Pennsylvania Sea Grant Final Report

Investigation of Chemical Signals for Improving Trapping Efficiency and Preventing Further Expansion of Invasive Crayfish Species

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Executive summary
Invasive crustacean species disturb existing food webs, macrophyte, benthic invertebrate and fish populations, and negatively affect overall ecosystem function (Hill and Lodge, 1999; Khulman and Hazelton, 2007). In Central Pennyslvania, the invasive *Orconectes rusticus* and *Orconectes obscurus* have invaded aquatic habitats, dominating the Susquehanna River and many of its tributaries while displacing native species (Lieb et al., 2011). Unfortunately, current practices to prevent further crustacean expansions have not been successful. Therefore, the main objectives our work have focused on 1) increasing public education about invasive species, 2) increasing our understanding of chemical communication in crayfish for potential development of pheromone baited traps that could enhance existing trapping methods, and 3) understanding distribution and possible hybridization of invasive crustaceans in local watersheds.

To address our first objective, we developed and held workshops for science teachers in the local schools. The workshops introduced teachers to the local invasive crustacean species *O. rusticus* and ways that these animals can be used as study organisms to address topics of biodiversity and biological invasions in specific lesson plans that meet the Pennsylvania Biology curriculum. Thirteen teachers attended the workshops and we have established an ongoing collaboration from teachers in two nearby schools.

To address our second objective, we have been conducting multiple studies that have help lay the groundwork for our ongoing research. We have designed and built a flow-through pheromone sampling tank that allows us to assess pheromone discrimination in crayfish. Using this behavioral paradigm, we have begun conducting experiments to assess how *O. rusticus* respond to same-sex and different-sex conspecific pheromones. We are expanding these pheromone discrimination assays to increase sample size and to include hetersospecific pheromones, as well as pheromones from individuals of different social status.

Furthermore, to understand the extent of the spread of invasive crayfish in Central Pennsylvania, we began sampling local watersheds and assessing the crustacean diversity in select Susquehanna River tributaries. We conducted molecular studies to better understand the specific geographical sources of the *O. rusticus* species that we found in our local watershed.

In conclusion, the funds from the Pennsylvania Sea Grant have allowed us to develop methods tailored to investigating crayfish chemical communication and to begin investigations, laying the groundwork for future development of pheromone-based traps to help manage invasive crayfish populations. Furthermore, with education workshops, we have been able to reach multiple schools with content that will increase awareness of biodiversity, expose students to environmental issues that are affecting their communities, and provide them with tools to help attenuate some of these impacts. Our sampling studies are an ongoing effort that we hope will give us insight about the abiotic factors that affect invasive and native species distribution. Finally, the funds from Pennsylvania Sea Grant have been used to provide research opportunities to several undergraduate science majors at Elizabethtown College. This research opportunity has given them experience designing, conducing, and presenting research; provided training in basic and applied behavioral ecology; and strengthened their preparation for graduate schools and future careers.

Introduction

Invasive crustacean species disturb existing food webs, macrophyte, benthic invertebrate and fish populations, and negatively affect overall ecosystem function (Hill and Lodge, 1999; Khulman and Hazelton, 2007). Originally introduced through aquaculture, aquarium and pond trades, and as live bait, crustacean species have invaded aquatic habitats globally, outcompeting native species and depleting aquatic vegetation (Olden et al., 2006, Lieb et al., 2011, Gherardi et al., 2011, Khulman and Hazelton, 2007). The crayfish species *Orconectes rusticus*, native to the mid-west, has invaded aquatic habitats throughout northeastern North America (Khulman and Hazelton, 2007) and Europe (Gherardi et al., 2011). In Pennsylvania, *O. rusticus* dominates the Susquehanna River and many of its tributaries, displacing native species (Lieb et al., 2011). Within the past century, introduced species, including *Orconectes obscurus* and *O. rusticus*, dramatically increased their geographic range while native species such as *Cambarus bartonii*, *Orconectes limosus*, and *Cambarus puncticambarus sp*. have declined and been classified as vulnerable (S3) on the conservation classification (Lieb et al., 2011). To date, no effective methods have been developed to manage and prevent range expansion of invasive crayfish species (Gherardi et al., 2011; Lieb et al., 2011). Manual trapping, while effective, requires multiple seasons, consistency, and lots of manpower. Besides manual trapping, there are no biological or chemical means to selectively target invasive crayfish species without affecting native populations (Freeman et al., 2010; Gherardi et al., 2011; Lieb et al., 2011).

To understand how local watersheds are affected by invasive *O. rusticus,* we set out to begin monitoring these streams, identifying and quantifying the crustacean species in this area. We also set out to conduct genetic studies, to assess the possibility of hybridization between invasive and local species. Hybridization events have been reported in other areas where *O. rusticus* were found.

The primary goal of our work has been to increase our understanding of the different behavioral responses that disparate chemical signals induce and to be able to apply that knowledge to development of baited traps in the future. We know that crayfish communicate chemically by releasing pheromones in their urine (Thiel and Breithaupt, 2011; Breithaupt, 2011), and that these pheromones likely carry different information about individuals (Adams and Moore, 2003; Bergman and Moore, 2005; Corkum and Belanger, 2007; Bergman and Moore, 2005; DelGaudio and Goldina, 2015). We hope to develop and improved trapping strategy that utilizes a two-pronged approach to enhance trapping by using chemical signals that attract invasive species, and other chemicals that repel approach to deter the re-establishment of the target species. Thus, one of our goals has been to develop effective methods for the prevention and eradication of aquatic invasions by *O. rusticus*, by increasing our understanding how *O. rusticus* distinguish between conspecific and heterospecific chemical signals that are produced in diverse social contexts (sex, alarm signal, and agonistic encounters) in its environment.

Furthermore, another way to minimize the spread of invasive crustaceans is through increasing public awareness and education. We believe that by increasing early student engagement, we can create more environmentally conscious public citizens. To that end, it was our goal to work with science teachers in the local middle schools and develop for them educational and engaging activities about invasive species and the effect of invasive species on local biodiversity.

Our studies have been conducted in the field and in the laboratory. In all aspects of this work, we have involved undergraduate students at Elizabethtown College. Furthermore, we have collaborated with faculty from the Education department, as well as established relationships with science teachers from the nearby Elizabethtown Middle School and Donegal Junior High School. Through these collaborations, we have been able to expand our efforts in increasing public awareness about the effects of invasive species on the local aquatic habitats. In the laboratory, we have developed and tested the experimental paradigms that we need to continue our exploration of chemical communication in crustaceans. In the field, we began monitoring crayfish distribution in the local watersheds. We address the specific methodologies and results to meet each objective below.

Objective 1: Assess crayfish distribution and possible hybridization of invasive crustaceans in local watersheds

Methods

During the summer of 2017 we sampled local streams, the Conoy Creek and the Conewago Creek. We used dichotomous keys to identify the crayfish we found to species. Crayfish were retained for behavioral studies. During our sampling, we were unable to find *O. obscurus*. The main species we found was *O. rusticus*, suggesting that hybridization events between *O. rusticus* and other *Orconectes* species might not currently be an important phenomenon in these populations. Instead, we focused DNA data collection on addressing the geographical sources of local *O. rusticus*.

We collected DNA sequence data from *O. rusticus* sampled from three local locations during the summer of 2018. Five individuals were collected from Conoy Creek, five from the Little Chiques Creek, and six from the Conestoga River in the city of Lancaster. DNA was extracted from one walking leg using the Qiagen Blood and Tissue kit. Specimens were preserved in 70% ethanol.

For the crayfish sampled, we obtained 576 bp of the 16S rRNA mitochondrial gene using the PCR primers ATAATGTGGTAGTTACAGCTCATGC and GAGTTCCTTGTAAAGTCCCTAACCAAC, as well as 831 bp of the COI mitochondrial gene, using the PCR primers GGAATTCGGCAAAAATTATTTCTGCCTG and CCTGGCTTACACCGGTCTGAACTC.

We compared the sequences obtained to those accessible through NCBI for *O. rusticus* from different locations within and outside the native range.

Results

We found that haplotypes of *O. rusticus* sampled from local waterways were identical to or similar to those documented for *O. rusticus* from Ohio, Iowa, Indiana, and Illinois, as well as from non-native *O. rusticus* collected in Toronto, Canada.

Haplotypes were less similar to those documented for *O. rusticus* from Kentucky, Tennessee, and North Carolina.

Table 1: Percentage identity of 16S haplotypes from sampled crayfish (shaded) and from *O. rusticus* with sequences available through NCBI. N is shown in parentheses.

Location	Haplotype								
	1	2	3	4	5	6	7	8	9
Conoy Creek, PA	100% (4)	99.81% (1)							
Conestoga River, Lancaster, PA	100% (4)	99.81% (2)							
Little Chiques Creek, PA	100% (2)	99.81% (3)							
Tippecanoe River, IN	100% (16)				99.42% (1)				
Portage River, South Bend, OH	100% (1)								
Huron River, Lovers Lane Bridge, OH	100% (1)								
Huron River, Monroeville, OH	100% (1)								
Dry Fork Whitewater River, OH	100% (2)								
Cedar River, IA	100% (3)		99.62% (1)						
Wapsipinicon River, IA	100% (1)		99.62% (5)						
Buffalo Creek, IA	100% (9)								
Pitman Creek, Campbellsville, KY				99.60% (1)		96.18% (5)	95.99% (1)	95.80% (2)	
Powell River at mouth of Gap, TN									95.03% (1)

Table 2: Percentage identity of CO1 haplotypes from sampled crayfish (shaded) and from *O. rusticus* with sequences available through NCBI. N is shown in parentheses.

Location	**Haplotype**					
	1	**2**	**3**	**4**	**5**	**6**
Conoy Creek, PA	100% (5)					
Conestoga River, Lancaster, PA	100% (4)	99.87% (2)				
Little Chiques Creek, PA	100% (5)					
Mill Creek, IL	100% (1)					
Little Rough Creek, Toronto	100% (1)					
Buffalo Creek at Hills Mill Road, IA	100% (1)					
Ohio River, KY					99.49% (1)	
Marion, NC			99.74% (1)	99.61% (1)		99.23% (1)

Results of this work will be presented at the Pennsylvania Academy of Sciences in spring (Baker and Bridge, 2019).

Conclusion

While our current sample sizes are small, our data imply that *O. rusticus* present in the locations sampled are descended from populations from the northeastern portion of the native range of *O. rusticus* (e.g. Ohio), rather than from the southwestern portion (e.g. Kentucky. This is not surprising, but transport of *O. rusticus* for bait could potentially have led to a different outcome. Since it is possible that there is some variation in pheromones or behavior over the range of *O. rusticus*, information about relatedness of different populations may be important in deploying pheromone-based control strategies.

Objective 2: Increase understanding of chemical communication in crayfish for enhancing trapping practices.

Methods

With technical assistance from the Engineering department at the college, we designed and built a water-flow enclosure system tank to assess crayfish ability to discriminate between chemical signals from individuals from different species, social status, and sex.

1. **Construction of a water-flow enclosure system for assessing discrimination behavior**
   We constructed a water-flow enclosure system to assess crayfish ability to discriminate between water-borne chemical signals (Fig. 1). Our design was modified from Kenning et al. (2015). The enclosure was constructed from plexiglass, measuring 1.5meters in length and 0.3 meters in width (Fig 2a and 2b). The enclosure was separated into three compartments; the holding compartments. The acclimation compartment 0.4 meters in length, was the location where the crayfish was originally placed at the beginning of the experiment. The acclimation compartment was then separated by a removable gate from the two remaining compartments, arms, of the enclosure. Each arm was connected to an independent water supply, allowing different chemical signals to be pumped into the arms (Fig. 1). Outflow openings on the opposite sides of the enclosure maintained constant water levels. The removable gate was made from mesh screening, allowing water to flow from the arms to the acclimation compartment. The divider between the arms was made out plexiglass, and attached to the bottom of the enclosure using plexiglass glue, ensuring that water signals pumped through each arm did not mix in the enclosure (Fig. 2). Water was not recirculated through the tanks. All water pumped through the tank was discarded through the outflow pipes, which connected to the drain in the room. The outflow pipes helped maintain a constant water depth in the tank.

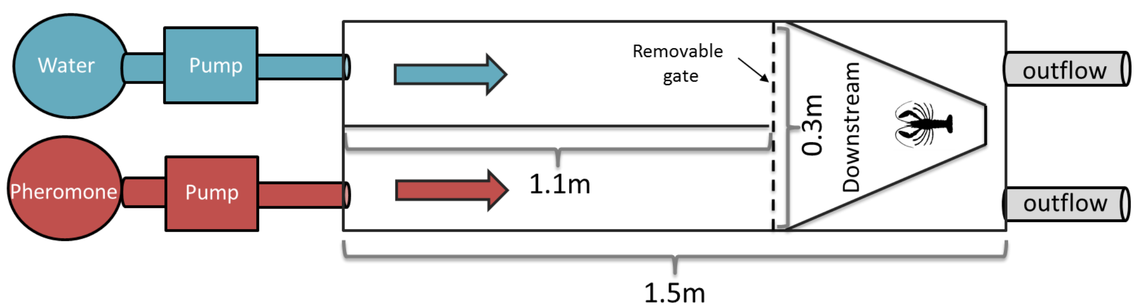

Figure #1a: Schematic of the water flow enclosure system

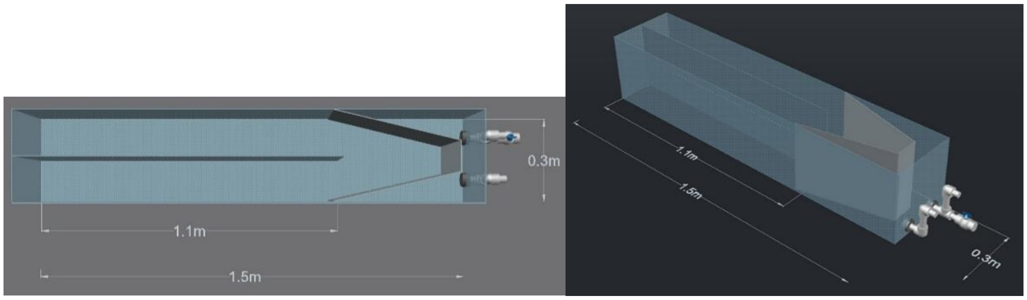

Figure 1b, c: Y-maze design. Top view. And side view

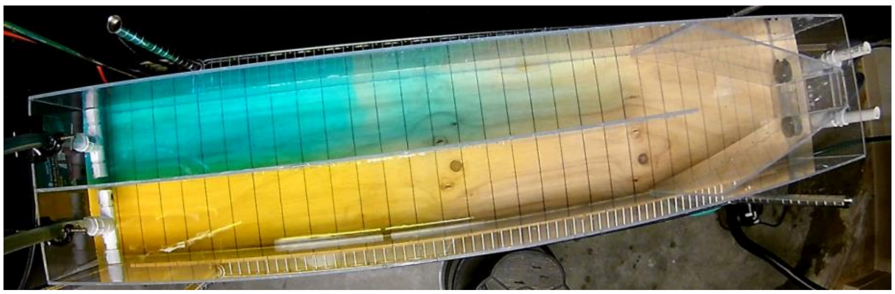

Figure 2. Quality control of the enclosure. There is no mixing between solutions pumped through each arm of the enclosure. Food dye was used to color the arms of the enclosure. Mixing of the dye would have been noted by appearance of green color in the water.

All water was pumped at 2cm/s, maintaining water depth at 12.7cm throughout the duration of the experiment. The inflow rate from each pump was maintained at 3.9L/min, while the outflow rate was 7.8L/min.

2.Validation of a water-flow enclosure system

To ensure that individuals don't exhibit side bias in the maze, sexually mature *O. rusticus* (n=5) were placed into the acclimation compartment. After a 10 minute acclimation period, the pumps were turned on and well water was pumped through both arms of the enclosure for 90 seconds. After 90 seconds, gates were removed and the animals were allowed to move freely through the tanks. Individual movement pattern and arm preference was recorded.

To assess if crayfish are capable of sensing a chemical signals pumped through the arms of the enclosure, we created a food pheromone by placings tilapia filets into a container of well water. The tilapia stock was then diluted to 50%. This concentration was chosen based on pilot data showing that it was most effective in eliciting a statistically significant response. Sexually mature *O. rusticus* (n=16) that were socially isolated for 1-2 weeks prior to the experiment, and unfed were placed into the enclosure. After a 10 minute acclimation period, inflow pumps were turned on and well water was pumped through one arm of the enclosure, while food-scented water was pumped through the other arm. The food/water treatments were randomly assigned to the arms.

3. Assessing crayfish discrimination behavior

After 1-2 weeks of social isolation, *O. rusticus* (n=25 males, n= 25 females) were exposed to same-sex and opposite sex conspecific pheromones (see pheromone extraction protocol below). For this experiment we followed the same protocol as for the validation procedure, 10 minute acclimation period, followed by a 90 seconds of pheromone signal pumped through a randomly chosen arm of the enclosure. Animals were videotaped during the entire duration of the experiment. We measured the duration of time animals spent in either compartment of the enclosure, as well as the arm that they ended up in at the end of the experiment. Individuals were believed to make a choice when they moved towards or away from an arm containing the chemical signal. Repeater measures ANOVA was used to measure difference in arm entry, where maze arms and center compartments was the within subjects factor. Where differences were significant ($p < 0.05$), LSD post-hoc analysis was used. Differences between male and female responses were analyzed using a two-tailed t-test.

4. Pheromone extraction protocol

To create a pheromone-loaded water, we socially isolated male and female O. rusticus for 7-8 days. During this isolation animals were kept on a 12:12 day-night light cycle, with an air bubbler in their tanks. The animals were not fed during that time. At the end of isolation, all animals were returned to their communal troughs, while the water from the container was combined and filtered to create *O.rusticus* female, and *O. rusticus* male pheromone stock. This stock was then used for behavioral assays described above.

Results

Enclosure validation

In the absence of a chemical signal in the water, individuals did not show preference for a particular arm of the maze (Fig 3). However, when a food signal was pumped through one of the arms, individuals consistently moved towards the arm containing the food signal (Fig. 3; n=16, $p < 0.01$).

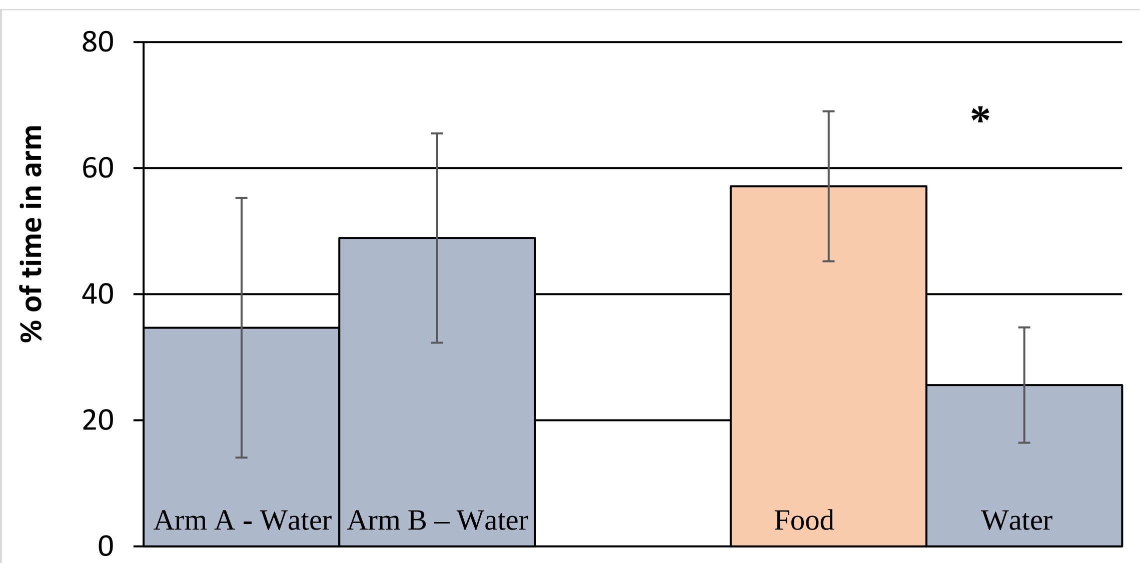

Figure 3: Assessment of side bias and sensitivity to chemical signals in the flow-through system. Water (blue) or food scent (orange) were pumped through the arms of the maze.

Crayfish did not show preference for either arm, when water was pumped through both, but consistently chose the arm with the food scent (orange) ($p<0.01$).

Pheromone discrimination assessment in *O. rusticus*

When *O. rusticus* were exposed to same-sex pheromones or water, they did not exhibit discrimination behavior (Fig. 4). However, overall, both males and females spent less time in the pheromone arm, relative to the water arm or the acclimation (center) compartment.

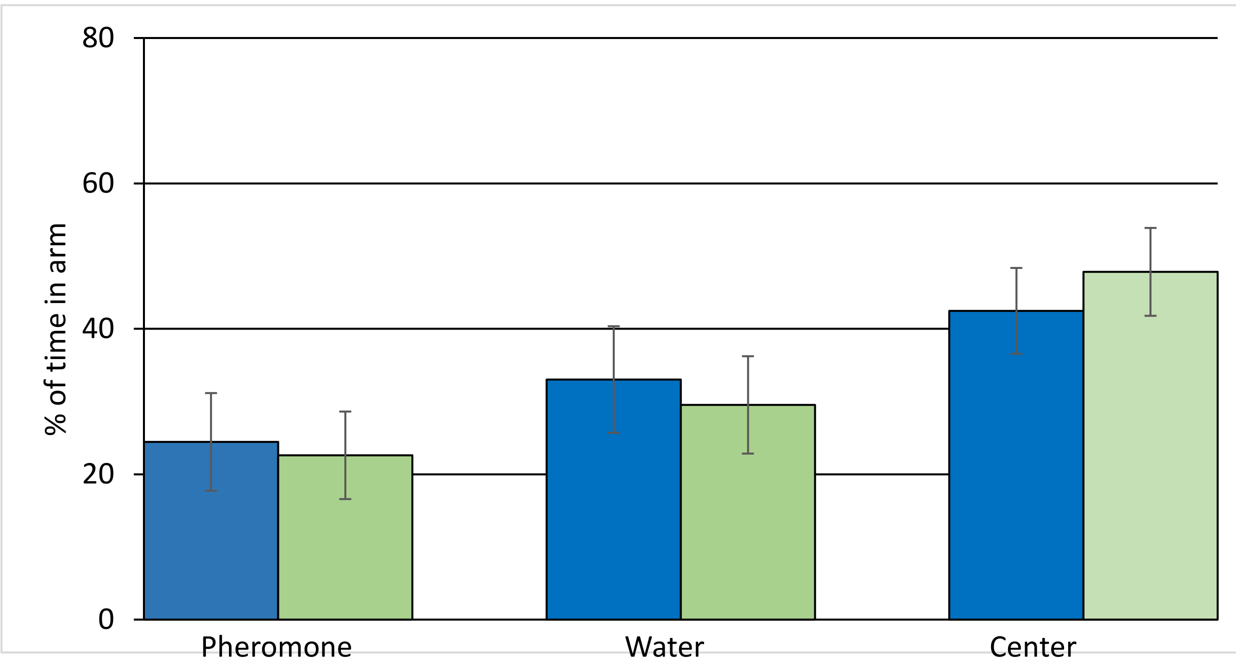

Figure 4: *O. rusticus* response to same-sex pheromones. Females (blue) and males (green) do not show preference or avoidance towards same-sex pheromones. Error bars represent standard error.

There was a sex-specific response to opposite-sex pheromones. Compared to males, female *O. rusticus* spent more time in the arm containing male pheromones (Fig. 5; $p<0.05$), entered the arm more frequently (Fig. 6), and walked a greater distance through the enclosure (Fig. 7).

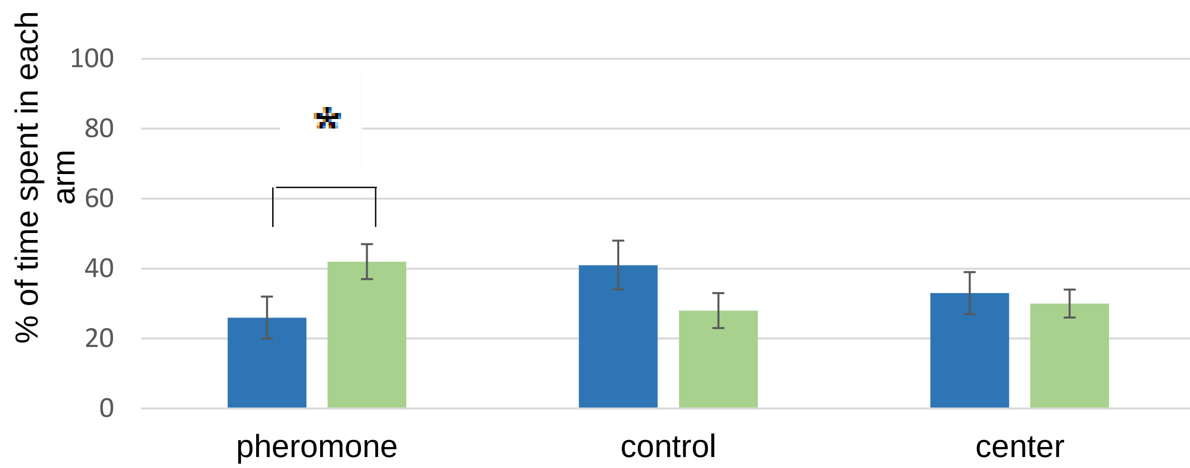

Figure 5: Sex difference in time spent in pheromone arm in O. rusticus. Compared to males (green), females (blue) are more attracted to opposite-sex conspecific pheromones and spend more time in the pheromone arm. Asterisk indicates statistically significant differences ($p<0.05$), error bars represent standard error.

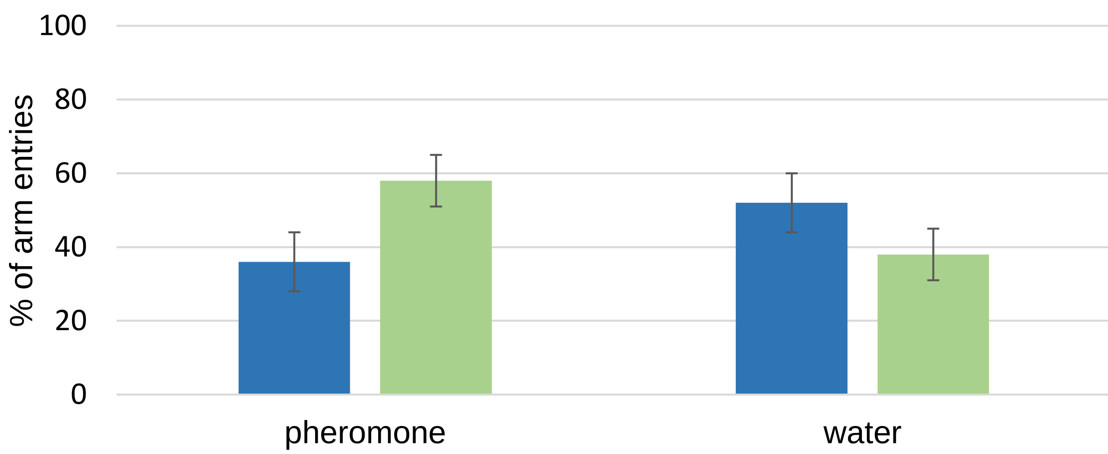

Figure 6: Sex difference entry frequency of pheromone arm. Females (blue) tend to enter the arm containing male pheromones more frequently than males (green). Error bars represent standard error.

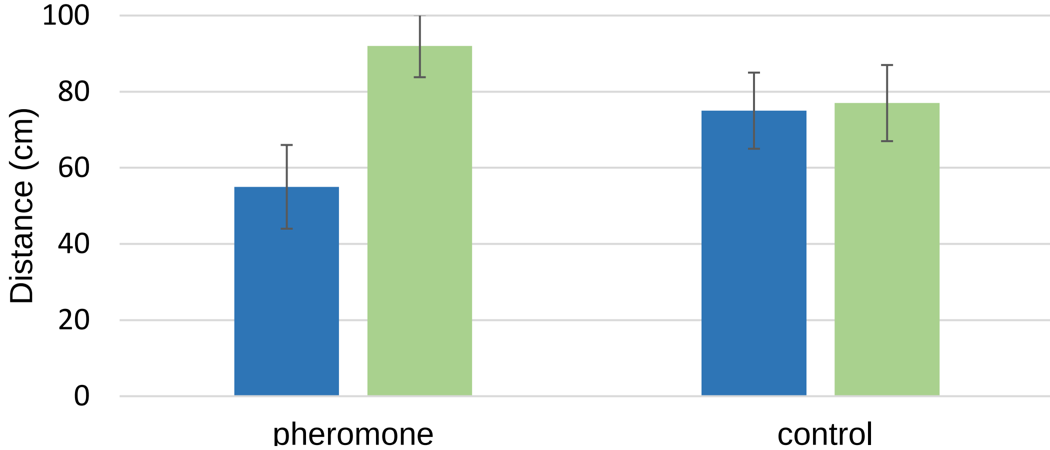

Figure 7: Distance traveled in the enclosure. Females (blue) tend to explore the enclosure more than males (green). Error bars represent standard error.

Conclusion

Our preliminary data show that at higher concentrations, males spent more time away from the same-sex pheromone, while females spent more time in the arm containing the chemical signal. Females also entered the pheromone arm significantly more than expected at the 50% concentration. These findings suggest that male and female *O. rusticus* exhibit sexually dimorphic behavior strategies towards same-sex pheromones. These findings would be an important consideration when designing chemical traps to limit invasion spread by *O. rusticus*. For example, based on our findings, male pheromones can be used to create baited traps that would attract females, which can help curb reproduction of *O. rusticus*. However, while the current findings are promising, we must also be able to understand how heterospecifics respond to the pheromones of *O. rusticus*. Traps that attract natives and invasives equally will not help reduce invasive populations. Therefore, we are currently in the process of expanding our pheromone collection and increasing sample sizes for the data we have collected.

The funding we received from the PA SEA Grant has allowed us to develop the experimental paradigms we need to investigate the species-specific chemical signals that allow the rusty crayfish to communicate with its environment. We have begun assessing the disparate behavioral responses, approach or avoidance, that crayfish exhibit in response to these signals. As we continue to further investigate this phenomenon, we hope to apply these findings in development of chemically-baited traps that can be used to enhance trapping efficiency of *O. rusticus*. We presented our preliminary findings at multiple conferences (Stonecipher et al., 2017; Henry and Goldina, 2017; Henry et al., 2018; Kalmbacher and Goldina, 2018).

Objective 3: Develop a public outreach program to educate local schools and residents about the dangers of invasive species and how to identify and trap invasive crayfish in the local watersheds

Methods

In collaboration with Science in Motion at Elizabethtown College, we developed specific lesson plans that address topics such as biodiversity, park management, animal behavior, ecosystems and food webs (Table 3). All the lesson plans were aligned with the Pennsylvania Environmental and Ecology and Biology Standards for grades 7 and 8 and had specific protocols for easy classroom implementation. The units include multiple inquiry-based activities that can be easily modified and adjusted for difficulty and relevance. Furthermore, each unit includes a required list materials, as well as sample handouts for teachers and students.

Table 3: Description of lesson plans developed and presented at the Crayfish Workshop.

Lesson Title	**Main lesson objectives**
Snapshot of ecosystems	Components of an ecosystem. Introduction of biotic and abiotic factors and food webs.
Invading national parks	Understanding how invasive species impact food webs.
Creating your own invasive species	Apply the knowledge about the characteristics of invasive species and dichotomous key to create an invasive species.
Characteristics of invasive species	Behavioral traits that make invasive species successful in their environments
Fight! Fight! Fight! Crayfish behavior	Observation and quantification of videotaped agonistic behavior in crayfish
Cray Madness	Use live crayfish to set up agonistic interactions to address a student-developed question.
Observations and a dichotomous key	Learn crayfish anatomy and how to identify crayfish species using a simplified dichotomous key.

We then conducted two summer workshops, June 16, 2017 and June 20, 2017 for science teachers in the Lancaster County using these materials.

Results

In total, 13 teachers attended the workshops we developed. During the workshop, teachers learned about the latest research on the rusty crayfish. Teachers spent a day learning how to maintain, handle, and identify *O. rusticus*. They then set up their own tanks, observed crayfish behavior, and quantified agonistic interactions.

At the end of the workshops participants were given their own “crayfish starter kits” which included holding tanks, air pumps, food, nets, and 1 crayfish. In addition, all teachers were provided with a folder that contained printed out lesson plans (student and teacher version), as well as the entire document in a USB drive. Based on an assessment survey completed at the end of each workshop, all participants felt that the information they learned was interesting, relevant, and could be implemented in their classrooms.

Based on the post-workshop survey, most of the teachers felt very positive about the material content and were enthusiastic about sharing it with their students. Some of the comments to the open-ended questions are shown below (Table 4).

Table 4: Workshop participants' response to open-ended question assessing value of the information gained.

What was most valuable about this workshop?
Perfect fit for our 7th grade CS curriculum and an excellent way to incorporate inquiry into our ecology unit.
I loved the fact that you were willing to adjust instruction based on the needs/desires of the participants!
The free equipment and having the crayfish fight. Thank you for the materials and food.
Hands on with the crayfish. Helpfulness of the instructors and the materials gained! I really enjoyed myself and learned a lot!
Seeing what studies are being done and actually getting to observe and handle the crayfish. Going through parts of the lessons as our students will. Thanks for everything, the workshop was amazing!
Lesson ideas
To just get another organism to relate to students concepts of animal behavior, evolution, etc.
The lesson plans and resources provided are excellent.
Hands on activities!
New ideas in ways to incorporate organisms into the curriculum to enhance learning.

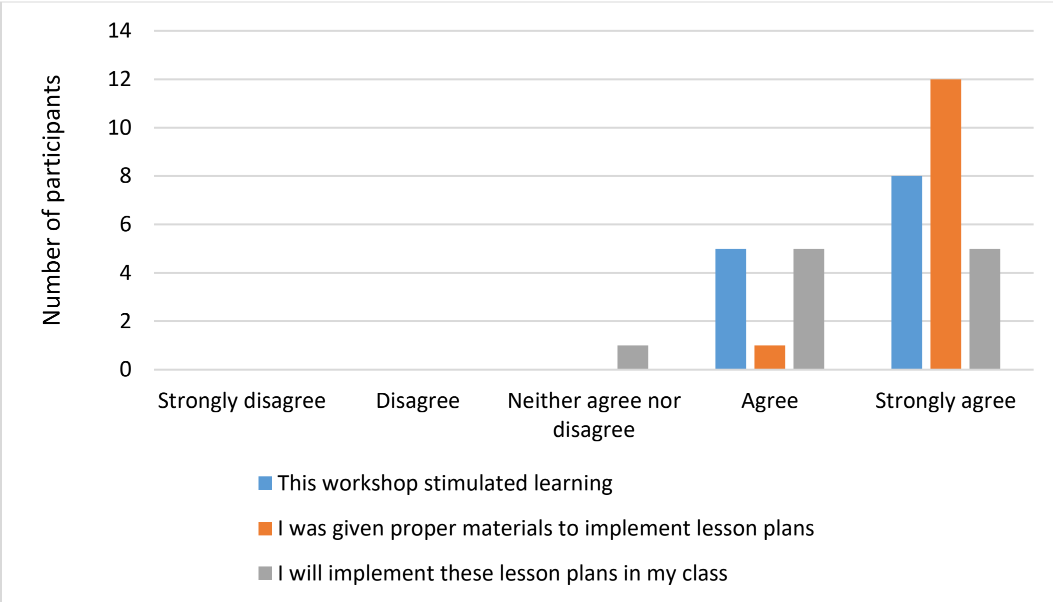

Figure 8: Assessment of crayfish workshops. Most of the participants agreed or strongly agreed that the content provided in our workshop would be appropriate for their classrooms and that they are likely to incorporate these lessons into their curriculum.

Following the workshops, we have been in contact with three local teachers from Elizabethtown College and Donegal Junior High School and Elizabethtown Middle School. These schools have recently received funding to establish a crayfish housing facility and began developing activities to implement some of the lesson plans we introduced during our workshops. We are currently developing ways to create projects that will allow Etown college students to work together with these schools on joint projects. The lesson plans we developed have been presented by Carly Egberts at the Pennsylvania Academy of Sciences (Egberts, Martin, and Goldina 2017) annual meeting. The lesson plans culminated in an honors thesis which is attached separately. A manuscript describing the lesson plans and assessment data is in preparation.

Conclusion

Prevention of further expansions of invasive species depends heavily on public education and buy-in. Since *O. rusticus* have been introduced as bait, it is important to educate the public about the dangers of invasive populations and how to interact with invasive species that they encounter in the local streams. This education must start early. The public outreach projects that we have initiation by collaborating with local schools will help develop a public that is aware of the local environmental challenges and will equip the students with strategies for recognizing and conserving the native species.

Future Research

This work laid the groundwork for multiple future projects. The findings of our study increased our understanding about how different chemical signals are perceived by crayfish, causing a change in their behavior. Evidence that *O. rusticus* can distinguish between conspecific and heterospecific signals, and exhibit differential approach and avoidance behaviors to chemical signals of different source or context allows us to begin identifying bait pheromones, which could be applied to traps to selectively trap *O. rusticus*, as well as repellant pheromones, which could be used to Additionally, we to create chemical barriers to prevent *O. rusticus* expansion into new habitats. Thus, our future research will focus on characterizing the specific chemical signals that can be used to develop chemical traps and expansion barriers. Ultimately, we hope to be able to test these traps in local creeks. We find that mitochondrial haplotypes of local *O. rusticus* are similar to those of *O. rusticus* from geographically distant sites within the current northeastern range of the species. This suggests that pheromone-based control strategies effective on locally collected animals may be applicable to *O. rusticus* across its northeastern range.

The development of a collaboration between the college and the Elizabethtown Middle School and the Junior High Donegal Schools is expected to increase science communication, student engagement, and awareness about their local environment. While we have not had the opportunity to develop an educational website for monitoring crayfish, we hope to be able to do that as a collaborative project between Elizabethtown College students and middle school science students. Furthermore, through increased communication between college and middle school science faculty, we hope to equip science teachers with support and materials they need to develop science curriculum that engages and challenges their students. We strongly believe that appreciation for the world around us, and the desire to conserve and protect the environment comes from early education and exposure. We hope that our collaboration with teachers in middle schools will help us reach young students and help maintain their excitement about science and environmental stewardship.

Citations

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Appendix A

Undergraduate Student Support

The funds from this grant helped support six undergraduate students at the Elizabethtown College.

Student name	Major	Graduation Year	Job
Marquise Henry	Environmental Science	2018	Food Safety and Quality technician at the Wilbur Factory
Jacob Stonecipher	Biology – Allied Health	2017	Applying to graduate schools in Physical Therapy
Carly Egberts	Biology Education	2017	Science teacher at the Hempfield School District
Mo Kalmbacher	Environmental Science	2017	GeoKids LINKS Fellow at Saint Joseph's University
Amanda Herzog	Environmental Science	Expected Spring 2019	----------------
Amelia Baker	Environmental Science	Expected Spring 2020	----------------

Faculty and staff support

- Anya Goldina = 0.16 FTE
- Diane Bridge = 0.04 FTE

Publications

- We currently have manuscripts in preparation, but none have been submitted yet.
- Carly Egberts completed her Honors Thesis in the major. Inquiry based activities to teach about invasive species in grades seven and eight.
- PDF files of posters resulting from Sea Grant-funded work are included at the end of this report.

Volunteer hours

- No volunteers were utilized in these projects.

Public and professional presentations:

Our findings were presented at the following conferences (* indicates undergraduate students):

- *Mo Kalmbacher and **Anya Goldina**. 2018. Behavioral response of the invasive crayfish *Orconectes rusticus* to opposite-sex chemical signals. Mid-Atlantic Chapter of the Ecological Society of America (MA-ESA) Annual Meeting. Newark, New Jersey.
- *Marquise Henry, *Jacob Stonecipher, **Anya Goldina**. 2018. Behavioral response to same-sex pheromones by the invasive crayfish *Orconectes rusticus*. Society for Integrative and Comparative Biology. San Francisco CA.
- *Marquise Henry and **Anya Goldina**. 2017. Development of a pheromone detection assay for regulation of invasive *Orconectes rusticus*. Summer Creative Arts and Research Program. Elizabethtown College, PA
- *Jacob Stonecipher, *Marquise Henry, **Anya Goldina**. 2017. Using a controlled water-flow enclosure system to assess ability of *Orconectes rusticus* to discriminate between different chemical cues. Pennsylvania Academy of Sciences. Wylkes Barre, PA.
- *Carly Egberts and **Anya Goldina**. 2017. Inquiry based activities for teaching about invasive species in grades seven and eight. Pennsylvania Academy of Sciences. Wylkes Barre, PA.

Project collaborators

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Appendix B

Impact Statement

Title: Investigation of Chemical Signals for Use in Control of the Invasive Rusty Crayfish

Collaborator: Wendy Martin from the Education department at Elizabethtown College

Recap: The focus of this project was to increase public awareness of the deleterious effects that the rusty crayfish have on local habitats by providing middle school science teachers with relevant, easy to implement science lessons that reflect current research.

Relevance: The invasive *O. rusticus* has been introduced into the local watersheds as bait.

Response: To address this issue we have established collaborations and hosted workshops for science teachers in the area to develop education strategies aimed to increase public awareness of the threat posed by invasive species to the local habitats.

Results: We developed multiple lesson plans, with specific activities that focus on a variety of topics about invasive species and biodiversity. These lesson plans were shared with 13 middle school science teachers that attended our workshops. Through these workshops we have established an ongoing collaboration with two local schools and will continue developing projects that will allow Elizabethtown College students to work with middle school science students.

Accomplishment Statement

Title: Investigation of Chemical Signals for Use in Control of the Invasive Rusty Crayfish

Collaborator: Mark Gatti from the Engineering and Physics Department at Elizabethtown College

Recap: This project examined chemical communication between crayfish, with the goal of identifying chemicals that could be used for environmentally-friendly control of the high-impact invasive rusty crayfish.

Relevance: The rusty crayfish, native to the mid-west, has invaded streams and rivers throughout northeastern North America and Europe. It causes major changes to freshwater ecosystems, affecting which other species, including fish, are present. No cost-effective methods are currently available to manage and prevent range expansion of this species. Manual trapping, while effective, is slow and labor-intensive. Chemicals that could be used to trap and to repel rusty crayfish without impacting other species would be valuable for conserving freshwater ecosystems.

Response: Continuing research using the methods developed during this project will focus on identifying specific pheromones that can be used to trap and to repel rusty crayfish.

Results: The research performed during this project showed that in some situations, rusty crayfish produce pheromones that attract other rusty crayfish. In other situations, they produce pheromones that repel other rusty crayfish. These results lay the foundation for species-specific chemical control tools.