PRELIMINARY STUDY OF CONSPECIFIC CHEMICAL CUEING IN AMERICAN EELS (*ANGUILLA ROSTRATA)*

By

Andrew K. Schmucker

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Fisheries and Wildlife - Master of Science

2016

ABSTRACT

PRELIMINARY STUDY OF CONSPECIFIC CHEMICAL CUEING IN AMERICAN EELS (*ANGUILLA ROSTRATA)*

By

Andrew K. Schmucker

Many species use conspecific chemical cueing to coordinate biological functions such as migration, reproduction, defense, and habitat selection. In this thesis, I hypothesized that conspecific chemical cueing could be used by American Eels as mechanisms for bidirectional migration coordination and danger avoidance as functions of cue concentration and life stage dependency. In chapter 1, I investigated conspecific chemical cueing in the youngest actively migratory life stage, glass eels, regarding inland migration coordination. I demonstrated conspecific glass eel washing affinity over a wide range of concentrations, characterized cue concentration preferences and differentiation capabilities, and observed no change in response during pigmentation into elvers. In chapter 2, I investigated conspecific chemical cueing in the oldest migratory life stage, silver eels, to help maintain aggregations during the downstream spawning migration and to avoid danger. I characterized their behavioral responses to both live and dead silver eel conspecific odors in a laboratory flume bioassay using multiple scoring metrics, but observed no significant responses to either cue. Combined, this thesis offers a survey of chemical ecology within and across life stages of the American Eel, supports conspecific chemical cueing as a likely mechanism for inland migration coordination in glass eels, and contributes useful information toward species management and restoration efforts.

ACKNOWLEDGEMENTS

I would like to thank my major advisor, Dr. Weiming Li, and the rest of my graduate committee, Dr. Nicholas Johnson and Dr. Heather Galbraith, for their assistance, inspiration, and mentorship during this project and throughout my Master of Science degree. I would also like to thank the Li laboratory, notably Dr. Tyler Buchinger, my friends and colleagues at Michigan State University, and the staff of the U.S. Geological Survey Northern Appalachian Research Laboratory and the Great Lakes Science Center Hammond Bay Biological Station for their additional assistance and support. The U.S. Geological Survey Fisheries Program, Great Lakes Fisheries Commission, and Michigan State University generously provided funding and facilities for this opportunity, and the Maryland Department of Natural Resources and local fishermen near Narrowsburg, NY, generously provided the research specimens.

TABLE OF CONENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION TO THESIS

In recent decades, many freshwater fish species have suffered significant population declines (Cumberland and Cronin 1986; Myers and Worm 2003; McCauley et al. 2015). Human impacts, such as pollution, overharvesting, climate change, habitat degradation and alteration, and introduction of invasive species, on fluvial ecosystems have had serious implications for fish recruitment, migration, and health. American Eels *Anguilla rostrata* are no exception to this. Although once constituting upward of 50% of the total fish biomass in eastern North American freshwater systems, some American Eel populations have declined by up to 99% (Ogden 1970; ASMFC 2000; DePhilip and Moberg 2010). As a catadromous species, American Eels can encounter man-made riverine structures, such as dams and hydroelectric turbines, many times during their lifetime (Huertas et al. 2008). These barriers can physically block migratory routes and inflict severe injury or death (Haro et al. 2002; Pohl 2002; Kocovsky et al. 2009; Pedersen et al. 2012; MacGregor et al. 2015). Current passive methods of addressing this issue include improving fish ladders and trap and transport initiatives at existing barriers (as opposed to active methods, such as structure removal), but are not highly effective in all cases (Richkus and Dixon 2003; Calles et al. 2012; Drouineau et al. 2014). As a once-popular sport fish, primary host species for juvenile freshwater mussels, valuable economic asset, and ecologically important predator, prey, and detritivore, American Eels are now a target for restoration (Hurley 1973; ASFMC 2000; Haro et al. 2000; Lellis et al. 2013).

Understanding the cues and mechanisms that impact American Eel behavior could help improve passage effectiveness at man-made riverine structures, especially those associated with olfaction and migration. Anguillids are known to use many cues to coordinate migrations, such

as tides, electromagnetic fields, salinity and temperature gradients, flow regime, and light cycles (Barbin 1998; Barbin et al. 1998; Hasler 1960; Parker and McCleave 1997; White and Knights 1997; Haro 2003; Bardonnet et al. 2005; August and Hicks 2008; DuColombier et al. 2009). However, conspecific chemical cueing is another possibility that has not been thoroughly investigated. Conspecific chemical cueing, which targets the olfactory system, involves the emission and detection of public chemical cues that can incite specific behavioral and physiological responses in distant conspecifics (Donahue 2006). A wide range of taxa uses conspecific chemical cueing, which increases fitness for many reasons (Atema 1986; French and Kline 1989; Morris 1992; Valone 2007; Huertas 2008). Addressing the knowledge gaps of whether and how conspecific chemical cueing exists and functions in the American Eel could provide information that explains how these fish coordinate such amazing migrations, avoid danger, and provide model systems for studying basic biological concepts.

Research in this thesis explored the hypotheses that American Eels use conspecific chemical cueing as a mechanism for bidirectional migration coordination, as well as danger avoidance in migratory adults, as a function of two major variable themes: cue concentration and life stage dependency. When migrating, American Eels could follow chemical cues released by conspecifics to reach preferable destinations if the cues are attractive. In contrast, they could use them as alarm cues to avoid danger if they are repulsive. Previous studies support the existence of conspecific chemical cueing in anguillids by demonstrating affinities to conspecific washings, bodily extracts, amino acids, and bile salts in laboratory and field settings (Pesaro et al. 1981; Saglio 1982; Sorensen 1986; Sola and Tosi 1993; Sola and Tongiorgi 1996; Briand et al. 2002; Huertas et al. 2007; Huertas et al. 2008). However, some behavioral responses differed by life stage, cue concentration, and some odors were repulsive (Sorensen 1986). Studies have also

demonstrated that conspecific chemical cues promote physiological or morphological changes in adult silver eels, such as sexual maturation and anatomical changes associated with the silvering process (ASMFC 2000; Liu et al. 2003; Huertas and Cerdá 2006; Huertas et al. 2007), and can function as alarm cues in fish species with similar traits (Brown et al. 1995; Chivers and Smith 1998; Chivers et al. 2007; Wagner et al. 2009, Imre et al. 2013).

One defining characteristic of the American Eel is its complex life history, which consists of five distinct life stages (ASFMC 2000). Leptocephali, the larval form, are born far offshore in the Sargasso Sea (Kleckner and McCleave 1985) and drift towards land in ocean currents over the course of several months. They eventually metamorphose into glass eels, a translucent, motile life stage, which completes the main inland migration into fresh and brackish water systems (ASFMC 2000; Huertas 2008). When glass eels arrive inland, typically in the spring, they transition into pigmented elvers and continue to penetrate inland. After spending two or three years as elvers, they are considered yellow eels, which are sexually immature, growth-phase resident eels (ASFMC 2000; Huertas 2008). Finally, after spending a lengthy time inland as yellow eels, they sexually mature into silver eels, typically in the fall, and migrate back to the Sargasso Sea to terminally spawn (ASFMC 2000; Huertas et al. 2008). Because this life cycle is central to American Eel biology, conspecific chemical cueing was analyzed in light of this characteristic. Research in this thesis focuses on the glass eel and silver eel stages, which are the two most-migratory life stages in either direction.

In Chapter 1, entitled American Glass Eels Respond to Conspecific Odor as a Function of Concentration (Schmucker et al., in press), I investigated the potential roles of conspecific chemical cueing in glass eels during the initial inland migration into fresh and brackish water systems and during pigmentation into elvers. I hypothesized that glass eels used conspecific

chemical cues as one mechanism to coordinate inland migrations, and would consistently be attracted to conspecific odors collected in glass eel washings following discernable relationships. By capturing their conspecific cues and introducing them to other glass eels in two-choice maze assays, I demonstrated the feasibility of conspecific chemical cueing during the inland migration, as well as characterized some specific concentration-response and preference relationships related to it. These results set the foundation for Chapter 2, whereby these concepts were assessed and characterized in older eels.

In Chapter 2, entitled American Silver Eels Do Not Show Behavioral Responses to Conspecific Odors in a Laboratory Flume Bioassay, I explored the potential roles and expression of conspecific chemical cueing in silver eels during their downstream migration to spawn. I hypothesized silver eels could use conspecific chemical cues as a mechanism for aggregation during this event, as well as danger avoidance. This investigation introduced many challenging conditions, namely that the fish were now swimming in the same direction as the river current and were migrating into ever-larger bodies of water. I applied concepts from the first chapter in a manner that adjusted for the biology and life stage of these fish. The silver eels, expected to instinctively swim downstream, passed a cue release point where I attempted to then influence their movements using conspecific cues toward particular areas of a laboratory flume. Silver eel responses to cues from both live and dead conspecific cues were assessed for attraction and repulsion in this assay using multiple scoring metrics. Results suggested that silver eels were not significantly attracted to or repulsed by either cue, their activity level and downstream swimming trajectories did not change, and they could not be consistently moved into targeted areas of the arena. However, these results do not negate all roles or the importance of conspecific chemical

cueing at this life stage, as positive responses may not have been readily apparent in this type of assay.

Appendix A, entitled Donor Life Stage Influences Juvenile American Eels *Anguilla rostrata* Behavioral Response to Conspecific Chemical Cues, addressed the potential role of conspecific chemical cueing in elvers during the completion of the main inland migration. Working with scientists in the U.S. Geological Survey, we expanded on concepts from the glass eel study and hypothesized that elvers used conspecific chemical cues as one mechanism to coordinate the completion of the inland migration, as some are still migratory. We predicted they would also be consistently attracted to conspecific odors following similar discernable relationships as those observed in glass eels. By capturing their conspecific odor at multiple temperatures, introducing them to other elvers in two-choice maze assays, and altering our methodology in some instances, we determined that elvers did not show the same response relationships to conspecific odors as glass eels. These results suggested that elvers may no longer use conspecific odors as a primary mechanism to coordinate inland migration because of an overall lack of affinity to the cue. This material was included in an appendix because it complements the research conducted for this thesis well and provides additional insight into American Eel conspecific chemical cueing.

Each manuscript in this thesis focused on a different life stage of the American Eel. The chapters appear in chronological order of completion and also in order of youngest-to-oldest life stage, with the exception of my work in Appendix A, which falls between the chapters in terms of chronology and life stage. Each manuscript has been submitted for consideration for publication in a peer-reviewed scientific journal and is formatted to follow the American Fisheries Society style guide. At the present time, the first chapter has been accepted and is being

published in *Transactions of the American Fisheries Society*. Copyright permission to use this manuscript in this thesis has been granted by the editors of the journal. The second chapter has also been submitted, and is under review in *Transactions of the American Fisheries Society.* The manuscript in Appendix A has been submitted and is under peer review in the *Journal of Fish Biology*. Results of these studies support the existence and function of conspecific chemical cueing as one mechanism of American Eel inland migration coordination in the glass eel stage and show that response to chemical cues varies with concentration. However, conspecific chemical cueing may be life stage dependent, as older migratory eels did not respond to conspecific cues. There was also no evidence of alarm or repulsion to dead eel odor. Combined, they expand our scientific knowledge in rarely addressed research areas regarding conspecific chemical cueing, migration coordination, and danger avoidance in the American Eel, and address several basic biological principles that may be of benefit to fisheries management strategies.

CHAPTER 1: AMERICAN GLASS EELS RESPOND TO CONSPECIFIC ODOR AS A FUNCTION OF CONCENTRATION (SCHMUCKER ET AL., IN PRESS)

ABSTRACT

American Eels *Anguilla rostrata* have experienced staggering population declines in recent decades and are the focus of restoration efforts. Studies have demonstrated that olfaction is critical to anguillid behavior, and that glass eels (the life stage which migrates inland from saltwater to freshwater) are attracted to conspecific washings. In this study, we evaluated conspecific chemical cueing as a potential mechanism for American glass eel inland migration coordination by assessing their affinity to conspecific washings, their concentration-response relationships, and changes in their responsiveness to washings during transition into elvers. We found that in two-choice maze assays, glass eels were attracted to glass eel washings over a wide range of concentrations (0.20 g glass eels$\cdot L^{-1} \cdot hr^{-1}$ to 0.40 g glass eels$\cdot L^{-1} \cdot hr^{-1}$) and the concentration-response relationship best fit a logarithmic function. When given a choice between higher and lower concentrations of conspecific washings, glass eels generally preferred the higher concentration washings. Stages 3 through 7 glass eels did not respond significantly different from each other to undiluted glass eel washings, however stage 7 eels were not attracted to the washings while the other stages were. Affinity to washings remained consistent over the course of several weeks. These results supported aspects of the conspecific chemical cueing hypothesis at the glass eel life stage under laboratory conditions, and suggested that conspecific chemical cueing may be an important component of juvenile American Eel migration coordination that warrants additional study.

INTRODUCTION

Freshwater eels of the family Anguillidae are important natural resources that have undergone up to 99% population declines in localized areas (Moriarty 1986, 2012; Haro et al. 2000; ICES 2013). Given their conservation status (Freyhof and Kottelat 2010), ecological and commercial importance (Hurley 1973; ASFMC 2000), and links to freshwater mussel distributions (Lellis et al. 2013), anguillids are the focus of restoration efforts (Haro et al. 2000). American Eels *Anguilla rostrata*, the only freshwater eel native to North America, have a complex catadromous life history that consists of five distinct stages (ASFMC 2000). Larval stage eels, known as leptocephali, are hatched in the Sargasso Sea and drift in ocean currents for several months (Kleckner and McCleave 1985). As they near coastal waters, they metamorphose into glass eels, the translucent life stage which actively migrates into estuaries and rivers every spring (ASMFC 2000). Within a few months of arrival, glass eels become pigmented elvers. Many elvers continue inland, while others remain in coastal waters and are still migratory (ASMFC 2000). The construction of dams and other riverine structures along the Atlantic coast has created nearly impassable blockades for migratory juvenile American Eels, which impedes their ability to reach critical habitats on the opposite side (Pohl 2002; Kocovsky et al. 2009). Modern devices, such as fish ladders and trap and transport operations, are present at many river barriers to aid American Eels with upstream trans-barrier movement, but their effectiveness could be improved (Calles et al. 2012; Drouineau et al. 2014). A better understanding of American Eel movements and migration coordination may help to remediate this ecological issue.

Fishes have been known to use multiple environmental correlates to facilitate migrations, but the role of conspecific chemical cueing is one potential mechanism that has not been fully

investigated in American Eels (Hasler 1960; White and Knights 1997; Bardonnet et al. 2005; August and Hicks 2008; DuColombier et al. 2009). Conspecific chemical cueing occurs in fishes when odors emitted by conspecifics provide public information that increases the probability of movement or settlement in particular areas (Donahue 2006). Conspecific chemical cueing yields higher fitness because conspecifics and the locations they occupy can be located distantly, and can transmit important chemical information to incite associated behaviors (Morris 1992; Valone 2007). According to this hypothesis, migratory fishes might utilize odor cues as a means to provide directionality and coordinate movements if these cues are detectable, attractive, and elicit locomotory behaviors. While widely tested in terrestrial species, empirical tests of conspecific chemical cueing in fishes are limited (Ralls 1971; Smith and Swink 2003; Rajchard 2006; Larsson and Svensson 2009; Mason and Parker 2010). Sea Lamprey *Petromyzon marinus* spawning migrations have fit predictions consistent with the conspecific chemical cueing hypothesis, and adult Sea Lampreys migrating upstream consistently prefer locations with higher concentrations of cues emitted by resident larvae (Wagner et al. 2009). Some salmonids have been shown to use conspecific chemical cues during return migrations to natal rivers (Nordeng and Bratland 2006), as have multiple species of larval pomacentrids when moving into settlement areas on coral reefs (Ben-Tzvi et al. 2010; Lecchini and Nakamura 2013). Given that American Eels migrate thousands of kilometers to reach inland waters and that conspecific chemical cueing is present among evolutionarily distanced species, American Eels may also use conspecific chemical cues to coordinate movements.

Studies have demonstrated that anguillids are attracted to conspecific odors and other organic molecules, such as bile salts, collected in washings (Pesaro et al. 1981; Sola and Tosi 1993; Sola and Tongiorgi 1996). However, many questions remain about the nature of these

responses as they pertain to migratory functions. Anguillid responses to conspecific washings have been shown to differ depending on the concentration of the washing applied and the life stage tested (Pesaro et al. 1981; Sorensen 1986), but the relationships by which concentration and development affect affinity have not been characterized. Adult eels have been known to utilize olfaction in estuarine environments during outmigrations (Barbin 1998; Barbin et al. 1998), but its role in juvenile glass eel inland migrations has not been examined. In field settings, releasing conspecific washings down fish ladders increased European Eel *Anguilla anguilla* passage rates 1.4 times (Briand et al. 2002) and lured them into traps (Saglio 1982), but whether these concepts could be ultimately utilized as restoration tools is still equivocal. Addressing these knowledge gaps would advance understanding of American Eel chemical ecology and indicate whether these cues may have further applications in improving American Eel passage efforts.

To date, no studies have systematically characterized glass eel behavioral responses when they are exposed to multiple concentrations of conspecific odor washings, or characterized glass eel washing preferences when exposed to different concentrations simultaneously. Furthermore, no studies have specifically examined the developmental transition from glass eels to elvers as a period when responsiveness to odors can change. We hypothesized that American glass eels used conspecific chemical cueing, in part, as a mechanism for inland migration coordination by following the odor of up-current individuals, but that cue responses could change during transition to elvers. Thus, we predicted that glass eels would have an affinity to conspecific washings that would incite locomotion following discernible concentration-response relationships, and would consistently prefer the higher concentration washings if two were presented simultaneously. We also predicted that attraction to glass eel washings would decrease during transition to elvers, as this transition could indicate the near-completion of the major

inland migration. To investigate these hypotheses, two-choice behavioral assays were conducted over a wide range of glass eel washing concentrations. Single washing assays were conducted to characterize glass eel affinity and concentration-response relationships to the washings, direct washing comparisons were used to characterize their concentration preferences, and single washing assays were repeatedly conducted during glass eel development to characterize potential changes in response.

METHODS

Animal collection and care

American glass eels were collected from a Maryland Department of Natural Resources (DNR) estuarine trapping site on the Assawoman Bay (8.5^{o}C, 6.5 ppt salinity water conditions) at Bishopville, MD, United States, on multiple dates. They were transported to a Maryland DNR building in Stevensville, MD, United States, in tanks of aerated brackish water from the collection site, then to the U.S. Geological Survey Northern Appalachian Research Laboratory in Wellsboro, PA, United States, over the course of 24 hrs. Upon arrival, they were placed in a 350 L flow-through (~5.0 L/min flow) social housing tank, in which heated and ambient temperature well water was proportioned to achieve 20^{o}C. This temperature was selected because it was the ambient temperature that the glass eels had acclimated to during travel and would also promote active feeding and swimming in captivity. One-liter plastic containers full of salt (Cargill top-flow evaporated salt, Minneapolis, MN, United States) were placed into the social housing tank to maintain salinity at 0.1 ppt (a typical salinity level for freshwater aquaculture). All glass eels received a 25 ppt standing bath salt treatment for 1 hr upon arrival to help control epidermal parasites, and again once per week thereafter. Glass eels were fed a combination of commercial fish mash (Bio-Oregon life stage size zero, East Westbrook, ME, United States), pink brine shrimp flakes (Zeigler, Gardners, PA, United States), store-bought chicken liver, and frozen daphnia (Fish King, Inc., Chicago, IL, United States) on belt feeders (Pentair AES, Apopka, FL, United States), and their tanks were cleaned daily to remove waste. Approximately 0.6% of the specimens expired daily, a percentage comparable to previous experiences with American glass eel culture in captivity.

Experimental apparatus

Two identical maze units, each containing two independent, unidirectional flow, two-choice mazes were constructed from lab grade PVC (United States Plastic Corp., Lima, OH, United States), eco-safe silicone (Pentair AES, Apopka, FL, United States), marine-grade white and dark gray epoxy paint (Pentair AES, Apopka, FL, United States), clear heavy duty PVC cement (Oatley SCS, Cleveland, OH, United States), screws, and 0.79 mm stainless-steel screen following a modified design of the mazes used by Li et al. (2002; Figure 1.1; Figure 1.2). Each arm of each maze was fed by a 0.64 cm diameter combination schedule 40 and 80 PVC pipe connected to a series of valves, which were fed by overhead heated and ambient temperature well water mains. Each maze had a 12.0(w) x 9.0(l) x 7.5(h) cm unidirectional flow block placed immediately downstream of the inflow constructed from 0.64 cm clear bubble tea straws, PVC glue, a 0.64 cm thick PVC base, rubber bands, and stainless steel bolts for added weight. Water depth in the maze was approximately 7.9 cm. An Axis Q1604 network camera was suspended approximately 60 cm above each maze to record glass eel behavior, and an IRLamp6 infrared light (Bat Conservation and Management, Inc., Carlisle, PA, United States) centered over each maze provided illumination of 5020 lux. A peristaltic pump (Cole-Parmer Masterflex L/S, Vernon Hills, IL, United States) with #14 tubing set and calibrated to pump 10.0 ± 1.0 mL/min per tube was used to dispense the washings into the maze arms. Dark gray lines were painted across the bottom of the arms to signify their lower boundary. Prior to testing in the bioassay, American glass eels were isolated for approximately 24 hrs in individual 1.4 L flow-through polystyrene aquaria maintained at 20^{o}C, without any food or salt, to prevent conspecific odor pre-exposure bias. Unidirectional flow in the bioassay was confirmed by pumping a 2-drops/L rhodamine dye (Bright Dyes Fluorescent FWT Red, Kingscote Chemicals, Miamisburg, OH,

United States) solution into the mazes prior to each night of testing and adjusting the pumping tubing as needed. Unidirectional flow was defined as the absence of dye being seen in the opposite-side arm from the dye release point and in the distant half of the opposite side of the maze below the arm at least 1 min after pumping had begun.

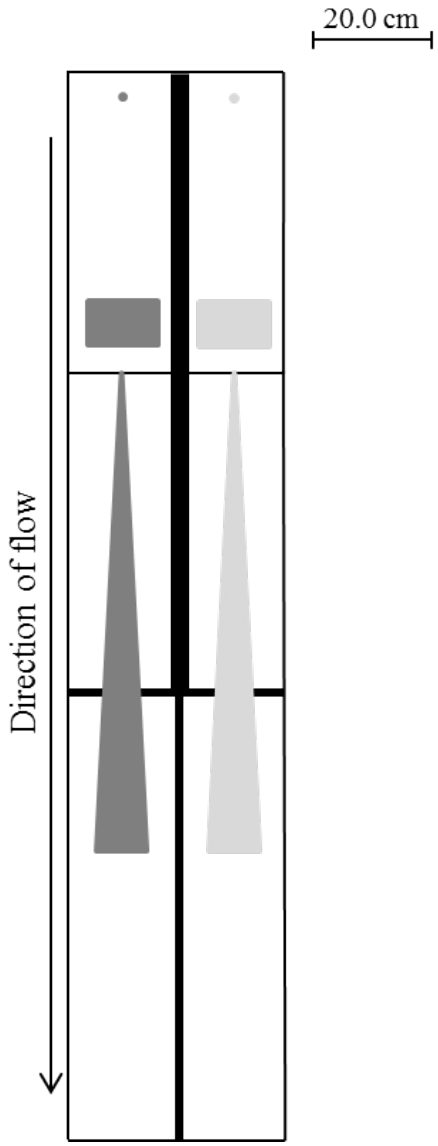

Figure 1.1: Diagram of one maze used for all two-choice maze assays at the Northern Appalachian Research Laboratory with glass eel washings based on the design of Li et al. (2002). The grey cones represent the expansion pattern of the washing plume according to dye tests (the washings were introduced at the upstream apex of the cones). The thickest vertical gridline represents the physical, impermeable center divider of the maze, and the three thinner gridlines represents painted lines on the bottom of the maze that delineated the arms and sides. The grey rectangles near the top of the apparatus represent the placement of the unidirectional flow blocks and the grey circles signify the inflow pipe locations. Figure is to scale.

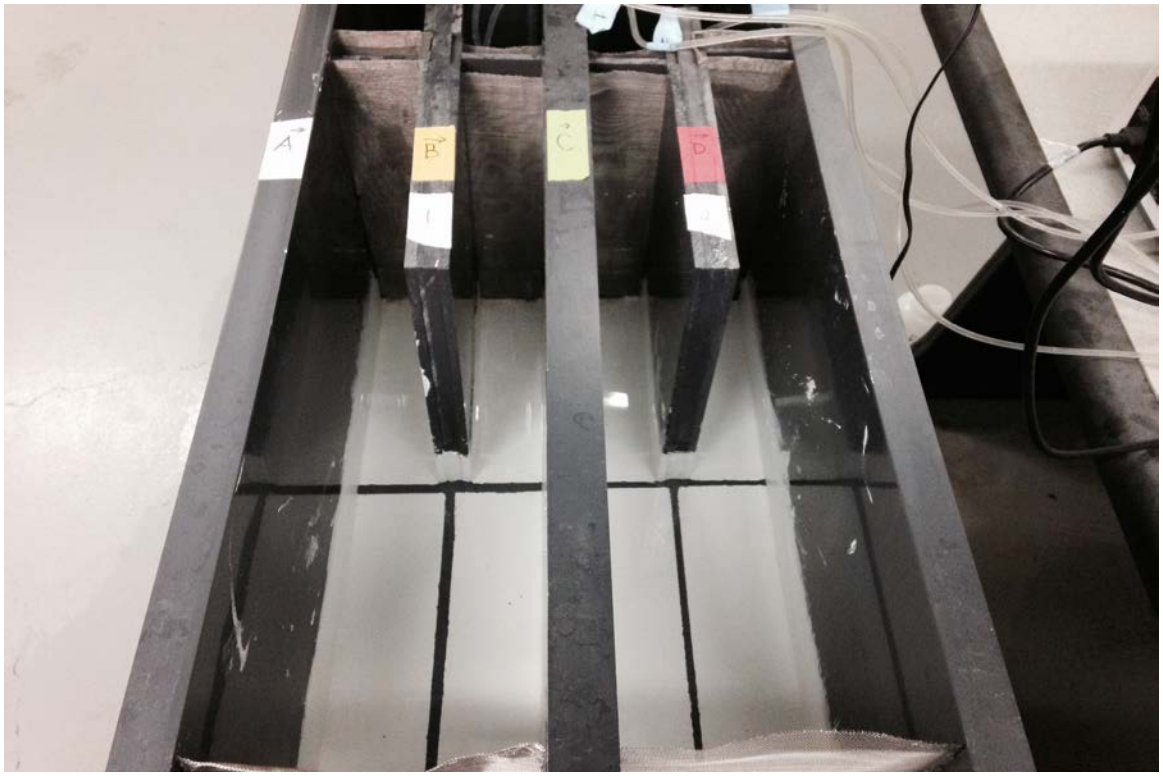

Figure 1.2: Photograph of a two-choice maze unit used for all American glass eel assays in the study at the Northern Appalachian Research Laboratory. Photograph taken by A. Schmucker.

Glass eel washing preparation

The first batch of American glass eels, collected March 13, 2015, was only used for preparing glass eel washings to collect conspecific odors. These glass eels received a 250 ppm standing bath formalin treatment for 45 min upon arrival to control epidermal parasites, and were reared for 24 days after treatment to allow for adequate recovery and increase pigmentation stage diversity. Four hundred-fifty glass eels (87.0 g total) were placed into a plastic tub containing 90.0 L of aerated well water at 23^{o}C for 25 min. They were then removed from the tub, and unfiltered washings were collected and proportioned with 9^{o}C well water in clean 1 L high-density polyethylene bottles. Undiluted, 1/2, 1/4, 1/8th, 1/16th, 1/100th, 1/500th, and 1/1000th dilution washings were created and promptly frozen at -20^{o}C in a walk-in freezer. The undiluted washing equated to a 2.1 glass eels$\cdot$L$^{-1}\cdot$hr^{-1} concentration and 0.40 g$\cdot$L$^{-1}\cdot$hr^{-1} by biomass. To confirm the developmental stage of the specimens washed as glass eels, 50 of them were sedated with a 200 mg/L MS-222 solution in Petri dishes and staged according to the pigmentation criteria of Haro and Krueger (1988) under a dissecting microscope for comparison to the glass eel stages used later in the study. Prior to use, large batches of glass eels were sampled for pigmentation stage diversity to confirm life stage (50 specimens each). Batches with samples less than 25% stage 7 glass eels were collectively considered to be glass eels. Stage 7 was the darkest stage in the criteria, and was considered the delineation point between glass eels and young elvers.

Single washing assays

To test whether American glass eels were attracted to undiluted, 1/2, 1/4, 1/8th, 1/16th, 1/100th, 1/500th, and 1/1000th dilution washings, and well water as a control (to determine the baseline response without a stimulus), a second batch of glass eels was collected from the Maryland DNR trapping site and transported to the Northern Appalachian Research Laboratory on April 6, 2015. They were maintained in the laboratory for 48 hrs as reported above, and 50 of those glass eels were staged to confirm they were glass eels and for later comparison to the glass eel stages used later in other assays. Three days later, experimentation began. Twenty-four hrs prior to each night of testing, 40 glass eels were randomly selected from the social housing tank and isolated for pre-exposure bias in individual 1.4 L aquaria. After isolation, glass eels were individually transferred into a maze with flowing well water (5.0 ± 0.5 L/min at 20.0^{o}C ± 2.0^{o}C) for individual testing. Upon the introduction of one glass eel, video recording began and each specimen was given a 5 min acclimation period with only well water pumping into both arms, followed by a 10 min period of only well water pumping as a control period. Glass eel washings were then randomly introduced into one arm with only well water pumping into the other, and another 5 min acclimation period was given. Ten min of footage with washings pumping into one arm was then recorded as the experimental period. The tip of the nose of the glass eel was used to determine its location given olfactory stimuli and associated responses were being tested. The location of the rest of the body was not considered in the analysis. Glass eels' whose noses were on the dark grey arm boundary lines were not considered to be in the arm. Upon completion of the trials, video recording ceased, the glass eels were removed, and the mazes and pump lines were flushed for 10 min with clean well water.

All trials were run in the dark between 1800-0000 hrs over six non-consecutive nights. Four mazes were run simultaneously, with 13-18 replicates collected for all washing concentrations and the control, with the exception of the 1/1000th dilution. Nine replicates were observed for this dilution only during a pilot study (see below). Frozen washings were thawed at ambient temperature one night in advance. After they thawed, they were kept in a refrigerator at 3^{O}C and were discarded if not used within 96 hrs. Trials were disqualified from use if the glass eel did not enter both arms of the maze during the control period, if the video quality was too poor to accurately score, if the glass eels did not receive at least 18 hrs of isolation, or if the temperature, flow rate, or unidirectional flow were outside of the listed ranges or definitions (Tables 1.1-1.3).

All dilution trials were fully randomized during the initial study with the exception of the 1/2 and 1/4 dilution trials. Those two dilutions were randomized independently on May 14 and 16, 2015 using glass eels from a third collection batch (collected April 27, 2015) due to a priori allocation of limited resources to lower concentration assays. The glass eel used for these trials were re-staged to confirm pigmentation comparability. Once all trials were recorded, an unbiased viewer scored the behavior of the glass eels during the 10 min control and experimental periods of each trial on a later date. The times spent in each arm were recorded both before and after washing introduction to the nearest five-second intervals. Indices of preference were obtained by calculating the difference between the proportion of time spent in each arm during the control and experimental periods using the equation $(T_{W2}/(T_{W1}+T_{W2})) - (T_{C2}/(T_{C1}+T_{C2}))$, where T is time, W is the washing arm, C is the control arm, 1 is the control period, and 2 is the experimental period (Siefkes et al. 2005). Wilcoxon signed-rank tests using the absolute values of the indices of preference as the response variables were used to determine whether glass eels

preferred the washing arm versus the control arm when exposed to the washings, and the standard error was calculated to represent variation given unequal sample sizes. Both one- and two-tailed statistical tests were used for the data analysis given the results of a pilot study (see below). Alpha was set at 0.05. These methods of preference calculation were selected to be consistent with the methods of Siefkes et al. (2005). The mean indices of preference were graphed against the washing dilution in SigmaPlot, and linear, exponential rise to maximum, logarithmic, and quadratic trend lines (four common curve shapes) were fit to the data to illustrate the concentration-response relationship. The R^2 and *P*-value was calculated for each curve, and the trend line with the highest R^2 and lowest *P*-value was considered the best fitting relationship.

Washing comparison assays

Following the single washing assays, washings were presented simultaneously (one in each maze arm during the experimental period) to determine whether American glass eels had preferences for higher or lower concentrations. The glass eels were staged again to confirm development comparability, and the methods used in the single washing assays were re-applied to the washing comparison assays. Randomized experimentation resumed on April 15, 2015 and occurred over three nights. Sixteen to 18 replicates were collected for four washing comparisons, which were undiluted washings to well water as a positive control, undiluted washings to 1/2 dilution as a similar-strength cue comparison, undiluted washings to 1/16th dilution as a stronger to weaker cue comparison, and undiluted washings to 1/100th dilution as a dilute background odor assay. The undiluted washings to 1/2 dilution comparison was conducted on May 22, 2015

after the initial comparisons took place (undiluted to 1/16th, 1/100th dilutions, and well water) due to the a priori allocation of limited resources towards more dilute comparisons. Glass eels used in this comparison were staged to confirm developmental comparability before use. The data were scored in the same fashion as the single washing assays, and Wilcoxon signed-rank tests were conducted to determine whether glass eels preferred one concentration arm to the other. The mean indices of preference toward the undiluted glass eel washings despite the simultaneous presence of other washings were graphed in SigmaPlot. Fifty glass eels were staged again after the completion of all comparison assays to confirm comparable developmental stage.

Developmental assays

To determine whether American glass eel affinity to glass eel washings changed during late stage glass eel transition into elvers, a third batch of glass eels was collected from the Maryland DNR trapping site, and individuals from that batch were exposed to undiluted washings in single washing assays every fourth night for three weeks total, beginning on April 30, 2015. Using the methods described for single washing assays, stages 3 through 7 glass eels were tested, and later individually staged according to Haro and Krueger (1988) in a 200 mg/L MS-222 solution under a dissecting microscope. If glass eels appeared to be on the border between pigmentation stages, they were considered to be the lower of the two stages. Seventy-two total replicates were collected over five nights. After determining their stages and calculating indices of preference to the washing, independent, one-way, weighted ANOVAs were conducted to determine whether there were any differences between the individual pigmentation stages' indices of preference and washing affinity by collection night. One-way, weighted ANOVAs

were selected given the evidence from the prior assays that consistently indicated an attraction to the washings across multiple glass eel stages with variable sample sizes. Graphs of mean indices of preference to glass eel washings throughout the glass eel-elver transition period by pigmentation stage and by collection night were plotted in SigmaPlot. A linear trend line was fit to the collection night data to quantify the basic trend in change of washing affinity over time.

Pilot study

Prior to the main study, on April 8 and 9, 2015, a pilot study was conducted using an abridged, live-viewed (i.e., not video-recorded, but scored during experimentation) version of the single washing assay methods to determine whether the conspecific washings incited any discernible behavioral response before using them in the main assay, and attempt to identify a lower boundary for washing sensitivity. Undiluted, 1/16th, and 1/1000th dilution single washing assays were conducted. American glass eels were attracted to the undiluted washings and the 1/16th dilution washings (Two-tailed Wilcoxon signed-rank tests: P=0.016, n=10; P<0.05, n=8, respectively). Washings diluted to 1/1000th concentration were not attractive to the glass eels and thus marked a suspected working lower boundary of response to the degree of washing dilution (Two-tailed Wilcoxon signed-rank test: P>0.05, n=9). The results led to adjustment of the planned experimental washing concentrations, and permitted the use of one-tailed tests for all single washing assays with a stimulus thereafter. Fifty glass eels used in this pilot study were staged to confirm their developmental comparability.

RESULTS

Qualitative behavioral observations

American glass eels exhibited regular search patterns when placed into the mazes. Glass eels consistently searched the perimeter of the mazes in an oblong pattern for several minutes at a time, then periodically switched direction and continued this pattern. The fish frequently paused in the corners of the mazes and alongside the screens and attempted to climb. Some glass eels were more active than others, and swam rapidly for the full 30 min period, while others were less active. In many trials, highly unequal arm times (the glass eel spent more than twice the time in one arm than the other) were observed during control periods. Some glass eels did not enter both arms during the control period (trials discarded from analysis), while others remained in the control arm well into the experimental period, and could have been unaware of washing introduction for extended portions of it. When washings were introduced, some glass eels actively swam upstream into the arms and thoroughly searched the screen near the washing source, while others did not pursue the washings as actively.

Single washing assays

American glass eels were attracted to undiluted and 1/2-dilution washings glass eel washings (One-tailed Wilcoxon signed-rank tests: P=0.032; P=0.028, respectively; Table 1.1). They were not attracted to 1/4, 1/8th, 1/16th, 1/100th, and 1/500th dilution washings (One-tailed Wilcoxon signed-rank tests: P=0.087; P=0.184; P=0.062; P=0.326; P=0.436, respectively; Table

1.1). The water control assay indicated that the glass eels were not arm biased and produced a slightly negative index of preference in the control mazes without any added stimulus (Two-tailed Wilcoxon signed-rank test: P=0.347; Table 1.1). The index of preference to conspecific washings decreased with decreasing concentration, best fitting a first-order logarithmic concentration-response relationship (R^2 = 0.87, P=0.002) (Figure 1.3). The R^2 values of the linear, exponential rise to maximum, and quadratic curves were 0.62, 0.76, and 0.74, and the P-values were 0.023, 0.007, and 0.30, respectively.

Table 1.1: Mean ± SE index of preference (i.p.) and associated data of American glass eels in response to glass eel washings presented as single washing assays in two-choice mazes at the Northern Appalachian Research Laboratory. Disqualified n indicates the number of trials that were eliminated from data analyses because glass eels did not meet the criteria for scoring.

Washing concentration	Undiluted	1/2	1/4	1/8	1/16	1/100	1/500	Well water Control
Mean i.p. ±	0.202 ± 0.106	0.198 ± 0.088	0.108 ± 0.079	0.080 ± 0.088	0.127 ± 0.082	0.029 ± 0.076	0.000 ± 0.063	-0.117 ± 0.108
P-value for attraction	0.032	0.028	0.087	0.184	0.062	0.326	0.436	0.347
Total n	18	15	15	16	14	15	16	13
Disqualified n	0	1	1	2	4	3	2	5

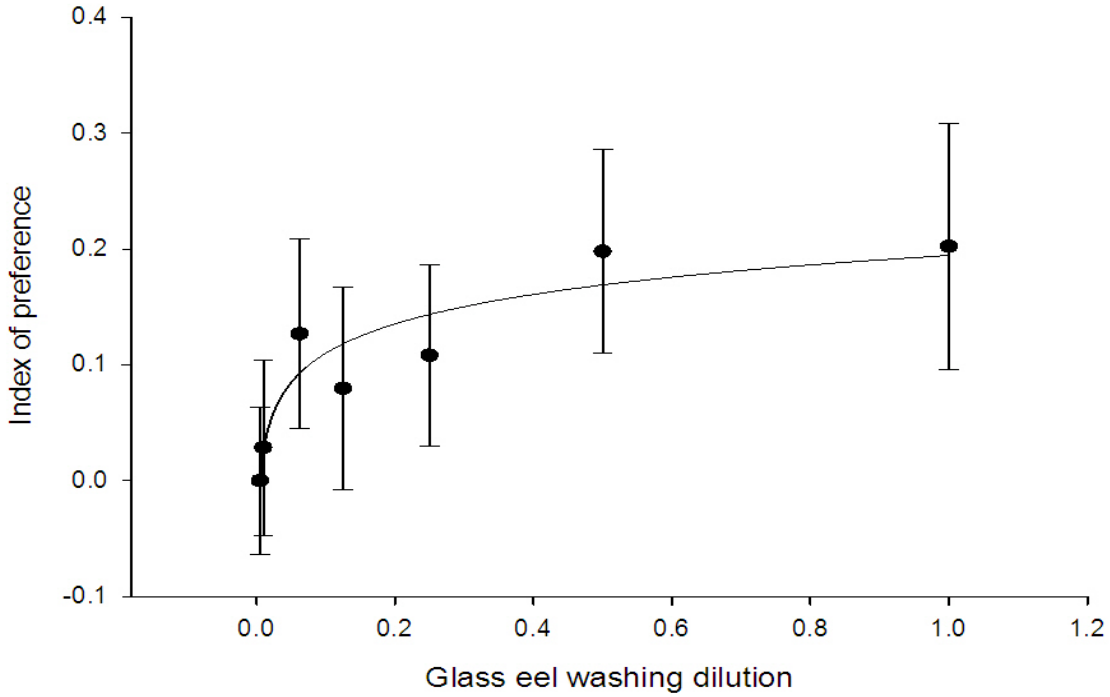

Figure 1.3: Logarithmic concentration-response curve (R^2=0.87, P=0.002) of the mean ± SE American glass eel indices of preference to various concentrations of glass eel washings presented in single washing two-choice maze assays at the Northern Appalachian Research Laboratory. Washing dilution denotes the dilution ratio of the undiluted glass eel washing to well water to achieve the desired testing concentrations.

Washing comparison assays

Undiluted washings were more attractive to American glass eels when directly compared to 1/100th dilution washings and to 1/16th dilution washings (Two-tailed Wilcoxon signed-rank tests: P=0.006; P=0.044, respectively; Table 1.2). Neither undiluted washings nor the 1/2-dilution washings were more attractive when directly compared (Two-tailed Wilcoxon signed-rank test: P=0.509; Table 1.2). Undiluted washings were still attractive when compared to well water (Two-tailed Wilcoxon signed-rank test: P=0.003; Table 1.2). A comparison of the indices of preference of undiluted washings indicated that the index of preference gradually decreased as the comparative washing concentration increased (Figure 1.4).

Table 1.2: Mean ± SE index of preference (i.p.) and associated data of American glass eels in favor of undiluted washings (ud) in response to combinations of glass eel washings presented in washing comparison assays in two-choice mazes at the Northern Appalachian Research Laboratory. Disqualified n indicates the number of trials that were eliminated from data analyses because glass eels did not meet the criteria for scoring.

Washing comparison	Ud to 1/2	Ud to 1/16th	Ud to 1/100th	Ud to well water
Mean i.p. of ud washings	0.091 ± 0.103	0.177 ± 0.082	0.244 ± 0.075	0.279 ± 0.078
P-value for ud preference	0.509	0.044	0.006	0.003
Total n	14	17	18	18
Disqualified n	2	1	0	0

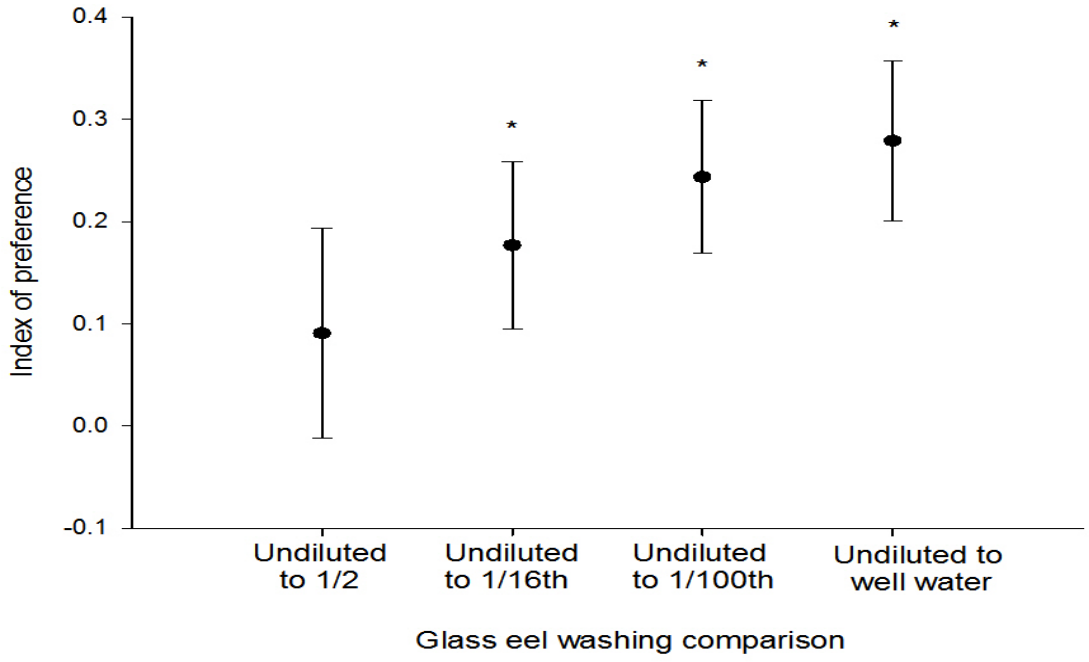

Figure 1.4: Comparison of the mean ± SE American glass eel indices of preference in favor of undiluted glass eel washings relative to the diluted washing during comparative washing two-choice maze assays at the Northern Appalachian Research Laboratory. Fractions correspond to those respective glass eel washing dilutions. Columns with an asterisk indicate a significant preference for the undiluted washing based on the *P*-value of the Wilcoxon signed-rank test at $\alpha \leq 0.05$.

Developmental assays

The indices of preference of stages 3 through 7 American glass eels to undiluted glass eel washings did not significantly differ from each other (ANOVA: $F_{4, 71}$=0.49, P=0.743; Figure 1.5). However, stages 4 through 6 glass eels were attracted to the washings (One-tailed Wilcoxon signed-rank tests: P=0.005; P=0.034; P= 0.003, respectively; Table 1.3) while stage 7 glass eels were not (One-tailed Wilcoxon signed-rank test: P=0.069; Table 1.3). Only two replicates of stage 3 glass eels were collected, precluding a Wilcoxon signed-rank test, but both were strongly positive and indicated that attraction at this stage was possible. Analysis of the data by test night indicated that the washings elicited consistent levels of attraction throughout the study, suggesting minimal washing degradation (ANOVA: $F_{4,71}$=1.36, P=0.257; Figure 1.6); However, there was a weak negative linear trend over time (R^2=0.49, P=0.116). The pigmentation stages of the glass eels used across the study were similar (Figure 1.7).

Table 1.3: Mean ± SE index of preference (i.p.) for attraction and associated data of American glass eels in response to undiluted glass eel washings repetitively presented in single washing assays by pigmentation stage (Haro and Krueger 1988) during elver transition in two-choice mazes at the Northern Appalachian Research Laboratory. Disqualified n indicates the number of trials that were eliminated from data analyses because glass eels did not meet the criteria for scoring

Glass eel stage	3	4	5	6	7
Mean i.p. for attraction	0.568 ± 0.130	0.193 ± 0.082	0.146 ± 0.072	0.312 ± 0.079	0.174 ± 0.105
P-value	n/a	0.005	0.034	0.003	0.069
Total n	2	24	22	14	10
Disqualified n	0	2	4	2	0

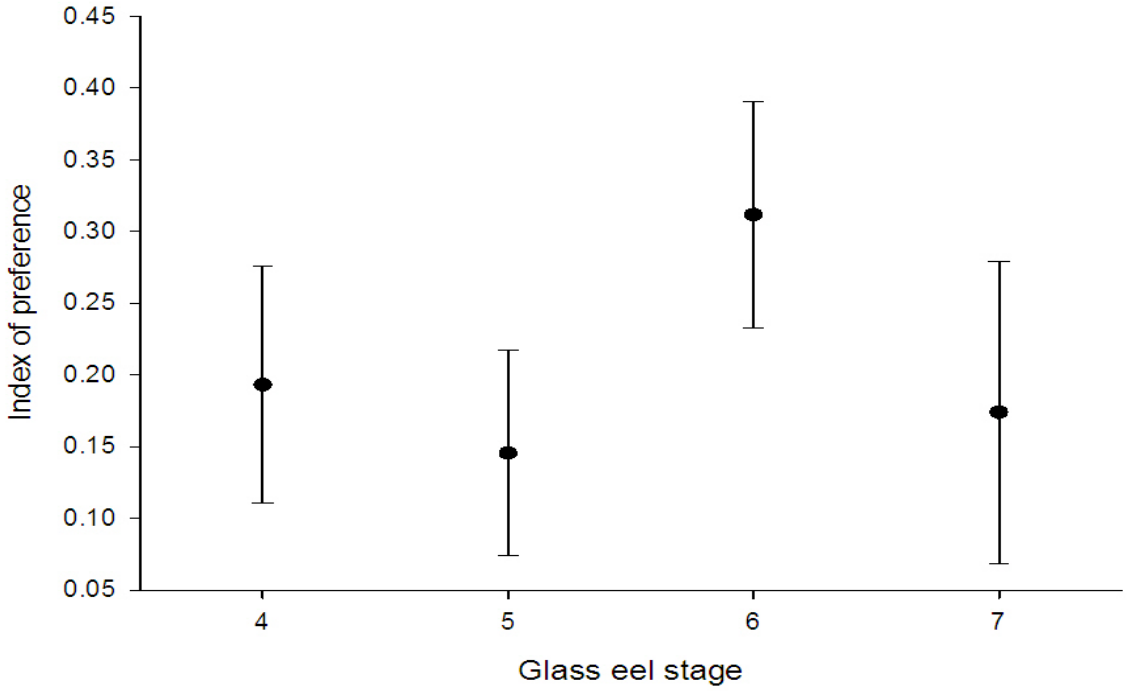

Figure 1.5: Behavioral responses of stages 4 through 7 American glass eels measured by index of preference during developmental two-choice maze assays to undiluted glass eel washings (Haro and Krueger 1988) during late-stage glass eel transition into elvers at the Northern Appalachian Research Laboratory.

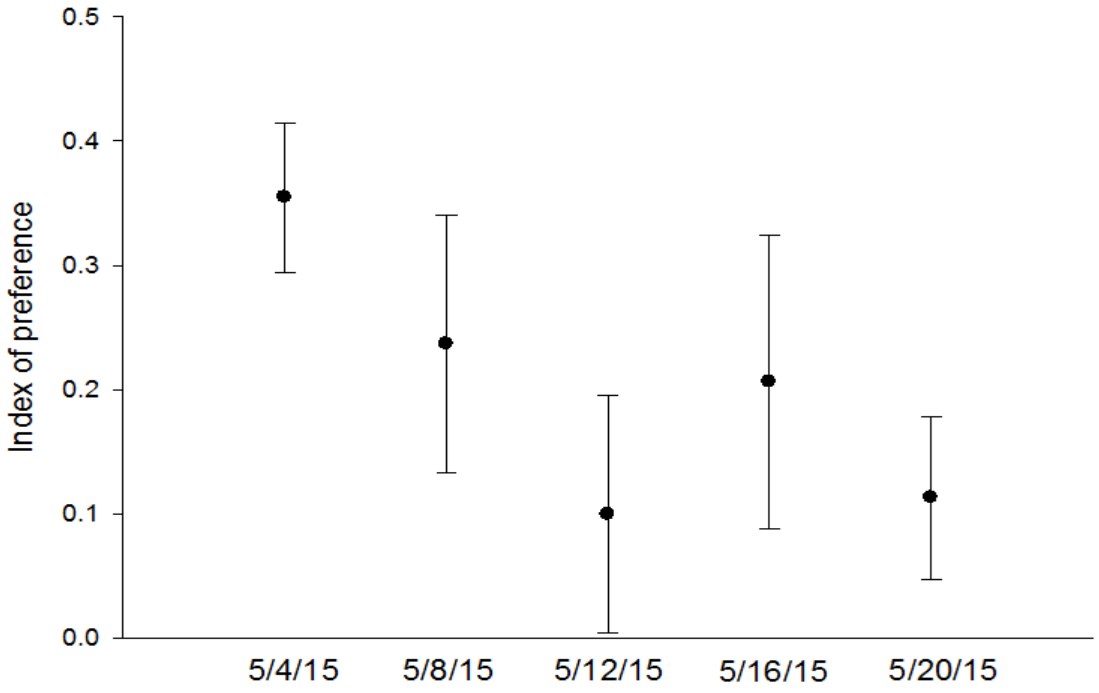

Figure 1.6: Behavioral responses of American glass eels to undiluted glass eel washings measured by index of preference during developmental two-choice maze assays illustrates washing affinity over the course of the experiment at the Northern Appalachian Research Laboratory.

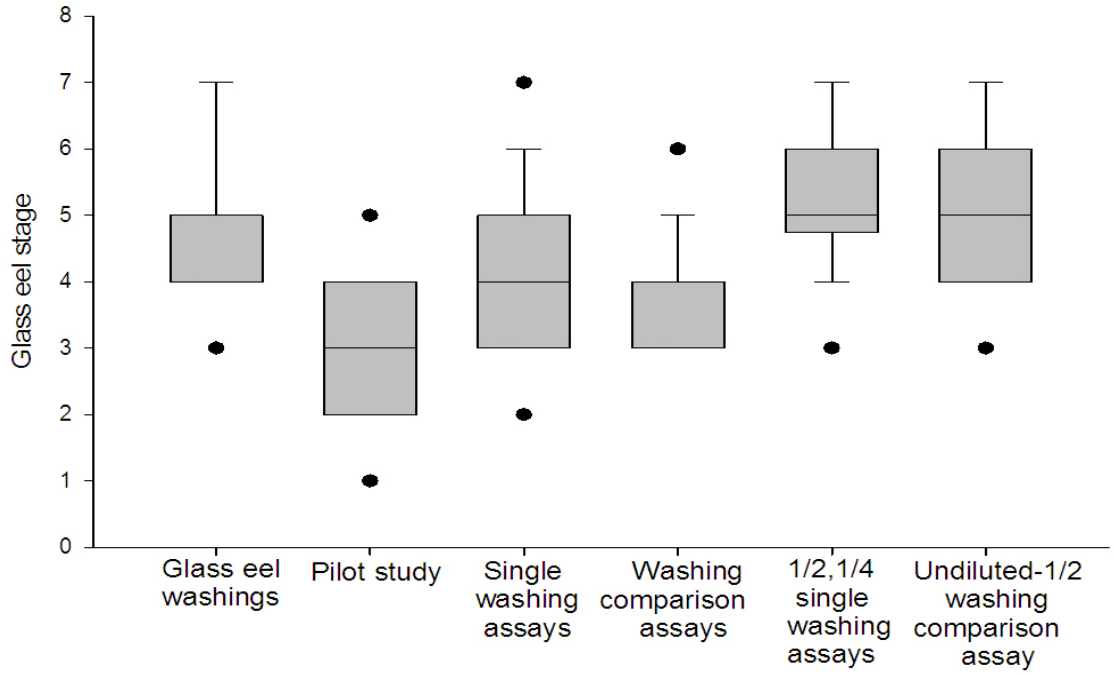

Figure 1.7: Box plot of American glass eel pigmentation stage variation (Haro and Krueger 1988) used across all two-choice maze assays with conspecific odors in the study and for making glass eel washings at the Northern Appalachian Research Laboratory. Center lines indicate median values, box ends are quartiles, whiskers are 10th and 90th percentiles, and points are outliers.

DISCUSSION

Results indicated that there was an odor in the American glass eel conspecific washings that produced a consistent, robust behavioral response in other glass eels at multiple concentrations that was not lost during transition through the later stages of pigmentation. The data supported several aspects of the conspecific chemical cueing hypothesis and was repeatable over several nights. The observed glass eel behavioral responses to conspecific washings were similar to those noted in older life stage anguillids (Pesaro et al. 1981; Sorensen 1986). The transitional range in the concentration-response curve where glass eels lost attraction in this study was roughly a 554-fold dilution of the most dilute, response-evoking washings used by Pesaro et al. (1981), and a 38-fold dilution of the most dilute, response-evoking washings used by Sorensen (1986). Behavioral assay and electroolfactogram data on anguillids using known substances suggest that anguillids can detect very dilute compounds down to 10^{-10} M concentrations (Sola and Tosi 1993; Sola and Tongiorgi 1996 Huertas et al. 2007; Crnjar et al. 2012), which could explain why our washings were still detectable at such great dilutions. Because it was beyond the scope of this study to determine the active components in the washings, we could not calculate molarity to confirm these results. We observed glass eel behaviors similar to those reported in studies that examined responses to molecules, such as bile salts (Sola and Tosi 1993; Sola and Tongiorgi 1996), and other studies that released conspecific washings near fish capture devices (Briand et al. 2002). However, these studies failed to isolate their specimens for pre-exposure bias, record specimen development by pigmentation stage, or calculate the index of preference for their stimulus, so the comparability of the results in these regards is limited.

In addition to showing an affinity to the odor, the data indicated that glass eels preferred areas with higher concentrations of odor, that they could differentiate between multiple odor plumes, and that they could make preferential decisions. The glass eel washings also incited consistent directional movements toward the odor sources based on the qualitative observations. These results were consistent with how the conspecific chemical cueing hypothesis presented in other species, whereby Sea Lampreys, salmonids, and pomacentrids preferred movement toward or settlement in areas containing higher concentrations of conspecific odor (Nordeng and Bratland 2006; Wagner et al. 2009; Ben-Tzvi et al. 2010; Lecchini and Nakamura 2013). This further substantiated conspecific chemical cueing as a likely mechanism for American Eel migration coordination.

The logarithmic best-fit concentration-response relationship had never before been documented in American glass eels and lends additional insight into the nature of this potential mechanism. Results indicated that responses to glass eel washings remained strong through late-stage glass eel transition into young elvers, but there could have been a loss of affinity by stage 7. This could be an early indication that affinity for this cue becomes less prominent in the elver stage, perhaps because the major inland migration is usually complete by this life stage transition (ASFMC 2000). However, there could have also been no such trend, as the individual glass eel stage indices of preference for the washings were not significantly different from each other and the immediately prior stage (stage 6) washing affinity was the highest of any tested. Given that glass eels and elvers can occupy the same habitats and elvers are still migratory to an extent, retention of some affinity to the cue might also make ecological sense (ASMFC 2000). Pigmentation stage according to Haro and Krueger (1988) was used to quantify glass eel development, but future studies should also consider whether other methods, such as those used

by Strubberg (1913) or Boetius (1976), which include different morphological or physiological criteria, may be more relevant to possible olfactory changes.

While the data supported that conspecific chemical cueing may be a mechanism of migration in American glass eels under laboratory conditions, how these results would differ under field conditions, the actual purposes of this cue, and the distance over which it is effective were not clear. Specifically, we could not infer how the small, artificial arena affected the response compared to how natural conditions would. Given the potential for large dilution factors of such odors in estuaries, the washing concentrations tested could have been unrealistically high. We could also not infer whether the observed response was a long-distance cue or some other type of more proximal cue, such as a schooling cue. Anguillids have been known to school to reduce energy consumption, provide protection, and obtain food (including from conspecific sources), and olfactory detection and conspecific odor affinity could simply be a mechanism of aggregation for such purposes (Burgerhout et al. 2013; Musumeci et al. 2014). Further experimentation would be needed to rigorously test these hypotheses.

Exposing glass eels to different concentrations of washings, as well as new types of washings, such as food, starved eel, dead eel, or individual bodily component washings, could continue to lend more insight into American Eel conspecific chemical cueing and chemical ecology. Assays could be conducted with glass eels from other locations to determine affinity variation across the range of the species, as well as with elvers and yellow eels to provide data about cue response shifts later in the eel life cycle. Applying this research model to other anguillids, such as European Eels, may also help characterize the role of chemical cues in migration, and assays designed to quantify the timeframe in which this affinity remains strong would also be helpful. Should these assays continue to yield meaningful results, chemical

analysis and electroolfactograms using preserved samples could potentially provide data regarding the molecular identity of the stimuli. Field assays in more lifelike settings could confirm laboratory results regarding cue attraction, determine more accurate distances the cue functions, and further refine its implications for use in species management.

In summary, odors emitted by glass eels were detected by other glass eels and triggered a consistent attraction over a wide range of concentrations that persisted throughout late-stage glass eel transition into elvers. Given the magnitude of the behavioral response and consistency of affinity toward higher odor concentrations, these cues may be one mechanism American Eels use to coordinate inland migrations that warrants further analysis. Because mixed statistical results in cue affinity were found during the glass eel transition into young elvers, conspecific chemical cueing may be conserved into the elver life stage. Combined, the results suggest that juvenile eels that encounter the odor of distant conspecifics may opt to actively move toward the largest or closest source under laboratory conditions. If multiple waves of juvenile eels were sequentially positioned throughout a confined area, perhaps a chain of odor gradients could be created that migratory individuals could follow (Jessop 2002). American Eels are proving to be a valuable species for advancing understanding of conspecific chemical cueing and chemical ecology in fishes, and future investigation of conspecific chemical cueing may ultimately provide a means to enhance population restoration efforts for this imperiled species.

ACKNOWLEDGEMENTS

Nicholas S. Johnson, Heather S. Galbraith, and Weiming Li were coauthors on this manuscript. The authors thank the staff of the Northern Appalachian Research Laboratory for their valuable assistance in performing this study, particularly David Dropkin, Jeffrey Henry, Carrie Blakeslee, and Sophie Weaver. We would also like to extend our gratitude to Keith Whiteford of the Maryland DNR for providing the specimens for this study, the anonymous reviewers, the Li Laboratory of Michigan State University, notably Tyler Buchinger, and staff of Hammond Bay Biological Station for their consultation, assistance, and commentary on the study. This manuscript is contribution number 2008 of the U.S. Geological Survey, Great Lakes Science Center. The U.S. Geological Survey Fisheries Program provided funding for this study. The U.S. Geological Survey Institutional Care Committee approved use of specimens, and the Michigan State University Animal Use and Care Committee granted an animal use exemption form because animal care was under the auspices of the U.S. Geological Survey. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

CHAPTER 2: AMERICAN SILVER EELS DO NOT SHOW BEHAVIROAL RESPONSES TO CONSPECIFIC ODORS IN A LABORATORY FLUME BIOASSAY

ABSTRACT

American Eel *Anguilla rostrata* populations have declined in recent decades. Sexually maturing silver eels, outmigrating from streams to their oceanic spawning grounds, frequently encounter migratory blockades and can experience high mortality at active hydroelectric turbines. In a search for tools to help improve downstream passage effectiveness, we investigated whether American silver eels use conspecific chemical cueing when they aggregate during downstream migration and to avoid danger. In a laboratory flume bioassay, silver eels were exposed to both live (putative attractant) and dead (putative repellent) silver eel conspecific washings to determine whether their trajectory of downstream movement, level of activity, and time spent in targeted areas of the arena changed after exposure to conspecific chemical cues. Silver eels were not strongly attracted to or repulsed by either odor according to our multiple scoring metrics, and the results did not support the hypothesis that conspecific chemical cueing plays a role in downstream silver eel migration or danger avoidance. Conspecific chemical cues may still play important roles in the silver eel life stage in ways not readily apparent in this type of assay and warrant further investigation.

INTRODUCTION

American Eels *Anguilla rostrata* are a scarce, but valuable economic, recreational, and ecological resource (Ogden 1970; Hurley 1973; ASMFC 2000; Haro et al. 2000; Lellis et al. 2013). They are catadromous, residing in fresh or brackish waters for much of their life, but migrate to the Sargasso Sea to spawn (Huertas et al. 2008; Béguer-Pon et al. 2015). During their migrations, American Eels can encounter structures, such as locks and dams, which physically block upstream and downstream movements critical for life history events, habitat access, and ecological health (Pohl 2002; Richkus and Dixon 2003; Kocovsky et al. 2009). Active hydroelectric turbines can inflict high mortality during the spawning outmigration of sexually maturing adults, known as silver eels (Haro et al. 2002; Pedersen et al. 2012; MacGregor et al. 2015). Management efforts, such as trap and transport operations and improving fish ladders, have been implemented to promote bidirectional anguillid passage near these structures, but are not highly effective in all cases (Richkus and Dixon 2003; Calles et al. 2012; Drouineau et al. 2014). A better understanding of the mechanisms driving anguillid migratory behavior would help advance restoration efforts.

Juvenile anguillids are known to use many cues and stimuli, such as light, electromagnetic fields, temperature, tides, salinity, and flow regime, to coordinate inland migration toward freshwater systems (Hasler 1960; Parker and McCleave 1997; White and Knights 1997; Bardonnet et al. 2005; August and Hicks 2008; DuColombier et al. 2009; Durif et al. 2013). However, few are known for the adult downstream migration. Olfaction and conspecific chemical cueing, recently identified as mechanisms for glass eel inland migration coordination (Schmucker et al., in press), could also apply to outmigrating adults. Conspecific chemical cueing is the emission and detection of public chemical cues targeting the olfactory

system that can incite behavioral and physiological responses in distant conspecifics (Morris 1992; Donahue 2006; Valone 2007). Some anadromous species use conspecific chemical cues to school during outmigration (McCormick et al. 1998; Nordeng and Bratland 2006), but disperse when they arrive at the ocean. Because silver eels travel in schools during parts of their migration (Burgerhout et al. 2013) and aggregate during the final spawning event, from an ecological perspective, it is possible that the catadromous American Eel would use chemical cues to coordinate this. Chemical cues that elicit behavioral responses are of interest to fisheries managers because they may have practical applications as attractants or repellants to enhance fish passage efforts.

Studies support the feasibility of conspecific chemical cueing during adult migration by demonstrating affinities to conspecific odors, cues, bodily extracts, and organic compounds across most life stages (Saglio 1982; Tesch 1991; Crnjar et al. 1992; Barbin 1998; Barbin et al. 1998; Briand et al. 2002; Huertas et al. 2007; Schmucker et al., in press). Chemical cues can also promote yellow eel sexual maturation into silver eels (Ghittino et al. 1975; Liu et al. 2003; Huertas and Cerdá 2006) and stimulate morphological and physiological changes suitable for saltwater environments (Pankhurst and Lythgoe 1983; Sorensen and Pankhurst 1988; Durif et al. 2005). Given the important roles conspecific cues can play in anguillids and the changes they can induce, American silver eels may have behavioral responses to conspecific chemical cues.

In contrast to releasing conspecific chemical cues to attract individuals to certain areas or promote sexual maturation, fishes can also transmit other types of chemical signals that repel conspecifics. Some fishes emit alarm cues if an individual is injured or killed to notify nearby conspecifics of danger and incite flight responses (Brown et al. 1995; Chivers and Smith 1998; Wisenden et al. 2010; Wagner et al. 2011; Imre et al. 2014; Sanches et al. 2015). While originally

evolved as microbial and predator defense mechanisms in some fishes (Chivers and Smith 1998; Chivers 2002; Chivers et al. 2007), these cues can be emitted during any other kind of physical altercation where a fish is damaged, such as injuries sustained while passing through riverine structures or fishing devices (Cada 2006). No studies to date have assessed the potential role of dead American silver eel odor as an alarm cue, which could be another way silver eels use conspecific chemical cueing to increase fitness.

A few obstacles make silver eel conspecific chemical cueing difficult to test, namely that the direction of silver eel spawning migration coincides with the direction of river flow (ASMFC 2000; Haro 2003), meaning that outmigrating fish should not be able to detect a cue until it moves downstream of its source. The dilution factor of such a cue would become ever larger as the silver eels neared the coast, potentially making detection more difficult. These predicaments do not negate the possibility or importance of conspecific cueing during migration to silver eels, but suggest that its expression may not be as straightforward and would be more difficult to evaluate in the laboratory. Anguillids could still detect conspecific chemical cues during their downstream migration if they are in close proximity to the source, in water bodies with low flow velocity, or in high population density areas.

We examined conspecific chemical cueing in American silver eels as a mechanism to aggregate during downstream migration and to avoid danger. Silver eel responses to both live and dead American silver eel conspecific washings were assessed while moving downstream in a laboratory flume. We hypothesized that conspecific chemical cues from live silver eels could help maintain aggregations because silver eels school while migrating and spawning, and would thus be attractive. We also hypothesized that conspecific chemical cues from dead silver eels could serve as alarm cues, and would thus be repulsive. These concepts were tested in a new

bioassay using multiple scoring metrics. The results offer insight into potential downstream migration coordination and defense mechanisms in the species, as well as provide a unique system for studying chemical ecology in large migratory fishes.

METHODS

American silver eel collection and care

Thirty-one female American silver eels (mean mass ± SE = 361.2 ± 41.1 g, mean length ± SE = 582 ± 19 mm, mean eye dia. ± SE = 6.8 ± 0.2 mm; Table 2.1) were purchased on October 15 and 20, 2015 from fishermen operating licensed eel weirs on the Delaware River near Hancock and Narrowsburg, NY, United States. Silver eels were held in holding cages after capture prior to purchase in a spring-fed 11^{o}C pond, and were transported to the laboratory over the course of 4 hrs in an ambient temperature, aerated freshwater (from the holding pond) tank. They were distributed into four 450 L flow-through (8 L/min inflow) covered communal housing tanks at 9^{o}C upon arrival at the laboratory. No acclimation period was given because the housing tank and transportation water conditions were similar. They were not given any food or salt during the entirety of the study because silver eels cease feeding at this life stage (Palstra et al. 2008), and were held under ambient lighting conditions. The sex of the fish used in the study could not be confirmed prior to purchase. The fishermen from whom they were purchased typically select for larger fish because they yield higher market prices, but are consequently almost all female.

Table 2.1: Morphological data and characteristics of all PIT tagged American silver eels used to test behavioral responses to live, dead, and control conspecific odor washings in laboratory flume bioassays.

Specimen	Total length (cm)	Total length (mm)	Mass (g)	Dorsal fin length (mm)	Eye diameter (mm)	Pectoral fin length (mm)	Gonad mass (g)	Gonado-somatic index	Fulton's condition factor (K)
3A2	73.3	733	735.4	490.0	8.5	34.0	26.8	3.6	0.187
3D5	56.0	560	259.9	370.0	6.5	27.0	7.4	2.8	0.148
D81	66.5	665	518.9	450.0	7.0	31.0	17.5	3.4	0.176
3AE	54.3	543	271.4	350.0	6.4	20.0	10.3	3.8	0.170
35A	69.1	691	485.1	462.0	7.7	25.0	17.4	3.6	0.147
6CB	54.0	540	271.0	350.0	6.1	30.0	8.6	3.2	0.172
271	59.8	598	431.6	388.0	6.0	25.0	15.6	3.6	0.202
310	51.5	515	212.0	340.0	4.6	21.0	8.0	3.8	0.155
B18	55.5	555	339.4	354.0	7.1	27.0	12.9	3.8	0.199
DB7	60.0	600	326.2	401.0	5.6	25.0	5.6	1.7	0.151
1D5	55.8	558	289.9	373.0	6.7	22.0	11.0	3.8	0.167
0C8	59.0	590	380.8	391.0	7.0	30.0	12.6	3.3	0.185
5EC	56.0	560	339.5	375.0	7.2	25.0	14.5	4.3	0.193
D7B	51.3	513	212.3	348.0	6.9	22.0	9.6	4.5	0.157
56E	49.5	495	191.7	328.0	7.9	22.0	10.1	5.3	0.158
CA9	47.0	470	185.3	305.0	6.9	22.0	8.3	4.5	0.178
640	71.5	715	690.3	466.0	7.6	31.0	27.0	3.9	0.189
Mean	58.2	582.4	361.2	360.3	6.8	25.8	13.1	3.7	0.173
SE	1.9	19.2	41.1	23.7	0.2	1.0	1.6	0.2	0.004

PIT tagging specimens

On October 21, 2015, 18 of the silver eels were randomly selected and implanted with 12mm Biomark (Boise, ID, United States) FDX-B HPT-12 Passive Integrated Transponder (PIT) tags. A Biomark MK 10 implanter was used to insert tags into the muscular region of the back, dorsal of the pectoral fin and lateral of the spine, while holding the eel in an ice bath. This method and location was chosen so that the silver eels could not bite or disturb the wound, and to avoid complications associated with gastric or body cavity implants (Baras and Jeandrain 1998; CATAG 2002; Økland and Thorstad 2013). We did not use chemical anesthesia during the tagging process because it had the potential to impair their olfactory apparatus and affect behavioral responses. After PIT tag insertion, the wound was gently massaged to secure the tag in place. The PIT tag implanter was sanitized in 75% ethanol between injections. Tagged specimens were given at least 120 hrs to recover from handling and allow the implant wound to close before experimentation. During the first 48 hrs, they were kept in pairs in 75 L covered flow-through (4 L/min inflow) aquaria at 9^{o}C to track individual recovery, and were then returned to the 450 L social housing tanks for the remaining 72 hrs. A Destron-Fearing 2001F-ISO (DFW Airport, TX, United States) reader was used to read the tags. The social housing tanks were then labeled with the tag numbers of their respective silver eels. All specimens retained the PIT tag and the surgery resulted in no obvious specimen illness or mortality.

Live silver eel conspecific washing collection

Live silver eel conspecific odors (washings; putative aggregation cues) were collected on October 22, 2015. Four untagged silver eels (mean mass ± SE = 362.4 ± 86.8 g, mean length ± SE = 564 ± 31 mm , mean eye dia. ± SE = 7.8 ± 0.2 mm) were randomly selected from the social housing tanks and placed into a clean, covered plastic container to soak in 55 L of 11^{o}C aerated well water for 25 min. The silver eels were then removed and returned to the social housing tanks, the washings were collected in clean plastic 1 L and 20 L bottles, and frozen at -20^{o}C.

Dead silver eel conspecific washing collection

Dead silver eel conspecific odors (washings; putative alarm cues) were collected on October 23, 2015. Three untagged silver eels (mean mass ± SE = 476.2 ± 121.2 g, mean length ± SE = 631 ± 58 mm, mean eye dia. ± SE = 5.8 ± 0.4 mm) were randomly selected from the social housing and chilled in an ice bath for 10 min. They were then euthanized by blunt force trauma to the head and immediately decapitated following USGS animal use protocols. They were coarsely homogenized to mimic the products of a predation event. 940 g of the product was placed into cheesecloth and secured into a ball with a rubber band. The ball was placed to soak in 55 L of 11^{o}C well water in a clean plastic tub for 25 min. The ball was then removed and washings were collected in clean plastic 1 L and 20 L bottles and frozen at -20^{o}C.

Arena construction

A flow-through 13.5(l) x 2.7(w) m flume was constructed in covered cement raceway to assess silver eel responses to live and dead conspecific washings (Figure 2.1). Rough-cut hemlock boards (4 cm thick) were used to dam the arena to the desired depth of 30 cm, and a 10 cm diameter schedule 80 PVC spray bar was constructed at the upstream terminus to supply an inflow of 270 ± 5 L/min (62-72 cm/min flow velocity) of 8^{o}C well water (Figure 2.2). The upstream dam board was sealed for water-tightness using black eco-safe silicone (Pentair AES, Apopka, FL, United States), neoprene/EPDM/SBR foam tape, wedges, and natural rope fiber, but the downstream dam board was not sealed, allowing the discharge to escape over, around, and underneath it. Two 15 cm high hemlock boards, with 0.25 cm metal washers between and below them as spacers, were stacked on top of each other and placed immediately downstream of the inflow to evenly distribute the current and promote unidirectional flow (Figure 2.2). An arena-wide piece of aluminum grating spanning the entire water column was placed 50 cm downstream of that unit for the same purpose. This blockade defined the upstream terminus of the experimental region of the arena (Figure 2.2).

A peristaltic pump (Cole-Parmer Masterflex L/S, Vernon Hills, IL, United States) with #25 (4.8 mm internal diameter) tubing, located beside the arena approximately halfway downstream, introduced the washings at a rate of 100 ± 5 mL/min (Figure 2.3). The two pump tubing lines were secured in place at the bottom of the arena, one on each side 10 cm distant from the wall facing downstream (Figure 2.3). Dark grey boxes (20(w) x 45(l) cm) were painted (Pentair AES marine-grade epoxy paint, Apopka, FL, United States) around the attachment points, so that the attachment points were centered along the upstream edge of the box (Figure 2.4). Another dark grey line, spanning the width of the arena, connected the top of these boxes

and represented the upstream maxima of washing presence. Initial downstream side choices were recorded at two dark grey lines 1 m downstream of the washing maxima line, each spanning approximately 1/3 the width of the flume (Figure 2.4).

A centered sandbag pseudo-divider 15(h) x 30(w) x 270(l) cm covered in 1 cm mesh net and small rocks was placed at the bottom of the arena approximately 4 m downstream of the washing maxima line. The divider created two equal-sized maze arms that abutted another aluminum grate with 1 cm mesh netting (Figure 2.4). The grate represented the bottom of the experimental region and was 1 m upstream of the lower dam board. Dark gray lines were painted across the top of the pseudo-divider to signify the upstream arm boundaries. Aluminum catwalks were placed over the arena at the top and bottom to provide access to both sides and to the upstream eel release point. A large drain located below the lower dam board collected the arena's discharge. Red spotlights and headlamps were used to provide limited arena lighting for maneuvering and safety. Flumes have been successfully used to test the impacts of flow regime, overhead cover, and physical barriers in downstream migrating fish behavior near man-made structures, and may be useful for testing behavioral responses to chemical odors as well (Amaral et al. 2003; Kemp et al. 2006; Russon and Kemp 2011; Vowles et al. 2014). These types of studies provided models for the arena and experimental design used in this study.

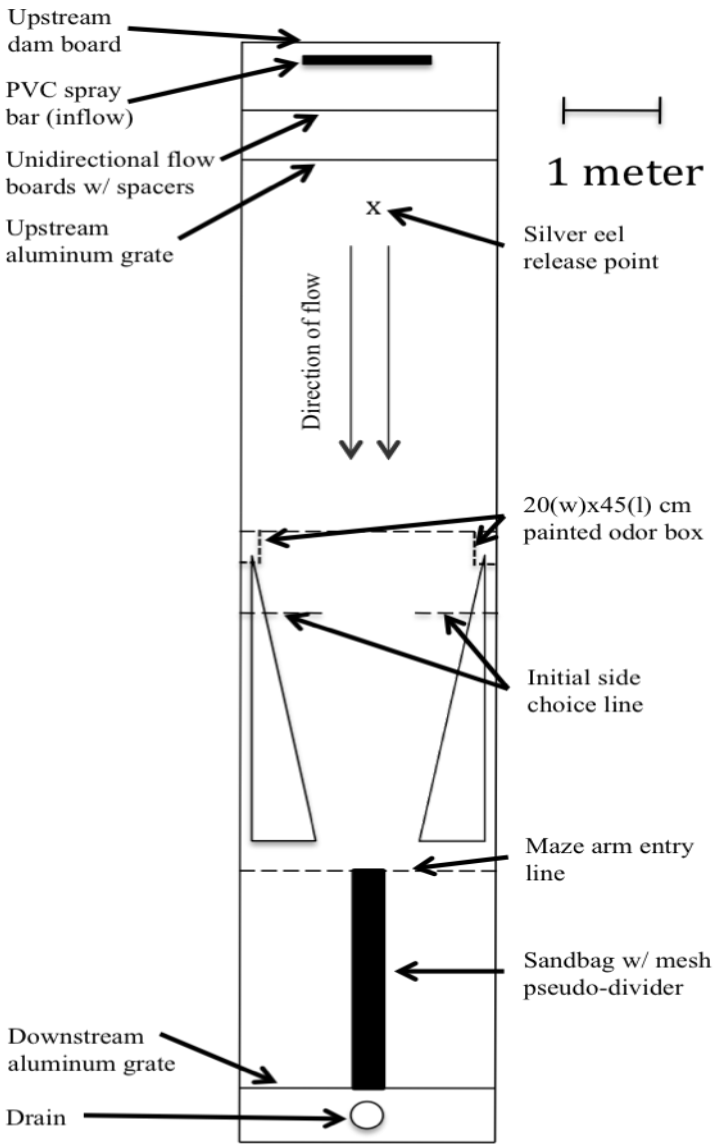

Figure 2.1: Annotated diagram of the flume used for testing American silver eel behavior responses to live, dead, and control conspecific washings in laboratory flume bioassays. Dotted lines represent dark grey painted lines on the bottom of the arena, and solid lines represent physical barriers. Conspecific washings were released at the apices of the triangles and dispersed in this pattern according to dye tests. Arrows represent direction of flow. Figure is to scale.

Figure 2.2: Annotated photograph of the upstream section of the arena used to test American silver eel behavior responses to live and dead conspecific washings in laboratory flume bioassays. Photograph taken by A. Schmucker.

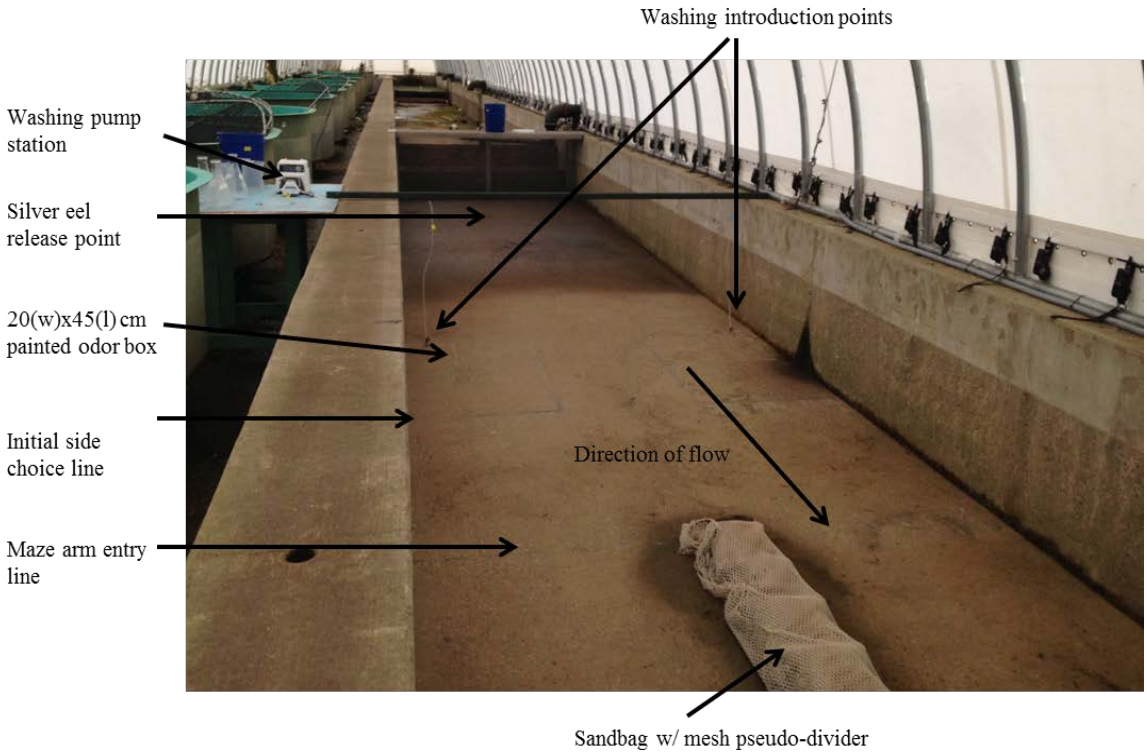

Figure 2.3: Annotated photograph of the middle reach of the arena used to test American silver eel behavior responses to live and dead conspecific washings in laboratory flume bioassays. Vertical lines connected to the initial side choice lines were not used in this assay. Photograph taken by A. Schmucker.

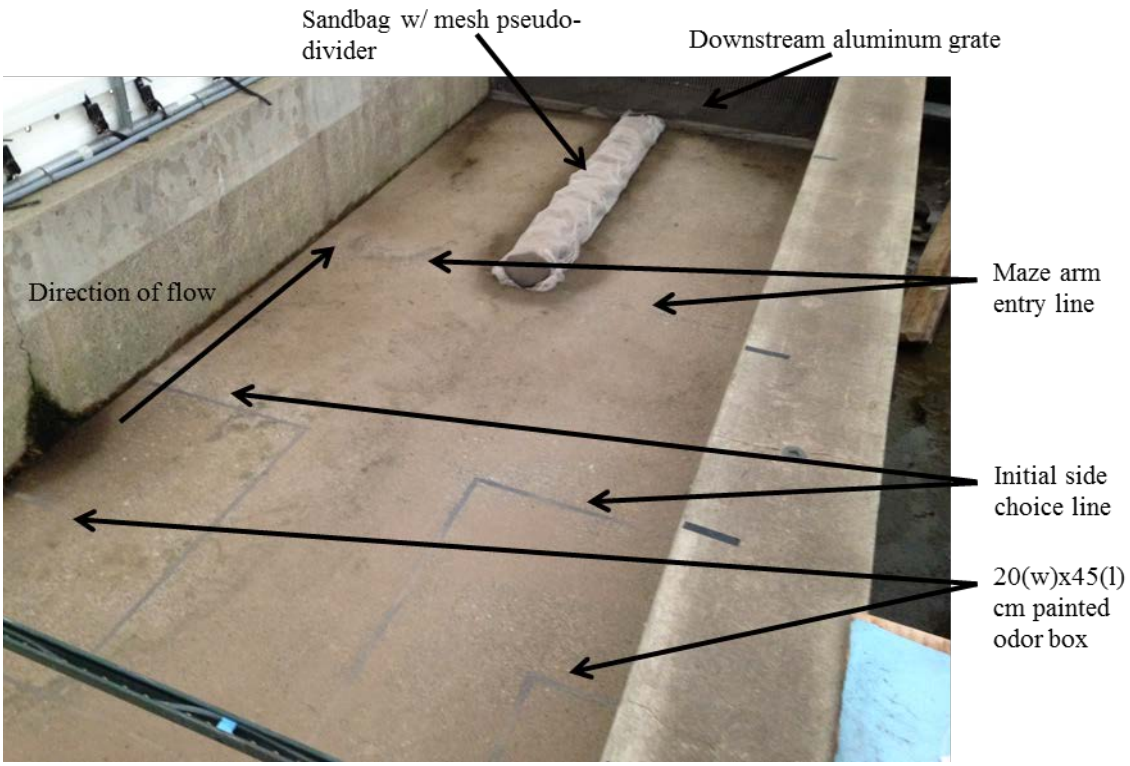

Figure 2.4: Annotated photograph of the downstream section of the arena used to test American silver eel behavior responses to live and dead conspecific washings in laboratory flume bioassays. Vertical lines connected to the initial side choice lines were not used in this assay. Photograph taken by A. Schmucker.

Experimental design

A repeated-measures design was used in this bioassay. Each PIT tagged silver eel was introduced to the three novel washings (live silver eel washing, dead silver eel washing, well water control washing) in randomized order. At least 24 hrs before experimentation, silver eels were randomly selected and individually isolated in flow-through (4 L/min) covered 75 L round tanks to prevent cue pre-exposure bias. After isolation, the silver eels were individually transported to the flume in a clean plastic bucket with 12^{o}C well water over approximately 5 min. Once there, they were then transferred into another clean, covered bucket with 8^{o}C well water to rest stationary for 8 min. During this time, the peristaltic pump started pumping well water for the control period of the assay.

Each silver eel was tested three on different nights, and during each individual night of testing they were tested twice. During the first test, they were exposed to well water control washings on both sides of the arena (nightly control), and during the second test, they were exposed to an experimental stimulus (live or dead silver eels washings, or well-water control). Each silver eel was released in the center of the arena below the upstream terminus and its location (maze arms, box near washing source, upstream of the washing maxima line) was continuously recorded for 12 min. After that period, the silver eel was captured with a net and placed back into its original bucket, but with fresh well water, to re-acclimate. Washings were then pumped randomly into one side of the arena for 8 min, with well water in the other tube, to establish washing plumes that extended into both maze arms. The silver eel was then re-released into the arena and its location was recorded for another 12 min. When the silver eel swam downstream for the first time during the experimental period, its flume side choice (left/right) at

the respective painted lines and the initial maze arm entered were recorded. A silver eel that swam between the two painted lines was considered a “middle” choice.

Upon completion of the trial, the silver eels were removed and returned to the social housing tanks for at least 24 hrs before being used again. Silver eels were given 24 hrs to prevent back-to-back isolation periods and allow for recovery from handling during the trials. A 35 min reset period was given to refresh the arena between tests with different individuals, during which the peristaltic pump lines were flushed with clean well water for at least 5 min remove residual odors. The nose of the silver eel was used to determine its location because olfactory stimuli were being tested, and silver eels resting on a line were considered to be outside of the scoring area it enclosed.

All silver eels were reused until they had been tested with the two experimental washings and the control. All trials were conducted at night between 1700 and 0100 hrs under red light between October 27 and November 20, 2015 because that was when the silver eels were expected to be most active. Washings were thawed in a warm water bath the afternoon before use and were transported to the raceway to acclimate to ambient temperature for at least 1 hr. Dye tests were conducted prior to experimentation with 4 drops/L rhodamine dye (Bright Dyes Fluorescent FWT Red, Kingscote Chemicals, Miamisburg, OH, United States) to confirm unidirectional flow and compare water velocities on both sides of the maze. Unidirectional flow was defined as no dye present in the opposite half of the maze after pumping for 8 min, and comparable water velocities was defined as one side of the arena flowing no more than 20% faster than the other after 8 min of pumping. Upon completion of the study, all tagged specimens were euthanized in a 200 mg/L MS-222 solution to obtain morphological data, determine sex, and calculate gonadosomatic indices to confirm life stage. Gonadosomatic index was calculated

by dividing the mass of the dissected gonads by the total mass of the fish. Specimens with a gonadosomatic index above 1.5 were considered to be a silver eel (Jessop 1987). Fulton's Condition Factor (K) was calculated using the methods of Fulton (1904).

Data collection, scoring metrics, and analysis

The assays were scored first-hand by an unbiased viewer who stood on a cement walkway next to the flume. Five data metrics were collected for each trial to determine silver eel behavioral responses to the washings. Alpha of the study was set at 0.05.

Metric 1: To test whether silver eels maintained steady downstream trajectories after encountering washings, the side of the arena selected by the silver eel was recorded after it passed the initial side choice line (1 m downstream of the washing introduction point). This was then compared to the lower arena maze arm that it entered first. If they were the same, the trajectory was considered steady. If they were opposite, then a deviation occurred. We predicted that if silver eels moving downstream deviated to the opposite side after encountering washings, it could indicate repulsion. Conversely, if silver eels maintained steady downstream trajectories after encountering the washings, it could indicate no response. The ratios of steady downstream trajectories to total recordable trajectories for live and dead silver eel washings were then compared to the control washing for significant differences using a generalized linear model with binary data. Trials where the silver eels made a "middle" side choice, failed to move downstream or enter at least one maze arm, or initially swam down the other side of the flume without any washings were not used for analysis due to the possible lack of washing encounter.

Metric 2: To test whether the silver eels were strongly repulsed by the washings, the time spent upstream of the washing maxima line was recorded before and after washing introduction. We predicted that if a silver eel swam downstream and encountered highly repulsive washings, it could influence the silver eel to move upstream of the washing introduction point altogether for significant periods of time. To test this prediction, correlated two-tailed t-tests were used to determine changes in time spent above the washing maxima line before and after live and dead silver eel washing introduction. One-way repeated measures ANOVA was then used to compare the ratios of time spent above the washing maxima line between the washings before and after washing introduction. One-way repeated measures ANOVA was then used to compare the ratios of time spent above the washing maxima line between the washings before and after washing introduction.

Metric 3: To test whether the silver eels were strongly attracted to the washings, the time spent inside the 20(w) x 45(l) cm boxes surrounding the washing introduction points was recorded before and after washing introduction (live and dead silver eel washings only). We predicted that if silver eels initially swam downstream and encountered washings that were highly attractive, they could suppress their instinct to swim downstream and move back upstream to close proximity of the odor source and remain there for significant periods of time. To evaluate this prediction, correlated two-tailed t-tests were used to quantify strong attraction live and dead silver eel washings by changes in time spent inside the painted boxes close to the odor source before and after washing introduction.

Metric 4: To test whether silver eels were weakly attracted to or repulsed by the washings, the times spent inside each lower arena maze arm before and after washing introduction were recorded. We predicted that silver eels could be drawn to, and would remain in, the side of the lower arena where an odor was present if it was attractive, but could not overcome their instinct to swim downstream. Conversely, if the washings were weakly repulsive, but could not overcome their instinct to swim downstream, they might move to and remain in the lower arena side without washing presence. To evaluate this prediction, preference indices to the washings were calculated and compared using the lower arena maze arm times in the equation $(B_2-B_1)-(A_2-A_1)$, where *B* was time spent in the stimulus arm, *A* was time spent in the control arm, *2* was during the period after stimulus introduction, and *1* was during the period before stimulus introduction. A positive preference index would suggest attraction to a stimulus while a negative preference index would suggest repulsion. Correlated two-tailed t-tests of preference indices were also conducted to determine whether silver eels were attracted or repulsed by each type of washing, and one-way repeated measures ANOVA was used to determine any response magnitude differences between the washings.

Metric 5: To test whether silver eel activity level changed during the experiment, the number of entry times into the odor-side maze arm was recorded before and after washing introduction. We predicted that the number of times the silver eels entered and exited the maze arms reflected their overall activity level, and that washing exposure could increase or decrease activity level relative to how excited the silver eels became. To evaluate this prediction, the number of times the silver eels entered the odor-side maze arm was recorded before and after live and dead silver eel washing introduction. Two-tailed correlated t-tests were conducted to calculate significant

changes for each individual washing, and a two-tailed independent t-test was used to determine any differences between the different washings.

RESULTS

A total of 48 trials were collected in this study, with 16 trials collected for each of the three novel washings. When silver eels were introduced into the flume, they remained stationary at first, but eventually swam downstream to the lower arena moving along the bottom of the flume. This generally occurred between 120 and 360 sec after introduction, but took as little as 27 sec or as much as 619 sec. Six of the 48 total trials failed to move downstream of the washing maxima line during the control and experimental periods. An additional five trials failed to move downstream of the washing maxima line during the control period only, and an additional four replicates failed to move downstream during the experimental period only. Three trials that moved downstream during either the control or experimental period failed to enter the 20(w) x 45(l) cm painted boxes around the washing introduction points. Two trials that moved downstream during either the control or experimental period failed to enter the maze arms. Seven out of the seven scorable live silver eel washing downstream swimming trajectories were steady, seven out of the seven scorable dead silver eel washing downstream swimming trajectories were steady, and 10 out of 11 scorable control silver eel washing downstream swimming trajectories were steady.

None of the five scoring metrics indicated significant silver eel behavioral response to live, dead, or control conspecific washings (Table 2.2). Silver eel downstream swimming trajectory was not significantly different before and after encountering live, dead, or control silver eel washings (Metric 1; Generalized linear model; T=0.003, P=0.998). The amount of time

spent upstream of the washing maxima line was not significantly different before and after live, dead, or control silver eel washing introduction (Metric 2; Correlated two-tailed t-tests; P=0.867, P=0.776, P=0.829, respectively). The ratios of time spent upstream of the washing maxima line were not significantly different among the washings (Metric 2; ANOVA $F_{2,47}$ =0.76, P=0.476). The amount of time spent within the 20(w) x 45(l) cm boxes around the washing introduction points were not significantly different before and after live and dead silver eel washing introduction (Metric 3; Correlated two-tailed t-tests; P=0.659, P=0.414, respectively). Silver eel maze arm preference indices were not significantly different before and after live, dead, and control washing introduction (Metric 4; Correlated two-tailed t-tests; P=0.398, P=0.268, P=0.659 respectively). Magnitudes of the silver eel preference indices to live, dead, and control washings were not significantly different from each other (Metric 4; One-way repeated measures ANOVA; $F_{2,47}$ =1.171, P=0.324; Figure 2.5). Silver eel activity, by comparison of the number of odor arm entries, was not significantly different before and after live or dead silver eel washing introduction (Metric 5; Correlated two-tailed t-tests; P=0.352, P=0.375, respectively). Silver eel activity by measure of odor arm entries before and after live and dead silver washing introduction were not significantly different from each other (Metric 5; Independent two-tailed t-test; P=0.658).

Table 2.2: Comparison of all scoring metric data collected to determine and compare PIT tagged American silver eel behavioral responses to live, dead, and control conspecific odor washings in laboratory flume bioassays.

Stimulus	Metric 1 ratio	Metric 2 (Mean ± SE) control	Metric 2 (Mean ± SE) experimental	Metric 2 ratio	Metric 3 (Mean ± SE) control	Metric 3 (Mean ± SE) experimental	Metric 3 ratio	Metric 4 (Mean ± SE)	Metric 5 ratio	Metric 5 (Mean ± SE) experimental	Metric 5 (Mean ± SE) control
Live silver eel washings	7/7	505 ± 35	497 ± 37	1.016/1	5 ± 1	5 ± 2	0.839/1	30 ± 35	0.737/1	1.188 ± 0.277	0.875 ± 0.221
Dead silver eel washings	7/7	465 ± 56	447 ± 53	1.039/1	6 ± 2	4 ± 1	1.455/1	66 ± 57	0.727/1	1.375 ± 0.350	1.000 ± 0.276
Well water control washings	10/11	502 ± 40	515 ± 60	0.977/1				-25 ± 55			

Figure 2.5: Comparison of American silver eel preference indices to conspecific odor washings (live silver eel, dead silver eel, and well water control) tested in laboratory flume bioassays. Center lines indicate median values, box ends are quartiles, whiskers are 10^{th} and 90^{th} percentiles, and points are outliers.

DISCUSSION

We hypothesized that American silver eels used conspecific chemical cues to aggregate during the downstream spawning migration and to avoid danger, and thus predicted live and dead conspecific cues would be attractive and repulsive, respectively. After analysis of their behavior during exposure to putative attractants and repellants in a laboratory flume bioassay, our results did not align with those from other anguillid conspecific cueing studies. Conspecific chemical cueing expression was not consistent with those observed in juvenile anguillids that typically migrate inland, suggesting it could be life stage dependent. Glass eels consistently showed affinity to live conspecific odors and organic compounds following consistent patterns and discernable relationships (Sola and Tosi 1993; Briand et al. 2002; Huertas et al. 2008; Schmucker et al., in press), but silver eels did not. Elvers and yellow eels had less affinity for conspecific chemical cues and their responses were more consistent with silver eels, but evidence of conspecific chemical cue affinity has still been reported for both (Pesaro et al. 1981; Sorensen 1986; Briand et al. 2002). The American silver eel olfactory system has been known to change during development, which may impact behavioral responses to conspecific chemical cues relative to other life stages (Sorensen and Pankhurst 1988; Churcher et al. 2015).

Studies have shown evidence of the importance of olfaction to yellow and silver eel behavior in estuarine environments, but they did not quantify affinity to specific odors by measure of preference index or their ability to impact fish location in an arena (Tesch 1991; Barbin 1998; Barbin et al.1998). Studies that assessed conspecific chemical cueing for sexual maturation or morphological development in anguillids often measured it over extended periods of time using other methods that involved dissection, observation, exchange of conditioned tank water, or microscopy; not behavioral assays (Ghittino et al. 1975; Pankhurst and Lythgoe 1983;

Sorensen and Pankhurst 1988; Liu et al. 2003; Huertas and Cerdá 2006). We did, however, observe some similar downstream swimming behaviors as other raceway flume studies testing anguillid behavioral responses to other types of stimuli (Amaral et al. 2003; Kemp et al. 2006; Russon and Kemp 2011; Vowles et al. 2014).

Potential silver eel alarm cue responses were not consistent with those observed in other species. Alarm cues in other fishes often presented robust panic responses that resulted in strong flight responses, which were not observed in American Eels (Brown et al. 1995; Wisenden et al. 2010, Wagner et al. 2011, Imre et al. 2014, Sanches et al. 2015). Many studies that addressed these concepts in other species used complicated, specific bodily component extract as stimuli to elicit such responses (Wisenden et al. 2010, Wagner et al. 2011, Imre et al. 2014, Sanches et al. 2015). In this study, generic dead eel washings were used as stimuli that incorporated all bodily components of the fish and were collected using simple extraction techniques. Phylogenetically, both primitive and derived fishes with similar traits use alarm cues, suggesting that anguillids evolved concurrently with this adaptation and should not be excluded from having them for these reasons. Alarm cues may also only still exist in the juvenile life stages given their lower stature in the food web and may not be present in the adults. Considering American silver eels are high-level predators and detritivores (Wattendorf 1979; Lookabaugh and Angermeier 1992), they may be frequently exposed to deathly odors in ways that do not reflect danger and hence are accustomed to it.

Even though the results of this study did not support the hypotheses that silver eels use conspecific chemical cueing to help maintain aggregations during the downstream migration and to avoid danger, silver eel behavioral responses to these types of stimuli may not have been readily apparent in this assay. They may also only serve as a secondary sensory cue during

outmigration. Studies have suggested other types of cues, such as electromagnetic fields, may be used instead of conspecific cues given the long distance and time span of the migration (Gill et al 2012; Durif et al. 2013). The direction of flume flow in this study was east-northeast, which could have affected silver eel downstream movement if they align with electromagnetic fields from the Earth's poles in other directions during outmigration. Other factors could have impacted silver eel behavior in this study as well, such as the metal roof of the flume (potential faraday cage), water temperature, and flow regime, but all were within the limits of those observed in the wild when silver eels outmigrate.

Water temperature used in the housing tanks was only 11^{o}C and was only 8^{o}C in the arena, which could have decreased silver eel activity (Scaion and Sebert 2008). Water for this study was pumped directly out of a well and was not tempered before use. Given the volume requirements for these assays it was the only available option, but was still within average seasonal river water temperature ranges near the collection point (6-19^{o}C; USGS 2016). Flow velocity in the arena was relatively low given the well pump size and water availability, and the fish may not have been able to discern flow direction. Low flow velocity could have impacted their instinct to swim downstream because silver eels have been primarily observed to outmigrate during floods when flows are higher (Haro 2003; Bultela et al. 2014), but low flows are not uncommon in much of their range during seasonal droughts and in tidal reaches.

All silver eels used in the study were female, which could have contributed to the lack of observed response. Given that males and females must come together to reproduce, sex of the specimen emitting or detecting the conspecific chemical cues could be a factor in behavior, with opposites theoretically being optimal. However, silver eels still school with members of the same sex during outmigration and males typically migrate separately than females (Jessop 1987;

Burgerhout et al. 2013), suggesting affinity to homosexual chemical cues could still be possible. Testing only females may have also been beneficial because it removed sex as an additional variable for attraction, thereby simplifying data analysis. Sex of the silver eel should not impact alarm responses to dead eel odor because this adaptation does not directly pertain to reproduction.

This study was a first attempt to establish an experimental system to examine chemical ecology and migration coordination concepts in large diadromous fishes. This type of arena and experimental design has not been reported for testing conspecific chemical cueing concepts, and may be useful for continuing to study these or related concepts in fishes where a large arena is necessary. A traditional two-choice maze assay with the arms at the upstream end of the arena was not selected for this study because silver eels would have had to constantly suppress their instinct to swim downstream while searching for odors upstream. This type of assay may have quantified affinity to washings better, but would not have been effective at indicating repulsion. However, aspects of a traditional design with upstream maze arms were still incorporated in this study through the use of painted boxes near the odor source, as well as overall low flow velocity to minimize the effect of rheotaxis. Future studies may test different types of odors or odor combinations to continue investigation into American Eel conspecific chemical cueing. Artificial fish passage devices could be constructed inside the arena to replicate riverine barriers, and multiple fish could be introduced simultaneously to assess silver eel chemical ecology when schooling.

To summarize, American silver eels were not attracted to or repulsed by either live or dead silver eel conspecific washings in this laboratory flume bioassay according to our multiple scoring metrics. While evidence suggests that conspecific chemical cues do not play critical roles

in aggregation during migration or danger avoidance in this life stage, chemical cues may still be critical to other aspects of anguillid behavior, sexual maturation, or fitness. This study lends insight into previously unexamined aspects of American Eel downstream migration coordination and defense mechanisms, and provides a unique model for studying large migratory fishes in a highly efficient and adaptable arena.

ACKNOWLEDGEMENTS

The authors thank the staff of the Northern Appalachian Research Laboratory for their support and assistance in performing this study, particularly David Dropkin, Jeffrey Henry, Daniel Spooner, Jeffrey Cole, Carrie Blakeslee, and Sophie Weaver. We would also like to extend our gratitude to Alex Haro with the USGS and Jessica Best with the Hudson River Estuary Program/Cornell University for assistance with obtaining the test subjects, the Li Laboratory of Michigan State University, notably Tyler Buchinger, the staff of Hammond Bay Biological Station, and Karen Limburg and Sarah Mount with SUNY-ESF for their consultation, assistance, and commentary on the study. This manuscript is contribution number X,XXX (to be replaced with a real number in the event of publication) of the U.S. Geological Survey, Great Lakes Science Center. The U.S. Geological Survey Fisheries Program provided funding for this study. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The U.S. Geological Survey Institutional Care Committee approved use of specimens and the Michigan State University Animal Use and Care Committee granted an animal use exemption form because animal care was under the auspices of the U.S. Geological Survey. This study was conducted under the Pennsylvania Fish and Boat Commission's Scientific Collector's Type II Permit #2015-02-0362.

CONCLUSION

In conclusion, this thesis addressed several previously unknown functions regarding American Eel olfaction, conspecific cueing, chemical ecology, and behavior as they pertained to migration coordination and danger avoidance. By demonstrating conspecific glass eel washing affinity, cue concentration preferences and differentiation capabilities, and discernable concentration-response relationships in American glass eels, I conclude that conspecific chemical cueing exists in the species and is a strong contributing factor to glass eel behavior. Chemical information transmitted by these conspecific cues is likely one mechanism by which they coordinate inland migrations, but the distance over which it functions could not be inferred. By demonstrating a lack of response to live and dead conspecific chemical cues in American silver eels, I conclude that conspecific chemical cueing may not be an important component of this life stage's behavior, particularly for aggregation during the downstream migration or avoiding danger. However, conspecific chemical cues may still play other important roles in silver eel biology, such as inciting sexual maturation, which may not appear in laboratory flume bioassays. Combined, I conclude that olfaction is an important sensory system to the American Eel and conspecific chemical cues can impact their behavior. However, the nature of its expression may be cue concentration and life stage dependent, it may exist or present only under certain conditions, or it may not have associated detectable behavioral responses. Conspecific chemical cueing warrants continued investigation to better understand all potential functions in the species.

Future studies could continue to address the role and expression of conspecific chemical cueing in the species by testing new types of chemical cues, different concentrations, different physical and environmental conditions, different arenas, and using field settings. Knowledge

obtained from this research could contribute to potential management tools or implications for species restoration goals, such as using chemical cues to make fish lures or repellants. Should this be the case, they could be applied in targeted areas to guide American Eels to safe passage while maintaining existing structures. Fisheries managers and aquatic engineers could also take this information into consideration when designing new riverine barriers within anguillid ranges to proactively facilitate passage before construction begins. Restoration of the American Eel is an ongoing and long-term project, but this thesis contributes information to that effort in a substantial way that sets the foundation for new approaches to this ecological issue. Adaptive restoration efforts must continue, and research initiatives such as this are one method by which restoration of the American Eel will be achieved.

APPENDIX

APPENDIX A: DONOR LIFE STAGE INFLUENCES JUVENILE AMERICAN EEL *ANGUILLA ROSTRATA* BEHAVIORAL RESPONSE TO CONSPECIFIC CHEMICAL CUES

ABSTRACT

American eel *Anguilla rostrata* populations have declined dramatically in the eastern United States, due in part to man-made riverine barriers that block migrations. Management efforts to facilitate eel movement around barriers, such as trap and transport operations, have had limited success and increasing passage effectiveness depends upon a better understanding of factors that influence migration. The present study investigated the potential role of conspecific chemical cues in juvenile eel migrations by assessing glass eel and one-year-old elver affinities to elver washings, and elver affinity to adult yellow eel washings. In two-choice maze assays, glass eels were attracted to elver washings, but elvers were neither attracted to nor repulsed by multiple concentrations of elver washings or to yellow eel washings. These results suggest that American Eel responses to chemical cues may be life stage dependent and that glass eels moving inland may use the odor of the previous year class as information to guide migration. The role of chemical cues and olfaction in eel migrations warrants further investigation as a potential restoration tool.

INTRODUCTION

Anadromous fish populations have declined globally, and American Eels *Anguilla rostrata* (Lesueur 1817) are no exception (Haro et al. 2000). American Eels historically comprised over half of the total fish biomass in eastern U.S. streams, but have declined to <1% of peak abundance in many locations (Ogden 1970; ASFMC 2000; DePhilip and Moberg 2010). Man-made barriers to migration, such as dams, are partially responsible for large-scale declines in eel abundance. Glass eels and elvers (migratory juvenile life stages) are unable to move past many barriers to reach upstream habitat, and adult silver eels (sexually maturing stage preparing to spawn) are vulnerable to high turbine mortality during downstream migration (Haro et al. 2000; Watene and Boubee 2005; Kocovsky et al. 2009). Management efforts, such as trap and transport operations, have been implemented to resolve these issues, but have met with limited success. Increased bidirectional fish passage effectiveness over riverine barriers could help sustainably restore American Eel populations.

Facilitating more effective eel passage depends on a better understanding of factors that influence their migration. Environmental correlates of eel migration in the wild include temperature, lunar phase, salinity, tides, and flow (Hasler 1960; White and Knights 1997; Bardonnet et al. 2005; August and Hicks 2008; DuColombier et al. 2009), but the use of olfaction and conspecific chemical cues have also been suggested, given eels' highly developed olfactory system (Sorenson 1986; Sola and Tongiorgi 1996; Barbin 1998; Barbin et al. 1998; Huertas et al. 2008). A wide range of freshwater and marine taxa release chemical cues, including insects, crayfish, lobsters, catfish, damselfish, sea lamprey, and round gobies (Atema 1986; French and Kline 1989; Breithaupt and Eger 2002; Corkum and Belanger 2007), which incite a variety of physiological or behavioral responses in conspecifics, such as sexual

maturation, species recognition, alarm or panic, and migration (Brown et al. 1995; Huertas et al. 2008; Wisenden et al. 2010).

In laboratory settings, specific amino acids, bile salts, and conspecific cues from elvers and yellow eels were attractive to migratory elvers (Miles 1968; Sorenson 1986; Sola and Tongiorgi 1996). Eels also responded to conspecific bile fluid and skin mucus in trace amounts (Huertas et al. 2007), suggesting detection in large water bodies or over long distances is feasible. In field studies, catches of European glass and yellow eels (inland resident life stage) *Anguilla anguilla* (Lineaus 1758) increased when researchers directed the outflow of an eel trap back onto the eel ladder itself (Briand et al. 2002) or when released near eel traps (Saglio 1982). Eels undergo morphological and physiological transformation throughout their life cycle, and a variety of chemical signals may influence eel migration depending on life stage and sex (ASFMC 2000). No studies to date have directly assessed juvenile eel (glass eel and elver) affinity to immediately older life stage cues as a potential mechanism by which they determine migration pathways. Addressing this knowledge gap would provide insight into unknown aspects of American Eel migration coordination that potentially leads to management applications.

Several hypotheses may explain the use of chemical cues among migratory fish species. The imprinting hypothesis suggests that anadromous fishes become imprinted on a bouquet of odors unique to their stream of origin, which they recognize during spawning migration (Hasler and Wisby 1951). This hypothesis was supported in migratory salmonids, but is not directly applicable to catadromous species such as eels. The pheromone hypothesis proposed by Nordeng (1977) predicts that migrating fishes respond to chemical cues secreted downstream from residents of previous year classes. The conspecific attraction hypothesis (Donahue 2006) expands on this to describe conspecific cuing in terms of either positive density dependence

(e.g., decreased predation, increased foraging success) or an indication of habitat quality as the primary driver behind the signaling. In the latter case, migration cues could be less precise than homing or imprinting to specific spawning grounds (Baker and Montgomery 2001).

The present study tested the attractiveness of conspecific washings to juvenile migratory stages of American Eel in light of (1) the pheromone hypothesis, which predicts newly migrating eels would have an affinity to chemical cues of a previous year class (Nordeng 1977), and (2) the conspecific attraction hypothesis, which predicts a positive density dependence among members of the same year class (Donahue 2006). To test the pheromone hypothesis in juvenile American Eels, the affinity of newly migrating glass eels to elver washings, and the affinity of elvers to yellow eel washings were systematically tested in two-choice maze assays. To test the conspecific attraction hypothesis as a result of the potential benefits of density dependence within the same year class, behavioral responses of elvers to elver washings were also tested at multiple conspecific washing concentrations.

METHODS

Animal collection and care

American glass eels used in behavioral assays were collected from Turville Creek (Ocean City, MD, United States) and Bishopville Prong (Bishopville, MD, United States) in the spring of 2014 and 2015 (Table 3.1). Glass eels were transported in coolers of oxygen-saturated brackish water from the trapping site to the laboratory. They were housed at ambient water temperature (from the collection location), which was gradually increased to 18-20^{o}C for testing, and were salt-treated (~20 ppt standing bath for 30 min) daily to minimize stress and severity of

parasite outbreaks. Housing tanks (370 L) were flow-through (~10-12 $L \cdot min^{-1}$) with natural ambient light/dark cycles. Glass eels were fed a combination of commercial fish mash (Bio-Oregon life stage size zero, East Westbrook, ME, United States), pink brine shrimp flakes (Zeigler, Gardners, PA, United States), chicken liver, and frozen daphnia (Fish King, Inc., Chicago, IL, United States). Housing tanks were cleaned daily.

Elvers used in behavioral assays and for preparing washings came from multiple sources (Table 3.1) including laboratory-reared individuals collected as glass eels in spring 2013, or from the eel ladder at the Conowingo Dam (Susquehanna River) in spring 2015 (Cecil County, MD, United States). Elvers were transported and maintained as reported above for glass eels. Yellow eels used to prepare conspecific washings had been collected as glass eels and reared in the laboratory for 3-4 yrs (Table 3.1). Yellow eels were housed under similar temperature and flow conditions as described for glass eels and fed a commercial fish diet (Bio-Oregon life stage size two, East Westbrook, ME, United States).

Table 3.1: Summary of two-choice maze behavioral assays conducted to assess the roles of conspecific chemical cuing in juvenile American Eels. Test Subject describes the life stage (and their collection information, Test Subject Source) on which behavioral assays were conducted. Test Washing describes the life stage (and their collection information, Washing Source) used to make the odorant washing used in behavioral assays.

Assay Date	Test Subject	Test Washing	Test Subject Source	Washing Source
May 2014	Glass eels (0.15 g/eel)	Elvers (3.02 g/eel)	Glass eels collected spring 2014 (in laboratory <10 days)	Laboratory-reared elvers collected as glass eels spring 2013
Jun/Jul- 2014	Elvers (3.02 g/eel)	Elvers (3.02 g/eel), 15^{o}C	Laboratory-reared elvers collected as glass eels spring 2013	Laboratory-reared elvers collected as glass eels spring 2013
Jul 2015	Elvers (3.02 g/eel)	Yellow eel (9.21 g/eel)	Elvers collected summer 2015 from Conowingo dam	Yellow eels reared in laboratory from 2011 and 2012 glass eels
Aug 2015	Elvers (1.62 g/eel)	Elvers (1.62 g/eel), 22^{o}C	Elvers collected summer 2015 from Conowingo dam	Elvers collected summer 2015 from Conowingo dam
Aug 2015	Elvers (1.62 g/eel)	Elvers (1.62 g/eel), 11^{o}C all dilutions	Elvers collected summer 2015 from Conowingo dam	Elvers collected summer 2015 from Conowingo dam
Apr 2014	Glass eels (0.15 g/eel)	Control	Glass eels collected spring 2014 (in laboratory <10 days)	Well water
Jul 2014	Elvers (3.02 g/eel)	Control	Laboratory-reared elvers collected as glass eels spring 2013	Well water
Aug 2015	Elvers (1.62 g/eel)	Control	Elvers collected summer 2015 from Conowingo dam	Well water

Stimulus preparation

Eel washings were collected from several life stages. Elver washings were prepared by placing 450 elvers in 90 L of aerated water (15^{o}C) in a large plastic tub for 25 min, equivalent to 2.1 eels (6.3 g) $L^{-1}{\cdot}hr^{-1}$ in the spring of 2014 and 2015. Yellow eel washings were collected in the spring of 2015 by placing 23 yellow eels in 90 L of aerated water (15^{o}C) in a large plastic tub for 25 min, equivalent to 0.11 eels (or 1.0 g) $L^{-1}{\cdot}hr^{-1}$. Washings were placed in 1 L polyethylene bottles, frozen, and stored at -20^{o}C until use in behavioral assays. Washings were thawed at ambient temperature 24 hrs prior to use and were refrigerated for no more than 96 hrs after thawing.

Behavioral assays

Glass eels and elvers were tested for affinity to conspecific washings using two-choice mazes constructed from sheet PVC based on the design of Li et al. (2002) and Siefkes et al. (2005), but scaled-down in size for larval fish (Figure 3.1, Figure 3.2). Behavioral assays were conducted in near-darkness between 1900 and 2400 hrs when the eels were most active (Tesch, 2003) using a combination of IRLamp6 infrared (Bat Conservation and Management, Inc., Carlisle, PA, United States) and visible red overhead lights and headlamps. Water depth in the mazes was 8 cm and water velocity was standardized in each arm to ~1 $cm{\cdot}s^{-1}$. Fluorescent dye (Bright Dyes Fluorescent FWT Red, Kingscote Chemicals, Miamisburg, OH, United States) was dripped via a peristaltic pump into each arm of the maze at the start and end of each night of trials to ensure that flow of the washing through each arm of the maze was rectilinear and

confined to the intended maze arm.

Each night, glass eels or elvers were randomly selected from a holding tank and individually placed into a two-choice maze for a 5 min acclimation period during which behavior was not observed. Following acclimation, eel behavior was observed and scored by quantifying time spent in each upstream section of the maze for 20 min with no washing present (control period). Following the control period, the stimulus was randomly introduced to one arm of the maze via peristaltic pump at a drip rate of 5 $mL \cdot min^{-1}$ while well water was dripped at the same rate into the opposite arm of the maze for 5 min without observing behavior. During the subsequent experimental period, behavior was observed and scored for 20 min as described in the control period. The tip of the nose of the eel was used to determine its location because olfactory stimuli and associated responses were being tested. Following each trial, experimental animals were removed and the maze was flushed with well water for 10 min prior to the next trial. A total of 10–16 replicate trials were completed for each washing stimulus, plus a negative control (well water applied to both arms of the maze; Table 3.1).

Behavioral assays were live-scored during experimentation. During the control period, if an individual eel did not enter both sides of the maze, the trial was terminated and not included in data analysis. A preference index was calculated as follows: $Ae/(Ae+Be)-Ac/(Ac+Bc)$, where A_e denotes the time spent in the experimental arm during the experimental period; A_c denotes time spent in the control arm during the experimental period; B_e denotes time spent in the experimental arm during the control period; and B_c denotes time spent in the control arm during the control period (Siefkes *et al.*, 2005). These differences were evaluated with two-tailed Wilcoxon-signed rank tests to determine whether the eels were attracted to (positive preference index; $p \leq 0.05$) or repulsed by (negative preference index; $p \leq 0.05$) each washing.

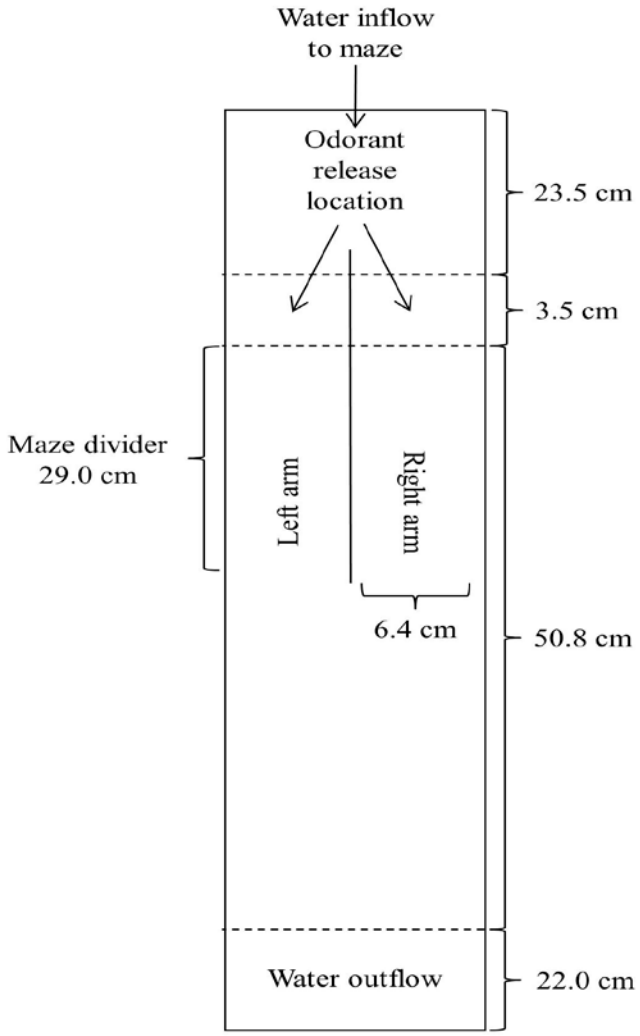

Figure 3.1: Illustration of a two-choice maze (design modified from Li et al. (2002) and Siefkes et al. (2005)) used for American Eel behavioral assays to the odor of conspecifics. Dashed lines indicate placement of stainless steel mesh to prevent animals from escaping. Solid line (maze divider) between left and right arms indicates an impermeable barrier separating maze arms.

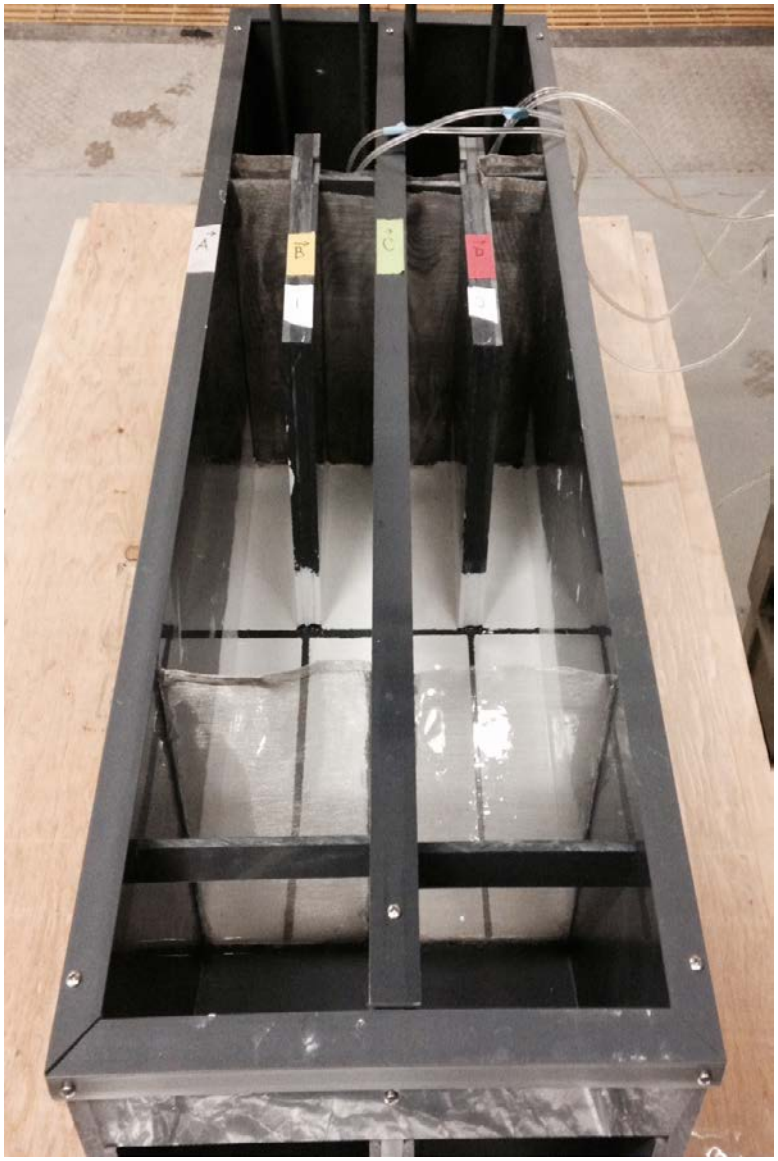

Figure 3.2: Photograph of two-choice maze apparatus used in all behavioral assays based on the design of Li et al. (2002) and Siefkes et al. (2005). Photograph taken by A. Schmucker.

Confirmative assays

After the initial elver response assays with elver washings elicited no behavioral response (see 'Results'), confirmative assays were used to further investigate effects of odor concentration. Additional assays were conducted in spring 2015 on elver response to elver washings collected using the methods described above (designated "concentrated" washings), but at four additional concentrations ($3/8^{th}$, $1/16^{th}$, $1/50^{th}$, and 100^{th} dilutions of concentrated elver washings; n=16 each). These trials were conducted and scored using the same apparatus and methods as described above with the following changes: washing preparations occurred at either 11 or 22^{o}C; the stimulus drip rate was increased to 10 mL·min^{-1}; the experimental scoring period was shortened to 10 min; test subjects were isolated in individual aquaria 24 hrs prior to testing to a reduce odor pre-exposure bias; and assays were video recorded to reduce human disturbance potentially caused by live-scoring. Video data were scored similarly, and the preference index was calculated and tested for significance with alpha Bonferroni-adjusted for multiple comparisons. One-way ANOVA was used to determine if changes in methodology affected elver response to elver washings (independent variables: 15^{o}C washings with original methodology; 11^{o}C washings with modified methodology; and 22^{o}C washings with modified methodology) and whether elver washing concentration (all collected at 11^{o}C with modified methodology) impacted elver washing affinity.

RESULTS

Glass eels spent more time in maze arms containing elver washings than in control arms (Two-tailed Wilcoxon signed-rank test; P=0.015; Table 3.2; Figure 3.3), but time spent did not

differ between washing and control arms for elvers exposed to elver washings at 15^{o}C (Two-tailed Wilcoxon signed-rank test, P=0.50) or yellow eel washings (Two-tailed Wilcoxon signed-rank test; P=0.50; Table 3.2; Figure 3.3). Changes in assay methodology (temperature of elver washings; pre-test isolation; drip rate; video scoring) did not significantly affect time elvers spent in washing or control arms (ANOVA; $F_{2,47}$ =0.59, P=0.56; Table 3.2; Figure 3.4). Varying the concentration of elver washings (undiluted to 1/100th dilution) during confirmative assays also did not significantly affect time elvers spent in washing and control arms (ANOVA; $F_{4, 79}$ =1.5, P=0.21; Table 3.2; Figure 3.5). During negative controls, glass eels (Two-tailed Wilcoxon signed-rank test; P=0.77) and elvers (Two-tailed Wilcoxon signed-rank test; P=0.80 for 2014 studies; P=0.78 for 2015 studies; Table 3.2) spent approximately equal amounts of time in each arm.

Table 3.2: Summary of juvenile American Eel (Test Subject) behavioral results in two-choice maze assays to conspecific washings (Test Washings). N is the number of individuals assessed per washing treatment. Preference Index calculations are explained in the text. SE is standard error.

Test Subject	Test Washing	N	Preference Index (SE)
Glass eels	Elvers	16	0.159 (0.058)
Elvers	Elvers, 15°C	16	-0.018 (0.089)
Elvers	Yellow eel	18	0.007 (0.054)
Elvers	Elvers, 22°C	16	0.058 (0.156)
Elvers	Elvers, 11°C Undiluted	16	-0.128 (0.113)
Elvers	Elvers, 11°C 3/8 dilution	16	0.18 (0.055)
Elvers	Elvers, 11°C 1/16th dilution	16	0.112 (0.111)
Elvers	Elvers, 11°C 1/50th dilution	16	0.072 (0.106)
Elvers	Elvers, 11°C 1/100th dilution	16	0.083 (0.075)
Glass eels	Control (well water)	10	-0.028 (0.051)
Elvers	Control (well water)	16	-0.018 (0.089)
Elvers	Control (well water)	16	-0.046 (0.110)

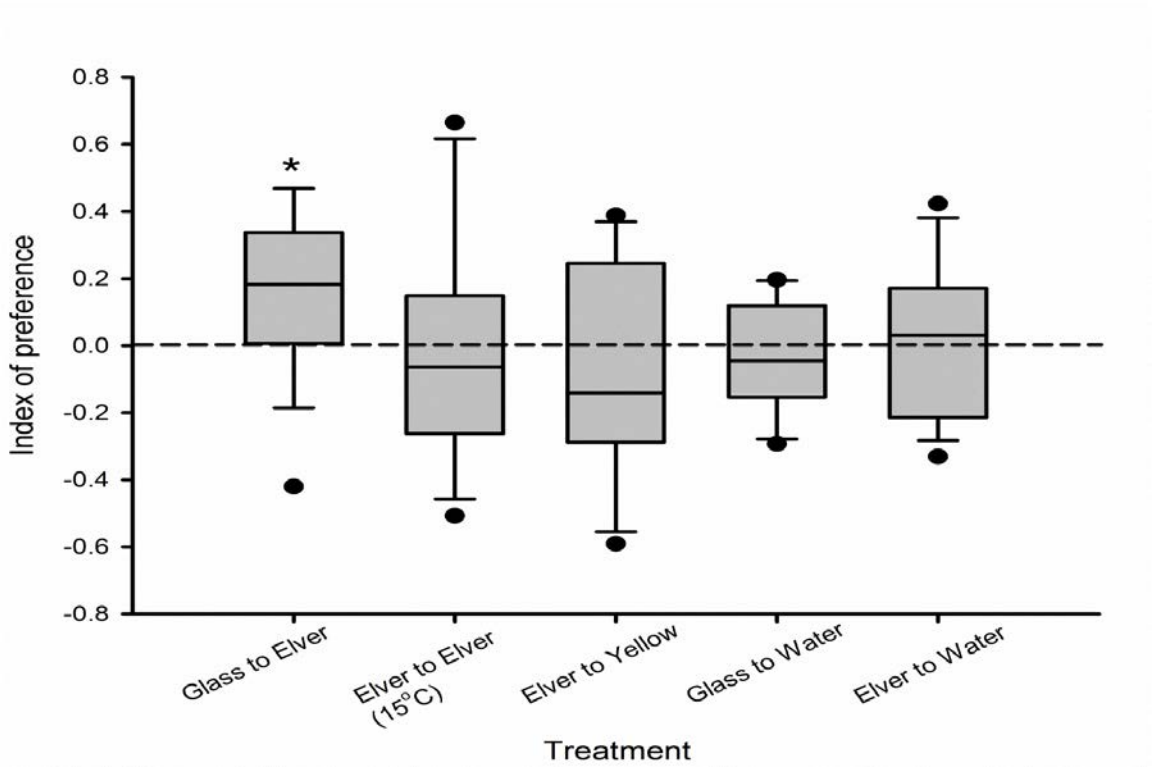

Figure 3.3: Glass eel and elver preference indices to conspecific washings and controls (water) tested in 2014 two-choice maze assays. Center lines indicate median values, box ends are quartiles, whiskers are 10^{th} and 90^{th} percentiles, and points are outliers. Asterisk denotes preference index that differs significantly from 0 ($p \leq 0.05$).

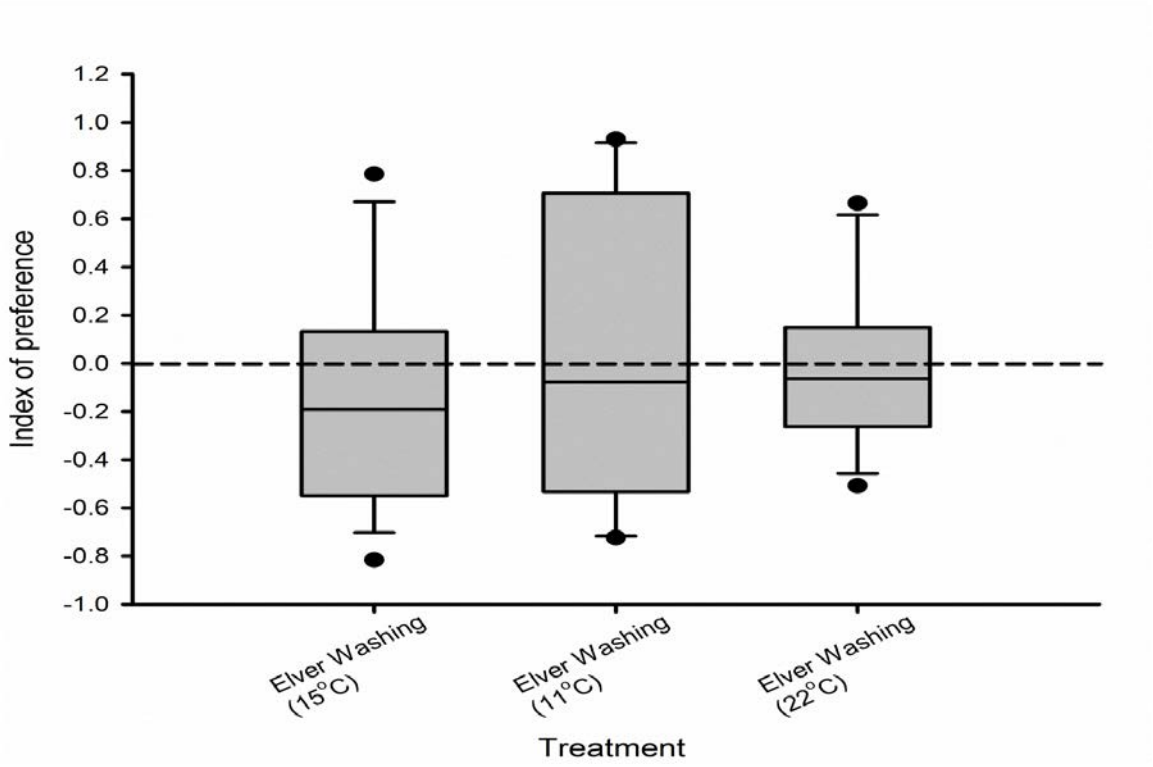

Figure 3.4: Comparison of elver preference indices to washings collected at three different temperatures (Note: methods used to test 11 and 22°C washings differed from those used to test 15°C washings; see methods for details). Center lines indicate median values, box ends are quartiles, whiskers are 10th and 90th percentiles, and points are outliers.

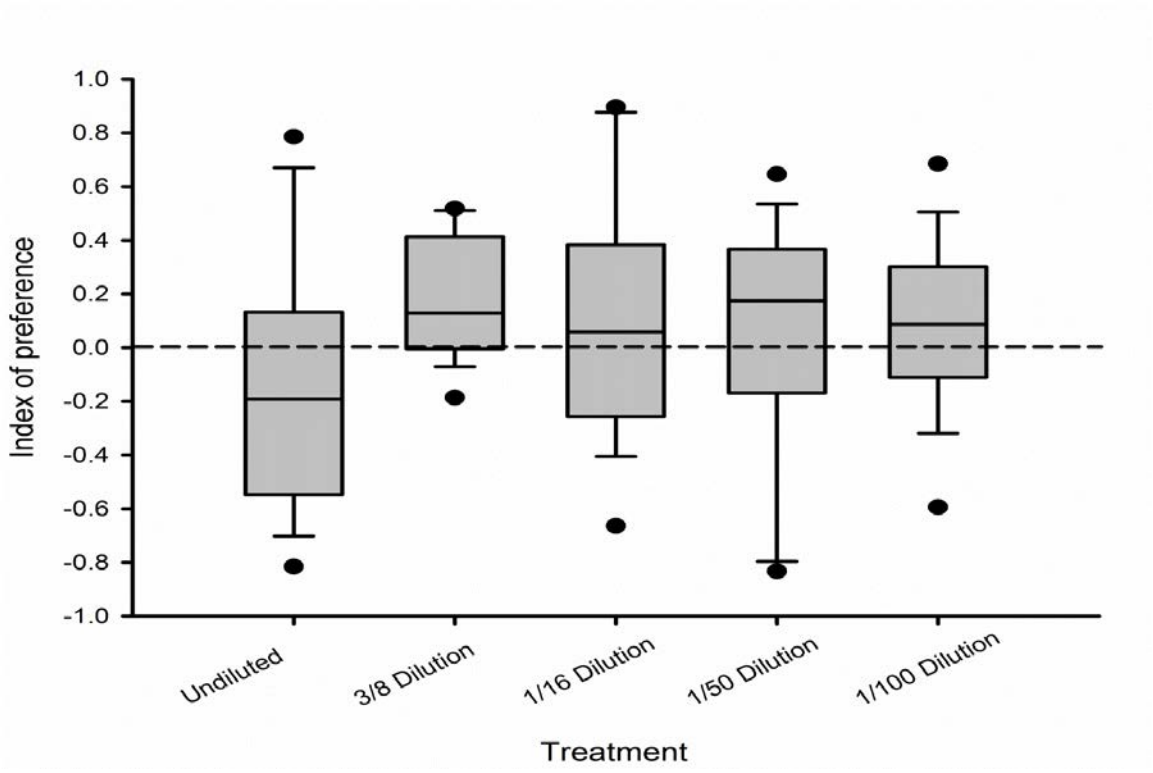

Figure 3.5: Comparison of elver preference indices to multiple concentrations of 11°C elver conspecific washings tested in confirmative two-choice maze assays. Center lines indicate median values, box ends are quartiles, whiskers are 10th and 90th percentiles, and points are outliers.

DISCUSSION

Glass eels were attracted to conspecific elver washings, which suggests that glass eels may follow chemical cues from previous year classes during migration. These results preliminarily support the pheromone hypothesis that glass eels use elvers to guide inland migration (Nordeng 1977; Donahue 2006). However, a lack of response of elvers to yellow eels implies that the role of the previous year class in guiding migration is life-stage dependent. The pheromone hypothesis was developed to explain homeward orientation in salmonids, where maturing fish return to freshwater by following pheromone trails left by related juvenile fish. This phenomenon has also been observed in other fish species, such as the amphidromous juvenile Banded Kokopu *Galaxias fasciatus* (Gray 1842), which were attracted to adult pheromones during migration upstream, possibly to aid juveniles in identifying accessible habitat (Baker and Montgomery 2001). Our results indicate the possibility for a similar behavioral attraction to conspecific glass eels, although our study cannot determine whether these cues serve as an indicator of habitat quality.

Grouping behaviors can provide a variety of ecological and evolutionary benefits such as predator avoidance, foraging efficiency, and better mate detection and selection opportunities (Sorenson and Baker 2015). However, elver washings did not elicit an attraction to other elvers, and confirmative assays did not demonstrate an effect of elver washing concentration on this lack of response. This refutes the conspecific attraction hypothesis. In contrast, Schmucker et al. (in press) observed an attraction of glass eels toward other glass eels. This again suggests life-stage dependence for the use of chemical cues in American Eels.

Eels undergo vast morphological and physiological changes in their lifetime, and chemicals emitted by eels vary with developmental stage and sex (Huertas et al. 2008). After

metamorphosis from glass eels to elvers, chemical cues emitted by glass eels may no longer remain attractive. If this is the case, experimental studies delineating a change from responsive glass eel to unresponsive elver are necessary. Elie et al. (1982) and Haro and Krueger (1988) have established methods for staging juvenile eels for use in future studies to delineate this change in eel responsiveness. Elvers responded positively to elver odor, but at a higher concentration than in the present study (Pesaro et al. 1981; Sorenson 1986). The concentration of stimuli presently used may not have been in the detectable range of test subjects, although the number of eel equivalents (number of $eels^{-1} \cdot L^{-1} \cdot hr^{-1}$) fell within the range of those reported in other studies (Miles 1968; Sorenson 1986).

Further testing is necessary to identify other potential chemical cues utilized during the elver life stage. Elvers were not attracted to yellow eel washings, as would be predicted by the pheromone hypothesis. Adult eels are known to prey on younger conspecifics, a factor that may elicit a negative response of elvers to odor produced by older conspecifics. Jessop (2000) suggested that predation by larger eels is likely the primary source of elver mortality in the East River, Nova Scotia. Lack of a significant eel response to conspecifics may not indicate a lack of chemical cueing, because laboratory conditions may have influenced our results. Test animals were held in captivity at densities reflective of eel culture conditions (~8000 $individuals \cdot m^{-2}$; (Tesch 2003), not native eel populations (~5 $individuals \cdot m^{-2}$; Jessop 2000) prior to testing. Habituation to odors of conspecifics may have produced false negative responses, although isolation of test individuals prior to odor exposure (24 hrs) did not affect results in elvers. Similarly, a non-significant behavioral response does not preclude a physiological or morphological reaction (e.g., density during glass eel culture affects growth and sex differentiation as a function of chemical signals (Huertas and Cerdà 2006; Huertas et al. 2008).

Additionally, glass eels migrate to freshwater in large numbers, whereas eels in this study were tested individually. The influence of group dynamics on migration behavior has yet to be addressed.

Results from this study are an initial step toward understanding the functional role of chemical cueing in American Eels. They allow us to narrow our focus on the odor source (elvers) to identify the active component that stimulated the response in one stage of eel development (glass eels). The invasive Sea Lamprey *Petromyzon marinus* (Linnaeus 1758) provides a model system where synthesized pheromones are being investigated for use in population control (Johnson et al. 2013). Results of the present study provide preliminary data for similar efforts applied to native species restoration. The use of chemical cues in eel restoration may be as complex as synthesizing compounds for field application (as in the case of the Sea Lamprey), or as simple as strategically manipulating populations of older conspecifics to guide migrating glass eels (e.g., upstream of fish ladders; Sorenson 2015). In combination with other fish passage technologies (e.g., fish ladders, electrical guidance, attraction flows), chemical cues may provide an effective and sustainable method to enhance American Eel restoration.

ACKNOWLEDGEMENTS

H. Galbraith was first author on this manuscript, and C. Blakeslee, N. Johnson, M. Hansen, and W. Li were coauthors. We thank D. Dropkin, J. Henry, M. Huertas, W. Lellis, G. Ricci, and S. Weaver for assistance with maze construction, experimental design, and data collection. We thank K. Whiteford and J. Devers for collection of experimental animals. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The U.S. Geological Survey Fisheries Program supplied funding for

this study. This paper is contribution X,XXX of the U.S. Geological Survey, Great Lakes Science Center. The U.S. Geological Survey approved use of specimens and the Michigan State University Animal Use and Care Committee granted an animal use exemption form because research was conducted under the auspices of the U.S. Geological Survey.

BIBLIOGRAPHY

BIBLIOGRAPHY

Amaral, S.V., F.C. Winchell, B.J. McMahon, and D.A. Dixon. 2003. Evaluation of angled bar racks and louvers for guiding silver phase American Eels. American Fisheries Society Symposium 33:367-376.

ASMFC (Atlantic States Marine Fisheries Commission). 2000. Interstate Fishery Management Plan for American Eel (*Anguilla rostrata*). Washington, D.C. Available: http://www.asmfc.org/uploads/file/amEelFMP.pdf. (September 2015).

Atema, J. 1986. Review of sexual selection and chemical communication in the lobster, *Homarus americanus*. Canadian Journal of Fisheries and Aquatic Sciences 43:2283-2290.

August, S.M., and B.J. and Hicks. 2008. Water temperature and upstream migration of glass eels in New Zealand: implications of climate change. Environmental Biology of Fishes 81:195-205.

Baker, C.F., and J.C. Montgomery. 2001. Species-specific attraction of migratory Banded Kokopu juveniles to adult pheromones. Journal of Fish Biology 58:1221-1229.

Baras, E., and D. Jeandrain. 1998. Evaluation of surgery procedures for tagging eel *Anguilla anguilla* (L.) with biotelemetry transmitters. Hydrobiologia 372:107-111.

Barbin, G.P. 1998. The role of olfaction in homing and estuarine migratory of yellow-phase American Eels. Canadian Journal of Fisheries and Aquatic Science 55:564-575.

Barbin, G.P., S.J. Parker, and J.D. McCleave. 1998. Olfactory clues play a critical role in the estuarine migration of silver-phase American Eels. Environmental Biology of Fishes 53:382-291.

Bardonnet, A., V. Bolliet, and V. Belon. 2005. Recruitment abundance estimation: role of glass eel (*Anguilla anguilla* L.) response to light. Journal of Experimental Marine Biology and Ecology 321:181-190.

Béguer-Pon, M., M. Castonguay, S. Shan, J. Benchetrit, and J.J. Dodson. 2015. Direct observations of American Eels migrating across the continental shelf to the Sargasso Sea. Nature Communications 6:8705.

Ben-Tzvi, O., D. Tchernov, and M. Kiflawi. 2010. Role of coral-derived chemical cues in microhabitat selection by settling *Chromis viridis*. Marine Ecology Progress Series 409:181-187.

Boetius, J. 1976. Elvers, *Anguilla anguilla* and *Anguilla rostrata* from two Danish localities. Size, body weight, developmental stage and number of vertebrae related to time of ascent. Meddelelser Fra Danmarks Fiskeri og Havunders~kelser 7:199-220.

Breithaupt, T., and P. Eger. 2002. Urine makes the difference chemical communication in fighting crayfish made visible. Journal of Experimental Biology 205:1221-1231.

Briand, C., D. Fatin, and A. Legault. 2002. Role of eel odor on the efficiency of an eel, *Anguilla anguilla*, ladder and trap. Environmental Biology of Fishes 65:473-477.

Brown, G.E., D.P. Chivers, and R.J.F. Smith. 1995. Fathead Minnows avoid conspecific and heterospecific alarm pheromones in the feces of Northern Pike. Journal of Fish Biology 7:387-393.

Bultela, E., Lasnea, E., Acoua, A., Guillaudeaua, J., Bertierc, C., and E. Feunteuna. 2014. Migration behavior of silver eels (*Anguilla anguilla*) in a large estuary of western Europe inferred from acoustic telemetry. Estuarine, Coastal and Shelf Science 137:23-31.

Burgerhout, E., C. Tudorache, and S.A. Brittijn. 2013. Schooling reduces energy consumption in swimming male European Eels, *Anguilla anguilla L*. Journal of Experimental Marine Biology and Ecology 448:66-71.

Cada, G.G. 2006. Efforts to reduce mortality to hydroelectric turbine-passed fish: locating and quantifying damaging shear stresses. Environmental Management 37:898-906.

Calles, O., S. Karlsson, M. Hebrand, and C. Comoglio. 2012. Evaluating technical improvements for downstream migrating diadromous fish at a hydroelectric plant. Ecological Engineering 48:30-37.

CATAG (Report of Concerted Action). 2002. Improvements of tagging methods for stock assessment and research in fisheries. Reykjavik, Iceland. Available: http://www.hafro.is/Bokasafn/Timarit/catag.pdf. (March 2016).

Chivers, D.P., R.S. Mirza, and J.G. Johnston. 2002. Learned recognition of heterospecific alarm cues enhances survival during encounters with predators. Behavior 139:929-938.

Chivers, D.P., and R.J.F. Smith. 1998. Chemical alarm signaling in aquatic predator-prey systems: A review and prospectus. Ecoscience 5:338-352.

Chivers, D.P., B.D. Wisenden, C.J. Hindman, T.A. Michalak, R.C. Kusch, S.G.W. Kaminskyj, C.L. Jack, M.C.O. Ferrari, R.J. Pollock, C.F. Halbgewachs, M.S. Pollock, S. Alemadi, C.T. James, R.K. Savaloja, C.P. Goater, A. Corwin, R.S. Mirza, J.M. Kiesecker, G.E. Brown, J.C. Adrian Jr., P.H. Krone, A.R. Blaustein, and A. Mathis. 2007. Epidermal 'alarm substance' cells of fishes maintained by non-alarm functions: possible defence against pathogens, parasites and UVB radiation. Proceedings of the Royal Society B 274:2611-2619.

Churcher, A.M., P.C. Hubbard, J.P. Marques, A.V.M. Canario, and M. Huertas. 2015. Deep sequencing of the olfactory epithelium reveals specific chemosensory receptors are expressed at sexual maturity in the European Eel *Anguilla anguilla*. Molecular Ecology 24:822-834.

Corkum, L.D., and R.M. Belanger. 2007. Use of chemical communication in the management of freshwater aquatic species that are vectors of human diseases or are invasive. General and Comparative Endocrinology 153:401-417.

Crnjar, R., G. Scalera, A. Bigiani, I. Tomassini Barbarossa, P.C. Magherini, and P. Pietra. 1992. Olfactory sensitivity to amino acids in the juvenile stages of the European Eel *Anguilla anguilla* (L.). Journal of Fish Biology 40:567-576.

Cumberland J.H. and L.E. Cronin. 1986. A Bioeconomic Approach to Improved Fisheries Management and Pollution Control for the Chesapeake Bay. Maryland University, College Park (USA). Sea Grant Coll. Program. Economics of Chesapeake Bay Management Conference Abstract, Durham, NH, 1985. Available: http://search.proquest.com.proxy1.cl.msu.edu/asfa/docview/14467930/20F87E2C3814467DPQ/1?accountid=12598. (October 2014).

DePhilip, M., and T. Moberg. 2010. Ecosystem flow recommendations for the Susquehanna River basin. The Nature Conservancy, Harrisburg, PA.

Donahue, M.J. 2006. Allee effects and conspecific cueing jointly lead to conspecific attraction. Oecologia 149:33-43.

Drouineau, H., C. Rigaud, A. Laharanne, R. Fabre, A. Alric, and P. Baran. 2014. Assessing the efficiency of an elver ladder using a multi-state mark-recapture model. River Research and Applications 31:291-300.

Ducolombier, S.B., V. Bolliet, and A. Bardonnet. 2009. Swimming activity and behavior of European *Anguilla anguilla* glass eels in response to photoperiod and flow reversal and the role of energy status. Journal of Fish Biology 74:2002-2013.

Durif, C.M.F., H.I. Browman, J.B. Phillips, A.B. Skiftesvik, L.A. Vollestad, and H.H. Stockhausen. 2013. Magnetic Compass Orientation in the European Eel. Plos One 8:1-6

Durif, C.M.F., S. Dufour, and P. Elie. 2005. The silvering process of *Anguilla anguilla*: a new classification from the yellow resident to the silver migrating stage. Journal of Fish Biology 66: 1025-1043.

Elie, P., R. Lecomte-Finiger, I. Cantrelle, and N. Charlon. 1982. Définition des limites des différents stades pigmentaires durant la phase civelle d'*Anguilla anguilla* L.(poisson téléostéen anguilliforme). Vie et milieu 32:149-157.

French, F.E., and D.L. Kline. 1989. l-Octen-3-ol, an Effective Attractant for Tabanidae (Diptera). Journal of Medical Entomology 2:459-461.

Freyhof, J., and M. Kottelat. 2010. *Anguilla anguilla*. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. Available: www.iucnredlist.org. (July, 2015).

Fulton, T.W. 1904. The rate of growth of fishes. Fisheries Board of Scotland, Annual Report 22 part 3, pp. 141-241.

Ghittino, G., J. Glenn, and F. Smith. 1975. Studies on hormonal induction of gonadal development in American Eel (*Anguilla rostrata*). Rivista Italiana di Piscicoltura e Ittiopatologia 10:75-83.

Gill, A.B., M. Bartlett, and F. Thomsen. 2012. Potential interactions between diadromous fishes of U.K. conservation importance and the electromagnetic fields and subsea noise from marine renewable energy developments. Journal of Fish Biology 81:664-695.

Haro, A. 2003. Downstream migration of silver-phase anguillid eels. Pages 215-222 in: Aida, K., K. Tsukamoto, and K. Yamauchi, eds. Eel Biology. Springer, Tokyo.

Haro, A., T. Castro-Santos, K. Whalen, G. Wippelhauser, and L. McLaughlin. 2002. Simulated effects of hydroelectric project regulation on mortality of American Eels. Biology, Management, and Protection of Catadromous Eels, American Fisheries Society Symposium 33:357-365.

Haro, A., and W. Krueger. 1988. Pigmentation, size, and migration of elvers (*Anguilla rostrata* (*Lesueur*)) in a coastal Rhode Island stream. Canadian Journal of Zoology 66:2528-2533.

Haro, A., W. Richkus, K. Whalen, A. Hoar, W.D. Busch, S. Lary, T. Brush, and D. Dixon. 2000. Population decline of the American Eel: implications for research and management. Fisheries 25:7-16.

Hasler, A.D. 1960. Guideposts of migrating fishes. Science 132:785-792.

Hasler, A.D., and W.J. Wisby. 1951. Discrimination of stream odors by fishes and its relation to parent stream behavior. The American Naturalist 85:223-238.

Huertas, M., A.V.M. Canario, and P.C. Hubbard. 2008. Chemical communication in the genus *Anguilla*: a minireview. Behavior 145:1389-1407.

Huertas, M. and J. Cerdá. 2006. Stocking density at early developmental stages affects growth and sex ratio in the European Eel (*Anguilla anguilla*). The Biological Bulletin 211:286-296.

Huertas, M., P.C. Hubbard, A.V.M. Canário, and J. Cerdá. 2007. Olfactory sensitivity to conspecific bile fluid and skin mucus in the European Eel *Anguilla anguilla* (L.). Journal of Fish Biology 70:1907-1920.

Hurley, D.A. 1973. The commercial fishery for the American Eel (*Lesueur*), in Lake Ontario. Transactions of the American Fisheries Society 102:369-377.

ICES (International Council for the Exploration of the Sea). 2013. Report of the joint EIFAAC/ICES working group on eels (WGEEL). ICES CM.2013/AC0M:18, Copenhagen, Denmark. Available: http://www.ices.dk/sites/pub/Publication%20Reports/Expert%20Group%20Report/acom/2013/WGEEL/wgeel_2013.pdf (March 2016).

Imre, I., R.T. Di Rocco, C.F. Belanger, G.E. Brown, and N.S. Johnson. 2014. The behavioral response of adult *Petromyzon marinus* to damage-released alarm and predator cues. Journal of Fish Biology 84:1490-1502.

Jessop, B.M. 1987. Migrating American Eels in Nova-Scotia. Transactions of the American Fisheries Society 116:161-170.

Jessop, B.M. 2000. Estimates of population size and instream mortality rate of American Eel elvers in a Nova Scotia river. Transactions of the American Fisheries Society 129:514-526.

Jessop, B.M. 2002. Annual and seasonal variability in the size and biological characteristics of the runs of American Eel elvers to two Nova Scotia rivers. American Fisheries Society Symposium 33:17-36.

Johnson, N.S., M.J. Siefkes, C.M. Wagner, H. Dawson, H. Wang, T. Steeves, M. Twohey, and W. Li. 2013. A synthesized mating pheromone component increases adult Sea Lamprey (*Petromyzon marinus*) trap capture in management scenarios. Canadian Journal of Fisheries and Aquatic Sciences 70:1101-1108.

Kemp, P.S., M.H. Gessel, B.P. Sandford, and J.G. Williams. 2006. The behavior of Pacific salmonid smolts during passage over two experimental weirs under light and dark conditions. River Research and Applications 22:429-440.

Kleckner, R.C., and J.D. McCleave. 1985. Spatial and temporal distribution of American Eel larvae in relation to North Atlantic Ocean current systems. Dana 4:67-92.

Kocovsky, P.M., R.M. Ross, and D.S. Dropkin. 2009. Prioritizing removal of dams for passage of diadromous fishes on a major river system. River Research and Applications 25:107-117.

Krueger, W.H., and K. Oliveira. 1999. Evidence for environmental sex determination in the American Eel, *Anguilla rostrata*. Environmental Biology of Fishes 55:381-389.

Larsson, M.C., and G.P. Svensson. 2009. Pheromone monitoring of rare and threatened insects: exploiting a pheromone-kairomone system to estimate prey and predator abundance. Conservation Biology 23:1516-1525.

Lecchini, D., and Y. Nakamura. 2013. Use of chemical cues by coral reef animal larvae for habitat selection. Aquatic Biology 19:231-238.

Lellis, W., B. St. John White, J. Cole, C. Johnson, J. Devers, E. van Snik Gray, and H. Galbraith. 2013. Newly documented host fishes for the Eastern Elliptio Mussel (*Elliptio complanata*). Journal of Fish and Wildlife Management 4:75-85.

Li, W., A.P. Scott, M.J. Siefkes, H. Yan, Q. Liu, S.S. Yun, and D.A. Gage. 2002. Bile acid secreted by male Sea Lamprey that acts as a sex pheromone. Science 296:138-141.

Liu, L., G. Feng, Z. Jeiming, and L. Dachun. 2003. Effects of exterior hormones and environmental factors on the ovarian development in Japanese Eel. Journal of Shanghai Fisheries University 12:6-13.

Lookabaugh, P.S., and P.L. Angermeier. 1992. Diet patterns of American Eel, *Anguilla rostrata*, in the James River drainage, Virginia. Journal of Freshwater Ecology 7:425-431.

MacGregor, R., T. Haxton, L. Greig, J.M. Casselman, J.M. Dettmers, W.A. Allen, D.G. Oliver, and L. McDermott. 2015. The demise of American Eel in the upper St. Lawrence River, Lake Ontario, Ottawa River and associated watersheds: implications of regional cumulative effects in Ontario. Managing the impacts of human activities on fish habitat: the governance, practices, and science, American Fisheries Society Symposium 78:149-188.

Mason, R.T., and M.R. Parker. 2010. Social behavior and pheromonal communication in reptiles. Journal of Comparative Physiology A-Neuroethology Sensory Neural and Behavioral Physiology 196:729-749.

McCauley, D.J, M.L. Pinksy, S.R. Palumbi, J.A. Estes, F.H. Joyce, and R.R. Warner. 2015. Marine defaunation: Animal loss in the global ocean. Science 347:247-256.

McCormick, S.D., L.P. Hansen, T.P. Quinn, and R.L. Saunders. 1998. Movement, migration, and smolting of Atlantic Salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 55:77-92.

Miles, S.G. 1968. Rheotaxis of elvers of the American Eel (*Anguilla rostrata*) in the laboratory to water from different streams in Nova Scotia. Journal of the Fisheries Research Board of Canada 25:1591-1602.

Moriarty, C. 1986. Variations in elver abundance at European catching stations from 1938 to 1985. Vie et Milieu 36:233-235.

Moriarty, C. 2012. The decline in catches of European elver 1980-1992. Archives of Polish Fisheries 20:215-217

Morris, D.W. 1992. Scales and costs of habitat selection in heterogeneous landscapes. Evolutionary Ecology 1:379-388.

Musumeci, V.L., K.W. Able, and M.C. Sullivan. 2014. Estuarine predator-prey interactions in the early life history of two eels (*Anguilla rostrata* and *Conger oceanicus*). Environmental Biology of Fishes 97:929-938.

Myers, R.A, and B. Worm. 2003. Rapid worldwide depletion of predatory fish communities. Nature 423:280-283.

Nordeng, H. 1977. A pheromone hypothesis for homeward migration in anadromous salmonids. Oikos 28:155-159.

Nordeng, H., and P. Bratland. 2006. Homing experiments with parr, smolt and residents of anadromous Arctic Char *Salvelinus alpinus* and Brown Trout *Salmo trutta*: transplantation between neighbouring river systems. Ecology of Freshwater Fish 15:499-499.

Ogden, J.C. 1970. Relative abundance, food habits, and age of the American Eel, *Anguilla rostrata* (LaSueur), in certain New Jersey Streams. Transactions of the American Fisheries Society 99:54-59.

Økland, F. and E.B. Thorstad. 2013. Recommendations on size and position of surgically and gastrically implanted electronic tags in European silver eel. Animal Biotelemetry 1:1-6.

Palstra, A. van Ginneken, V., and G. van den Thillart. 2008. Cost of transport and optimal swimming speed in farmed and wild European silver eels (*Anguilla anguilla*). Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 151:37-44.

Pankhurst, N.W., and J. Lythgoe. 1983. Changes in vision and olfaction during sexual maturation in the European Eel *Anguilla anguilla* (L.). Journal of Fish Biology 23:229-240.

Parker, S.J., and J.D. McCleave. 1997. Selective tidal stream transport by American Eels during homing movements and estuarine migration. Journal of the Marine Biological Association of the United Kingdom 77:871-889.

Pedersen, M.I., N. Jepsen, K. Aarestrup, A. Koed, S. Pedersen, and F. Okland. 2012. Loss of European silver eel passing a hydropower station. Journal of Applied Ichthyology 28:189-193.

Pesaro, M., M. Balsamo, G. Gandolfi, and P. Tongiori. 1981. Discrimination among different kinds of water in juvenile eels, *Anguilla* (*L*.). Monitore Zoologico Italiano 15:183-191.

Pohl, M.M. 2002. Bringing down our dams: trends in American dam removal rationales. Journal of the American Water Resources Association 38:1511-1519.

Rajchard, J. 2006. Antipredator pheromones in amphibians: a review. Veterinarni Medicina 51:409-413.

Ralls, K. 1971. Mammalian scent marking. Science 171:443-449

Richkus, W.A., and D.A. Dixon. 2003. Review of research and technologies on passage and protection of downstream migrating catadromous eels at hydroelectric facilities. American Fisheries Symposium 33:377-388.

Russon, I.J., and P.S. Kemp. 2011. Advancing provision of multi-species fish passage: behavior of adult European Eel (*Anguilla anguilla*) and Brown Trout (*Salmo trutta*) in response to accelerating flow. Ecological Engineering 37:2018-2024.

Saglio, P. 1982. Use of intraspecific biological extracts to trap eels (*Anguilla L.)* in the field. demonstration of the pheromonal attractivity of the skin mucus. Acta Oecologica 3:223-231.

Sanches, F.H.C., C.A. Miyai, C.F. Pinho-Neto, and R.E. Barreto. 2015. Stress responses to chemical alarm cues in Nile Tilapia. Physiology and Behavior 149:8-13.

Scaion, D., and P. Sebert. 2008. Glycolytic fluxes in European silver eel, *Anguilla anguilla*: sex differences and temperature sensitivity. Comparative Biochemistry and Physiology Part A Molecular and Integrative Physiology 151:687-690.

Schmucker, A.K., N.S. Johnson, H.S. Galbraith, and W. Li. 2016. American glass eels respond to conspecific odor as a function of concentration. Transactions of the American Fisheries Society, in press.

Schulte, E. 1972. Studies of the regio olfactoria or the eel, *Anguilla anguilla* L. I. Fine strucutre of the olfactory epithelium. Zeitschrift für Zellforschung Mikroskopische Anatomie 125:210-228.

Siefkes, M.J., S.R. Winterstein, and W. Li. 2005. Evidence that 3-keto petromyzonol sulphate specifically attracts ovulating female sea lamprey, *Petromyzon marinus*. Animal Behavior 70:1037-1045.

Smith, J.W., and W.D. Swink. 2003. Boll weevil eradication: A model for sea lamprey control? Journal of Great Lakes Research 29:445-455.

Sola, C., and P. Tongiorgi. 1996. The effect of salinity on the chemotaxis of glass eels, *Anguilla*, to organic earthy and green odorants. Environmental Biology of Fishes 47:213-218.

Sola, C., and L. Tosi. 1993. Bile salts and taurine as chemical stimuli for glass eels, *Anguilla*: A behavioral study. Environmental Biology of Fishes 37:197-204.

Sorensen, P.W. 1986. Origins of the freshwater attractant(s) of migrating elvers of the American Eel, *Anguilla rostrata*. Environmental Biology of Fishes 17:185-200.

Sorenson, P.W., and C. Baker. 2015. Species-specific pheromones and their roles in shoaling, migration, and reproduction: A critical review and synthesis. *in* P. W. Sorenson, editor. Fish Pheromones and Related Cues. John Wiley and Sons, Inc.

Sorensen, P.W., and N.W. Pankhurst. 1988. Histological changes in the gonad, skin, intestine and olfactory epithelium of artificially-matured male American Eel, *Anguilla rostrata* (LeSueur). Journal of Fish Biology 32:297-307.

Strubberg, A. 1913. The metamorphosis of elvers as influenced by outward conditions. Meddelelser fra Kommissionen for Havundersøgelser. Serie: Fiskeri. Copenhagen, Denmark.

Tesch, F.W., Westerberg, H., and L. Karlsson 1991. Tracking studies on migrating silver eels in the central Baltic. Meeresforschung-Reports On Marine Research 33:183-196.

USGS (U.S. Geological Survey). 2016. USGS Surface-Water Monthly Statistics for USGS 01428500 Delaware River above Lackawaxan River near Barryville, NY. Available: http://waterdata.usgs.gov/nwis/monthly?referred_module=sw&site_no=01428500&por_01428500_1=1049425,00010,1,1975-10,2014-09&format=html_table&date_format=YYYY-MM-DD&rdb_compression=file&submitted_form=parameter_selection_list **(**February 2016).

Valone, T.J. 2007. From eavesdropping on performance to copying the behavior of others: a review of public information use. Behavioral Ecology and Sociobiology 62:1-14.

Vowles, A.S, J.J. Anderson, M.H. Gessel, J.G. Williams, and P.S. Kemp. 2014. Effects of avoidance behavior on downstream fish passage through areas of accelerating flow when light and dark. Animal Behavior 92:101-109.

Wagner, C.M., M.B. Twohey, and J.M. Fine. 2009. Conspecific cueing in the Sea Lamprey: do reproductive migrations consistently follow the most intense larval odor? Animal Behavior 78:593-599.

Watene, E.M., and J.A.T. Boubee. 2005. Selective opening of hydroelectric dam spillway gates for downstream migrant eels in New Zealand. Fisheries Management and Ecology 12:69-75.

Wattendorf, R.J. 1979. Cannibalism in elvers. The Progressive Fish Culturist 41:218.

White, E.M., and B. Knights. 1997. Environmental factors affecting migration of the European Eel in the rivers Severn and Avon, England. Journal of Fish Biology 50:1104-1116.

Wisenden, B.D., C.L. Binstock, K.E. Knoll, A.J. Linke, and B.S. Demuth. 2010. Risk-sensitive information gathering by cyprinids following release of chemical alarm cues. Animal Behavior 79:1101-1107

Yamamoto, M. and K. Ueda. 1978. Comparative morphology of fish olfactory epithelium. 4. Anguilliformes and Myctophiformes. Bulletin of the Japanese Society for the Science of Fish. 44:1207-1212.