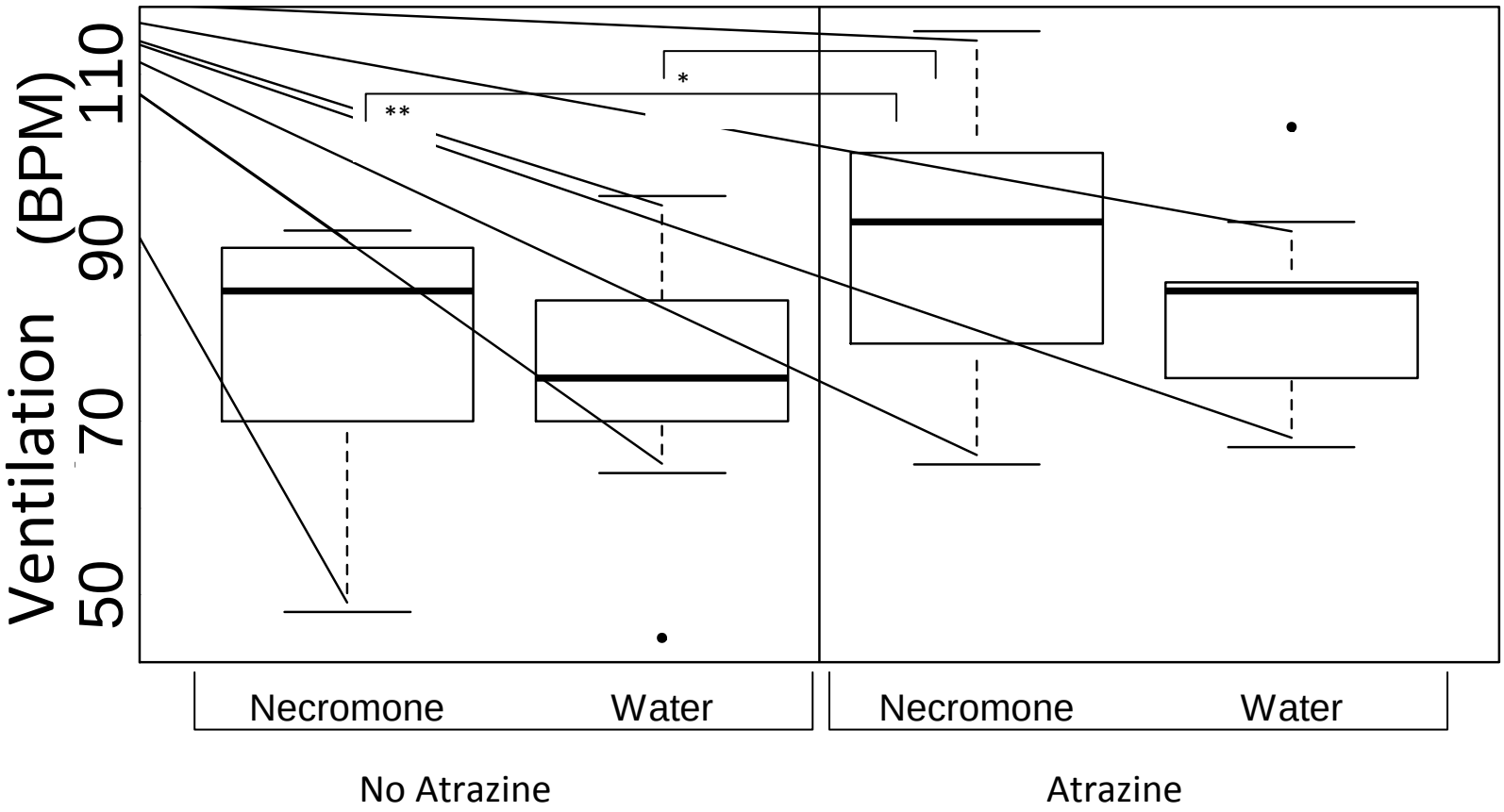

Figure 2.6. Box and whisker plots of the maximum ventilation (beats per minute) of adult lamprey in reaction to a necromone (a homogenized lamprey that died of natural causes) or well water. Lamprey were tested after being treated with 100 ug/l of atrazine for 24 hours (Atrazine) or after being subjected to an identical treatment setup but without atrazine (No Atrazine). Boxes represent the interquartile range (25^{th} to 75^{th} percent). Horizontal line in the middle of the box is the median. Whisker ends indicate 1.5 time the interquartile range. Outliers are represented as cicles beyond.Brackets indicate a significant difference. * P <0.01, **P<0.05

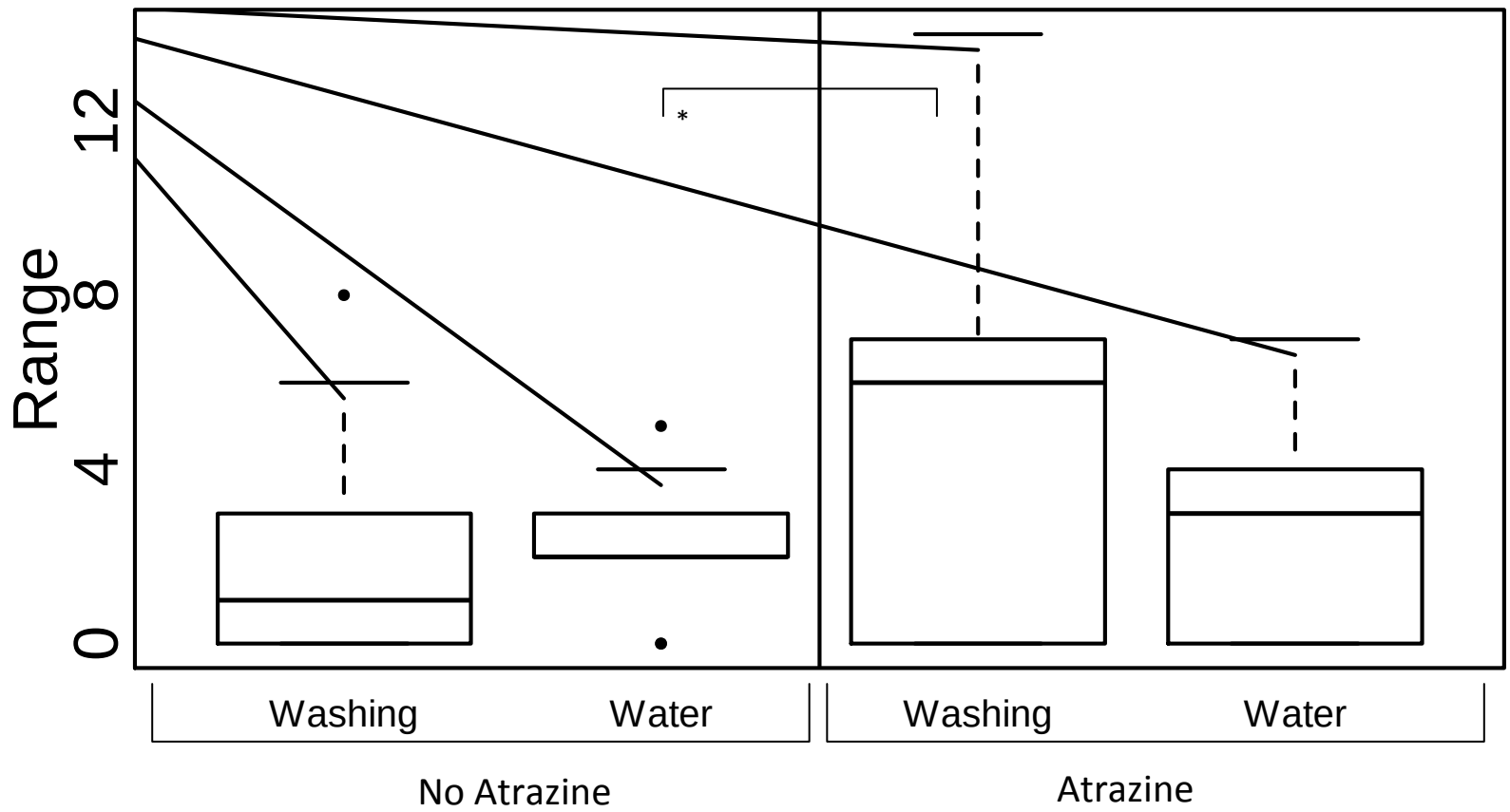

Figure 2.7. Box and whisker plots of the range in ventilation (beats per minute) of adult Pacific lamprey in reaction to adult odor (the resulting water from 4 adult lamprey placed in a static container for 3 hours) or well water. Lamprey were tested after being treated with 100 ug/l of atrazine for 24 hours (Atrazine) or after being subjected to an identical treatment setup but without atrazine (No Atrazine). Boxes represent the interquartile range (25^{th} to 75^{th} percent). Horizontal line in the middle of the box is the median. Whisker ends indicate 1.5 time the interquartile range. Outliers are represented as cicles beyond. Brackets indicate a significant difference. * $P < 0.1$

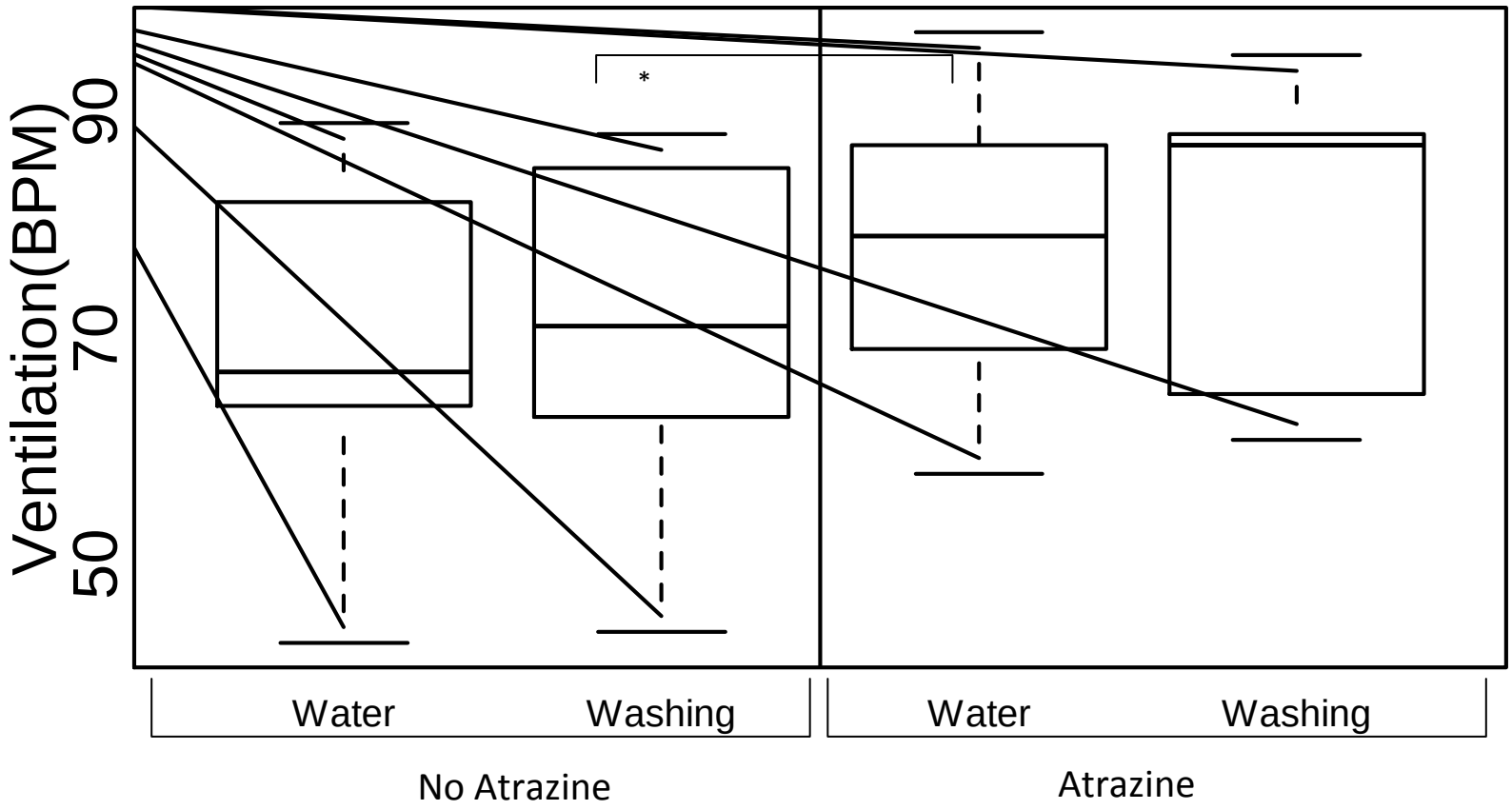

Figure 2.8. Box and whisker plots of the maximum ventilation (beats per minute) of adult lamprey in reaction to adult odor (the resulting water from 4 lamprey placed in a static container for 3 hours) or well water. Lamprey were tested after being treated with 100 ug/l of atrazine for 24 hours (Atrazine) or after being subjected to an identical treatment setup but without atrazine (No Atrazine). Boxes represent the interquartile range (25^{th} to 75^{th} percent). Horizontal line in the middle of the box is the median. Whisker ends indicate 1.5 time the interquartile range. Outliers are represented as empty cicles beyond. Brackets indicate a significant difference. * $P < 0.1$

Table 2.1. Difference in the amount of time adult lamprey spent in arm conditioned with effluent (water from adult lamprey holding tank) minus arm unconditioned with effluent. Negative numbers show a preference for non-pheromone arm. Ripe: Showed obvious sexual characteristics, regardless of sex, and were found actively searching their tank at night. Active: Lamprey actively searching their tank at night but had no sexual chartacteristics. Non-Active: Lamprey that did not search their tank at night and had no sexual characteristics. Groupings are mutually exclusive. Lamprey were tested after being treated with 100 ug/l of atrazine for 24 hours (Atrazine) or after being subjected to an identical treatment but without atrazine (No Atrazine). Within groups each row is one lamprey. Order was randomly assigned and clearance time was allowed between trials. Final number indicates median for column. Brackets indicate signigicant comparisons. * P <0.10, ** P<0.05

Ripe		Active		Non-Active	
No-Atrazine	Atrazine	No-Atrazine	Atrazine	No-Atrazine	Atrazine
71	-1022	0	-1800	1800	-1735
-1784	0	1260	-1570	1800	-1567
-1161	0	-1080	-141	-15	-1287
1757	0	1749	-15	297	-83
1800	0	0	-13	170	-4
1800	0	-864	0		
-1310	64	-138	0		
850	310	1800	0		
0	843	1800	0		
-7	1800	-26	1061		
35.5	32	0	-6.5	297	-1287

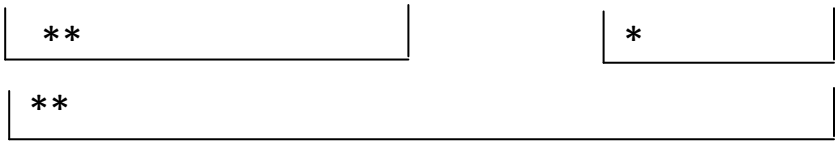

Table 2.2 Difference in the number of times adult lamprey crossed into arm conditioned with effluent (water from adult lamprey holding tank) minus arm unconditioned with effluent. Negative numbers show a preference for non-pheromone arm. Ripe: Showed obvious sexual characteristics, regardless of sex, and were found actively searching their tank at night. Active: Lamprey actively searching their tank at night but had no sexual chartacteristics. Non-Active: Lamprey that did not search their tank at night and had no sexual characteristics. Groupings are mutually exclusive. Lamprey were tested after being treated with 100 ug/l of atrazine for 24 hours (Atrazine) or after being subjected to an identical treatment but without atrazine (No Atrazine). Within groups each row is one lamprey. Order was randomly assigned and clearance time was allowed between trials. Final number indicates median for column. Brackets indicate signigicant comparisons.* P <0.10, ** P<0.05

Ripe		Active		Non-Active	
No-Atrazine	Atrazine	No-Atrazine	Atrazine	No-Atrazine	Atrazine
-1	0	-47	-20	-1	-2
-1	0	1	-13	-1	-1
0	0	-3	-3	12	-1
1	0	0	-1	17	-1
1	0	0	-1	1	2
32	0	-20	0		
-1	1	-3	0		
0	1	1	0		
1	1	1	0		
19	7	54	0		
0.5	0	0	-0.5	1	-1

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Chapter 3. Conclusions

We were interested in finding out if herbicides used to manage forestland, specifically atrazine, could be influencing population declines of Pacific lamprey (*Entosphenus tridentatus*). To better understand this we conducted an odorant response experiment and odorant detection experiment.

Our odorant response experiment showed that adult Pacific lamprey which have been exposed to environmentally realistic concentrations of atrazine have altered behaviors. We conducted a y-maze experiment where lamprey were given two arms to chose from, one with well water scented with effluent from their holding tanks and the other a well water control. We found that lamprey exposed to atrazine had a depressed response to the effluent which was likely a spawning or migratory pheromone. Their preference for the effluent arm was abolished after exposure to atrazine. They spent significantly less time in the effluent arm after exposure and showed no preference for it versus the control arm. Their activity level was also altered, although they showed no preference in the number of times the crossed into the effluent arm versus the control arm prior to being exposed to atrazine, afterwards the number of times they crossed into the effluent arm was significantly reduced. We also found activity level post exposure to atrazine differed between adult life history stages, something which was not significantly different during control trials.

Our odorant detection experiment showed that adult Pacific lamprey which have been exposed to environmentally realistic concentrations of atrazine have a hypersensitive response to biologically significant odorants. Lamprey exposed to

atrazine had a significantly higher ventilation rate in response to a necromone from conspecifics, when compared to controls. They also had a slight increase in ventilation to a conspecific odor obtained from other adults, likely a pheromone.

The response which lamprey exhibited to atrazine after exposure is likely the result of inhibition by atrazine on transduction in the olfactory epithelium, reducing their ability to smell minute concentrations of behaviorally relevant odors. Moore and Wang (1998) found that atrazine was able to inhibit transduction within olfactory receptor cells of Atlantic salmon and effectively prevent males from detecting and responding to pheromones. Additionally Tierney et al. (2007) found that only a 30 minute exposure to 1 ug/l of atrazine effectively reduced rainbow trout's elecotro-olfactory response to an amino acid and reduced their preference behavior towards the amino acid as well. It is likely that lamprey, which have very similar olfactory structure as teleosts (Laframboise et al. 2007), would also have a similar toxic mode of action in reaction to atrazine in the environment.

Forestry Best Management Practices

Best management practices are tools which are practical and effective at reducing non-point source pollution to standards compatible with a set goal. The goal of this research was to increase lamprey populations by modifying current forest management practices. With this in mind the following is an explanation of the development of three best management practices, provided below, which would be invaluable in aiding the goal of increasing lamprey populations.

Limiting exposure to atrazine in the environment would reduce both behavioral and physiological effects that atrazine could potentially have on lamprey populations. We are specifically interested in how to effectively manage forests, while limiting negative effects that these management strategies may have on lamprey populations. On forested land atrazine is used to control competing vegetation during the first several years of sapling growth, usually starting with an application pre - planting. It has been demonstrated in numerous studies (Harrington et al. 1995; Stein 1999; Ares et al. 2007; Maguire et al. 2009) the importance of removal of competing vegetation to increase the yield of Douglas fir (*Psuedotsuga menziesii)*, the most commonly harvested tree species in Oregon (ODF 2010). The effectiveness of different removal strategies is of utmost importance to forest managers tasked with creating the largest harvestable trees possible in 40 - 65 years, while only actively managing the trees during the first 5 years of growth (Haynes et al. 2003).

Several studies have looked at the method of removal of competing vegetation surrounding Douglas fir saplings and the effect that it had on tree volume. Maguire et al. (2009) found that of five different herbicide regimes tested five consecutive years of application, the most aggressive, yielded an increase in tree volume equivalent to 1.1 years of tree growth compared to controls. However, Stein et al. (1999) found a four-fold increase in tree volume after one manual cutting when compared to controls. Stein et al. also found it to be the most cost effective method, even more so than herbicide application. Yuldez et al. (2011) found that the benefits from manually removing 50% of the shrubs surrounding saplings of manual vegetation removal were

still effective 14 years later. They also found that 50% removal of only shrubs had the same effect on tree volume at year 14 as heavier manual removal methods. These studies suggest that not only is manual removal effective at increasing Douglas fir growth but it is also more cost effective when compared to herbicide application.

Aerial application of herbicides on forested land

Aerial application is the most common form of herbicide application on forested land, though stem injection, and manual spot-spraying are used as well with little risk to non-target species (Shepard et al. 2004). The obvious benefits from removing aerial spraying of herbicides include reducing exposure to non-target areas and species, such as: small streams, wildlife, songbirds, insects, native understory plants and of course lamprey.

The not so obvious harm that can occur from exposure to aerially applied herbicides include: reduction of soil acidification caused by biomass export, disruption of nutrient cycles (Flueck and Smith-Flueck 2006), altered plant species composition and diversity, changes in spatial interspersion of habitat types, reduced yield in crop species, alterations in soil microorganisms abundance and diversity, diet shifts in mammals which result in decreased survival and lower reproductive output (Freemark and Boutin 1995) and reduced movement of fish in streams (Davies et al. 1994).

The negative effects of aerially spraying can have ramifications across trophic levels and between biotic and abiotic interactions. Shifting biomass export and disrupting nutrient cycles can affect food availability to ammocoetes (Denoyelles et al. 1982; Munoz et al. 2001). Altering plant species composition can alter channel

morphology, degrade aquatic habitat and cause deteriorated water quality (Bunn et al. 1999).

Recommendation

The following best management practices are suggested to be incorporated into forest management plans:

1. Where herbicides are necessary to manage timber land, use stem injection, and spot spraying as first choice for application.
2. Reduce the number of aerial applications of atrazine, amount of reduction and timeline for reduction should be determined by forest managers with a goal of complete remove of aerial application within a determined time frame.
3. Removal of 50% of shrubby/ woody competition vegetation surrounding each sapling by hand. Implementation as a primary method of removal of competing vegetation will be within the same time frame as best management practices #2.

Conclusion

The results of our study suggest that if lamprey encounter atrazine in the environment they will have altered responses to biologically relevant odors, most likely through inhibition of transduction in the olfactory epithelium. This will reduce their ability to smell minute concentrations of behaviorally relevant odors, altering olfactory-mediated behaviors such as migration and spawning. Lamprey which are

migrating towards spawning grounds, or searching for other spawning individuals, may have altered success at detecting attractants in the water column. For a lamprey that must obtain all of its nutrients to survive for up to 2 years before spawning, having a hypersensitive reaction or missing a chemical cue could result in the consumption of energy which would otherwise be used in gamete production. If the effect on gamete production occurs population wide, a reduction in population would result. If we are concerned about lamprey populations we must be concerned about their contact with atrazine in the environment.

References

Ares A, Terry T, Harrington C, Devine W, Peter D, Bailey J. 2007. Biomass removal, soil compaction, and vegetation control effects on five-year growth of Douglas fir in coastal Washington. Forest Science 53:600-610.

Bunn SE, Davies PM, Mosisch TD. 1999. Ecosystem measures of river health and their response to riparian and catchment degradation. Freshwater Biology 41:333-345.

Davies PE, Cook LSJ, Barton JL. 1994. Triazine herbicide contamination of tasmanian streams - sources, concentrations and effects on biota. Australian Journal of Marine and Freshwater Research 45:209-226.

Denoyelles F, Kettle WD, Sinn DE. 1982. The responses of plankton communities in experimental ponds to atrazine, the most heavily used pesticide in the United States. Ecology 63:1285-1293.

Flueck WT, Smith-Flueck JAM. 2006. Herbicides and forest biodiversity: An alternative perspective. Wildlife Society Bulletin 34:1472-1478.

Freemark K, Boutin C. 1995. Impacts of agricultural herbicide use on terrestrial wildlife in temperate landscapes - a review with special reference to North America. Agriculture Ecosystems & Environment 52:67-91.

Harrington TB, Wagner RG, Radosevich SR, Walstad JD. 1995. Interspecific competition and herbicide injury influence 10-year responses of coastal Douglas fir and associated vegetation to release treatments. Forest Ecology and Management 76:55-67.

Haynes R, Adams D, Mills J. 2003. Contemporary management regimes in the pacific northwest: Balancing biophysical and economic concerns. In: Monserud R, Haynes R, Johnson A, editors. Compatible forest management. Norwell, MA: Kluwer Academic Publishers.

Laframboise AJ, Ren X, Chang S, Dubuc R, Zielinski BS. 2007. Olfactory sensory neurons in the sea lamprey display polymorphisms. Neuroscience Letters 414:277-281.

Maguire DA, Mainwaring DB, Rose R, Garber SM, Dinger EJ. 2009. Response of coastal Douglas fir and competing vegetation to repeated and delayed weed control treatments during early plantation development. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 39:1208-1219.

Moore A, Waring CP. 1998. Mechanistic effects of a triazine pesticide on reproductive endocrine function in mature male atlantic salmon (*Salmo salar* L.) parr. Pesticide Biochemistry and Physiology 62:41-50.

References (continued)

Munoz I, Real M, Guasch H, Navarro E, Sabater S. 2001. Effects of atrazine on periphyton under grazing pressure. Aquatic Toxicology 55:239-249.

ODF.2010. 25-Year Harvest History Data [1986-2010] (xls): Annual Timber Harvest. Oregon Department of Forestry. Available: http://www.oregon.gov/ODF/STATE_FORESTS/FRP/Charts.shtml [accessed 24 January 2012].

Shepard JP, Creighton J, Duzan H. 2004. Forestry herbicides in the United States: An overview. Wildlife Society Bulletin 32:1020-1027.

Stein WI. 1999. Six-year growth of Douglas fir saplings after manual or herbicide release from coastal shrub competition. PNW-RP-500. USDA Forest Service Pacific Northwest Research Station Research Paper. Available: http://www.fs.fed.us/pnw/pubs/pnw_rp500.pdf [accessed 24 January 2012].

Tierney KB, Singh CR, Ross PS, Kennedy CJ. 2007. Relating olfactory neurotoxicity to altered olfactory-mediated behaviors in rainbow trout exposed to three currently-used pesticides. Aquatic Toxicology 81:55-64.

USEPA. 2010. Pesticide reregistration status. Available: http://www.epa.gov/opp00001/reregistration/status.htm [accessed 1 June 2010].

Yildiz O, Cromack K, Radosevich SR, Martinez-Ghersa MA, Baham JE. 2011. Comparison of 5th-and 14th-year Douglas fir and understory vegetation responses to selective vegetation removal. Forest Ecology and Management 262:586-597.

APPENDICES

Appendix 1: Risk Assessment-Which herbicides may have a negative effect on Pacific lamprey (*Entosphenus tridentatus*) olfaction?

Introduction

The Confederated Tribe of Siletz Indians (CTSI) who live and own timber property in the Siletz Basin, OR are interested in researching causes for Pacific lamprey (*Entosphenus tridentatus*) population declines. In addition to the tribes forestland, the basin is more than 90% forested, which is largely managed for timber products by both private industry and the state and federal governments (Siletz basin: 2000). The CTSI received a grant to look at the effects of herbicides on Pacific lamprey's ability to migrate and spawn (Van de Wetering, S., Oregon State University, 2011, personal communication).

The main goal for this research project is to determine if herbicides used in forestry management are a causal factor in lamprey population declines. In order to begin this study, a risk assessment was done to determine which herbicide would be used in the research study. This determination was based on a water quality report review of herbicides detected in Oregon waters, as well as on personal communication with property owners in the Siletz basin, and databases such as those provided by the National Pesticide Information Center (EXTOXNET 2010), International Union of Pure and Applied Chemistry (IUPAC 2010), and the U.S. Environmental Protection Agency's (USEPA) pesticide reregistration status (USEPA 2010)

Methods

For this risk assessment we assume that environmental conditions, such as water temperature or pH of the lamprey intestines, are similar and that the nature of the organism at the individual level is also similar across the population we are interested in. Thus we will only be looking at the qualities of the contaminant to determine if the herbicide can negatively affect lamprey.

Due to lack of data many of the characteristics gathered had to be substituted with attributes for a teleost. These "surrogate species" are the best options for estimating whether an herbicide will also have an effect on lamprey.

Ten reports and two emails on water quality from reference sites in Oregon were reviewed for contaminants from 1997 to 2010 (Table 4.1). Percent detection data were taken from six of these reports as they were the only ones that listed this information. The other four reports and two emails were used for supportive information. The two emails were exchanged with forest hydrologists from two timber companies in the Siletz basin.

Carpenter et al. (2008) compiled data from four different USGS studies which were conducted between 2000 and 2005 in the Clackamas River Basin. The studies were done on different types of water, i.e. source water for drinking vs. storm water, as well as different locations within the basin. The data sets from these four studies were treated as independent data sets, bringing the total data sets to be reviewed to nine (Table 4.1).

In order to select the herbicide to be used in this study we looked at both quantitative and qualitative features. We compared indicators of the potential for sub-lethal effects on Pacific lamprey. They indicators are: a calculated risk quotient, registered use site includes forestry, octanol water partitioning coefficient (K_{ow}), water solubility (S_w), significant findings in other species of fish, target organs, and half life.

The herbicides of interest for this study were calculated by first averaging percent detections across the nine data sets for all 68 herbicides detected and then weighting the resulting percentages by the number of studies which detected the compound. The top 10 herbicides based on this weighted percent detection were then reduced further to only those whose USEPA registered sites included forestry (Table 4.2).

From this reduced list we calculated a unitless number called a risk quotient (RQ) for each herbicide. A RQ is the maximum detected concentration for each herbicide, divided by the USEPA's (US Environmental Protection Agency) NOAEC (no observable adverse effect concentration) for freshwater fish (Table 4.2) for that herbicide. According to USEPA, anything with an RQ greater than 1 is something which warrants further investigation for exposure (USEPA 2010). We reduced the list of herbicides again down to the four highest RQ's and used the other indicators of the potential of sub-lethal effects to pick which herbicide is of greatest concern for our research study.

Indicators of the potential for sub-lethal effect

The octanol-water partitioning coefficient (K_{ow}) is a test of how much of a contaminant is no longer in solution after having been mixed with a known amount of n-octanol, an alcohol, and is reported on a log scale. N-octanol acts as a surrogate for organic matter and shows the ability of the contaminant to bind to organic matter. This has implications for a contaminants capacity to bind to fat molecules inside the body of an organism, e.g., to be lipophilic (Newman 2010).

Water solubility (S_w) is the maximum amount of a substance that can be dissolved in water at equilibrium. It is used to determine the fate of a contaminant, e.g., whether it has the potential to leach into the ground water, be carried away by surface water, or stay in the soil (Newman 2010).

Target organs are the areas inside an organism where a contaminant tends to concentrate. Half - life is the amount of time needed for a chemical concentration to decay by fifty percent.

Herbicides of Concern

In the 10 reports and two emails listed in Table 4.1 we found 68 unique herbicides. After reducing the herbicides to the top 10 by weighted percent detections, this list was further reduced to only the seven herbicides that were registered for use on forested sites (atrazine, 2,4-D, triclopyr, glyphosate, simazine, deethylatrazine, and metalochlor). We then calculated the RQ's and the four highest, atrazine, 2,4-D, triclopyr, and glyphosate, were further investigated the indicators of potential sub-lethal effects.

Atrazine

Atrazine is a triazine herbicide registered for use in forestry, food crops and right of way areas. It is currently the second most widely used pesticide in North America and is used to control broadleaf and grassy weeds. Atrazine had the highest calculated risk quotient in this assessment, 1.38. It has a low solubility (S_w) of 30 mg/l, suggesting that it does not break down readily and it tends to persist in the environment (half life 742 days aquatic, 146 days terrestrial). Its moderate K_{ow}, 2.75, suggests there is some potential for it to bioaccumulate in fish. Once inside an organism it tends to target the lungs, kidneys, liver, spleen, brain, and heart (Gilliom et al. 2006). Atrazine is considered slightly to moderately toxic to fish by the USEPA (96-hour LC_{50} 4500 ug/l)(USEPA 2010).

Atrazine has been shown to change enzyme levels and testosterone binding sites in the testes of fish. It has also been shown to significantly reduce male response to female priming pheromones as well as cause reduced olfactory sensitivity and migratory responses (Tierney et al. 2008; USEPA 2010).

Takacs et al. (2002) summarized findings that atrazine causes sublethal physiological effects to several fish species at concentrations between 5 and 40 ug/l including kidney damage, liver damage, and metabolic and hormonal alterations. Moor and Lower (2001) showed that male Atlantic salmon parr which were exposed for 5 days to atrazine (0.04 ug/l) had a significantly reduced response to female priming pheromones. It also caused a significant effect on migratory activity and

olfactory sensitivity in salmon smolts, as well as inhibited the secretion of testosterone in the testes (Moore and Waring 1998).

Due to its persistence and mobility in the environment, its ability to bioaccumulate and the references showing atrazine can have an effect on olfaction, testosterone secretion, and migration, atrazine is considered of high importance for our study.

2, 4-D

With over 660 products registered for use in the United States 2, 4-D (2, 4-Dichlorophenoxyacetic acid) is the third most common herbicide used in North America (USEPA 2010). It is mainly used as a post-emergence herbicide for controlling monocots in food crops. 2, 4-D had the second highest calculated risk quotient in this study, 0.70. It has a high solubility, 890 moles/l, thus it easily disperses throughout the environment. Its moderate K_{ow}, 2.81, makes it likely that it will bioaccumulate in fish. 2, 4-D tends to target the liver. It has a very long half life (>732 days aquatic, 47 days terrestrial) so the potential for chronic exposure is high(Gilliom et al. 2006). 2,4-D is considered non-toxic to moderately toxic to freshwater fish by the USEPA (96-hour LC_{50}, Ester- 2.09 mg/l, Salt 384 mg/l)(USEPA 2010).

In bleak, *Alburnu alburnus*, 2, 4-D has been found to cause high egg mortality, slow embryogenesis, and halt the morula to gastrula stage of embryonic development. Those eggs which did survive to the larval stage showed malformations and reduced mobility at 100mg/l, the LC_{50} for freshwater fish. At exposure concentrations of 800mg/l the embryos were immobile (Biro 1979). Larvae were also highly susceptible

to 2,4-D in studies at concentrations of 400mg/l and up; no larvae survived to the 48-hour end point (WHO 2009)

A chronic study of 86 days with 2,4-D ester found that mortality rates from alevin stage to fry stage was 47.6% greater for exposures of 118 ug/l in Chinook salmon (*Oncorhynchus tshawytscha*). They also found that exposed Chinook were significantly smaller in size (WHO 2009). In a separate study done with rainbow trout, *Oncorhynchus mykiss*, there was a decrease of more than 50% in their ability to swim upstream in flowing water, after being exposed to 2,4-D for 24 hours at concentrations greater than 7mg/l (NOAEC 14.2mg/l) (WHO 2009).

Due to 2, 4-D's persistence in the environment, its potential to be highly toxic comparably high risk quotient, long half life and the references showing reduced development, high mortality rates in fry and reduced ability to swim upstream, 2, 4-D is considered of high importance to our study.

Triclopyr

Triclopyr is a selective herbicide used to control woody and herbaceous weeds. It enters the plant through actively growing surfaces such as green bark, leaves, roots and cut surfaces. Triclopyr can be purchased in two different formulations, either as an ester or a salt. Triclopyr ester will break down to triclopyr acid in roughly 12 hours and triclopyr salt will break down to triclopyr avid in roughly 1 minute. Thus triclopyr acid is the dominant chemical found in the environment. We will only analyze triclopyr acid and will refer to it as triclopyr throughout this assessment.

Triclopyr had the third highest calculated risk quotient in this assessment, 0.058. It degrades slowly in water with an average half - life of 30 day and does not tend to bioaccumulate in fish (K_{ow}=0.63). Based on these indicators, which suggest it will not have a sub-lethal effect, triclopyr is of low interest to our study. Triclopyr is considered practically non-toxic by the USEPA to freshwater fish, the 96-hour LC50 for is 117 ug/l(USEPA 2010)

Glyphosate

Glyphosate is a broad spectrum herbicide used to control broadleaf weeds and grasses in numerous types of crops. It is one of most widely used pesticides by volume in the world (USEPA 2010). One feature unique to glyphosate is its strong adsorption, an estimated 24,000 ug/g, which is orders of magnitude larger than most other herbicides. It will adhere to mineral, organic and clay soils alike, making it very immobile compound despite having high solubility (12,000 mg/l). It has an extremely low (K_{ow}) of (-3.20) so it does not tend to bioaccumulate in fish. It tends persist in the soil, having a half life of 70 days aquatic, 47 days terrestrial. Glyphosate is considered practically non-toxic by the USEPA to freshwater fish, the 96-hour LC50 for is 86 mg/l(USEPA 2010)

Studies have shown that the surfactants added to glyphosate products are possibly potent compounds (Takacs et al. 2002). Glyphosate by itself, is not rated as toxic to aquatic life by the USEPA, but some products contain harmful surfactants which are rated as toxic. One of these is nonylphenoxy polyethoxylates found in Roundup Pro,

it breaks down into nonylphenol. Nonylphenol is a known potent estrogenic compound (Takacs et al. 2002).

The risk quotient calculated in this study was only 0.025, this by itself would tell us that glyphosate may not warrant further investigation. Although, due to the known potency of some surfactants and its ability to stay in the environment for long periods of time glyphosate is considered of moderate importance to our study.

Conclusion

We conducted a review of water quality reports in Oregon between 1997 and 2010 and found four herbicides worthy of further investigation for their potential to be a causal factor in Pacific lamprey population declines. We determined that atrazine will be the first herbicide used in our study due to its persistence and mobility in the environment, its ability to bioaccumulate, and research that shows that atrazine can affect olfaction, testosterone secretion, and migration in teleosts. As time and money allow the remaining herbicides will be tested in the following order: 2, 4-D, triclopyr, and glyphosate (Table 4.3).

References

Biro P. 1979. Acute effects of the sodium salt of 2,4-d on the early developmental stages of bleak, a*lburnus alburnus*. Journal of Fish Biology 14:101-109.

EXTOXNET. 2010. Pesticide information profiles (pips). Corvallis, OR: Oregon State University. Available: http://extoxnet.orst.edu/pips/ghindex.html [accessed 1 June 2010].

Gilliom RJ, Barbash JE, Crawford CG, Hamilton PA, Martin JD, Nakagaki N, Howell LH, Scott JC, Stackelberg PE, Thelin GP and others. 2006. The quality of our nation's waters- pesticides in the nation's streams and ground water, 1992-2001: US Geological Survey circular 1291. Reston, VA: USGS. 172 p.

IUPAC. 2010. Global availability of information on agrochemicals- pesticide properties database: A to Z list of pesticide active ingredients. Pesticide Information Database. Available: http://sitem.herts.ac.uk/aeru/iupac/index.htm [accessed 1 June 2010.]

Moore A, Lower N. 2001. The impact of two pesticides on olfactory-mediated endocrine function in mature male atlantic salmon (S*almo salar* L.) parr. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology 129:269-276.

Moore A, Waring CP. 1998. Mechanistic effects of a triazine pesticide on reproductive endocrine function in mature male atlantic salmon (*Salmo salar* L.) parr. Pesticide Biochemistry and Physiology 62:41-50.

Newman MC. 2010. Fundamentals of ecotoxicology. Boca Raton, FL: CRC Press Taylor & Francis Group. 541 p.

2000. Siletz basin: Dlcd generalize land use zoning. Corvallis, OR: Wetland & Watershed Assessment Group. Green Point Consultants, Inc. Available: http://www.midcoastwatershedscouncil.org/Assessments/2000/report/figures/ownership_Siletz.jpg [accessed 22 April 2010]

Takacs P, Martin PA, J. S. 2002. Pesticides in ontario: A critical assessment of potential toxicity of agricultural products to wildlife, with consideration for endocrine disruption. Technical Report Series Number 369. Available: http://publications.gc.ca/collections/Collection/CW69-5-369E.pdf [accessed 1 June 2010].

Tierney KB, Sampson JL, Ross PS, Sekela MA, Kennedy CJ. 2008. Salmon olfaction is impaired by an environmentally realistic pesticide mixture. Environmental Science & Technology 42:4996-5001.

References (continued)

USEPA. 2010. Pesticide reregistration status. Available: http://www.epa.gov/opp00001/reregistration/status.htm [accessed 1 June 2010].

WHO. 2009. 2,4-dichlorophenoxyacetic acid (2,4-d)- environmental aspects. In: INCHEM I, editor. Environmental Health Criteria Monographs (EHCs): World Health Organization (WHO).

Table 4.1 Water quality reports and emails from 1997 to 2010 containing information on water contaminants in the state or Oregon. Detection rates: paper lists detection concentration not just occurrences that can be used in calculating risk quotient. Summary: Summary of paper findings. Region: location in Oregon that paper focused on. Emails exchanged with representative from private timber companies in the Siletz Basin *Forest Capital and ^Plum Creek.

Water Quality Report	Detection Rates	Summary	Region
(USEPA 2009)		DDT, DDE, Mercury, and PCB's are pesticides of interest detected.	Columbia River, OR
(Agriculture 2008)	X	Herbicides listed broken down by region or major land use type.	Oregon State
(Agriculture 2009)	X	Herbicides listed broken down by region or major land use type.	Oregon State
(Anderson et al. 1997)	X	Found 36 pesticides detected basin wide and 5 in 99% of the samples	Willamette Basin, OR
(Gilliom and Hamilton 2006)		Found that 90% of water samples had more than 1 pesticide in them and 20% more than 10 pesticides	United States
(Carpenter et al. 2008)	X	Herbicide concentrations detected in four studies.	Clackamas River, OR
(Wentz et al. 1998)	X	Listed pesticides detected, was able to tease out herbicides	Willamette Basin, OR
(ODEQ, 2009)	X	Listed herbicide concentrations	Willamette Basin, OR
(Garono and Brophy 2001)		Describes Water Quality information for comparable stream	Alsea River, OR
(Garono and Brophy 1999)		Necessity of monitoring for non-point pollution	Rock Creek (Siletz Basin, OR)
(Anderson 2010)*		Herbicides that Forest Capital is applying to their timberland	Siletz Basin, OR
(Light 2010)^		Herbicides commonly used by private timber companies in the Oregon Coast Range	Siletz Basin, OR

Table 4.2. Risk quotient for herbicide which had high weighted percent detections and are registered for use in forestry land in Oregon. Risk quotient is produced by dividing the maximum detected concentration and dividing it by the NOAEC (no observable adverse effect concentration). Maximum Detected Concentration: the maximum concetration found in nine water quality reports from 1997 to 2010 in Oregon.

Chemical	Risk Quotient	Maximum Detected Concentration (ug/l)	NOEC (ug/l)
Atrazine	1.38	90.00	65.00
2,4-D	0.70	10.00	14.20
Triclopyr	0.058	6.00	104.00
Glyphosate	0.025	45.80	1800.00
Simazine	0.0094	9.00	960.00
Deethylatrazine	0.0060	0.012	2.00
Metolachlor	0.0045	4.50	1000.00

Table 4.3. Summary of herbicides and their importance to this risk assessment, Concentration range is from the maximum detected in water quality reports to the NOAEC (no observed adverse effect concentration) from a life-cycle or early life stage test as reported by EPE. * Glyphosate isopropylamine salt

Herbicide	Importance	Concentration (mg/l)
Atrazine	Very High	65-100
2,4-D	High	10-79.2
Triclopyr	Low	6-180
Glyphosate*	Moderate	45.8-1800

Appendix 2: Change of response with subsequent doses of odorants

During our odorant detection pilot study we attempted to find a threshold at which lamprey first reacts to an odorant. We found interesting results during the first trial while placing multiple dosages of different odorants (Figure 4.1). We found that the response was diminished with subsequent exposures and time to recovery decreased as well. We then tested three more lamprey to see if we could find a similar response. It appears as though each of these lamprey had a strong response to the first or second dosage but with each subsequent dose their response (Figure 4.2) (shape of curve) and time to recovery (length of line) diminished.

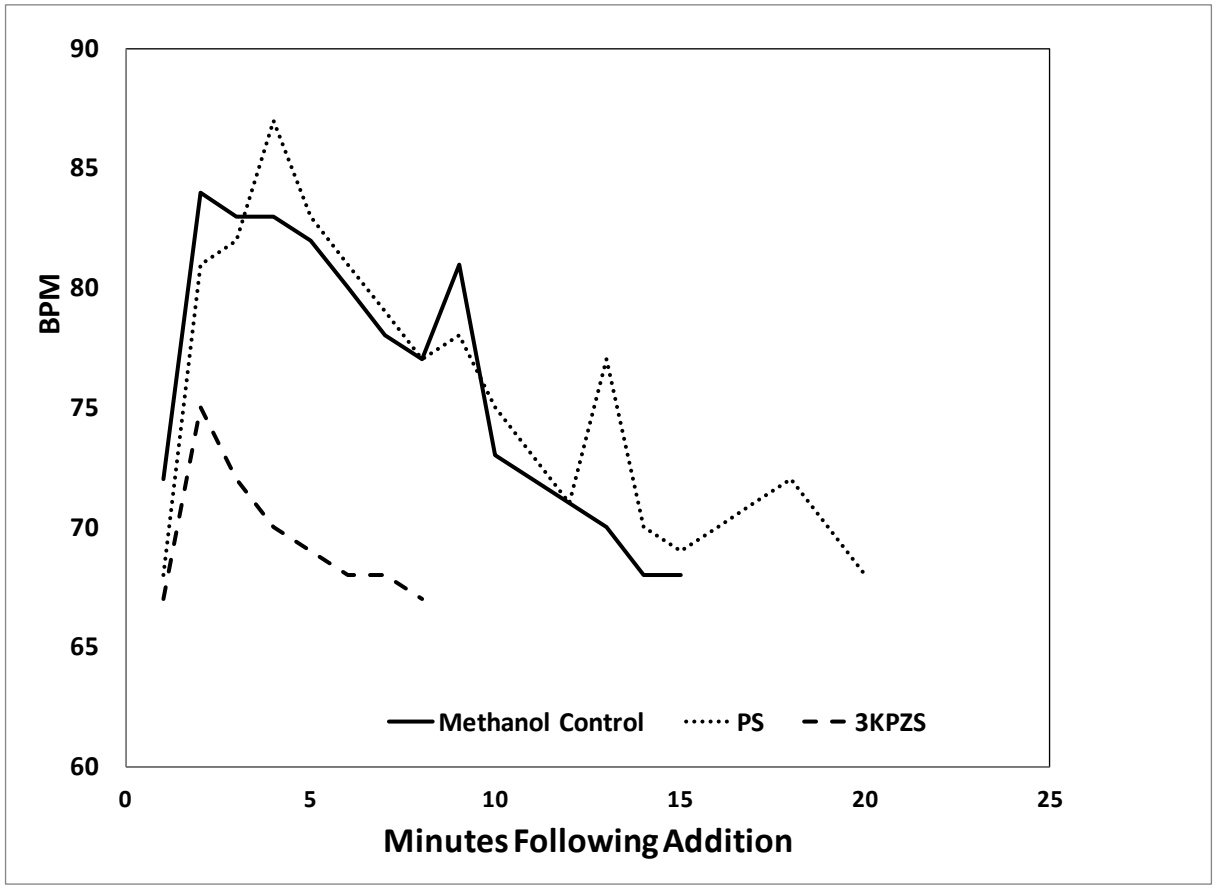

Figure 4.1 Plot of the ventilation (beats per minute) ofadult lamprey in reaction to Adult odor (the resulting water from 4 adult lamprey placed in a container for 3 hours) during pilot study. Lamprey were given methanol control first, petromyzenol sulfate (PS) 2nd and 3 keto Petromyzenol sulfate 3rd. Length of line is equivalent to time to recovery to reaction from odorant. All odorants were the same volume.

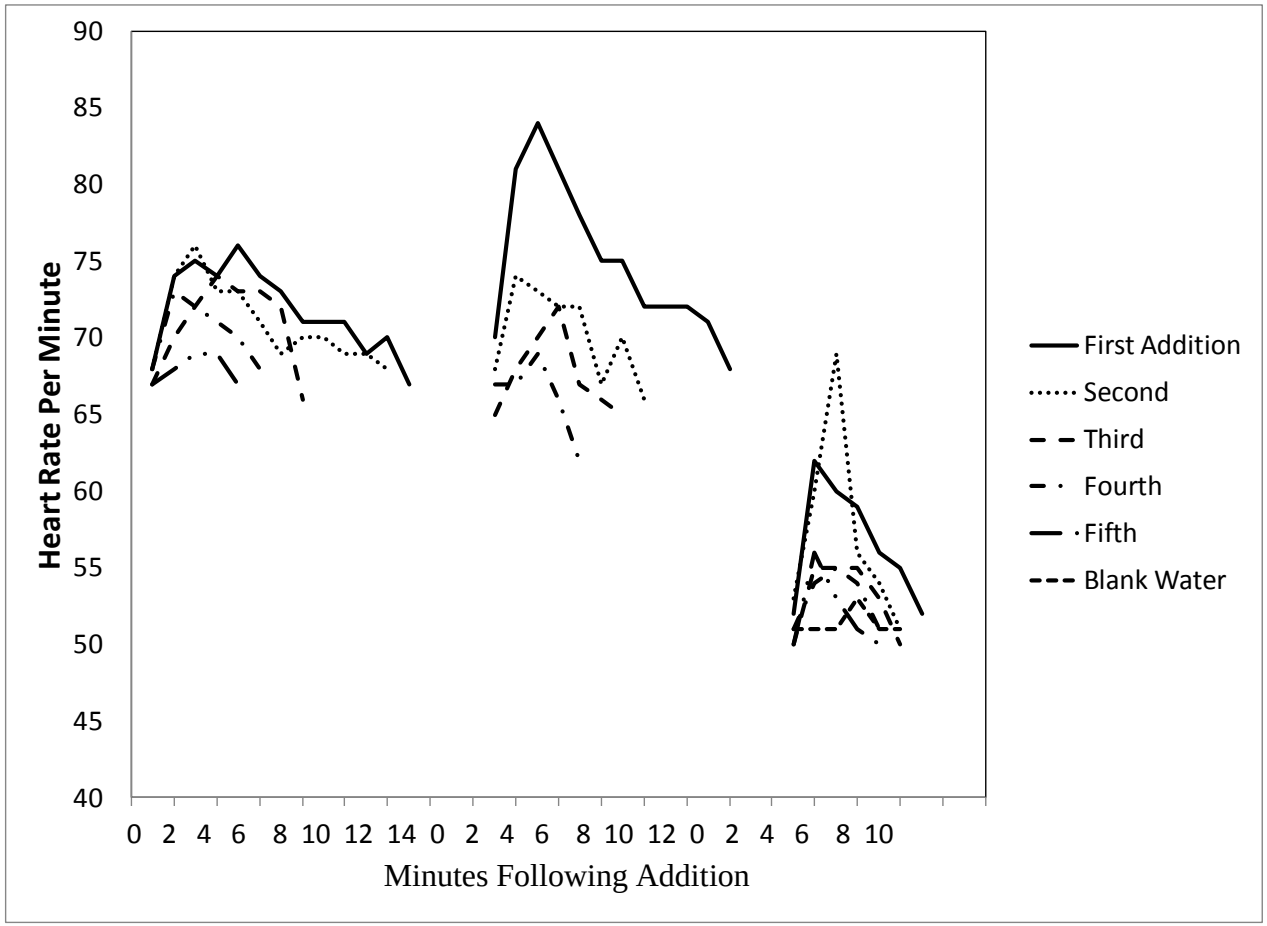

Figure 4.2. Plot of the ventilation (beats per minute) ofthree different adult lamprey in reaction to Adult odor (the resulting water from 4 adult lamprey placed in a container for 3 hours) during pilot study. Lamprey were given concentration of Adult odor repeatedly. Length of line is equivalent to time to recovarry from reaction to odorant. All odorants were the same volume.

Bibliography

2000. Siletz basin: DLCD generalize land use zoning. Corvallis, OR: Wetland & Watershed Assessment Group. Green Point Consultants, Inc. Available: http://www.midcoastwatershedscouncil.org/Assessments/2000/report/figures/ownership_siletz.jpg [accessed 22 April 2010].

Anderson C, Wood T, Morace J. 1997. Distribution of dissolved pesticides and other water quality constituents in small streams and their relation to land use, in the Willamette River basin, Oregon, 1996. Portland, OR.

Ares A, Terry T, Harrington C, Devine W, Peter D, Bailey J. 2007. Biomass removal, soil compaction, and vegetation control effects on five-year growth of Douglas fir in coastal Washington. Forest Science 53:600-610.

Barton BA. 2002. Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology 42:517-525.

Biro P. 1979. Acute effects of the sodium salt of 2,4-d on the early developmental stages of bleak, A*lburnus alburnus*. Journal of Fish Biology 14:101-109.

Bjerselius R, Li WM, Teeter JH, Seelye JG, Johnsen PB, Maniak PJ, Grant GC, Polkinghorne CN, Sorensen PW. 2000. Direct behavioral evidence that unique bile acids released by larval sea lamprey (*Petromyzon marinus*) function as a migratory pheromone. Canadian Journal of Fisheries and Aquatic Sciences 57:557-569.

Bunn SE, Davies PM, Mosisch TD. 1999. Ecosystem measures of river health and their response to riparian and catchment degradation. Freshwater Biology 41:333-345.

Carpenter KD, Sobieszczyk S, Arnsberg AJ, Rinella FA. 2008. Pesticide occurrence and distribution in the lower Clackamas River basin, Oregon 2000-2005. Reston, VA.

Clemens B, Mesa M, Magie R, Young D, Schreck C. 2012. Pre-spawning migration of adult Pacific lamprey, Entosphenus tridentatus, in the Willamette River, Oregon, U.S.A. Environmental Biology of Fishes 93:245-254.

Clemens BJ, Binder TR, Docker MF, Moser ML, Sower SA. 2010. Similarities, differences, and unknowns in biology and management of three parasitic lampreys of North America. Fisheries 35:580-594.

Bibliography (continued)

Close D, Fitzpatrick M, Li H, Parker B, Hatch D, James G. 1995. Status report of the Pacific lamprey, *Lampetra tridentata*, in the Columbia River basin. Portland, OR: U.S. Department of Energy. Available: https://pisces.bpa.gov/release/documents/documentviewer.aspx?doc=39067-1 [accessed 1 June 2010].

Close DA, Fitzpatrick MS, Li HW. 2002. The ecological and cultural importance of a species at risk of extinction, Pacific lamprey. Fisheries 27:19-25.

Davies PE, Cook LSJ, Barton JL. 1994. Triazine herbicide contamination of Tasmanian streams - sources, concentrations and effects on biota. Australian Journal of Marine and Freshwater Research 45:209-226.

Dawson J. 1905. The breathing and feeding mechanism of the lampreys. Biological Bulletin 9:1-21.

Denoyelles F, Kettle WD, Sinn DE. 1982. The responses of plankton communities in experimental ponds to atrazine, the most heavily used pesticide in the United States. Ecology 63:1285-1293.

Elefritz M, Atkinson M, Fitzgerald S. 2006. The care and planting of tree seedlings on your woodland. Available: http://extension.oregonstate.edu/catalog/pdf/ec/ec1504.pdf [accessed 24 January 2012].

EXTOXNET. 2010. Pesticide information profiles (pips). Corvallis, OR: Oregon State University. Available: http://extoxnet.orst.edu/pips/ghindex.html [accessed 1 June 2010].

Flueck WT, Smith-Flueck JAM. 2006. Herbicides and forest biodiversity: An alternative perspective. Wildlife Society Bulletin 34:1472-1478.

Freemark K, Boutin C. 1995. Impacts of agricultural herbicide use on terrestrial wildlife in temperate landscapes - a review with special reference to North America. Agriculture Ecosystems & Environment 52:67-91.

Garono R, Brophy L. 1999. Rock creek (Siletz) watershed assessment final report. Corvallis, OR: Earth Designs consulting Inc. Available: http://www.earthdesign.com/rockcreek/DRAFT.PDF [accessed 22 April 2010].

Bibliography (continued)

Garono R, Brophy L. 2001. MidCoast watersheds council sixth field watershed assessment. Corvallis, OR: MidCoast Watersheds Council. Available: http://www.midcoastwatershedscouncil.org/Assessments/MCWC_Assessment.pdf [accessed 22 April 2010].

Gibson AK, Mathis A. 2006. Opercular beat rate for rainbow darters, *Etheostoma caeruleum*, exposed to chemical stimuli from conspecific and heterospecific. Journal of Fish Biology 69:224-232.

Gilliom RJ, Barbash JE, Crawford CG, Hamilton PA, Martin JD, Nakagaki N, Howell LH, Scott JC, Stackelberg PE, Thelin GP and others. 2006. The quality of our nation's waters- pesticides in the nation's streams and ground water, 1992-2001: US Geological Survey circular 1291. Reston, VA: USGS. 172 p.

Harding JS, Benfield EF, Bolstad PV, Helfman GS, Jones EBD. 1998. Stream biodiversity: The ghost of land use past. Proceedings of the National Academy of Sciences of the United States of America 95:14843-14847.

Hardisty MW, Potter IC, editors. 1971. The biology of lampreys, Volume 1. New York: Academic Press. 1-7 p.

Hardisty MW, Potter IC, editors. 1972. The biology of lampreys, Volume 2. New York: Academic Press. 393-399 p.

Harrington TB, Wagner RG, Radosevich SR, Walstad JD. 1995. Interspecific competition and herbicide injury influence 10-year responses of coastal Douglas fir and associated vegetation to release treatments. Forest Ecology and Management 76:55-67.

Haynes R, Adams D, Mills J. 2003. Contemporary management regimes in the pacific northwest: Balancing biophysical and economic concerns. In: Monserud R, Haynes R, Johnson A, editors. Compatible forest management. Norwell, MA: Kluwer Academic Publishers.

Heath AG. 1995. Water pollution and fish physiology. 2nd edition. Water pollution and fish physiology. 3-9, & 11-13 chpt.

Howard JK, Close DA. 2005. Pacific lamprey research and restoration project. 2003 annual report, project no. 199402600. BPA Report DOE/BP 00005455-7:1-132.

Bibliography (continued)

IUPAC. 2010. Global availability of information on agrochemicals- pesticide properties database: A to Z list of pesticide active ingredients. Pesticide Information Database. Available: http://sitem.herts.ac.uk/aeru/iupac/index.htm [accessed 1 June 2010]

Keefer ML, Moser ML, Boggs CT, Daigle WR, Peery CA. 2009. Variability in migration timing of adult Pacific lamprey (*Lampetra tridentata*) in the Columbia river, USA. Environmental Biology of Fishes 85:253-264.

Kleerekoper H, Mogensen J. 1963. Role of olfaction in the orientation of *Petromyzon marinus* L. Response to a single amine in prey's body odor. Physiological Zoology 36:347-360.

Kleerekoper H, Van Erkel G. 1960. The olfactory apparatus of *Petromyzon marinus* L. Canadian Journal of Zoology 38:209-223.

Laframboise AJ, Ren X, Chang S, Dubuc R, Zielinski BS. 2007. Olfactory sensory neurons in the sea lamprey display polymorphisms. Neuroscience Letters 414:277-281.

Luzier CW, Brostrom J, Cook-Tabor C, Goodman D, Nelle RD, Ostrand KG, Schaller H, Streif B. Proceedings of the Pacific lamprey conservation initiative work session- October 28-29, 2008; 2008 October 28-29, 2008; Portland, OR. US Fish and Wildlife Service.

Maguire DA, Mainwaring DB, Rose R, Garber SM, Dinger EJ. 2009. Response of coastal Douglas fir and competing vegetation to repeated and delayed weed control treatments during early plantation development. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 39:1208-1219.

Moore A, Lower N. 2001. The impact of two pesticides on olfactory-mediated endocrine function in mature male Atlantic salmon (*Salmo salar* L.) parr. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology 129:269-276.

Moore A, Waring CP. 1998. Mechanistic effects of a triazine pesticide on reproductive endocrine function in mature male Atlantic salmon (*Salmo salar* L.) parr. Pesticide Biochemistry and Physiology 62:41-50.

Moring, J.R. Lantz RL. 1975. The Alsea watershed study: effects of logging on aquatic resources of three headwater streams of the Alsea River, Oregon. Part I. Biological studies. Fish. Res. Rep. No. 9. Oregon Department of Fish and Wildlife, Corvallis, OR.

Bibliography (continued)

Moser ML, Close DA. 2003. Assessing Pacific lamprey status in the Columbia River basin. Northwest Science 77:116-125.

Moser ML, Zabel RW, Burke BJ, Stuehrenberg LC, Bjornn TC, Spedicato MT, Lembo G, Marmulla G. 2005. Factors affecting adult Pacific lamprey passage rates at hydropower dams: Using "Time to event" Analysis of radio telemetry data. Aquatic telemetry: advances and applications. Proceedings of the Fifth Conference on fish telemetry held in Europe, Ustica, Italy, 9-13 June 2003.:61-70.

Munoz I, Real M, Guasch H, Navarro E, Sabater S. 2001. Effects of atrazine on periphyton under grazing pressure. Aquatic Toxicology 55:239-249.

Myllynen K, Ojutkangas E, Nikinmaa M. 1997. River water with high iron concentration and low ph causes mortality of lamprey roe and newly hatched larvae. Ecotoxicology and Environmental Safety 36:43-48.

Newman MC. 2010. Fundamentals of ecotoxicology. Boca Raton, FL: CRC Press Taylor & Francis Group. 541 p.

ODEQ. 2009. Watershed-based toxics monitoring program 2008 progress report: Willamette River basin. Hillsboro, OR: Oregon Department of Environmental Quality Laboratory and Environmental Assessment Division. Available: http://www.deq.state.or.us/lab/wqm/docs/10-LAB-007.pdf [accessed 13 May, 2010]

ODF.2010. 25-Year Harvest History Data [1986-2010] (xls): Annual Timber Harvest. Oregon Department of Forestry. Available: http://www.oregon.gov/ODF/STATE_FORESTS/FRP/Charts.shtml [accessed 24 January 2012].

PURS-Oregon 2007. 2008. Pesticide Use Reporting System. Salem, OR: Oregon Department of Agriculture. Available: http://www.Oregon.gov/ODA/PEST/docs/pdf/pursreportweb2007.pdf [accessed 22 April 2010].

PURS-Oregon 2008. 2009. Pesticide Use Reporting System. Salem, OR: Oregon Department of Agriculture. Available: http://www.Oregon.gov/ODA/PEST/docs/pdf/pursreport08web.pdf [accessed 22 April 2010].

Bibliography (continued)

R Development Core Team (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.Bibliography (continued)

Robinson TC, Bayer JM. 2005. Upstream migration of Pacific lampreys in the John Day River, Oregon: Behavior, timing, and habitat use. Northwest Science 79:106-119.

Robinson, T. C., P. W. Sorensen, et al. (2009). "Olfactory Sensitivity of Pacific Lampreys to Lamprey Bile Acids." Transactions of the American Fisheries Society 138: 144-152.

Rohr JR, McCoy KA. 2010. A qualitative meta-analysis reveals consistent effects of atrazine on freshwater fish and amphibians. Environmental Health Perspectives 118:20-32.

Shepard JP, Creighton J, Duzan H. 2004. Forestry herbicides in the United States: An overview. Wildlife Society Bulletin 32:1020-1027.

Soimasuo M, Kervinen J, Sinkkonen S, Paasivirta J. 2004. Bioaccumulation of pops from contaminated sediment to lamprey (Lampetra fluviatilis L.) larva. Journal of Soils and Sediments 4:75-83.

Solomon KR, Carr JA, Du Preez LH, Giesy JP, Kendall RJ, Smith EE, Van Der Kraak GJ. 2008. Effects of atrazine on fish, amphibians, and aquatic reptiles: A critical review. Critical Reviews in Toxicology 38:721-772.

Sorensen PW, Fine JM, Dvornikovs V, Jeffrey CS, Shao F, Wang JZ, Vrieze LA, Anderson KR, Hoye TR. 2005. Mixture of new sulfated steroids functions as a migratory pheromone in the sea lamprey. Nature Chemical Biology 1:324-328.

Stein WI. 1999. Six-year growth of Douglas fir saplings after manual or herbicide release from coastal shrub competition. PNW-RP-500. USDA Forest Service Pacific Northwest Research Station Research Paper. Available: http://www.fs.fed.us/pnw/pubs/pnw_rp500.pdf [accessed 24 January 2012].

Takacs P, Martin PA, J. S. 2002. Pesticides in Ontario: A critical assessment of potential toxicity of agricultural products to wildlife, with consideration for endocrine disruption. Technical Report Series Number 369. Available: http://publications.gc.ca/collections/Collection/CW69-5-369E.pdf [accessed 1 June 2010].

Bibliography (continued)

Tierney KB, Baldwin DH, Hara TJ, Ross PS, Scholz NL, Kennedy CJ. 2010. Olfactory toxicity in fishes. Aquatic Toxicology 96:2-26.

Tierney KB, Sampson JL, Ross PS, Sekela MA, Kennedy CJ. 2008. Salmon olfaction is impaired by an environmentally realistic pesticide mixture. Environmental Science & Technology 42:4996-5001.

Tierney KB, Singh CR, Ross PS, Kennedy CJ. 2007. Relating olfactory neurotoxicity to altered olfactory-mediated behaviors in rainbow trout exposed to three currently-used pesticides. Aquatic Toxicology 81:55-64.

Tillitt DE, Papoulias DM, Whyte JJ, Richter CA. 2010. Atrazine reduces reproduction in fathead minnow (Pimephales promelas). Aquatic Toxicology 99:149-159.

USEPA. 2009. Aquatic life benchmarks for pesticides. Available: http://www.epa.gov/oppefed1/ecorisk_ders/aquatic_life_benchmark.htm [accessed 1 June 2010].

USEPA. 2010. Pesticide reregistration status. Available: http://www.epa.gov/opp00001/reregistration/status.htm [accessed 1 June 2010].

USEPA. 2010. Pesticide reregistration status. USEPA.

Vrieze LA, Bjerselius R, Sorensen PW. 2010. Importance of the olfactory sense to migratory sea lampreys Petromyzon marinus seeking riverine spawning habitat. Journal of Fish Biology 76:949-964.

Wagner CM, Stroud EM, Meckley TD. 2011. A deathly odor suggests a new sustainable tool for controlling a costly invasive species. Canadian Journal of Fisheries and Aquatic Sciences 68:1157-1160.

Wentz DA, Bonn BA, Carpenter KD, Hinkle SR, Janet ML, Rinella FA, al. e. 1998. Water quality in the Willamette basin, Oregon 1991-1995. Report nr USGS Circular 1161. Available: http://pubs.usgs.gov/circ/circ1161/circ1161.pdf [accessed 22 April 2010].

WHO. 2009. 2,4-dichlorophenoxyacetic acid (2,4-d)- environmental aspects. In: INCHEM I, editor. Environmental Health Criteria Monographs (EHCs): World Health Organization (WHO).

Bibliography (continued)

Yildiz O, Cromack K, Radosevich SR, Martinez-Ghersa MA, Baham JE. 2011. Comparison of 5th-and 14th-year Douglas fir and understory vegetation responses to selective vegetation removal. Forest Ecology and Management 262:586-597.

Yun SS, Scott AP, Siefkes MJ, Li WM. 2002. Development and application of an ELISA for a sex pheromone released by the male sea lamprey (Petromyzon marinus L.). General and Comparative Endocrinology 129:163-170.

Yun SS, Wildbill AJ, Siefkes M J, Moser ML, Dittman AH, Corbett SC, Li W, Close DA. 2011. Identification of putative migratory pheromones from Pacific lamprey (Lampetra tridentata). Canadian Journal of Fisheries and Aquatic Sciences 68:2194-2203.