EFFECTS OF OLFACTORY AND AUDITORY STIMULI ON LOCOMOTION OF *PROCAMBARUS CLARKII*

By

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ABSTRACT

EFFECTS OF OLFACTORY AND AUDITORY STIMULI ON LOCOMOTION OF *PROCAMBARUS CLARKII*

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This study addressed solutions for the population control of invasive *Procambarus clarkii* (Red Swamp Crayfish) in Michigan. The infestation was reported to the Michigan Department of Natural Resources in 2015. *P. clarkii* outcompetes native species of crayfish, is highly fecund and causes bank erosion through burrowing. Intensive trapping allows slowing the spread of the population and detecting new spread, but the practice is costly. In this study, auditory stimuli were tested as a means of affecting a locomotive response in *P. clarkii*. These trials tested various pure tone sounds and pink and white noise ranges in artificial habitats. Following those results, a white noise frequency band was played underwater during the trapping season. The results indicated that a high frequency range of white noise (10-15 kHz) was most effective at eliciting a locomotory response. When used during the trapping season, a combination of sound and food bait performed at the highest capture per unit effort (CPUE), 0.820. Traps with only food and only sound performed at 0.644 and 0.675, respectively. Moreover, traps without sound or food bait performed at a high baseline CPUE of 0.487. Artificial refuge traps performed at a higher CPUE than other trap types, despite the lack of food bait. The results suggest that the benefits of refuge, sound, and food bait are additive. A novel trap design was created using the advantages of artificial refuge, food bait, and acoustic stimuli. The implications of this study span from the control of invasive species in the Great Lakes region to increasing profits of crayfish farming in the southern United States.

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CHAPTER 1: INTRODUCTION

The invasion of *Procambarus clarkii* (Girard, 1852), better known as Red Swamp Crayfish (RSC) has been witnessed worldwide. The average observer might notice a "small lobster" crawling across a pond-side lawn, while the ecologist might see a destructive aquatic invader come to change the ecological landscape forever. Although extreme, the effects of an invasive species on an ecosystem can be deleterious. The RSC is no exception; they are typically more aggressive than native species, fecund, and mobile. The introduction of the invasive crayfish results in the loss of biodiversity and reductions in populations of finfish and crabs (Moonga & Musuka, 2014). Additionally, RSC can burrow under civil infrastructure causing damage to dams, reservoirs, and levees (Booy, Cornwell, Parrott, Sutton-Croft, & Williams, 2017). The RSC was reported to be present in Michigan in 2015 and was listed as an invasive species by the Michigan Department of Natural Resources (MDNR). As of October 29th, 2019, over 20 water bodies were infested. The MDNR previously developed a Red Swamp Crayfish Response Plan which helped to inform them on response efforts should *P. clarkii* invade Michigan. The goal of this research is to investigate and conclude engineering designs to assist the MDNR in control and eradication of the Red Swamp Crayfish (RSC). Knowledge gaps were found during a literature review covering the understanding of the biology, ecology, invasive habits, and current trapping techniques.

RESEARCH GAPS

It was critical to understand the biology and ecology of RSC, so as to utilize these properties to conclude an engineering solution. A method of luring RSC would be useful and would require an understanding of their sensory organs. No studies were found related to the use of sensory stimulus as a lure for RSC or any other crayfish, aside from different food baits. Sensilla which could be tested included olfactory, optical, thermal, and auditory. In addition,

predator prey studies are lacking information on Michigan native species. The Fisheries and Wildlife department at MSU is already conducting trials to determine which native fish species will consume RSC.

LITERATURE REVIEW

A literature review was completed to better understand the context of the engineering problem of controlling invasive populations and provide some information to guide solutions. The topics studied were biology and ecology, including sensory stimuli, and past control efforts relating to RSC. From the literature review, the knowledge gaps were identified, and research objectives were created.

Biology and Ecology

RSC are invertebrates under the order *Decapoda*, family *Cambaridae*, genus *Procambarus* and species *clarkii*. The RSC typically has a unique phenotype from other crayfish species. They have a dark red carapace, with claws (chelae) reaching out in front of them as seen in Figure 1.1. Red spots are common on the chelae, but the color is not a reliable predictor of species for this crayfish as many juvenile RSC are not red (Boets, Lock, Cammaerts, Plu, & Goethals, 2009). The adult length ranges from 5.5 to 12 centimeters.

Figure 1.1: Adult male crayfish. The red raised spots on the sickle shaped chela (claws) and black stripe on the underside of the tail are characteristic of Procambarus clarkii. Photography by Douglas Clements.

Native to the United States, RSC have habitat along the Gulf of Mexico between Mexico and Florida. However, as of 2019, infestations have begun along the west coast, east coast, and in the northern mid-west (Nagy, Fusaro, Conard, & Morningstar, 2019). Worldwide distribution of crayfish has led to infestations in Europe, Africa, South America, and Asia (Hobbs III, 1993; Holdich, Gydemo, & Rogers, 2017). Many means of introduction exist, including live fish bait, aquarium trade, biological supply to laboratories and classrooms, and live seafood markets (Kilian et al., 2012).

RSC have an omnivorous diet and consume plants, snails, macrophytes, insects, and detritus (Gherardi & Barbaresi, 2007; Hobbs III, 1993). Studies have found that adult RSC preferentially feed on plants and detritus, by volume. Conversely, juveniles consume mostly animal matter in the form of insects, gastropods (snails), and fish (Correia, 2003). Due to the aggressive nature of the species, RSC out-competed native crayfish for territory and food (Gherardi & Cioni, 2004).

Crayfish are ecosystem engineers characterized by their burrowing. The RSC can dig burrows extending up to 90 cm below the water table (Ingle, 1997). Burrows are most commonly found in areas with fine sediment and are less prevalent in areas with sand and harder soil substrates (Barbaresi, Tricarico, & Gherardi, 2004).

The RSC are nocturnal, mostly active in the nighttime immediately after the sun sets. Male crayfish exhibit dimorphism, expressing a form 1 sexually active physicality or a form 2 sexually inactive physicality. Form 1 males can be characterized by the stiffness and definition of their gonopods, and usually grow, through calcification, sharp hooks on their walking legs. Form 2 males do not have hooks on their walking legs and have less shapely definition in their gonopods. Males show different behaviors characterized by their locomotion. One behavior type is that of form 1 males in a mate-seeking phase. This phase is characterized by bursts of highspeed movement. The second behavior type is characterized by an immobile stage during which the crayfish hides in its burrow only coming out at night to forage (Nagy et al., 2019).

Sensory Stimuli

It is important to understand what motivates RSC, in order to inform an engineering solution to the eradication and control of the species. The crayfish eat and mate of course, but even those motivating activities are sensed and communicated beforehand. Therefore, and understanding of the sensory organs and processes should reveal testable solutions to affecting locomotory responses.

Though it is known that marine crustaceans create and respond to sounds, little is known about the effects of sound on crayfish locomotion (Edmonds, 7AD). RSC do not have hollow, air-filled organs to hear with, but instead perceive sound through hair-like sensory structures called mechano-receptors shown in Figure 1.2 (Popper, Salmon, & Horch, 2001). Therefore, the pressure of sound are not the direct cause of stimuli to the sensilla, but instead the particle

velocity is responsible (Goodall, Chapman, & Neil, 1990). RSC emit sounds composed of wide-band frequency pulses lasting 0.4 millisecond with a 20 kHz RMS bandwidth, peaking at 28 kHz. Maximum SPL_{PK} (intensity) of the signal is 146 dB relative to 1 µPa. Such sounds occur during tail flips, fighting, and encountering events. Since the sound carries efficiently through water, it is thought that dominance and territory may be communicated at distance through acoustics (Buscaino et al., 2012). This is of importance, since peak sensitivity to hydroacoustic stimuli was determined to be at frequencies below 150 Hz (Breithaupt & Tautz, 1990).

An experiment was conducted by an undergraduate engineering group at Michigan State University, to determine the effect sound stimuli had on RSC locomotion. Various pure tone frequencies between 20 Hz and 500 Hz were tested using a speaker modified for underwater use and it was found that the sound had a significant attraction effect on crayfish locomotion, especially in the 500 Hz trials. Since the highest locomotory response came from the maximum frequency tested, the group recommended further testing with a higher range of frequencies(Ausmus, L, Kontorousis, A, Li, B, Tang, 2018).

Figure 1.2: Mechanoreceptors can be seen on an antenna under a Keyence microscope at 100x magnification. Photograph by Amy Albin.

The age of a crayfish and light exposure affect which parts of the spectrum crayfish most readily see. An electroretinogram study showed that juvenile crayfish had a higher response (voltage presence) to ultraviolet and blue light than do the adults. Adult crayfish have a higher response to red and green light. Spectral sensitivity is dependent on whether crayfish have been exposed to dark or light; adults only respond to ultraviolet light when dark-adapted and shifts to red sensitivity when light-adapted. The study suggests that short wavelength and long wavelength receptor cells change proportion as juveniles become adults (Fanjul-Moles & Fuentes-Pardo, 1988).

The RSC primarily communicate dominance hierarchies through use of olfactory sensory organs. Crayfish emit olfactory stimuli through urination. Olfactory sensors on the antennules detect the excreted chemicals. Crayfish with the sensory organs removed fight each other more often, while crayfish with the sensory organs avoided fights, thus proving that olfactory stimuli control aggressive behavior (Horner, Schmidt, Edwards, & Derby, 2008). Olfactory stimuli are

enhanced through the creation of jets of water which draw odors in toward the antennules, which have a high concentration of sensilla. The odors would otherwise be limited to naturally occurring currents and molecular diffusion (Denissenko, Lukaschuk, & Breithaupt, 2007).

The effects of heat stimuli on the heartrate of crayfish (*Cherax destructor*) were studied in cooling and warming environments. The heartrate drops significantly faster than body temperature during cooling events and increased slower than temperature during heating events (Goudkamp, Seebacher, Ahern, & Franklin, 2004). No literature was found considering locomotory response of crayfish with respect to heat or infrared light.

Control Efforts

Current control strategies vary due to the nature of the infested water bodies. Rivers and streams with continuous flow are not necessarily a good fit for chemical treatments as the residuals would wash down stream. Likewise, a large lake may be costly to trap intensively. Each water body will bring a different set of challenges to control strategies. The MDNR response efforts include the ultimate goal of developing a framework to classify waters for different treatment types.

Intensive trapping is the practice of placing traps in a high-density arrangement to trap a large quantity of the population. A common type of trap used for trapping is a semi-cylindrical minnow trap, although many geometries exist. Between dip-netting, Fyke-netting, cylindrical traps, and semi-cylindrical traps, the semi-cylindrical wire mesh traps have the highest capture rates per unit effort (CPUE). However, variations in habitat also influenced CPUE. Sex selectivity can be an issue with trap geometries; however, the semi-cylindrical trap does not appear to select one way or the other (Paillisson, Soudieux, & Damien, 2011). One research team tested collapsible mesh netting traps, among many 10 other trap types, and found that each trap captures distinct size categories of crayfish, sex ratios, and quantity of bycatch. The team

concluded that a combination of trap types might be useful for intensive trapping to cover all size ranges (De Palma-Dow, Curti, & Emi Fergus, 2020). Artificial refuge traps were tested based on an indigenous method of crayfish capture using brush piles; the design used PVC tubes to mimic a crayfish burrow. The benefits are that artificial refuge traps do not require bait, and can catch egg-bearing females when they seek shelter for protection (Green, Bentley, Stebbing, Andreou, & Britton, 2018; O'Connor, Brennan, & Baars, 2018; Parkyn, Distefano, & Imhoff, 2011). Intensive trapping requires a large amount of human effort to reduce populations and must be maintained or populations will return to previous levels within a couple of breeding cycles (Holdich et al., 2017).

Sterile Male Release Technique (SMRT) was tested as a means to control populations. In SMRT, male crayfish are exposed to X-rays, which reduce the size of their testes and alter spermatogenesis. The result of SMRT is a 43% reduction of offspring from females that mated with irradiated males (Aquiloni et al., 2009). The efficacy of such a treatment is yet to be field tested, but the expected impact is low compared to other control strategies (Holdich et al., 2017).

Pesticides have been used with some success. Biocide is the term used to describe pesticides targeting invasive organisms. Biocides work best for smaller bodies of water were biocide quantity does not have to be costly to achieve lethal doses for invasive crayfish. Biocides are not specific to a species and can harm native crayfish and other organisms. In addition, accumulation and magnification of toxins can cause undesirable results. Some biocides include organophosphate, rotenone, surfactants, and pyrethroids insecticides. Trends in chemical treatment lean toward low environmental persistence, since selectivity of biocides does not yet exist for crayfish (Holdich et al., 2017). One promising biocide is emamectin benzoate which has been used to force molting of egg-bearing American lobster, thus aborting the eggs in the

process. However, experimental trials have not been concluded (Freeman, Turnbull, Yeomans, & Bean, 2010).

Predator prey studies with various organisms have been documented. A potentially effective predator is the European eel (*Anguilla anguilla*). European eels tend to consume crayfish under 45 mm in length, of which normally tend to be trap-shy (Aquiloni et al., 2010). While they may be effective in controlling the population in combination with traps, biological controls come with a caution. The eels themselves may create another ecological problem if introduced. Therefore, studies should be completed to test for each site's specific consequences, with preference given to native species.

RESEARCH OBJECTIVES

In order to assist the MDNR with their goals of RSC population control and eradication, this study seeks to develop a better understanding of locomotory responses to auditory, olfactory. and thermal stimuli. Specifically, the objectives for this study are to: i) determine the locomotory response of various sound frequencies on RSC; ii) test the ability of sound to enhance intensive trapping of RSC; iii) test the ability of a heat source to attract RSC in lower temperature water; and iv) design a solution to enhance the control of RSC. The results of this study will provide options for an engineering solution to controlling RSC as an invasive species. Additionally, the benefits of this research could reach beyond control measures for invasive populations; a novel lure could provide innovation to crayfish farmers worldwide.

CHAPTER 2: ACOUSTIC FREQUENCY RESPONSE TRIALS

INTRODUCTION

It was clear from the literature review that hydroacoustics, or underwater sound, would be a good starting point for experimentation. RSC create and sense a variety of different frequency sounds; a locomotory response using different frequencies was needed to consider hydroacoustics as a possible solution to RSC population control. Little research on the subject of sound stimulus on RSC exists, but what does exist seems contradictory. Specifically, the high sensitivity of RCS to detect sound in a lower band of frequency (<150 Hz) is at odds with what the *Crayfish Will* group found to be the best frequency to attract a locomotory response (500 Hz) (Breithaupt & Tautz, 1990). However, if one considers the physics of sound propagation, an explanation may exist. Higher sensitivity at lower frequencies may be required to sense the lower energy sounds. As frequency increases, the energy required to produce the sound increases. The speaker power drives the wave intensity, which drives the pressure level of the waves, which in-turn drives the particle velocity of the water. This particle velocity is the measureand of the crayfish mechanoreceptors, unlike the pressure level, which humans perceive. So, at higher frequencies, higher energy levels are required, and therefore, the crayfish may not need a high sensitivity at such high particle velocities.

Since the attraction trial results showed the highest level of attraction at the maximum frequency tested, further testing was completed to expand the range of frequencies. An experiment was designed to verify the results of *Crayfish Will*, and to expand the range of frequencies tested. Additionally, many naturally made noises are in a classification called colored noises. Two such noises were studied: white noise, and pink noise. Pink noise is characteristic of waterfalls and other sounds which have equal energy per octave. White noises

are characteristic of a randomly generated noise within a spectrum, and usually require added energy such as a fan or car tires driving across a road.

Using a modified method to the *Crayfish Will* study, an experiment was designed to further test sound frequencies. In a long aquarium, sound was played at one end, to stimulate the crayfish. After a period of time, the population distribution was recorded and compared to the pre-stimulus distribution. The hypothesis was that at higher frequencies, a higher population distribution would occur nearest the speaker, thus, showing a sort of attraction effect to the sound treatment. The null hypothesis was thus a population distribution similar to that in a silent condition, which was used as the control. The second hypothesis is that the noises will have a higher impact on population distribution than the pure tone frequencies. Both hypotheses were tested using the variance from the population distributions recorded during the silent condition. The conclusion from a frequency response trial would address Objective i), determine the locomotory response of various sound frequencies on RSC.

MATERIALS AND METHODS

Experimental Design

Laboratory trials were conducted in 3 artificial habitats made from cattle bunk feeders. Pea gravel was used to level out the slope in the habitat. Each habitat contained 3 sponge filters with air sponges attached to clean the water and maintain suitable oxygen levels for the crayfish. Extruded polystyrene lids were used to prevent RSC from escape and to block out light. Each habitat was divided into 3 zones of equal area. Three zones were chosen for ease of recording population distribution, because of the difficulty of counting a large quantity of moving crayfish. Three 8 cm lengths of 3.8 cm diameter clear flexible tube were added to each zone for artificial habitat. Preliminary habitat set-up is shown in Figure 2.1.

Figure 2.1: The cattle bunk feeders provided a long and narrow habitat shape for conducting the lab-scale research. This picture was taken during cleaning of the sponge filter in zone 2, which explains its absence. A layer of door sealing insulation was used to create a seal around the uneven top of the bunk feeders, to prevent escape.

Between 16 and 20 crayfish were placed in each habitat, and randomly exchanged between habitats after each trial. RSC were sourced from Carolina Biological Supply Company. RSC were unsexed and varied in size from juvenile to adult. A Lubell Labs UW30 30-Watt speaker was placed into Zone 1 in each tank. The speaker was powered by a Bogen CC4301 amplifier. A Dell PC sent audio signals to the amplifier using Windows Media Player. Sound files were generated using Audacity (Figure 2.2). Pure tones sound files used a sine waveform and were exported as an MP3 file with 320 kbps quality. Noise files were generated using a built-in function and both high-pass and low-pass virtual 5^{th} order Butterworth filters were added

to each. Sound treatments included the following pure tones: 500 Hz, 1 kHz, 2 kHz, 3 kHz, 4 kHz, 5 kHz, 6 kHz, 7 kHz, 8 kHz, 9 kHz, 10 kHz, 11 kHz, 12 kHz, 13 kHz, 14 kHz, and 15 kHz. Frequency bands of white noise were tested in 4 different frequency ranges, 1-5 kHz, 5-10 kHz, 10-15 kHz, and 1-15 kHz. Each treatment was tested a number of times as shown in Appendix A. The quantity of RSC in each zone was recorded before the treatment and immediately after the treatment. Two habitats were chosen to use the same treatment, while the third habitat was the control without a sound treatment. The control alternated habitats for each 24-hour trial. Treatments lasted for 24 hours and crayfish were fed and left without sound for another 24 hours. Habitats were cleaned according to a strict schedule (Appendix A).

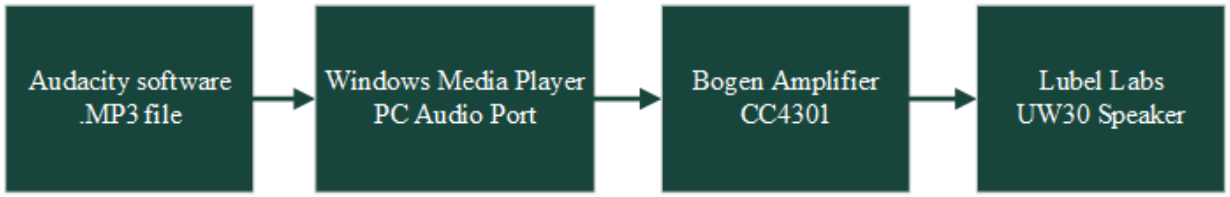


Figure 2.2: Flowchart of the process used to play sounds in habitats.

Analysis Methods

The response variable for the analysis was the percent of total population of RSC in each habitat (population distribution), for each zone. The independent variable was the sound treatment used during the trial. The population distribution for each zone was tested for a normal distribution using a histogram for visual reference, a Shapiro Wilk test for normality, and a Q-Q plot. Data in zones 2 and 3 were not normally distributed as seen in Appendix A. Therefore, a non-parametric test (Kruskal Wallis Rank Sum) was used to determine if frequency affected population distribution. Significance was set to $P<0.05$. A Conover Test was used for zones 1 and 2 to determine which frequencies had the largest impact upon population distribution as compare between treatments with and without sound. The statistical analysis was completed in the programming language R.

RESULTS

Overall, sound treatments had a statistically significant effect on Zones 1 and 2, but not on Zone 3. The respective p-values were 0.008784, 0.01737, and 0.1557. Figure 2.3 shows a bar chart of the population distributions of individual sound treatments. The Conover Test values with $P<0.05$ are listed in Table 2.1. Note that adjustments were made to the p-values using the Conover Test. Adjustments using the Benjamini & Hochberg method (1995) changed some previously significant values to nearly significant ($p\sim0.05$) and some values as not significant. It is important to note that near significance should not be dismissed in the case of false negatives in the analysis. However, type I error was introduced when a method for adjustment was not used. The full analysis was completed using R Markdown (Appendix A).

Table 2.1: Conover Test Results for Zones 1 and 2.

Treatment (Hz)	P	P-adjust	Zone
2000-control	0.0053	0.0514	1
3000-control	0.0248	0.1333	1
6000-control	0.0188	0.1132	1
11000-control	0.0115	0.0912	1
12000-control	0.0040	0.0508	1
WN6k10k-control	0.0046	0.0528	1
WN10k15k-control	0.0052	0.0531	1
2000-control	0.0247	0.1098	2
6000-control	0.0146	0.0820	2
8000-control	0.0234	0.1078	2
14000-control	0.0228	0.1069	2
PN1k15k-control	0.0178	0.0902	2

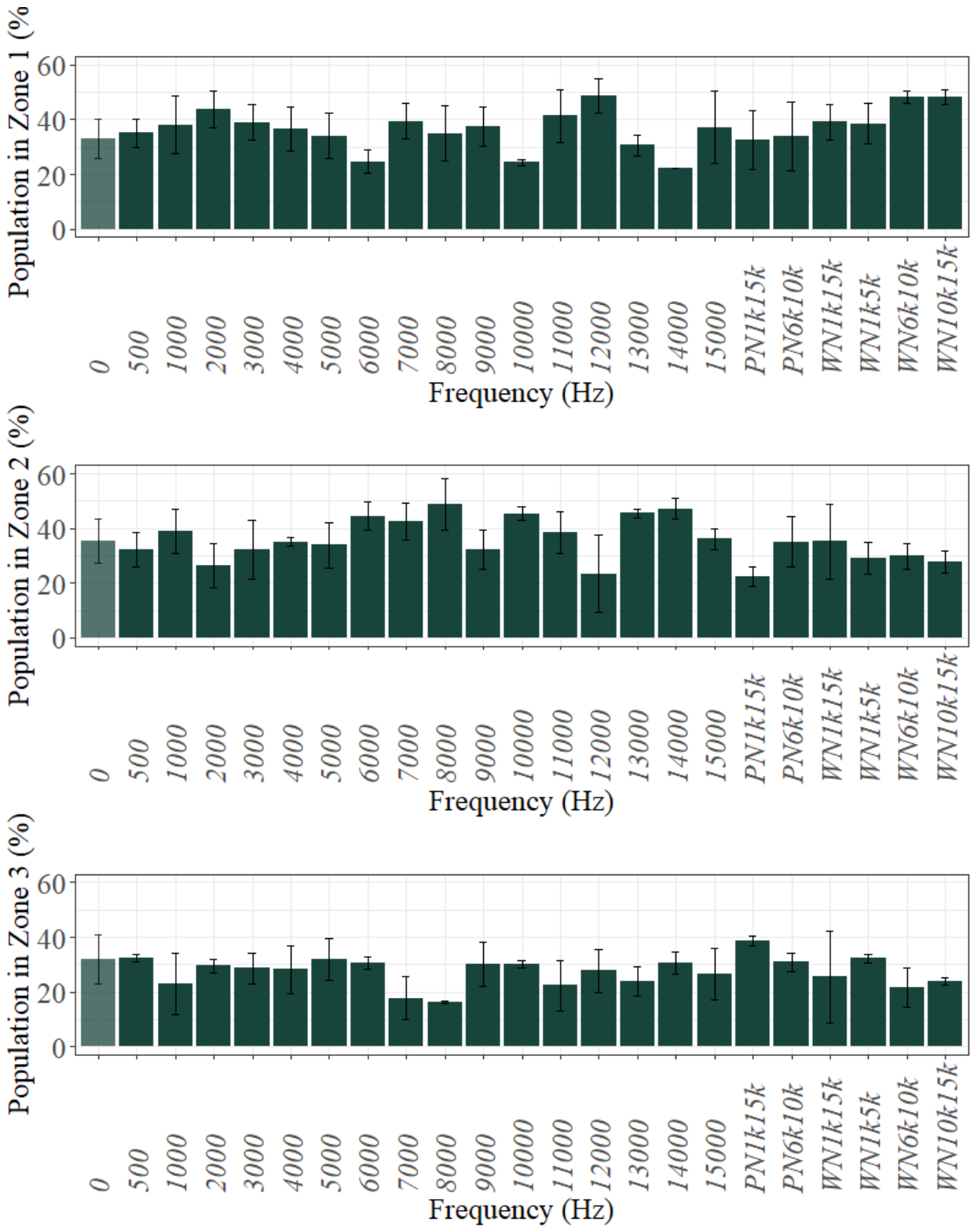


Figure 2.3: The population distribution for sound treatment is represented for each zone. The zero denotes the silent condition (control).

DISCUSSION

From the pure tone frequencies, 12 kHz had the highest population distribution in Zone 1. Both high and medium ranges of white noise had the highest population distribution of all noise treatments. The high and medium frequency ranges of white noise showed a locomotory response that hints at an attraction, since a higher population distribution was closer to the speaker and a lower distribution was in the farthest zone from the speaker. The high range showed a lower population distribution in Zone 2 than the medium range. Zone 3 showed high variability in the medium range, but a lower mean distribution than in the high range.

Aside from 2 kHz and 12 kHz, pure tone frequencies and pink noises underperformed compared to the two highest white noise ranges. The attraction type effect of white noise may come from an interest in catching prey who also make such noises, such as turbulent movement through water. Further investigation is required to determine the cause. For the first hypothesis, the null hypothesis was not rejected, however, 12 kHz was near the higher end of frequencies tested.

The research once again showed that the highest population distribution differences occurred near the top of the range of frequencies tested. The speakers used had a poor frequency response above 15 kHz. Thus, further testing should be done with speakers that have a higher frequency response range. For the second hypothesis, the null hypothesis was not rejected. However, at medium and high frequency ranges, white noise had a higher population distribution than the pure tone frequencies within the range, except for the 12 kHz pure tone.

The locomotory response findings bring question to their relationship with frequency sensitivity findings using methods such as Offutt's use of a heart rate-conditioning technique. Offutt found peak sensitivity at 75 Hz for the American lobster (*Homarus americanus*) between 10-150 Hz (Offutt, 1970). Similarly, Breithaupt used a vibration chamber to test statocyst

vibration sensitivity of *Orcenectes limnosus* and found optimal sensitivity at the lowest frequency tested 3 Hz (Breithaupt & Tautz, 1990). Since the locomotory response was maximized at relatively high frequencies, the frequency may play less of a role than the particle velocity. A similar test with variety of particle velocities could elude to the relationship. Indeed, Mark Plumber and Jürgen Tautz studied the effect of water vibrations on 9 interneurons and found that RSC were not sensitive to high frequency sounds (greater than 400 Hz). All low pass interneurons were inhibited by stimulus above 100 Hz. Broad band neurons were sensitive up to 80 Hz, but high pass neurons respond poorly above 60 Hz (Plummer, Tautz, & Wine, 1986). Therefore, the high frequency white noise may be inhibiting the ability to sense lower frequency noises nearby. Without the ability to sense predators or prey with their mechanosensors, RSC are limited to olfactory and optical sensory input. Since crayfish are more active in the darkness, even vision becomes unreliable, since the experiment was performed in complete darkness. An on-going study using manganese-enhanced magnetic resonance imaging to study the nervous responses of RSC to food and sound will shed light on mechanisms of different capture strategies.

No consideration was made to varying the amplitude (particle velocity of water) during the experiment. The habitats used had very limiting boundary conditions for sound propagation. The plastic walls and pea gravel floor, as well as the shallow water surface allowed sound to spread in an unorganized fashion. A hydrophone was used to record the 1000 Hz signal at various distances from the speaker. Noise was present at relatively low levels outside of the 1000 Hz frequency. However, the sensitivity at which RSC will show a locomotory response is unknown and the unintended sound reflections could have been a factor in the response variable.

CONCLUSION

The best frequencies for attracting crayfish were the medium and high range of white noise and the 12 kHz pure tone. Since the high range white noise had less variability than the medium range white noise and 12 kHz pure tone, and nearly identical population distribution, it was chosen to be used for the acoustic locomotion response trials. Thus, research objective i) was completed.

Because the high range of white noise performed the best, higher pure tone frequencies and ranges of white noise should be tested to find an optimal frequency. However, testing may be limited by the frequency response range of the speakers used. If similar speakers are used for further testing, the signal should first be recorded with a hydrophone and a spectral analysis completed across each pure tone to confirm the quality of the signal above the speaker response range. Additionally, particle velocity should be varied and recorded to create a relationship between locomotory response and signal amplitude.

CHAPTER 3: ACOUSTIC LOCOMOTORY RESPONSE TRIALS

INTRODUCTION

After testing out sound frequencies in a laboratory setting, a more realistic test was needed to understand how the population distribution in an infested pond will be affected by underwater sounds. The laboratory habitats did not create a realistic spatial crayfish density (crayfish per area) or boundary conditions for sound propagation. In particular, the habitats were too small to view a gradient in the amplitude of the soundwave. Sound intensity drops as it travels away from the source or reflects off a boundary such as the surface of the water or bottom of the pond. In designing a solution that involved sound emission, it would be critical to know the effective amplitude needed in order to attract crayfish, as this information could help to determine power consumption and speaker specifications.

Trap research was already being conducted since the beginning of the infestation by the MDNR in collaboration with Michigan State University's Fisheries and Wildlife Department. The research focused on early detection, trap densities, and testing various trap types. The most common trap used for the research was a modified Gee's Minnow trap, which had an expanded opening to allow for larger crayfish to enter. The ongoing research was an opportunity to test the ability of sound to attract crayfish outside of a laboratory setting.

To address objective ii), test the ability of sound to enhance intensive trapping of RSC, an experiment was designed to determine the effects of sound stimuli on intensive trapping of RSC. Originally, two infested ponds in Michigan were used as the testing site. The response variable for the research was the mean capture per unit effort (CPUE). A unit of effort was defined as one crayfish trap, used for a duration of one day. Therefore, the CPUE was the daily amount of crayfish caught in one trap over one day. The manipulated variable was the bait treatment used. Standard baited traps use dogfood as an inexpensive but effective bait. Four treatments were

used, including no bait, sound, sound and food bait, and only food bait. Other uncontrollable variables included weather, water temperature, water depth, and the ponds physiological characteristics. The hypotheses are as follows: 1) Sound will have a statistically significant impact on mean CPUE for both treatments which include sound ($p < 0.05$). 2) Traps closer to the underwater speaker will have a higher CPUE than traps further from the speaker.

Because different trap types will be tested, another hypothesis can be made about how trap types are affected by sound. Three trap types are not baited while two trap types are baited with dog food. The trap types which do not use bait are refuge style traps, which create safe places for crayfish to hide. The third hypothesis is as follow: 3) Refuge traps will increase in CPUE nearer to the speaker. While the number of each trap types are uneven (the vast majority are Gee's Minnow Trap) the result will help to inform the engineering solution design.

MATERIALS AND METHODS

Experiment Design

Field trials took place in an infested stormwater retention pond in Novi, Michigan (42.442431, -83.434977). The pond was characterized by a soft, silt bottom and the bank was a spatial mixture of clay and cobble. The pond was shallow, estimated at less than 1.5 m depth at the center. Heavy microbial matting suggested nutrient-rich water. A population of *Pimephales promelas* (fathead minnows) was present in the pond, in addition to the RSC. The shape of the pond was a long oval with an approximate major diameter of 118 m and minor diameter of 32 m. Intensive trapping using Gee's Minnow Traps was performed for the previous 3 trapping seasons. The RSC present had sizes ranging from juvenile to large adult (> 50 mm carapace length). The perimeter of the pond was lined with 57 crayfish traps, spaced 5 m apart (Figure 3.1).

Figure 3.1: Trap locations spaced in a linear density of 5 meters. Distance from shore was dependent on water depth; traps were completely submerged.

Five trap types were used including a modified Gee's Minnow Trap with expanded openings, a pyramid shaped trap with a single opening at the top, and three custom designed artificial refuge type traps. Five of each trap were used, with the exception of Gee's Minnow Traps, which filled in the remaining 37 trap locations in the pond. Two of the artificial refuge trap types were constructed from varying lengths and diameters of PVC pipe, attached together with adhesive and a fine mesh screen to allow water to flow out of one end. One configuration of the trap which had pipes attached horizontally in a "pan flute" arrangement was denoted artificial refuge trap (ART). The second configuration was made of two horizontal configurations stacked on top of one another and was referred to as APART (short for artificial apartment). The third trap was made from a deconstructed luffa and a stainless-steel nut, with the intent to capture juvenile crayfish, by providing a safe place for them to hide from predation. These were called juvenile traps. All three custom traps were designed to trap crayfish by means of providing an artificial protective space; these traps were not baited with food during any treatment. Trap types are shown in Figure 3.2.

Figure 3.2: Trap types include APART (top left), ART (top right) (Green et al., 2018), pyramid trap (bottom left), Gee's Minnow Trap™ (Stancliffe-Vaughan, 2015) shown in bottom middle, and a deconstructed luffa (bottom right).

Stakes were labeled by trap location number 1-57 beginning at the northeastern most point and numbered in a clockwise manner. Figure 3.3 shows the process for sound generation. An LL916C-100 UW underwater speaker was placed 1 meter from a designated trap location (Lubell Labs, Inc). The speaker was powered by 3 Duracell SLI31MDC 12-volt deep cycle batteries through a CA-160R TOA 60-Watt amplifier (TOA Electronics, Inc). A SanDisk Clip Jam MP3 Player was connected to the amplifier and contained a 10-minute sound file with a 10-15 kHz band of white noise. The white noise sound file was generated in Audacity software using a high-pass and low-pass 5th order Butterworth filter for 10 and 15 kHz, respectively, with a sound quality of 320 kbps. The sound file was looped for the duration of the sound treatments. The speaker location was moved 3 times throughout the trapping season.

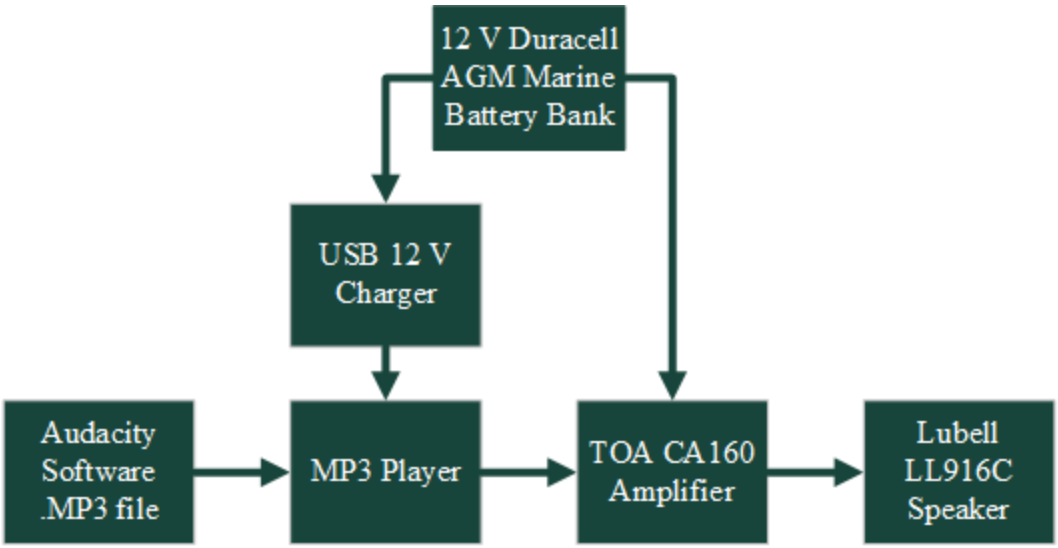


Figure 3.3: Process flow for sound generation using batteries as a remote power source.

Distance to each trap location was measured from the speaker location using Google Maps in combination with Autodesk AutoCAD 2020; measuring accuracy was confirmed using a meter stick to measure objects on either end of the major diameter with overall readings ±10 cm (Figure 3.4). Since traps moved around freely on 0.5-meter twine, the accuracy of trap location was accurate within 1 m.

Figure 3.4: Map of trap locations, courtesy of Google Maps.

Four treatments were tested: 1) Sound and Food bait, 2) Food bait only, 3) Sound only, and 4) No treatment. Dog food was used as a food bait in treatments 1 and 2. Each treatment was performed for a 24-hour period, starting in the morning, and ending the next morning. Traps were emptied each day and the trap type, number of crayfish caught, and trap location were recorded. Methods were approved by the MDNR before experimentation began.

Analysis Methods

Due to differences in trap types (baited versus not baited), treatment analysis was only performed using a subset of the data with only Gee's Minnow Traps. The first step of the analysis was to determine which metric to use to analyze efficacy of each treatment. Since the initial population of RSC in the pond was unknown, CPUE was used as the response variable. The second step was to determine if the daily catch data were normally distributed. A histogram was used to visualize the CPUE and a Shapiro Wilk test was used to test normality of the data. Since the number of samples was suitably large ($n>20$), the Central Limit Theorem justified the use of parametric tests such as the analysis of variance (ANOVA). However, non-parametric tests were also used to confirm results. The Kruskal Wallis Rank Sum Test was used to verify the ANOVA. Significance was defined as $p<0.05$. Given significance, a post hoc test (pairwise Wilcoxon Test) was used to compare treatments. The third step was to determine role of the variables: treatment, distance to the speaker, and trap type as a factor of CPUE.

In addition to treatment, distance from the speaker was also important to analyze, as this would help to determine the minimum particle velocities that RSC could perceive acoustic signals with. Calculations were performed to see if a zoned analysis was necessary, due to any abrupt changes in particle velocity over the length of the pond. Sound intensity, J was calculated using an inverse square law relationship (Equation 1). P is the power of the speaker in Watts, while r is the radius from the speaker.

$$J = \frac{P}{4\pi r^2} \quad \text{Eq. 1}$$

Then, acoustic impedance, Z was calculated using Equation 2, where $\rho_{water,20℃}$ is the density of water at 20ºC and c is the speed of sound in fresh water at 20ºC. 1482.66 m/s was used for c (Greenspan & Tschiegg, 10AD) and 998 kg/m^3 was used for $\rho_{water,20℃}$ (Moore & Fierro; Nyer, 2008).

$$Z = c\rho_{water,20℃} \quad \text{Eq. 2}$$

Finally, particle velocity, $v_{particle}$ was calculated using Equation 3. Using MatLab® (Mathworks, Natick, MA), Particle velocity was plotted versus radius from the speaker in meters to determine if any abrupt drop-off would occur that would justify using distance intervals for analysis (Figure 3.5).

$$v_{particle} = \sqrt{\frac{J}{Z}} \quad \text{Eq. 3}$$

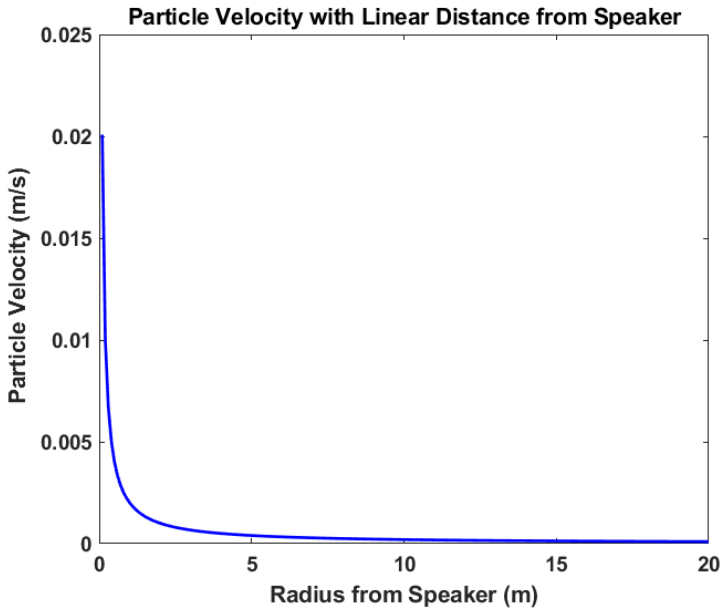


Figure 3.5: The particle velocity is asymptotic about zero as radius from the speaker increases.

The only notable change in particle velocity happens between 0 and 5 m radius from the speaker. There was not any logical reason to run a zoned analysis, because traps were spaced 5 m

apart, and a separate zone for the significant change would only contain one trap. Therefore, a single factor ANOVA and Kruskal Wallis Rank Sum Test was used to determine if distance from the speakers had a significant effect on CPUE on a subset of data using sound treatments. The coding language R was used to perform all statistical analysis (Appendix B).

Data Recording and Manipulation

Data were intended to be recorded following a biweekly schedule. During the first week, the *sound and food bait* treatment was used Monday through Tuesday, and sound was turned off for Wednesday and Thursday. In the second week, food bait was removed for Monday and Tuesday, and the *sound* treatment was used until Wednesday, where the *no treatment* condition was used until Friday. Weekend data was not used for analysis because they were not actively trapped until Monday morning. Due to numerous schedule changes, the actual trapping schedule was modified as challenges arose.

Equipment failure and unforeseen events compromised much of the collected data. Originally, an additional infested pond was used to provide a different type of physiography; however, the water level dropped so low during the dry season that the perimeter of the pond was reduced and the linear trap density became too variable for a reasonable comparison. Therefore, only one pond was used for the analysis. The intended trapping schedule was modified when flooding events washed traps into unsafe depths of the pond. July 8th and 9th saw 30 traps missing from analysis. Treatments changed schedule when technological failures occurred. When the MP3 player failed to loop the 10-minute sound file on the first week of testing, the treatment type was switched to *food* instead of *food and sound*. The trapping schedule can be found in Appendix B.

RESULTS

A single factor ANOVA showed the effect of treatment on daily catch rate was statistically significant (p =3.57e-4). Similarly, the Kruskal Wallis Rank Sum Test confirmed statistical significance (p=6.892e-6). Table 3.1 shows a data summary of the treatment type with associated CPUE. The pairwise test results can be viewed in Appendix B. The largest difference occurred between *no treatment* and *food and sound*.

Table 3.1: Summary of Treatment and CPUE for Gee's Minnow Traps.

Treatment	Total RSC	CPUE	n
Food	711	0.6745731	1054
Sound	56	0.6436782	87
Food & Sound	547	0.8200900	667
No Treatment	136	0.4874552	279

Distance from the speaker was not statistically significant as a factor of daily catch rate for *Sound, Sound & Food,* or a combination of both treatments as seen in table 3.2. The Kruskal Wallis Rank Sum Test results agree in all ANOVA tests.

Table 3.2: Statistical Results of Speaker Proximity as a Factor of Daily Catch.

Treatment	Test	p-value	χ^2	df	p<0.05?
Sound	ANOVA	0.5960	-	43	No
Sound	Kruskal Wallis	0.3579	45.769	43	No
Sound &Food	ANOVA	0.4760	-	96	No
Sound &Food	Kruskal Wallis	0.3505	100.73	96	No
Both	ANOVA	0.0971	-	96	No
Both	Kruskal Wallis	0.0710	117.06	96	No

Trap type was found to be a statistically significant factor of daily catch rate during conventional use (Food Treatment). The ANOVA and Kruskal Wallis Rank Sum Tests yielded p=4.22e-11 and p=8.94e-15, respectively. The CPUE for each trap was calculated as shown in Table 3.3. Note that under conventional use, only Gee's Minnow traps and pyramid traps are

baited. A pairwise comparison was completed using a Wilcoxon Rank Sum Test. Every trap comparison was significantly different from each other($p<0.05$), excluding the pyramid and Gee's Minnow Traps, whose p-value was 0.153.

Table 3.3: CPUE During Conventional Use.

Trap Type	CPUE	σ
Apartment	1.69	1.56
ART	1.10	1.49
Gee's Minnow	0.67	1.12
Juvenile	0.53	1.95
Pyramid	0.84	1.23

DISCUSSION

The treatment had a remarkable impact on capture rates. Consider that an empty Gee's Minnow Trap without sound still captures crayfish with a CPUE of 0.487. This implies that without bait incentive (food, sound or otherwise), RSC are still captured effectively. Food bait showed a 38% increase in CPUE, while sound alone added a 32% increase. The food and sound combination increased CPUE by 68% from the control. Therefore, the increase in CPUE from sound and food seem to be additive.

Both ART and Apartment trap types had high CPUE relative to the baited traps. The creator of the ART trap found similarly larger CPUE relative to baited traps. However, she was trapping signal crayfish, which also tend to be aggressive. Despite the aggression, multiple adults, namely females and smaller crayfish would be found sharing the same refuge tube (Green et al., 2018). This was not true for the RSC adults, who were not observed sharing tubes. Juvenile crayfish were found beginning in early August, and late into September, sharing tubes. This wave of juveniles may have skewed the mean CPUE. However, the refuge traps show the value of refuge as an attractant. Similarly, the *no treatment* of the Gee's Minnow Traps shows a similar attractant, since no food or sound bait was used.

CONCLUSION

Two conclusions were drawn from this analysis. First, the effectiveness of refuge as a means of attraction is important to RSC capture, shown by the refuge trap types and further reinforced by the control treatment for Gee's Minnow Traps. Second, both sound and food bait contributed to a seemingly additive increase in CPUE beyond the baseline for Gee's Minnow traps. These conclusions will inform the design process to determine an enhanced capture solution.

Further studies should measure particle velocity as an analog for sound intensity, to find out what the minimum energy requirements will be for a speaker system in the future. Additionally, more variety of ponds should be used to determine the effects of refuge and sound in the presence of abundant refuge, vegetation, and effects on non-target species.

CHAPTER 4: ENGINEERING DESIGN OF SOFTVALVE TRAP

DATA DRIVEN DESIGN

Based on the data from both laboratory and field tests, an engineering solution of RSC capture was designed. It is important to note that the Gee's Minnow Trap is not the industry standard for crayfish farmers in the southern United States. Crayfish farmers need more storage volume and an increased speed of harvest. They use a galvanized steel pyramid geometry trap with holes where the corners would be, and a smooth, PVC tube coming out of the top to keep crayfish from escaping (Figure 4.1). Traps can be emptied in one fluid motion, turning them upside-down and giving them a shake. These traps work well for uniform depth water levels, such as flooded rice fields. However, the water depths of infested Michigan sites vary greatly, and their ability to stack for transportation and seasonal storage is poor.

Figure 4.1: Rice farmer showing pyramid style trap (Boyd, n.d.).

The results of the pond trials show that Gee's Minnow traps have a high catch rate without any bait, perhaps because they supply shelter. Additionally, the PVC traps show how

effective refuge traps can be, but their design leaves a major limitation: trapping capacity. Therefore, the design process used principals from both the metal mesh style baited trap types, and the PVC refuge trap types.

The first step was to generate many potential solutions which can be found in Appendix C. After considering each, a novel trap design, which was termed the “SoftValve”, was prototyped. A concept model was made, to be tested in an aquarium. The SoftValve model was made from clear PVC hose, soft PVC bristles, and a waterproof epoxy as seen in Figure 4.2. The concept is that a crayfish could enter the non-bristled side, pass the bristles as they easily bend out of the way, but cannot re-enter the bristled ends, as the bristles support each other to resist the movement. The SoftValve was attached to an aquarium divider to test the efficacy. Food was placed on bristled side of the aquarium and crayfish were placed on the other side of the divider. While no formal experiment was conducted, the SoftValve was able to allow crayfish to enter the side with food without returning.

Figure 4.2: The top view shows two RSC that moved through the SoftValve to the bristle side of the aquarium (left). The profile view (middle) show the directionality of the Softvalve. A 3D rendering was created using Autodesk Inventor™ (right).

INITIAL DESIGN

The initial design was a box-shaped trap made from galvanized steel frame and surrounded by a galvanized steel wire mesh. The major diameter sides contained 11 SoftValves

for points of entry into the trap. This trap is already an improvement on the previously used artificial refuge traps, because there is now storage for the captured crayfish, so as soon as a tube is emptied, it can be filled again. It also utilized the added benefit of the food bait. Using this trap in conjunction with the speakers, will also lend the added benefit of the auditory stimuli. The basic shape and SoftValve placement can be seen in Figure 4.3. The design process is iterative, however, and after preliminary testing, more modifications will be tested.

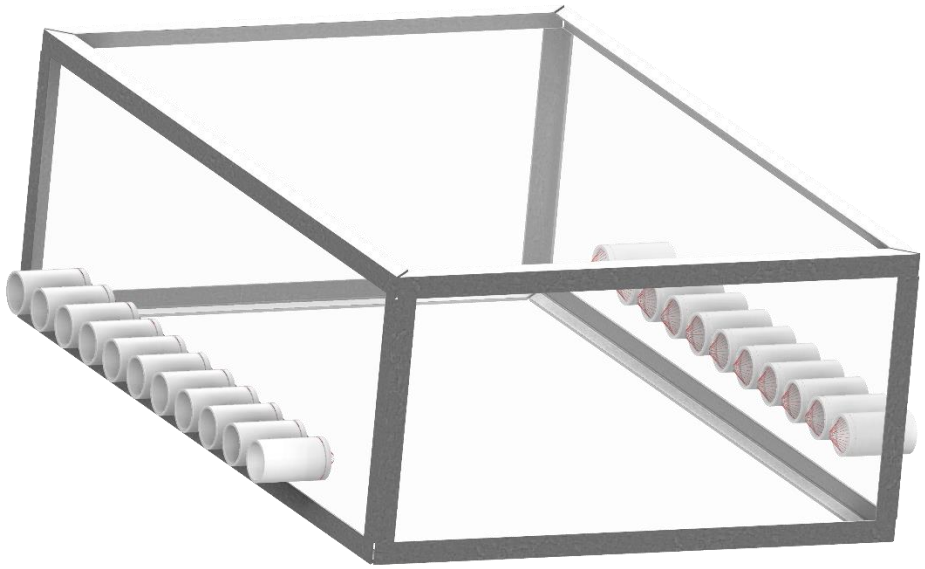


Figure 4.3: An AutoCAD rendering of the initial trap design. SoftValves are placed low enough on the frame for crayfish to have access. The galvanized steel mesh is shown as a clear surface to better view the SoftValves.

VISION FOR FINAL DESIGN

The full design incorporated the SoftValve, and can include other modular components, such as speakers, heat sources, space for food bait, a smooth-tube chimney for fish to escape, and drone trap retrieval systems. Figure 4.4 shows a rendering of what these components look like. Manual labor is time consuming and expensive, especially when the invasive species spreads

rapidly, as they have in Michigan. Drone retrieval will allow for rapid trap emptying and potentially lower costs as infestations continue across Michigan's numerous water bodies.

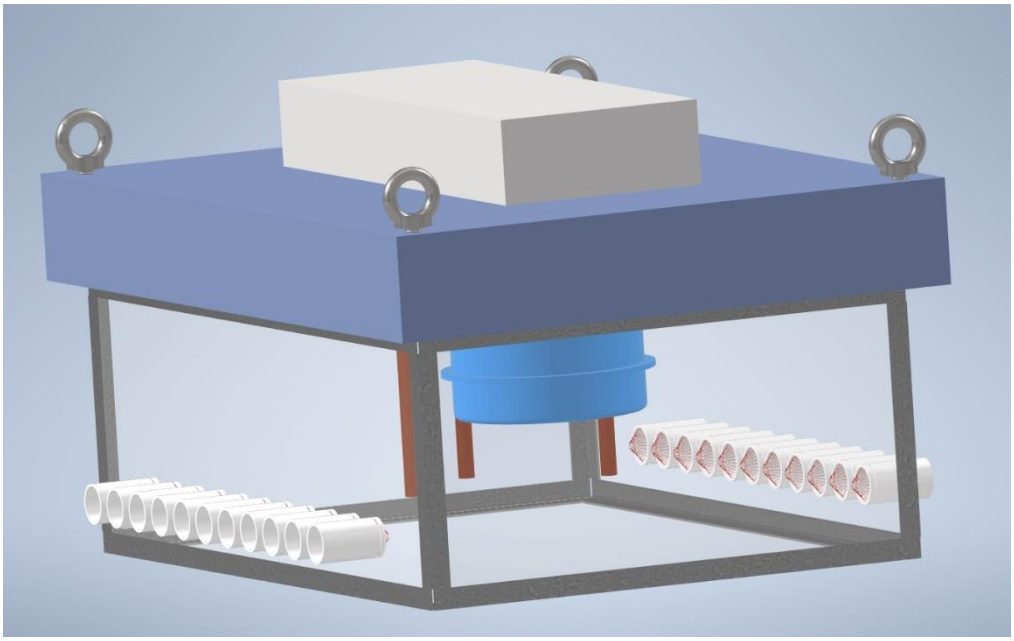


Figure 4.4: A floating mat is attached to top of the trap. Modular systems such as battery bank, heating elements, speakers and anchors for drone retrieval are attached.

CHAPTER 5: CONCLUSIONS

Objectives i) and ii) were completed by research on frequency testing and locomotion responses. High range white noise and 12 kHz pure tone were most able to affect a locomotion response in RSC. Additionally, sound was found to enhance CPUE with or without baited traps. A baseline CPUE was found to exist for Gee's Minnow Traps without food or sound, which helped to inform the engineering design of a solution.

This thesis has addressed a possible solution to controlling invasive populations of RSC. While the solutions to the RSC infestation will be varied, depending on the characteristics of the water body, this research shows that improvements can be made on population control using acoustic stimuli, food bait, and different trapping gears. Such improvements led to a preliminary engineering design of next-generation large trap, which will alleviate the expensive practice of intensive trapping. Thus, objective iv) was met.

Objective iii) was not able to be completed because of the unforeseen circumstances surrounding the pandemic which began to spread rapidly in the United States in March 2020. Most university research was halted during the spring, which was when cold water heat trials were to resume. Research on heat stimuli has therefore been moved to future work in Chapter 6.

CHAPTER 6: CURRENT AND FUTURE WORK

While this thesis helped lay the groundwork for a solution to the RSC infestations in Michigan, it cannot be discussed without the context of events in the 2020 year. The pandemic has hampered much of the research which was planned for the spring and summer trapping season. Much of that research has been moved to Chapter 8 and will be brought into another thesis or dissertation. More work is necessary to inform the engineering solutions to the RSC infestation; the design process is iterative and will continue to be updated with new information.

CURRENT WORK

Investigations are already underway to use manganese enhanced magnetic resonance imaging (ME-MRI) to determine how crayfish are affected by each sensory stimuli, including heat, sounds, and olfactory. Crayfish are injected with the contrast agent, then stimulated, before being anesthetized on ice prior to the imaging (Figure 6.1). The results should elucidate the cause of their behavior and help to further inform the engineering design process.

Figure 6.1: A canula is installed using a gel glue (left) and the crayfish is placed in a 50 mL tube before anesthetizing.

During the acoustic frequency response trials (Chapter 2), the HVAC system failed for one week in early February, causing a temporary halt to trials. Small 300-Watt aquarium heaters were placed each habitat to slow the heat loss. During this time, it was observed that most of the crayfish were in close proximity to the heaters. The anecdotal evidence suggests that heat may be

an effective attractant in colder temperature waters. The possibility of extending the crayfish capture season would also increase the total seasonal capture of RSC; this could also aid in achieving the goal of the research.

Heat stimuli trials began in the late fall of 2019 with promising preliminary observations. Aquarium heaters were placed inside Gee's Minnow Traps and powered by a propane generator (Figure 6.2). After the first 24-hour trial, ten RSC were captured in 3 heated traps. However, more time will be needed to collect sufficient data to draw any conclusions. If successful, it may be possible to extend the trapping season in Michigan by use of a thermal stimulus, and therefore increase yearly capture.

Figure 6.2: The propane generator (left) powers the aquarium heaters inside Gee's Minnow Traps (right).

FUTURE WORK

Additional frequency testing should be completed, upwards of 15 kHz to help find the optimum frequency for locomotion response. In addition, the sound intensity and pressure levels should be better tested to find the minimum amounts necessary to enhance trapping. Finding this quantity will help to reduce energy usage for crayfish capture using a sound system, and therefore lower costs of associated trapping methods.

APPENDICES

APPENDIX A: CHAPTER 2 SUPPLEMENTAL MATERIALS

ADDITIONAL TABLES AND FIGURES

Table A.1: Number of Trials.

Sound Treatment	Number of Trials
0	35
1000	8
10000	2
11000	7
12000	2
13000	2
14000	2
15000	2
2000	4
3000	6
4000	4
500	2
5000	4
6000	4
7000	4
8000	2
9000	6
PN1k15k	2
PN6k10k	2
WN10k15k	2
WN1k15k	4
WN1k5k	2
WN6k10k	2

ACOUSTIC FREQUENCY RESPONSE ANALYSIS IN R

The following is an R Markdown code output for the frequency response trials.

Lab Trial Analysis
Douglas Clements & Wei Liao

7/13/2020

Setup

```
library (MASS)
library(ggplot2)
library(grid)
library(gridExtra)
library(ggpubr)
library(readxl)
library(conover.test)
```

Add windows fonts to get Times New Roman

```
windowsFonts(A = windowsFont("Times New Roman"))
```

Add in a custom color for Kelley Green (MSU green)

```
color<-rgb(24,69,59, maxColorValue = 255)
```

Set the working directory and add in the two data files. One is used for frequency analysis, and another used for a grouped frequency analysis.

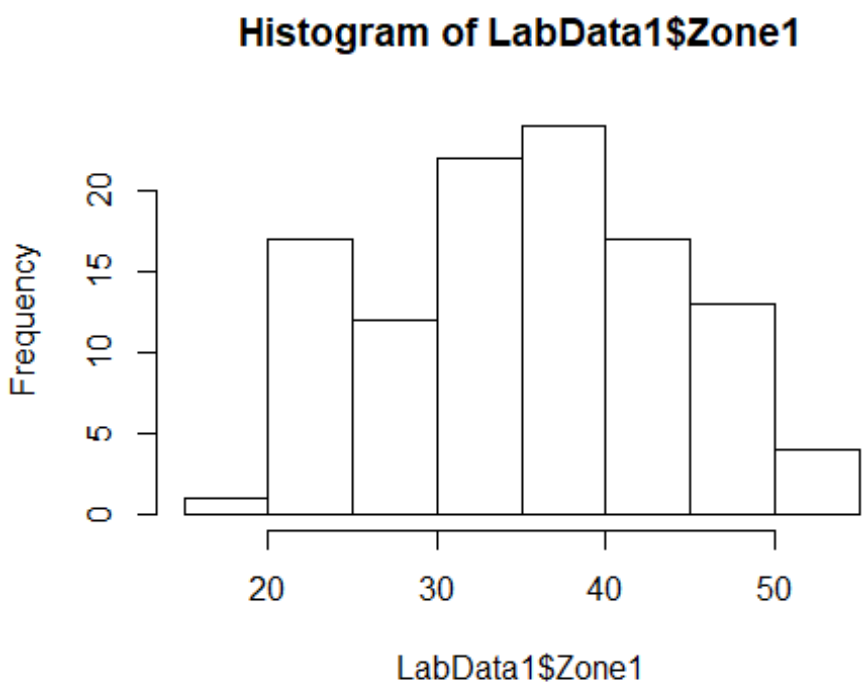
```
setwd("G:/School/Thesis Work/Sound Pond Trials/Data Files")
LabData1<-as.data.frame(read_xlsx("FrequencyRangeAnalysis.xlsx",sheet = "Sheet1"))
head(LabData1)
```

```
##   row.names Frequency Frequency_range Hours    Zone3    Zone2    Zone1 Sound
## 1         1         0         Control    24 27.77778 38.88889 33.33333 FALSE
## 2         2         0         Control    24 38.88889 27.77778 33.33333 FALSE
## 3         3         0         Control    24 27.77778 38.88889 33.33333 FALSE
## 4         4         0         Control    24 33.33333 33.33333 33.33333 FALSE
## 5         5         0         Control    24 27.77778 33.33333 38.88889 FALSE
## 6         6         0         Control    24 41.17647 29.41176 29.41176 FALSE
```

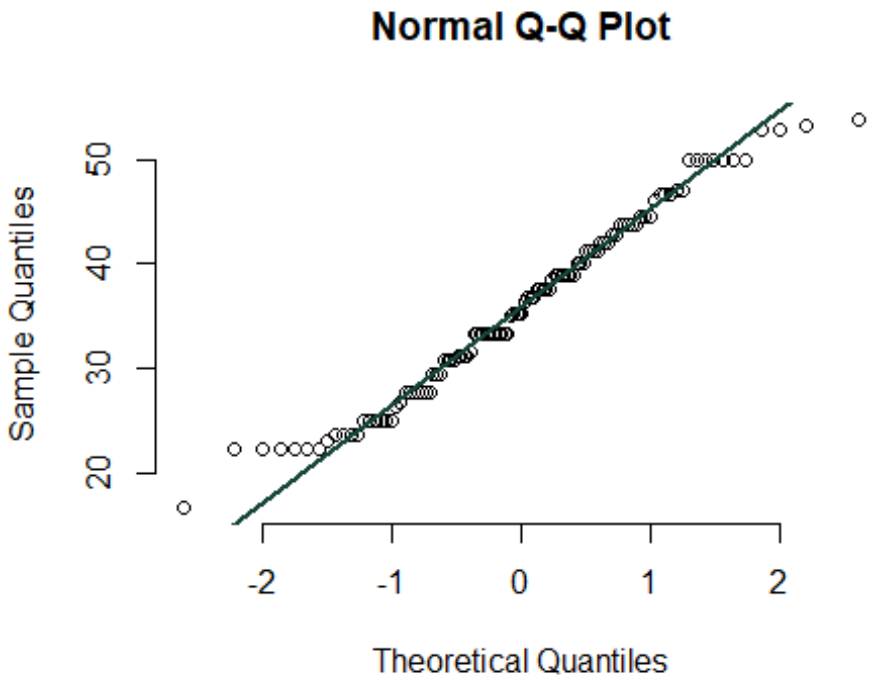
Data Visualization

Check the distribution of population distribution data for normal distribution.

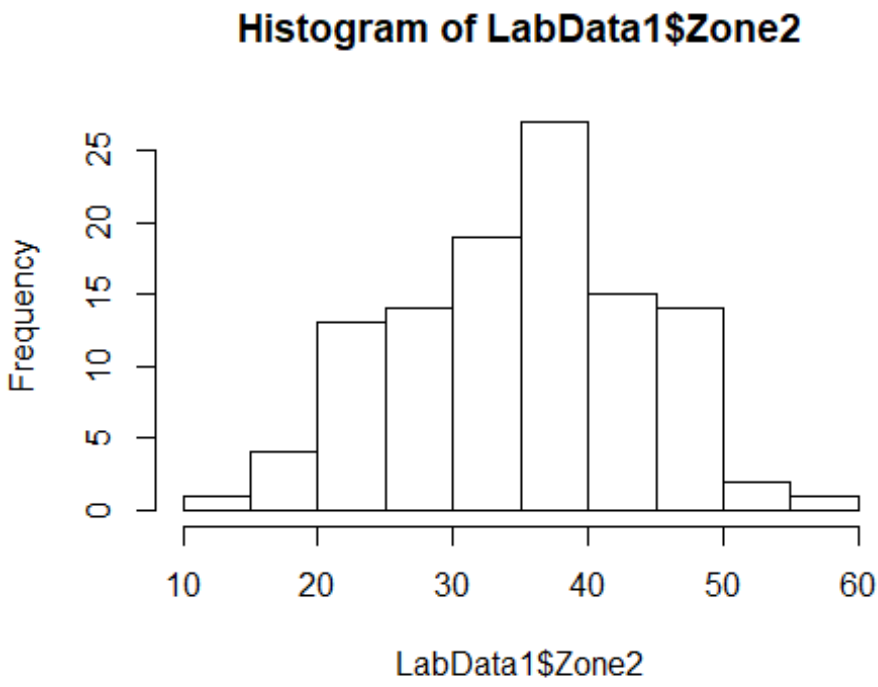
```
# Check distribution using a histogram, Shapiro test, and Q-Q plot

hist(LabData1$Zone1)# Looks Normal
```




```
shapiro.test(LabData1$Zone1)# Confirmed Normal

##
##  Shapiro-Wilk normality test
##
## data:  LabData1$Zone1
## W = 0.97639, p-value = 0.04777

qqnorm(LabData1$Zone1,pch=1,frame=FALSE)
qqline(LabData1$Zone1,col=color,lwd=2)
```



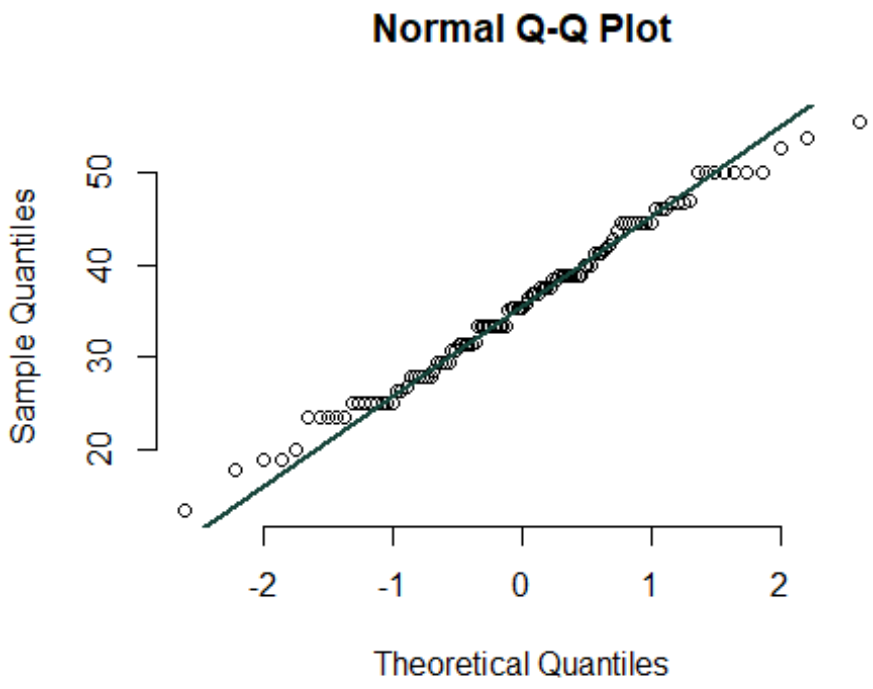

```
hist(LabData1$Zone2)# Looks Normal
```



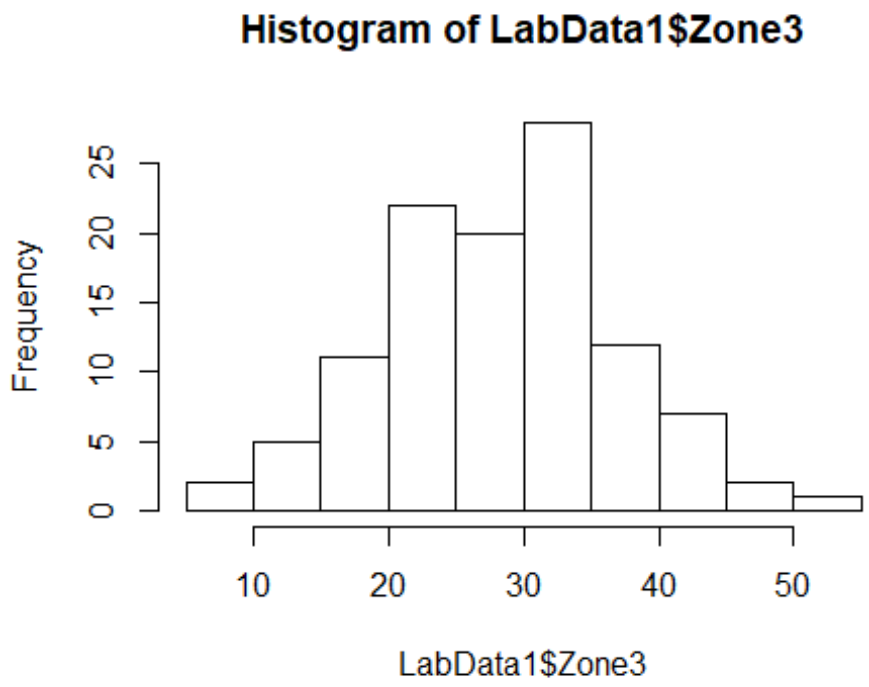

```
shapiro.test(LabData1$Zone2)# Confirmed Normal
```

```
##
##  Shapiro-Wilk normality test
##
## data:  LabData1$Zone2
## W = 0.98725, p-value = 0.3853
```

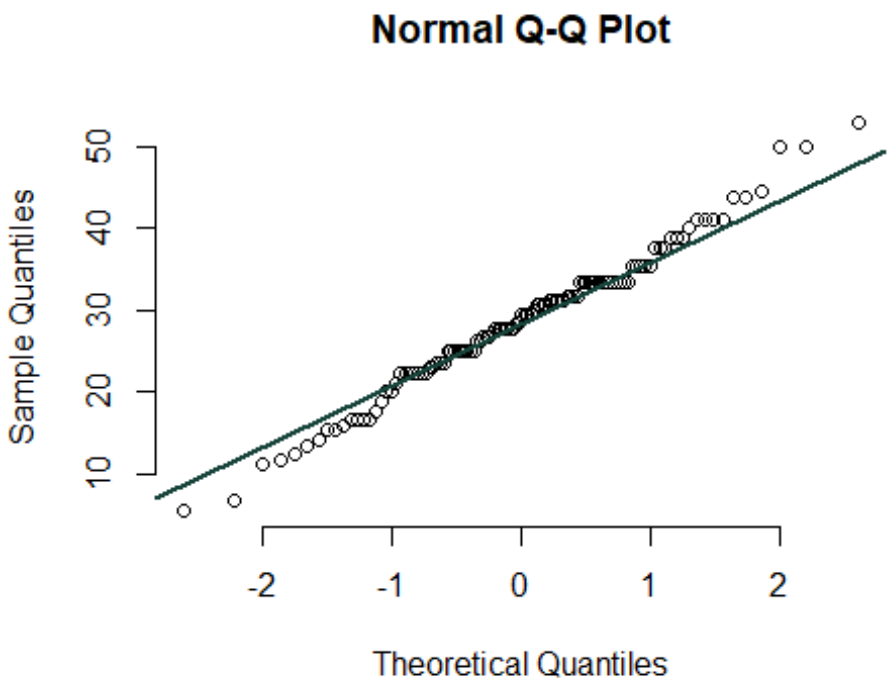
```
qqnorm(LabData1$Zone2,pch=1,frame=FALSE)
qqline(LabData1$Zone2,col=color,lwd=2)
```




```
hist(LabData1$Zone3)# Looks Normal
```




```
shapiro.test(LabData1$Zone3)# Confirmed Normal

##
##  Shapiro-Wilk normality test
##
## data:  LabData1$Zone3
## W = 0.9881, p-value = 0.4451

qqnorm(LabData1$Zone3,pch=1,frame=FALSE)
qqline(LabData1$Zone3,col=color,lwd=2)
```



Analysis

Kruskal-Wallis Rank Sum Test

Zone 1

```
kruskal.test(Zone1 ~ as.factor(Frequency), data = LabData1)

##
##  Kruskal-Wallis rank sum test
##
## data:  Zone1 by as.factor(Frequency)
## Kruskal-Wallis chi-squared = 40.767, df = 22, p-value = 0.008784
```

Zone 2

```
kruskal.test(Zone2 ~ as.factor(Frequency), data = LabData1)

##
##  Kruskal-Wallis rank sum test
##
## data:  Zone2 by as.factor(Frequency)
## Kruskal-Wallis chi-squared = 38.206, df = 22, p-value = 0.01737
```

Zone 3

```
kruskal.test(Zone3 ~ as.factor(Frequency), data = LabData1)

##
##  Kruskal-Wallis rank sum test
##
## data:  Zone3 by as.factor(Frequency)
## Kruskal-Wallis chi-squared = 28.633, df = 22, p-value = 0.1557
```

Zone 1 and 2 have p values < 0.05. Zone 3 does not.

Conover Tests

```
#ZONE1

CoZ1<-conover.test(LabData1$Zone1,as.factor(LabData1$Frequency),method="bh",kw=FALSE)

##
##                          Comparison of x by group
##                            (Benjamini-Hochberg)
## Col Mean-|
## Row Mean |        0      1000     10000     11000     12000     13000
## ---------+------------------------------------------------------------
##     1000 |  -1.620464
##          |    0.1764
##          |
##    10000 |   1.539085   2.218642
##          |    0.1853     0.1023
##          |
##    11000 |  -2.313149  -0.623523  -2.590093
##          |    0.0912     0.3955     0.0508
##          |
##    12000 |  -2.713330  -1.691995  -3.091630  -1.265847
##          |    0.0508     0.1779     0.0338     0.2425
##          |
##    13000 |   0.357182   1.131731  -0.859278   1.518385   2.232352
##          |    0.4433     0.2558     0.3473     0.1884     0.1018
##          |
##    14000 |   1.965545   2.610827   0.310048   2.976792   3.401679   1.169327
##          |    0.1356     0.0498     0.4519     0.0341    0.0256*     0.2504
##          |
##    15000 |  -0.739429   0.123257  -1.656547   0.524016   1.435083  -0.797268
##          |    0.3743     0.4836     0.1778     0.4181     0.2084     0.3654
##          |
##     2000 |  -2.613019  -1.215138  -2.884567  -0.672343   0.685340  -1.892358
##          |    0.0514     0.2482     0.0417     0.3834     0.3795     0.1474
##          |
##     3000 |  -1.991857  -0.453811  -2.448359   0.139512   1.338099  -1.395962
```

```
##         |    0.1333    0.4290    0.0690    0.4808    0.2309    0.2168
##         |
##    4000 | -0.917841  0.245920 -1.851442  0.755124  1.718465 -0.859232
##         |    0.3218    0.4658    0.1525    0.3739    0.1711    0.3449
##         |
##     500 | -0.520107  0.324952 -1.497093  0.722890  1.594537 -0.637814
##         |    0.4155    0.4472    0.1918    0.3801    0.1723    0.3909
##         |
##    5000 | -0.288444  0.788393 -1.467856  1.285124  2.102051 -0.475646
##         |    0.4595    0.3673    0.2004    0.2390    0.1131    0.4276
##         |
##    6000 |  2.111659  2.857022 -0.005114  3.306192  3.564793  0.987094
##         |    0.1132    0.0423    0.4980    0.0217*   0.0752*    0.2991
##         |
##    7000 | -1.689903 -0.419512 -2.321974  0.104990  1.247933 -1.329765
##         |    0.1760    0.4340    0.0921    0.4852    0.2390    0.2320
##         |
##    8000 | -0.410446  0.425800 -1.417366  0.822327  1.674264 -0.558088
##         |    0.4360    0.4355    0.2130    0.3580    0.1740    0.4086
##         |
##    9000 | -1.350308  0.071078 -2.101177  0.649037  1.685281 -1.048780
##         |    0.2282    0.4912    0.1107    0.3924    0.1751    0.2827
##         |
##  PN1k15k | -0.020539  0.784368 -1.133893  1.175880  1.957737 -0.274614
##         |    0.4957    0.3668    0.2569    0.2518    0.1353    0.4614
##         |
##  PN6k10k | -0.252046  0.571468 -1.302205  0.965958  1.789425 -0.442927
##         |    0.4651    0.4045    0.2387    0.3021    0.1624    0.4297
##         |
## WN10k15k | -2.615854 -1.602353 -3.020762 -1.177458  0.070868 -2.161484
##         |    0.0531    0.1739    0.0322    0.2532    0.4892    0.1031
##         |
##  WN1k15k | -1.614375 -0.354415 -2.275944  0.168590  1.293963 -1.283734
##         |    0.1762    0.4424    0.0970    0.4808    0.2376    0.2374
##         |
##   WN1k5k | -1.056228 -0.168079 -1.886869  0.236754  1.204761 -1.027590
##         |    0.2815    0.4768    0.1464    0.4656    0.2441    0.2877
##         |
##  WN6k10k | -2.664592 -1.647174 -3.056196 -1.221653  0.035434 -2.196918
##         |    0.0528    0.1787    0.0314    0.2477    0.4917    0.1021
## Col Mean-|
## Row Mean |     14000      15000       2000       3000       4000        500
## ---------+-------------------------------------------------------------------
##    15000 | -1.966596
##         |    0.1381
##         |
```

```
##    2000 |  -3.242581  -0.971751
##         |    0.0236*     0.3038
##         |
##    3000 |  -2.828090  -0.419512   0.773095
##         |    0.0408     0.4362     0.3699
##         |
##    4000 |  -2.209455   0.061373   1.265314   0.612987
##         |    0.1018     0.4911     0.2405     0.3959
##         |
##     500 |  -1.807142   0.159453   1.155872   0.614802   0.122747
##         |    0.1618     0.4784     0.2519     0.3974     0.4818
##         |
##    5000 |  -1.825869   0.444959   1.735109   1.127621   0.469795   0.260838
##         |    0.1582     0.4309     0.1760     0.2555     0.4281     0.4633
##         |
##    6000 |  -0.363128   1.907701   3.526595   3.090095   2.261280   1.723580
##         |    0.4427     0.1453    0.0427*     0.0309     0.0976     0.1746
##         |
##    7000 |  -2.679988  -0.409158   0.689032  -0.018298  -0.576281  -0.593279
##         |    0.0530     0.4344     0.3847     0.4947     0.4045     0.4008
##         |
##    8000 |  -1.727415   0.239180   1.247933   0.712447   0.214808   0.079726
##         |    0.1760     0.4666     0.2411     0.3828     0.4711     0.4916
##         |
##    9000 |  -2.480908  -0.072329   1.212250   0.490990  -0.173832  -0.267619
##         |    0.0656     0.4927     0.2473     0.4226     0.4827     0.4624
##         |
##  PN1k15k |  -1.443942   0.522653   1.575260   1.059629   0.542135   0.363200
##         |    0.2072     0.4165     0.1768     0.2822     0.4117     0.4470
##         |
##  PN6k10k |  -1.612254   0.354341   1.380909   0.853490   0.347784   0.194887
##         |    0.1748     0.4403     0.2205     0.3452     0.4411     0.4756
##         |
## WN10k15k |  -3.330811  -1.364215  -0.603508  -1.251303  -1.636633  -1.523669
##         |    0.0230*     0.2249     0.3982     0.2419     0.1753     0.1886
##         |
##  WN1k15k |  -2.633957  -0.363128   0.745408   0.043458  -0.519906  -0.547249
##         |    0.0526     0.4449     0.3738     0.4944     0.4133     0.4116
##         |
##   WN1k5k |  -2.196918  -0.230322   0.705798   0.137426  -0.327326  -0.389775
##         |    0.0995     0.4663     0.3789     0.4796     0.4483     0.4391
##         |
##  WN6k10k |  -3.366245  -1.399649  -0.644424  -1.294701  -1.677549  -1.559103
##         |    0.0240*     0.2177     0.3923     0.2396     0.1753     0.1803
## Col Mean-|
## Row Mean |       5000       6000       7000       8000       9000    PN1k15k
```

```
## ---------+-------------------------------------------------------------
##     6000 |   1.791485
##          |     0.1644
##          |
##     7000 |  -1.046077  -2.837562
##          |     0.2817     0.0421
##          |
##     8000 |  -0.168777  -1.631519   0.685340
##          |     0.4828     0.1748     0.3818
##          |
##     9000 |  -0.688466  -2.650940   0.457453  -0.365264
##          |     0.3826     0.0524     0.4295     0.4483
##          |
##  PN1k15k |   0.158548  -1.304192   1.012667   0.283473   0.712447
##          |     0.4768     0.2402     0.2921     0.4596     0.3804
##          |
##  PN6k10k |  -0.035801  -1.498542   0.818316   0.115161   0.506307  -0.168312
##          |     0.4956     0.1934     0.3575     0.4829     0.4175     0.4788
##          |
## WN10k15k |  -2.020220  -3.482961  -1.166101  -1.603395  -1.598486  -1.886869
##          |     0.1277    0.0246*     0.2497     0.1757     0.1731     0.1438
##          |
##  WN1k15k |  -0.989701  -2.781186   0.056375  -0.639310  -0.395696  -0.966636
##          |     0.3002     0.0442     0.4912     0.3925     0.4385     0.3040
##          |
##   WN1k5k |  -0.710912  -2.173654   0.143205  -0.469502  -0.209756  -0.752976
##          |     0.3787     0.1026     0.4813     0.4260     0.4712     0.3725
##          |
##  WN6k10k |  -2.061135  -3.523877  -1.207017  -1.638830  -1.641883  -1.922303
##          |     0.1188    0.0287*     0.2452     0.1769     0.1782     0.1435
## Col Mean-|
## Row Mean |    PN6k10k   WN10k15k    WN1k15k     WN1k5k
## ---------+----------------------------------------
## WN10k15k |  -1.718557
##          |     0.1737
##          |
##  WN1k15k |  -0.772286   1.212132
##          |     0.3679     0.2452
##          |
##   WN1k5k |  -0.584663   1.133893   0.097175
##          |     0.4027     0.2589     0.4864
##          |
##  WN6k10k |  -1.753991  -0.035434  -1.253047  -1.169327
##          |     0.1720     0.4937     0.2434     0.2525
##
```

```
## alpha = 0.05
## Reject Ho if p <= alpha/2

#ZONE2

CoZ2<-conover.test(LabData1$Zone2,as.factor(LabData1$Frequency),method="bh",kw=FALSE)

##
##                          Comparison of x by group
##                            (Benjamini-Hochberg)
## Col Mean-|
## Row Mean |         0      1000     10000     11000     12000     13000
## ---------+------------------------------------------------------------
##     1000 |  -1.155327
##          |    0.2319
##          |
##    10000 |  -1.824661  -1.105317
##          |    0.1330    0.2424
##          |
##    11000 |  -0.792000   0.241200   1.245551
##          |    0.3222    0.4615    0.2206
##          |
##    12000 |   1.440252   1.897186   2.373687   1.714957
##          |    0.1921    0.1247    0.0835    0.1458
##          |
##    13000 |  -1.884458  -1.160308  -0.043474  -1.299773  -2.417161
##          |    0.1262    0.2317    0.4944    0.2095    0.0863
##          |
##    14000 |  -2.027971  -1.292286  -0.147812  -1.429905  -2.521499  -0.104337
##          |    0.1069    0.2105    0.4752    0.1920    0.0899    0.4834
##          |
##    15000 |  -0.222102   0.368439   1.165106   0.207591  -1.208580   1.208580
##          |    0.4637    0.4318    0.2351    0.4680    0.2256    0.2274
##          |
##     2000 |   1.992147   2.456356   2.745918   2.200716   0.005019   2.796118
##          |    0.1098    0.0921    0.0773    0.0836    0.4980    0.0732
##          |
##     3000 |   1.149751   1.779015   2.246927   1.502556  -0.660234   2.300172
##          |    0.2323    0.1403    0.0799    0.1800    0.3610    0.0815
##          |
##     4000 |   0.072955   0.802220   1.576267   0.584611  -1.164630   1.626467
##          |    0.4864    0.3216    0.1667    0.3711    0.2318    0.1618
##          |
##      500 |   0.603095   1.127313   1.765049   0.955852  -0.608637   1.808523
##          |    0.3727    0.2374    0.1424    0.2789    0.3742    0.1356
##          |
```

```
##    5000 |  0.262403   0.965504   1.691726   0.744141  -1.049171   1.741926
##         |    0.4563     0.2768     0.1454     0.3336     0.2522     0.1435
##         |
##    6000 | -2.216897  -1.171383   0.180718  -1.343616  -2.560179   0.230918
##         |    0.0822     0.2344     0.4714     0.2062     0.1028     0.4640
##         |
##    7000 | -1.796816  -0.809319   0.436736  -0.989876  -2.304161   0.486936
##         |    0.1370     0.3224     0.4074     0.2705     0.0829     0.3989
##         |
##    8000 | -2.016011  -1.281288  -0.139117  -1.419061  -2.512804  -0.095643
##         |    0.1078     0.2110     0.4729     0.1921     0.0874     0.4850
##         |
##    9000 |  0.952972   1.618018   2.140437   1.346273  -0.766724   2.193682
##         |    0.2783     0.1607     0.0907     0.2071     0.3294     0.0832
##         |
##  PN1k15k |  2.133897   2.535081   2.877987   2.343929   0.504299   2.921461
##         |    0.0902     0.1030     0.0708     0.0844     0.3951     0.1123
##         |
##  PN6k10k | -0.030752   0.544410   1.304224   0.381101  -1.069463   1.347698
##         |    0.4976     0.3812     0.2115     0.4303     0.2511     0.2084
##         |
## WN10k15k |  1.452211   1.908184   2.382382   1.725801   0.008694   2.425856
##         |    0.1937     0.1279     0.0876     0.1445     0.5025     0.0914
##         |
##  WN1k15k | -0.182388   0.582142   1.420648   0.369593  -1.320249   1.470848
##         |    0.4727     0.3703     0.1934     0.4334     0.2092     0.1890
##         |
##   WN1k5k |  1.236942   1.710217   2.225875   1.530603  -0.147812   2.269349
##         |    0.2203     0.1436     0.0822     0.1743     0.4773     0.0856
##         |
##  WN6k10k |  1.081470   1.567241   2.112842   1.389626  -0.260844   2.156317
##         |    0.2499     0.1659     0.0895     0.1970     0.4550     0.0891
## Col Mean-|
## Row Mean |     14000      15000       2000       3000       4000        500
## ---------+-------------------------------------------------------------------
##    15000 |  1.312918
##         |    0.2101
##         |
##    2000 |  2.916597   1.400569
##         |    0.0949     0.1968
##         |
##    3000 |  2.427959   0.819968  -0.841872
##         |    0.0948     0.3197     0.3121
##         |
##    4000 |  1.746946   0.230918  -1.432523  -0.727378
##         |    0.1439     0.4619     0.1929     0.3390
```

```
##         |
##     500 |  1.912861   0.599943  -0.707814  -0.085191   0.461836
##         |    0.1288     0.3721     0.3437     0.4853     0.4062
##         |
##    5000 |  1.862405   0.346377  -1.291115  -0.572473   0.141407  -0.346377
##         |    0.1303     0.4335     0.2092     0.3707     0.4759     0.4355
##         |
##    6000 |  0.351397  -1.164630  -3.141715  -2.599703  -1.709191  -1.857385
##         |    0.4374     0.2335     0.1452     0.1067     0.1421     0.1297
##         |
##    7000 |  0.607415  -0.908612  -2.828158  -2.256219  -1.395634  -1.601367
##         |    0.3728     0.2894     0.0735     0.0820     0.1967     0.1642
##         |
##    8000 |  0.008694  -1.304224  -2.906557  -2.417310  -1.736906  -1.904167
##         |    0.5005     0.2097     0.0838     0.0897     0.1430     0.1269
##         |
##    9000 |  2.321469   0.713479  -0.976572  -0.150598   0.592678  -0.021297
##         |    0.0866     0.3432     0.2741     0.4781     0.3714     0.4994
##         |
##  PN1k15k |  3.025799   1.712880   0.577295   1.277873   1.746946   1.112937
##         |    0.1375     0.1446     0.3705     0.2105     0.1459     0.2412
##         |
##  PN6k10k |  1.452036   0.139117  -1.239930  -0.649585  -0.070279  -0.460825
##         |    0.1918     0.4749     0.2210     0.3618     0.4855     0.4026
##         |
## WN10k15k |  2.530194   1.217275   0.005019   0.670883   1.174670   0.617332
##         |    0.0982     0.2259     0.5000     0.3582     0.2350     0.3723
##         |
##  WN1k15k |  1.591327   0.075299  -1.623117  -0.936162  -0.190593  -0.617455
##         |    0.1655     0.4874     0.1610     0.2816     0.4733     0.3743
##         |
##   WN1k5k |  2.373687   1.060768  -0.175698   0.479202   0.993952   0.460825
##         |    0.0864     0.2510     0.4715     0.4004     0.2706     0.4046
##         |
##  WN6k10k |  2.260654   0.947736  -0.306217   0.340766   0.863433   0.347793
##         |    0.0852     0.2787     0.4411     0.4319     0.3048     0.4370
## Col Mean-|
## Row Mean |      5000       6000       7000       8000       9000    PN1k15k
## ---------+-------------------------------------------------------------------
##    6000 |  -1.850599
##         |    0.1277
##         |
##    7000 |  -1.537042   0.313556
##         |    0.1740     0.4399
##         |
##    8000 |  -1.852365  -0.341357  -0.597375
```

```
##         |    0.1291     0.4337     0.3713
##         |
##    9000 |  0.437773   2.465004   2.121519   2.310820
##         |    0.4089     0.0944     0.0893     0.0839
##         |
##  PN1k15k |  1.631487   3.142495   2.886477   3.017104   1.384362
##         |    0.1622     0.2898     0.0777     0.1058     0.1970
##         |
##  PN6k10k | -0.185738   1.325269   1.069251   1.443341  -0.543096  -1.573763
##         |    0.4733     0.2092     0.2495     0.1929     0.3798     0.1657
##         |
## WN10k15k |  1.059211   2.570219   2.314201   2.521499   0.777372  -0.495605
##         |    0.2500     0.1072     0.0856     0.0949     0.3267     0.3970
##         |
##  WN1k15k | -0.332001   1.518598   1.205041   1.581287  -0.801463  -1.902565
##         |    0.4338     0.1764     0.2252     0.1669     0.3201     0.1253
##         |
##   WN1k5k |  0.878493   2.389501   2.133483   2.364992   0.585691  -0.652112
##         |    0.3002     0.0892     0.0886     0.0827     0.3726     0.3627
##         |
##  WN6k10k |  0.747974   2.258982   2.002964   2.251960   0.447255  -0.765144
##         |    0.3338     0.0834     0.1091     0.0808     0.4067     0.3282
## Col Mean-|
## Row Mean |    PN6k10k   WN10k15k    WN1k15k     WN1k5k
## ---------+----------------------------------------
## WN10k15k |   1.078158
##         |    0.2494
##         |
##  WN1k15k |  -0.085339  -1.330289
##         |    0.4873     0.2092
##         |
##   WN1k5k |  0.921651  -0.156506   1.149570
##         |    0.2858     0.4776     0.2307
##         |
##  WN6k10k |  0.808618  -0.269539   1.019052  -0.113032
##         |    0.3208     0.4553     0.2623     0.4818
##
## alpha = 0.05
## Reject Ho if p <= alpha/2
```

```
#ZONE3
CoZ3<-conover.test(LabData1$Zone3,as.factor(LabData1$Frequency),method="bh",kw=FAL
SE)
```

```
##
##                          Comparison of x by group
##                            (Benjamini-Hochberg)
```

```
## Col Mean-|
## Row Mean |        0      1000     10000     11000     12000     13000
## ---------+-------------------------------------------------------------
##     1000 |  2.003124
##          |    0.2908
##          |
##    10000 |  0.167726 -0.838694
##          |    0.4919    0.4154
##          |
##    11000 |  2.386555  0.392503  1.080323
##          |    0.4043    0.4783    0.3808
##          |
##    12000 |  0.549360 -0.487732  0.277459 -0.734271
##          |    0.4562    0.4665    0.4850    0.4454
##          |
##    13000 |  1.447323  0.338058  0.930304  0.079970  0.652845
##          |    0.3909    0.4875    0.3836    0.4875    0.4529
##          |
##    14000 | -0.056764 -1.045142 -0.163211 -1.283883 -0.440670 -1.093515
##          |    0.4930    0.3858    0.4874    0.3610    0.4775    0.3770
##          |
##    15000 |  0.672830 -0.374186  0.367225 -0.622312  0.089766 -0.563079
##          |    0.4480    0.4747    0.4756    0.4607    0.4895    0.4545
##          |
##     2000 |  0.393385 -0.942825  0.098941 -1.245246 -0.221440 -0.975281
##          |    0.4804    0.3799    0.4939    0.3555    0.4878    0.3854
##          |
##     3000 |  0.608414 -0.955734  0.179903 -1.292886 -0.159913 -0.959482
##          |    0.4592    0.3760    0.4931    0.3711    0.4845    0.3773
##          |
##     4000 |  0.772192 -0.616334  0.329805 -0.926262  0.009423 -0.744417
##          |    0.4369    0.4609    0.4816    0.3794    0.5042    0.4463
##          |
##      500 | -0.404724 -1.365136 -0.416188 -1.599402 -0.693648 -1.346493
##          |    0.4826    0.3768    0.4794    0.3774    0.4489    0.3706
##          |
##     5000 | -0.147767 -1.409240 -0.230863 -1.700938 -0.551246 -1.305086
##          |    0.4856    0.3734    0.4881    0.3547    0.4580    0.3860
##          |
##     6000 | -0.008614 -1.289304 -0.146056 -1.583760 -0.466438 -1.220279
##          |    0.5025    0.3627    0.4842    0.3606    0.4668    0.3481
##          |
##     7000 |  2.967727  1.275978  1.667872  0.922542  1.347490  0.593649
##          |    0.2450    0.3559    0.3681    0.3782    0.3760    0.4613
##          |
##     8000 |  2.423858  1.236106  1.640273  0.965457  1.362814  0.709969
```

```
##         |    0.4409    0.3475    0.3674    0.3875    0.3720    0.4494
##         |
##    9000 |  0.602257 -0.960771  0.176571 -1.297775 -0.163245 -0.962814
##         |    0.4596    0.3799    0.4924    0.3849    0.4895    0.3856
##         |
##  PN1k15k | -1.414933 -2.294151 -1.150639 -2.515423 -1.428099 -2.080944
##         |    0.3835    0.3825    0.3637    0.4341    0.3890    0.3405
##         |
##  PN6k10k | -0.000641 -0.993530 -0.122408 -1.232993 -0.399867 -1.052713
##         |    0.4997    0.3931    0.4881    0.3450    0.4798    0.3933
##         |
## WN10k15k |  1.537120  0.420637  0.995589  0.161394  0.718129  0.065284
##         |    0.3762    0.4825    0.3957    0.4860    0.4514    0.4915
##         |
##  WN1k15k |  1.723075  0.203223  0.909320 -0.125547  0.588938 -0.164902
##         |    0.3608    0.4871    0.3823    0.4909    0.4579    0.4910
##         |
##   WN1k5k | -0.292479 -1.261912 -0.334583 -1.497622 -0.612042 -1.264887
##         |    0.4850    0.3501    0.4867    0.3710    0.4602    0.3530
##         |
##  WN6k10k |  1.705488  0.575473  1.117997  0.314064  0.840538  0.187693
##         |    0.3624    0.4535    0.3790    0.4819    0.4178    0.4919
## Col Mean-|
## Row Mean |     14000     15000      2000      3000      4000       500
## ---------+-------------------------------------------------------------
##    15000 |  0.530436
##         |    0.4578
##         |
##     2000 |  0.287401 -0.325093
##         |    0.4850    0.4790
##         |
##     3000 |  0.379795 -0.269854  0.094817
##         |    0.4795    0.4838    0.4936
##         |
##     4000 |  0.518265 -0.094230  0.282749  0.214919
##         |    0.4560    0.4917    0.4848    0.4863
##         |
##      500 | -0.252977 -0.783414 -0.579515 -0.689628 -0.810378
##         |    0.4894    0.4338    0.4542    0.4480    0.4216
##         |
##     5000 | -0.042403 -0.654899 -0.403927 -0.537297 -0.686676  0.249709
##         |    0.4949    0.4549    0.4803    0.4598    0.4465    0.4886
##         |
##     6000 |  0.042403 -0.570092 -0.300060 -0.423516 -0.582809  0.334516
##         |    0.4969    0.4536    0.4862    0.4837    0.4553    0.4842
##         |
```

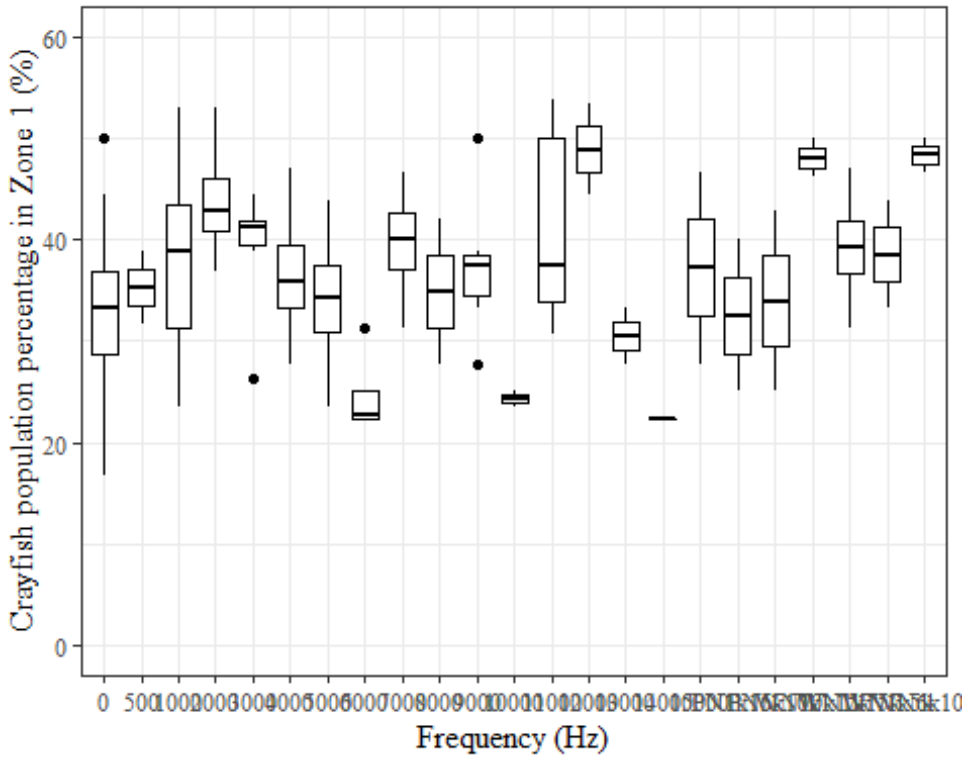
```
##    7000 |  1.856332   1.243837   1.921540   2.010124   1.638791   2.148446
##         |    0.3520     0.3518     0.3187     0.3005     0.3585     0.3632
##         |
##    8000 |  1.803485   1.273048   1.795083   1.829013   1.564219   2.056462
##         |    0.3378     0.3528     0.3320     0.3446     0.3656     0.3379
##         |
##    9000 |  0.376463  -0.273186   0.090603  -0.004711  -0.219133   0.686296
##         |    0.4760     0.4846     0.4912     0.5021     0.4866     0.4435
##         |
##  PN1k15k |  -0.987428  -1.517865  -1.427586  -1.589143  -1.658449  -0.734450
##         |    0.3856     0.3649     0.3819     0.3751     0.3644     0.4487
##         |
##  PN6k10k |   0.040802  -0.489633  -0.240286  -0.329822  -0.471150   0.293780
##         |    0.4935     0.4683     0.4860     0.4840     0.4670     0.4868
##         |
## WN10k15k |   1.158800   0.628363   1.050665   1.039439   0.819801   1.411777
##         |    0.3631     0.4604     0.3864     0.3814     0.4229     0.3785
##         |
##  WN1k15k |   1.097780   0.485285   0.992507   0.992420   0.709758   1.389893
##         |    0.3827     0.4624     0.3900     0.3864     0.4462     0.3731
##         |
##   WN1k5k |  -0.171371  -0.701808  -0.485285  -0.589682  -0.716148   0.081605
##         |    0.4925     0.4475     0.4651     0.4605     0.4492     0.4888
##         |
##  WN6k10k |   1.281208   0.750772   1.192010   1.189358   0.961147   1.534186
##         |    0.3576     0.4460     0.3562     0.3535     0.3830     0.3698
## Col Mean-|
## Row Mean |      5000       6000       7000       8000       9000    PN1k15k
## ---------+-------------------------------------------------------------------
##    6000 |  0.103867
##         |    0.4939
##         |
##    7000 |  2.325467   2.221600
##         |    0.4043     0.3324
##         |
##    8000 |  2.124888   2.040081   0.226152
##         |    0.3292     0.3119     0.4880
##         |
##    9000 |  0.533083   0.419302  -2.014338  -1.832345
##         |    0.4592     0.4804     0.3133     0.3558
##         |
##  PN1k15k |  -1.097780  -1.182587  -2.996517  -2.790913  -1.585811
##         |    0.3786     0.3533     0.4501     0.2724     0.3681
##         |
##  PN6k10k |   0.089518   0.004711  -1.809217  -1.762682  -0.326490   1.028231
##         |    0.4876     0.5001     0.3461     0.3435     0.4807     0.3841
```

```
##         |
## WN10k15k |   1.380470   1.295663  -0.518265  -0.644684   1.042771   2.146228
##         |    0.3729     0.3748     0.4587     0.4544     0.3833     0.3371
##         |
##  WN1k15k |   1.396434   1.292567  -0.929033  -0.984704   0.996634   2.237964
##         |    0.3753     0.3659     0.3810     0.3836     0.3990     0.3514
##         |
##   WN1k5k |  -0.155479  -0.240286  -2.054216  -1.974856  -0.586350   0.816056
##         |    0.4843     0.4907     0.3196     0.2959     0.4563     0.4217
##         |
##  WN6k10k |   1.521816   1.437009  -0.376920  -0.522276   1.192690   2.268637
##         |    0.3702     0.3904     0.4784     0.4594     0.3600     0.3621
## Col Mean-|
## Row Mean |    PN6k10k   WN10k15k    WN1k15k     WN1k5k
## ---------+-------------------------------------------
## WN10k15k |   1.117997
##         |    0.3748
##         |
##  WN1k15k |   1.050665  -0.240286
##         |    0.3905     0.4883
##         |
##   WN1k5k |  -0.212174  -1.330172  -1.295663
##         |    0.4853     0.3754     0.3805
##         |
##  WN6k10k |   1.240406   0.122408   0.381631   1.452580
##         |    0.3493     0.4902     0.4812     0.3951
##
## alpha = 0.05
## Reject Ho if p <= alpha/2
```

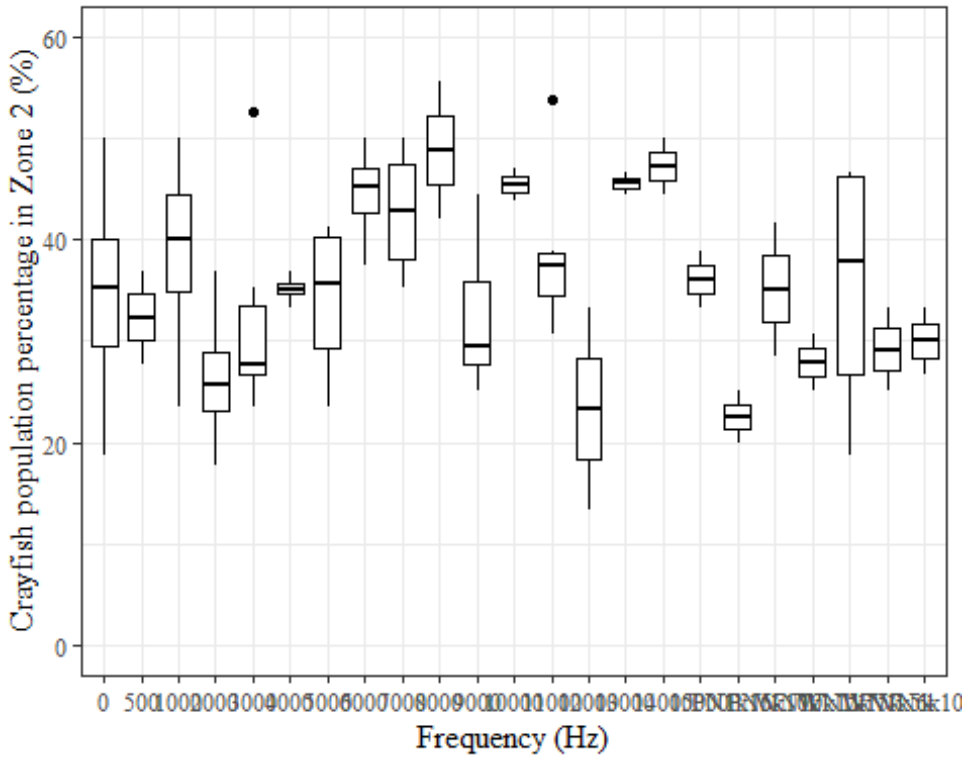
…

Plot box and whisker for each fit

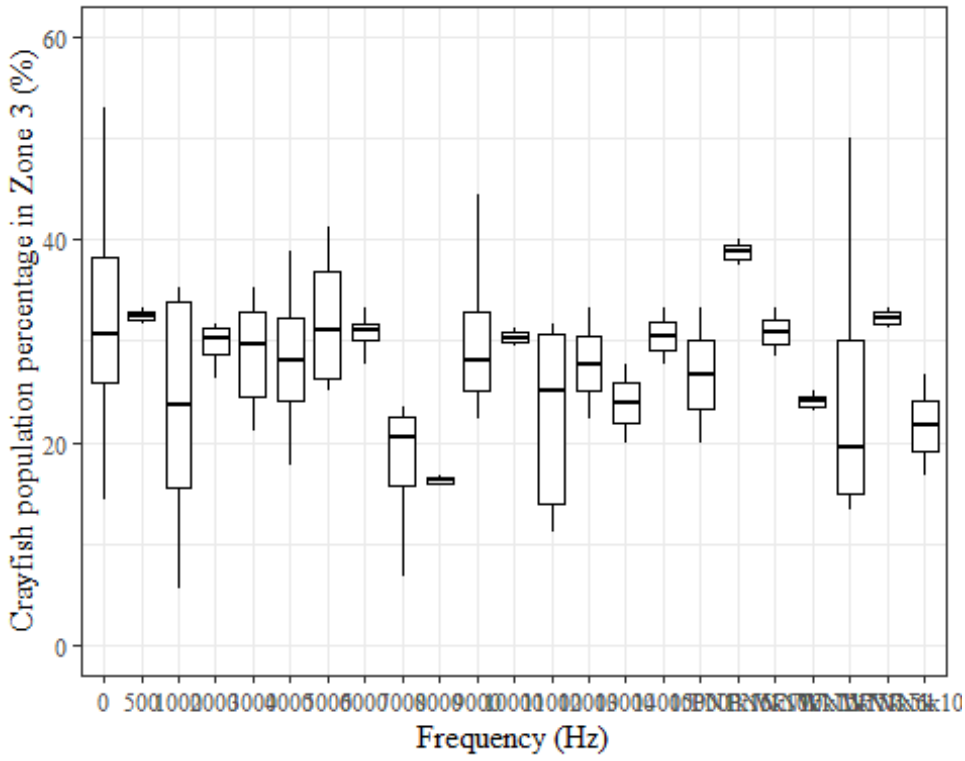
```
box_1 <- ggboxplot(LabData1, x = "Frequency", y = "Zone1")+ xlab("Frequency (Hz)") +
  ylab("Crayfish population percentage in Zone 1 (%)") +
  ylim(0, 60)+
  theme_bw()+
  theme(text=element_text(family="A", size=12))
  #geom_hline(yintercept = 33.33,color ='red')
box_1
```



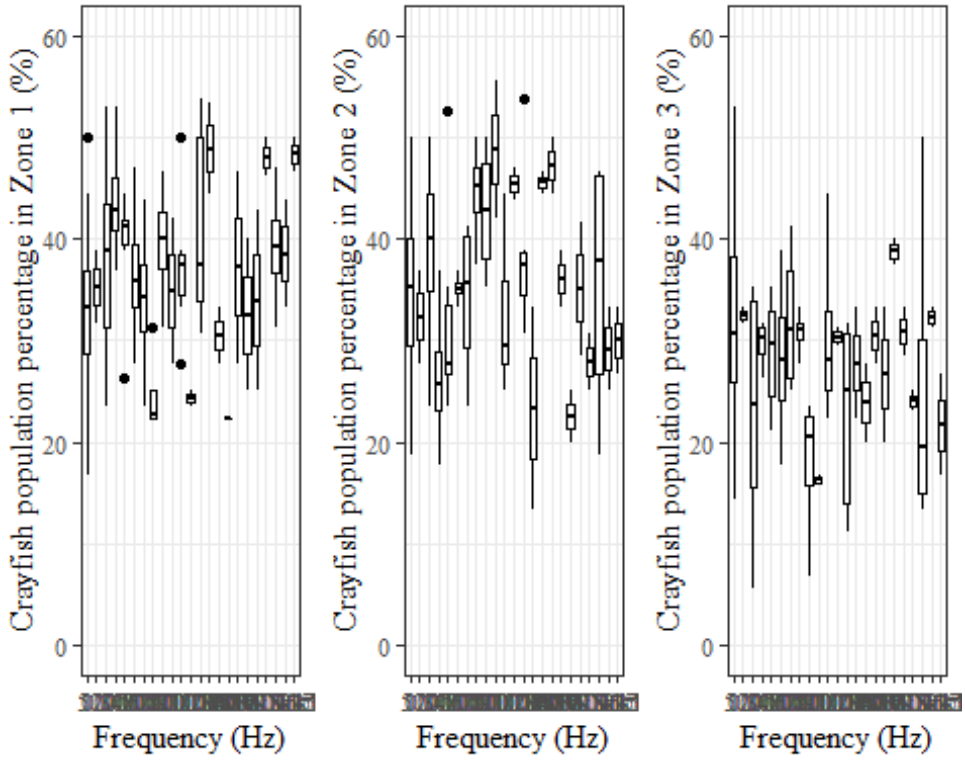

```
box_2 <- ggboxplot(LabData1, x = "Frequency", y = "Zone2")+
  xlab("Frequency (Hz)") +
  ylab("Crayfish population percentage in Zone 2 (%)") +
  ylim(0, 60)+
  theme_bw()+
  theme(text=element_text(family="A", size=12))
  #geom_hline(yintercept = 33.33,color ='red')
box_2
```




```
box_3 <- ggboxplot(LabData1, x = "Frequency", y = "Zone3")+
  xlab("Frequency (Hz)") +
  ylab("Crayfish population percentage in Zone 3 (%)") +
  ylim(0, 60)+
  theme_bw()+
  theme(text=element_text(family="A", size=12))
  #geom_hline(yintercept = 33.33,color ='red')
box_3
```



ga<- **grid.arrange**(box_1, box_2, box_3, ncol=3)



…

Create bar charts

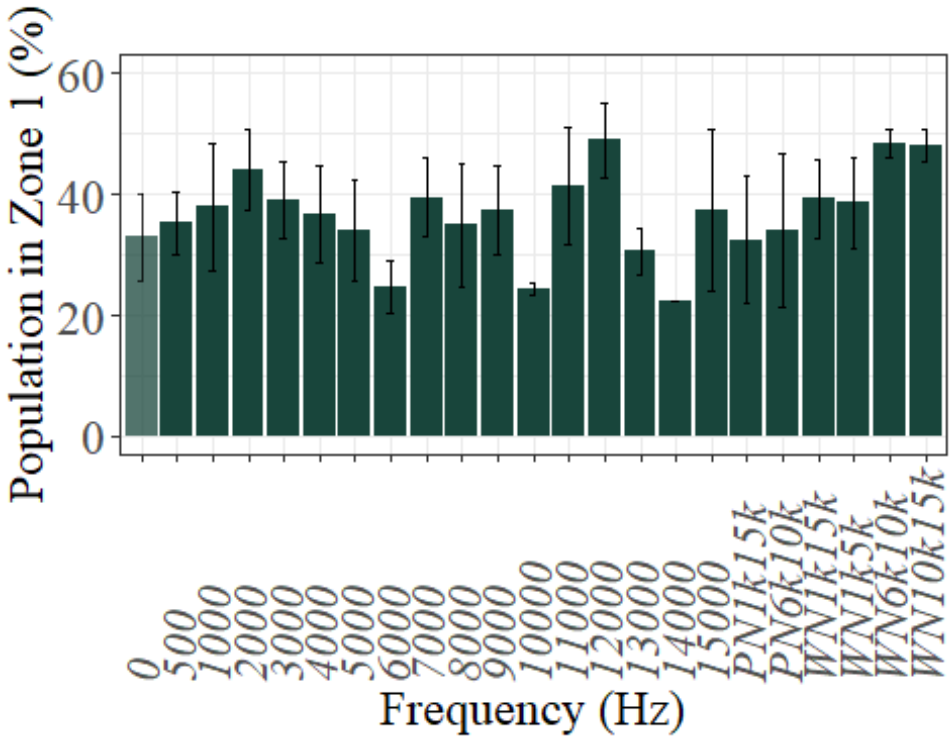
First, calculate sd using the following function.

```
data_summary <- function(data, varname, groupnames){
  require(plyr)
  summary_func <- function(x, col){
    c(mean = mean(x[[col]], na.rm=TRUE),
      sd = sd(x[[col]], na.rm=TRUE))
  }
  data_sum <- ddply(data, groupnames, .fun=summary_func, varname)
  data_sum <- rename(data_sum, c("mean" = varname))
  return(data_sum)
}
```

Zones by frequency treatment

Zone 1 by Frequency

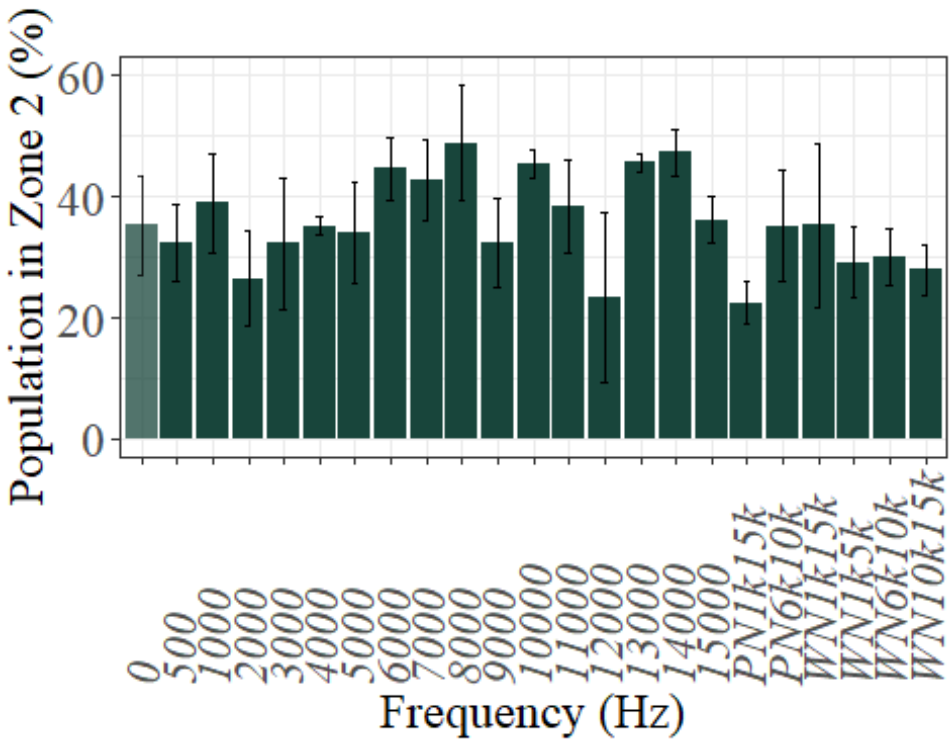
```
MovementToZone1 <- data_summary(LabData1, varname="Zone1", groupnames=c("Frequency"))

MovementToZone1$Frequency<-factor(MovementToZone1$Frequency,levels=c("0","500","1000","2000","3000","4000","5000","6000","7000","8000","9000","10000","11000","12000","13000","14000","15000","PN1k15k","PN6k10k","WN1k15k","WN1k5k","WN6k10k","WN10k15k"))

box_11 <- ggplot(MovementToZone1, aes(x=Frequency, y=Zone1,alpha=Frequency != 0)) + geom_bar(stat="identity", position=position_dodge(0.9), fill=color)+
  geom_errorbar(aes(ymin=Zone1-sd, ymax=Zone1+sd), width=0.2, position=position_dodge(0.9))+
  xlab("Frequency (Hz)")+ ylab("Population in Zone 1 (%)") + labs(fill="")+
  ylim(0, 60)  + labs(title = "", subtitle=NULL) +
  #geom_hline(yintercept = 33.33,color ='red')+
  scale_alpha_manual(values = c(0.75,1))+
  guides(alpha=F)+
  theme_bw()+theme(text=element_text(family="A", size=18))+
  theme(title=element_text(size=18), axis.text.x = element_text(size=18, face="italic", angle=90, vjust=0.5, hjust=0), axis.text.y=element_text(size=18), axis.title.y = element_text(size =18), axis.title.x=element_text(size=18))
box_11
```



Zone 2 by Frequency

```
MovementToZone2 <- data_summary(LabData1, varname="Zone2",
                      groupnames=c("Frequency"))

MovementToZone2$Frequency<-factor(MovementToZone2$Frequency,levels=c("0","500","10
00","2000","3000","4000","5000","6000","7000","8000","9000","10000","11000","12000","130
00","14000","15000","PN1k15k","PN6k10k","WN1k15k","WN1k5k","WN6k10k","WN10k15k
"))
head(MovementToZone2)

##   Frequency    Zone2       sd
## 1         0 35.26773  8.092236
## 2      1000 38.88889  8.195106
## 3     10000 45.40441  2.339692
## 4     11000 38.36354  7.584991
## 5     12000 23.33333 14.142136
## 6     13000 45.55556  1.571348

box_21 <- ggplot(MovementToZone2, aes(x=Frequency, y=Zone2,alpha=Frequency != 0)) + ge
om_bar(stat="identity", position=position_dodge(0.9), fill=color)+
  geom_errorbar(aes(ymin=Zone2-sd, ymax=Zone2+sd), width=0.2, position=position_dodge(
0.9))+
  xlab("Frequency (Hz)")+ ylab("Population in Zone 2 (%)") + labs(fill="")+
  ylim(0, 60)  + labs(title = "", subtitle=NULL) +
```

```
  scale_alpha_manual(values = c(0.75,1))+
  guides(alpha=F)+
  #geom_hline(yintercept = 33.33,color ='red')+
  theme_bw()+theme(text=element_text(family="A", size=18))+
  theme(title=element_text(size=18), axis.text.x = element_text(size=18, face="italic", angle=90, vjust=0.5, hjust=0), axis.text.y=element_text(size=18), axis.title.y = element_text(size =18), axis.title.x=element_text(size=18))
box_21
```

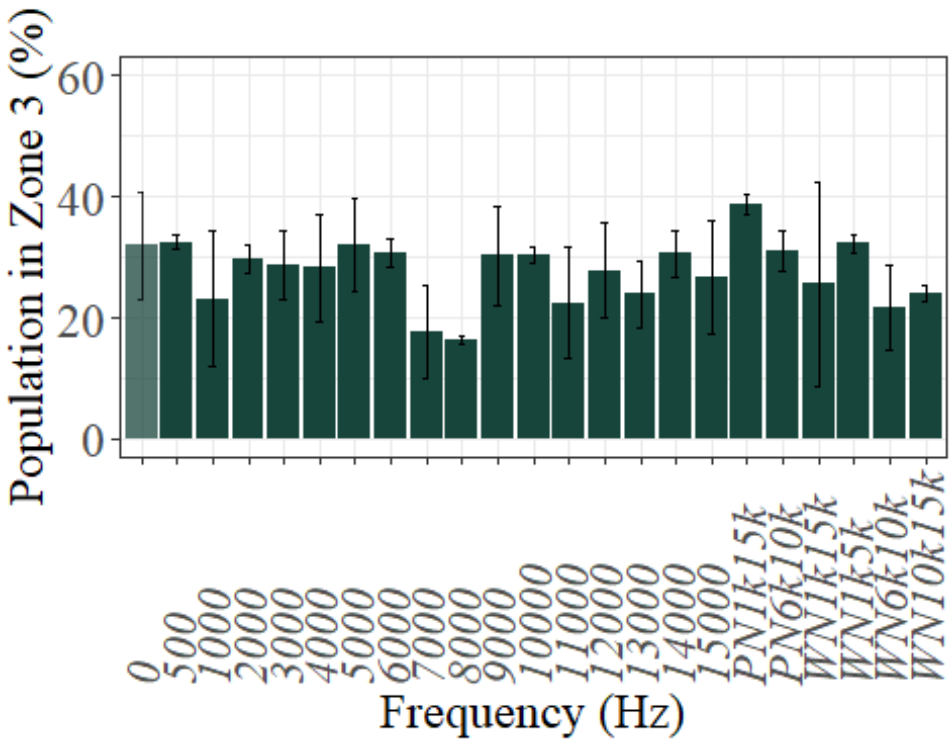


Zone 3 by Frequency

```
MovementToZone3 <- data_summary(LabData1, varname="Zone3",
                        groupnames=c("Frequency"))

MovementToZone3$Frequency<-factor(MovementToZone3$Frequency,levels=c("0","500","1000","2000","3000","4000","5000","6000","7000","8000","9000","10000","11000","12000","13000","14000","15000","PN1k15k","PN6k10k","WN1k15k","WN1k5k","WN6k10k","WN10k15k"))
head(MovementToZone3)

##   Frequency    Zone3       sd
## 1         0 31.93596  8.842344
## 2      1000 23.14134 11.238741
## 3     10000 30.33088  1.299829
## 4     11000 22.44473  9.181065
```

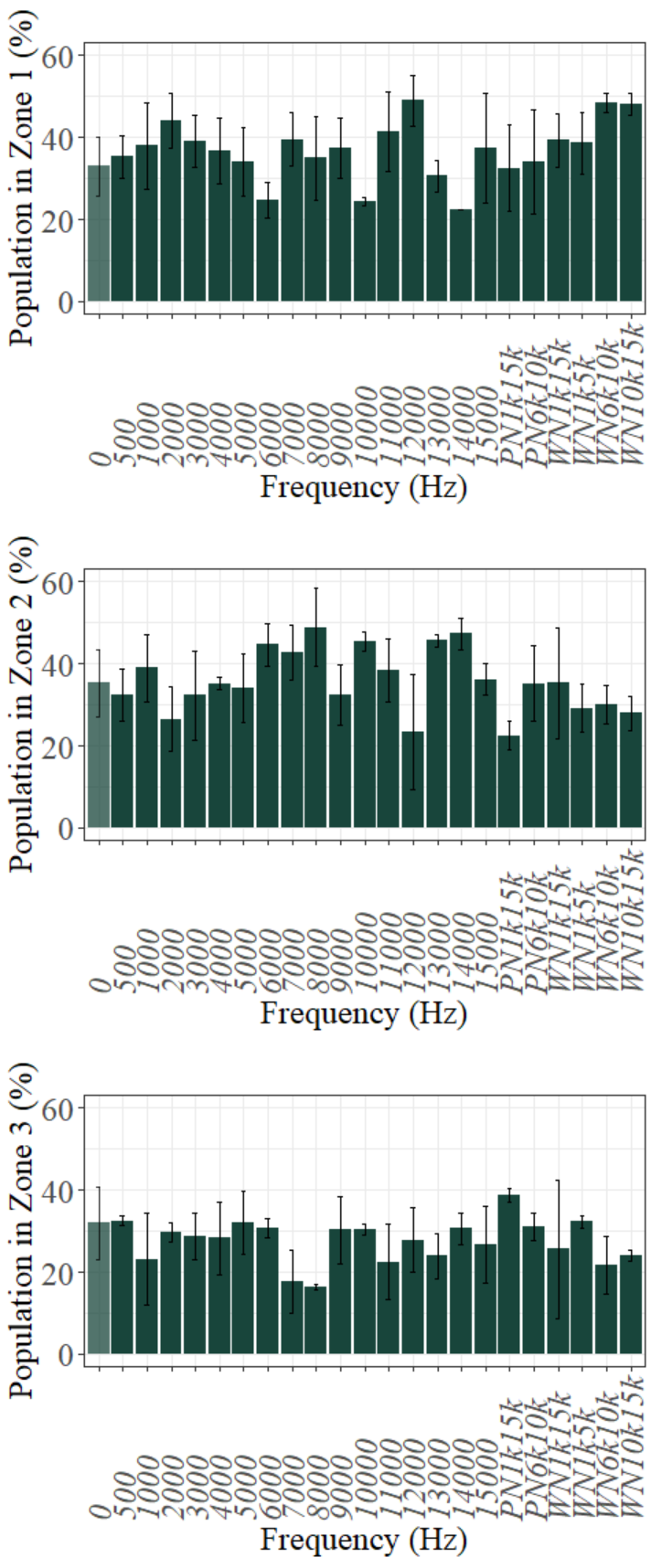
```
## 5     12000 27.77778  7.856742
## 6     13000 23.88889  5.499719
```

```
box_31 <- ggplot(MovementToZone3, aes(x=Frequency, y=Zone3,alpha=Frequency != 0)) + geom_bar(stat="identity", position=position_dodge(0.9), fill=color)+
  geom_errorbar(aes(ymin=Zone3-sd, ymax=Zone3+sd), width=0.2, position=position_dodge(0.9))+
  xlab("Frequency (Hz)")+ ylab("Population in Zone 3 (%)") + labs(fill="")+
  ylim(0, 60)  + labs(title = "", subtitle=NULL) +
  scale_alpha_manual(values = c(0.75,1))+
  guides(alpha=F)+
  #geom_hline(yintercept = 33.33,color ='red')+
  theme_bw()+theme(text=element_text(family="A", size=18))+
  theme(title=element_text(size=18), axis.text.x = element_text(size=18, face="italic", angle=90, vjust=0.5, hjust=0), axis.text.y=element_text(size=18), axis.title.y = element_text(size =18), axis.title.x=element_text(size=18))
box_31
```



Side-by-side visualization

```
ka<- grid.arrange(box_11, box_21, box_31, nrow=3,ncol=1)
```


Population in Zone 1 (%)
Population in Zone 2 (%)
Population in Zone 3 (%)
0
20
40
60
0
500
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
11000
12000
13000
14000
15000
PN1k15k
PN6k10k
WN1k15k
WN1k5k
WN6k10k
WN10k15k
Frequency (Hz)

LABORATORY CRAYFISH STANDARD OPERATING PROCEDURES

Standard Operating Procedure for Red Swamp Crayfish Research

Created by Douglas Clements 10/01/18

All individuals working with Red Swamp Crayfish (*Procambarus clarkii*) must read and agree to follow the standard operating procedure (SOP) outlined below.

Requirements

- All personnel working with *P. clarkii* must be trained in proper handling procedure.
- If personnel are uncomfortable holding crayfish, they may use a skimmer/net provided for catching crayfish safely.
- Rubber gloves are provided for protection against claw pinching if required.
- Personnel must check the water temperature daily; safe levels are between 6°C and 22°C.
- The habitats must be gravel syphoned weekly; syphon and pump are provided. Effluent must be disposed of in the designated sink.
- Sponge filters will be cleaned weekly; rinsed in the designated sink.
- Crayfish must be fed every two days according to instructions on food label; sinking algae pellets will be provided.
- Dead crayfish must be disposed of in a biohazard bag and placed in designated freezer prior to EHS incineration disposal. The same applies to euthanization.
- Lids on habitats must remain sealed to prevent unintended escape of *P. clarkii*. Weights must be placed on the lid to prevent movement.
- Close attention must be paid to open habitats. If personnel must leave the area, lids must be closed.
- First aid kits are available in the adjacent laboratory.
- Habitats must be monitored every day (including weekends and holidays) by personnel

Facility Information

- ADREC is on a septic system which is pumped and fed into the South Campus Anaerobic Digestor (SCAD).
- ADREC uses well water not treated with chlorine or fluoride.
- ADREC has a power redundancy and back-up generator available.

Experimental Procedure

1. Open the container by lifting all lids simultaneously; record the population distribution of the crayfish among the three zones.
2. Check and record water temperature. Make sure all air hoses are making bubbles.
3. Cover with lids and play sounds for 5 minutes, record population distribution.
4. Cover with lids and play sounds for 24 hours, record population distribution.
5. Feed *P. clarkii* according to instructions on container and allow 24 hours of silence before beginning the next experiment.

HABITAT CLEANING PROCEDURE

Last updated 6/21/2019

Two cleaning regimes were completed weekly to control nutrient levels and pH in the crayfish habitats. The first was a gravel siphon and 50% water change, while the second was sponge filter cleaning. Two of the sponge filters in each habitat were new, while the third was “pre-charged” with microbes from established aquariums in Preuss Pets, a Lansing, MI pet store. This ensured the establishment of a healthy microbial colony to help manage filtering and nutrient levels. The order of cleaning was important to prevent escape of Red Swamp Crayfish (RSC). Since the tap water was untreated well-water, there was no need to dechlorinate water prior to refilling habitats. The following include the steps taken during each weekly cleaning.

1. Following an experimental trial on Monday or Tuesday, all lids would be removed from Habitat #1.
2. Any crayfish remains would be removed (i.e. chela, legs, molt) and placed in a freezer bag prior to incineration.
3. Gravel siphoning would be completed until bottom area was complete or until 50% of water was removed (which ever happened first).
4. Sponge filters would be removed and rinsed under tap water and returned to their original position.
5. The number of RSC were counted and checked against the total number recorded for the habitat, during the course of the experimental trial.
6. Lids would be replaced, and steps 1-6 would be repeated for Habitat #2 and #3.

APPENDIX B: CHAPTER 3 SUPPLEMENTAL MATERIALS

TREATMENT SCHEDULE

	Week #	Monday	Tuesday	Wednesda	Thursday	Friday	Saturday	Sunday
		Sheraton	Sheraton	Sheraton	Sheraton	Sheraton	Sheraton	Sheraton
June	1							
	2							
	3	17-Jun	18-Jun	19-Jun	20-Jun	21-Jun	22-Jun	23-Jun
	4	24-Jun	25-Jun	26-Jun	27-Jun	28-Jun	29-Jun	30-Jun
July	1	1-Jul	2-Jul	3-Jul	4-Jul	5-Jul	6-Jul	7-Jul
	2	*7/8/2019	*7/9/2019	10-Jul	11-Jul	12-Jul	13-Jul	14-Jul
	3	15-Jul	16-Jul	17-Jul	18-Jul	19-Jul	20-Jul	21-Jul
	4	22-Jul	23-Jul	24-Jul	25-Jul	26-Jul	27-Jul	28-Jul
	5	29-Jul	30-Jul	31-Jul	1-Aug	2-Aug	3-Aug	4-Aug
August	1	5-Aug	6-Aug	7-Aug	8-Aug	9-Aug	10-Aug	11-Aug
	2	12-Aug	13-Aug	14-Aug	15-Aug	16-Aug	17-Aug	18-Aug
	3	19-Aug	20-Aug	21-Aug	22-Aug	23-Aug	24-Aug	25-Aug
	4	26-Aug	27-Aug	28-Aug	29-Aug	30-Aug	31-Aug	1-Sep
September	1	2-Sep	3-Sep	4-Sep	5-Sep	6-Sep	7-Sep	8-Sep
	2	9-Sep	10-Sep	11-Sep	12-Sep	13-Sep	14-Sep	15-Sep
	3	16-Sep	17-Sep	18-Sep	19-Sep	20-Sep	21-Sep	22-Sep
	4	23-Sep	24-Sep	25-Sep	26-Sep	27-Sep	28-Sep	29-Sep

Treatment
Food
Food + Sound
Sound
No Trapping
Empty Trap

* 30 traps missing

Figure B.1: Recorded schedule used for tracking the treatment being used.

ACOUSTIC POND TRIAL ANALYSIS IN R

The following is an R Markdown code output for the statistical analysis of the field trials.

Pond Trial Analysis
Douglas Clements
8/4/2020

Set-up

Load in libraries

```
library(readxl)  #reads excel documents
library(ggplot2) #makes plots more easily customizable
library(doBy)    #adds the summaryby function
library(dplyr)   # Data manipulation
library(tidyverse)
library(ggpubr)
library(writexl) # Writes excel files
library(extrafont) #Adds in Times New Roman
```

Set Theme and add custom color

```
#Theme
theme_set(theme_pubr())
# Creates Kelly Green (Spartan Green)
SpartanGreen <-rgb(24,69,59, maxColorValue = 255)
# Import Times New Roman
windowsFonts(Times=windowsFont("Times New Roman"))
```

Read in Data

```
#setwd("H:/School/Thesis Work/Sound Pond Trials/Data Files")
DALL<-as.data.frame(read_xlsx("MasterDataCrayfish.xlsx",sheet = "Sheet1"))
head(DALL) #shows a sampleof data
```

```
##   Trap_Number row.name Sample_Number   Set_Date  Pull_Date Day_Number
## 1           1        1       19ST005 2019-07-09 2019-07-10          1
## 2           1       58       19ST006 2019-07-10 2019-07-11          2
## 3           1      114       19ST007 2019-07-11 2019-07-12          3
## 4           1      171       19TR260 2019-07-15 2019-07-16          5
## 5           1      228       19TR813 2019-07-17 2019-07-18          6
## 6           1      274       19TR810 2019-07-18 2019-07-19          7
##       Trap_Type Daily_Catch Sound Food Week_Number Volume Amp AnySp_InBerry
## 1 GeeMinnowTrap           0     1    1           1    0.5   1             0
## 2 GeeMinnowTrap           1     0    1           1    0.5   1             0
## 3 GeeMinnowTrap           1     0    0           1    0.5   1             0
## 4 GeeMinnowTrap           0     0    1           2    0.5   1             0
```

```
## 5 GeeMinnowTrap         0    0   1         2   0.5  1          NA
## 6 GeeMinnowTrap         1    0   1         2   0.5  1          NA
##   CommentsTrap CommentsSample WaterTemp   VolTreat   VolLevel  Treatment
## 1           NA           NA       NA Treatment1 Treatment1 Food+Sound
## 2           NA           NA       NA Treatment1        NA       Food
## 3           NA           NA     <NA> Treatment1        NA       None
## 4           NA           NA       77 Treatment1        NA       Food
## 5         <NA>         <NA>     82.4 Treatment1        NA       Food
## 6         <NA>         <NA>     80.3 Treatment1        NA       Food
##   Distance
## 1       22
## 2       22
## 3       22
## 4       22
## 5       22
## 6       22
```

Subset data by trap type

```
geesonly<-DALL[which(DALL$Trap_Type=="GeeMinnowTrap"),]#Gees Traps only, all treatments
PVConly<-DALL[which(DALL$Trap_Type=="Apartment"|DALL$Trap_Type=="ART"),]# PVC refuge traps
ARTonly<-DALL[which(DALL$Trap_Type=="ART"),]# ART traps
APARTonly<-DALL[which(DALL$Trap_Type=="Apartment"),] # APART traps
```

Subset Gee's Traps by Treatment

```
soundgees<-geesonly[which(geesonly$Treatment=="Sound" | geesonly$Treatment=="Food+Sound"),]#Both Sound and Sound+Food
soundonlygees<-geesonly[which(geesonly$Treatment=="Sound"),]#Sound
soundfoodgees<-geesonly[which(geesonly$Treatment=="Food+Sound"),]#Food+Sound
FoodGees<-geesonly[which(geesonly$Treatment=="Food"),]#Food
NoneGees<-geesonly[which(geesonly$Treatment=="None"),]#Silence
```

Subset data by Treatment

```
FoodOnly<-DALL%>%
  filter(Treatment=="Food")
FoodandSound<-DALL%>%
  filter(Treatment=="Food+Sound")
SoundOnly<-DALL%>%
  filter(Treatment=="Sound")
None<-DALL%>%
  filter(Treatment=="None")
```

…

Data summary

Combined Data Summary

```
# Basic summaryBy with Daily catch as a factor against trap type (Daily catch mean is CPUE)
DataSummary<-summaryBy(Daily_Catch~Treatment+Trap_Type,data = DALL,FUN = c(mean
,length,sum))
DataSummary
```

```
##     Treatment     Trap_Type Daily_Catch.mean Daily_Catch.length Daily_Catch.sum
## 1        Food     Apartment        1.6891892                74             125
## 2        Food           ART        1.1014493                69              76
## 3        Food GeeMinnowTrap        0.6745731              1054             711
## 4        Food      Juvenile        0.5333333                60              32
## 5        Food       Pyramid        0.8378378                74              62
## 6  Food+Sound     Apartment        1.4255319                47              67
## 7  Food+Sound           ART        1.2888889                45              58
## 8  Food+Sound GeeMinnowTrap        0.8200900               667             547
## 9  Food+Sound      Juvenile        0.2857143                42              12
## 10 Food+Sound       Pyramid        1.2830189                53              68
## 11       None     Apartment        1.5882353                17              27
## 12       None           ART        1.4444444                18              26
## 13       None GeeMinnowTrap        0.4874552               279             136
## 14       None      Juvenile        2.2307692                13              29
## 15       None       Pyramid        0.9230769                13              12
## 16      Sound     Apartment        1.5000000                 6               9
## 17      Sound           ART        1.6666667                 6              10
## 18      Sound GeeMinnowTrap        0.6436782                87              56
## 19      Sound      Juvenile        0.0000000                 4               0
## 20      Sound       Pyramid        1.7000000                10              17
```

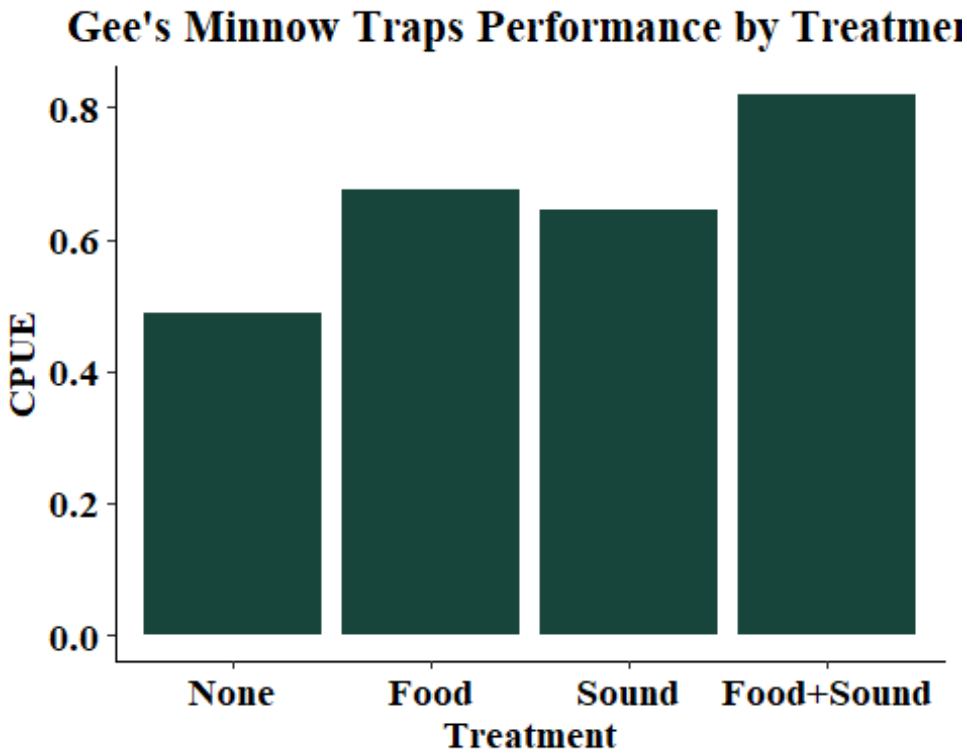
Summarize Treatments for Gee's Minnow Traps

```
# Basic summaryBy with Daily catch as a factor against trap type (Daily catch mean is CPUE)
GeesSummary<-summaryBy(Daily_Catch~Treatment,data = geesonly,FUN = c(mean,length,su
m))
GeesSummary
```

```
##    Treatment Daily_Catch.mean Daily_Catch.length Daily_Catch.sum
## 1       Food        0.6745731              1054             711
## 2 Food+Sound        0.8200900               667             547
## 3       None        0.4874552               279             136
## 4      Sound        0.6436782                87              56
```

```
ggplot(data=GeesSummary,aes(x=Treatment,y=Daily_Catch.mean))+
  geom_col(fill=SpartanGreen)+labs(y="CPUE",title = "Gee's Minnow Traps Performance by Tr
eatment")+
  theme(plot.title = element_text(hjust = 0.5),
```

```
    text=element_text(family="Times", face="bold", size=14))+
scale_x_discrete(limits = c("None","Food","Sound","Food+Sound"))
```



Daily_Catch.mean is CPUE. The highest CPUE on a treatment basis is *Food & Sound*. Note that *Food* and *Sound* are approximately equal. *None* represents an empty trap and is still nearly 2/3 of *Food* or *Sound*. Also, note that when *Food*-*None* and *Sound*-*None* are added to *None*, the results are approximately equal to *Food & Sound*. It seems the effects are addative, and if so, likely work on different mechanisms.

Analysis on Treatment

Visualize Treatment Data on Gee's Minnow Traps

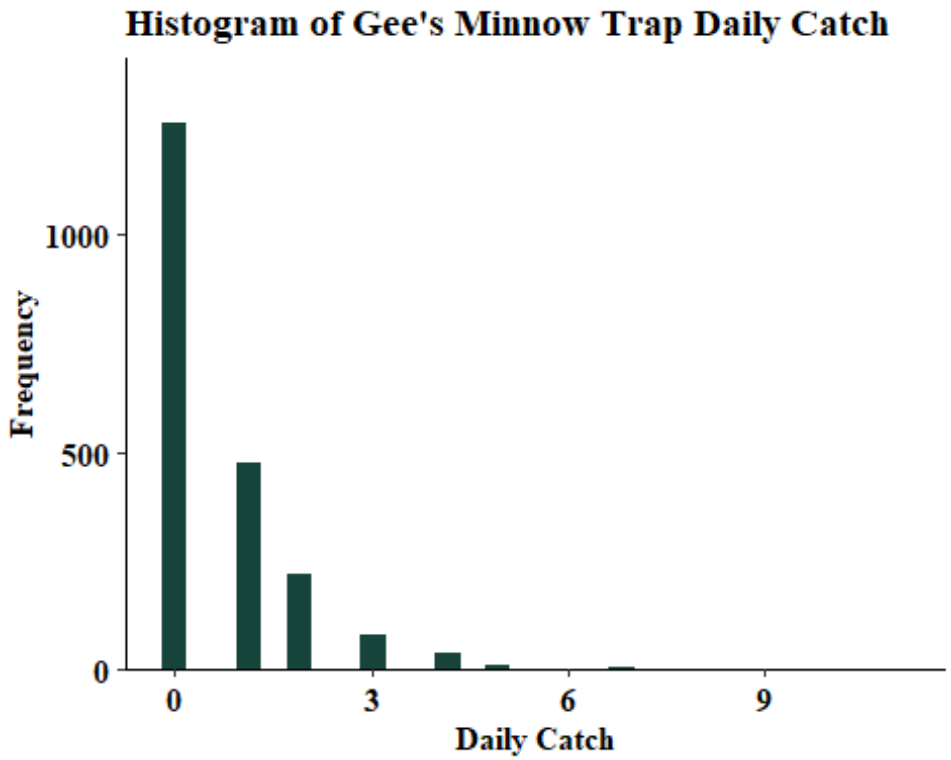
```
table(geesonly$Daily_Catch)
```

```
##
##    0    1    2    3    4    5    6    7    8   11
## 1252  474  219   82   37   10    4    5    3    1
```

Note the high number of Zeros in the daily catch data. Daily catch data should be checked for normality.

```
ggplot(data=geesonly,aes(x=Daily_Catch))+
  geom_histogram(fill="#18453b")+
  labs(x="Daily Catch", y="Frequency",title = "Histogram of Gee's Minnow Trap Daily Catch")+
```

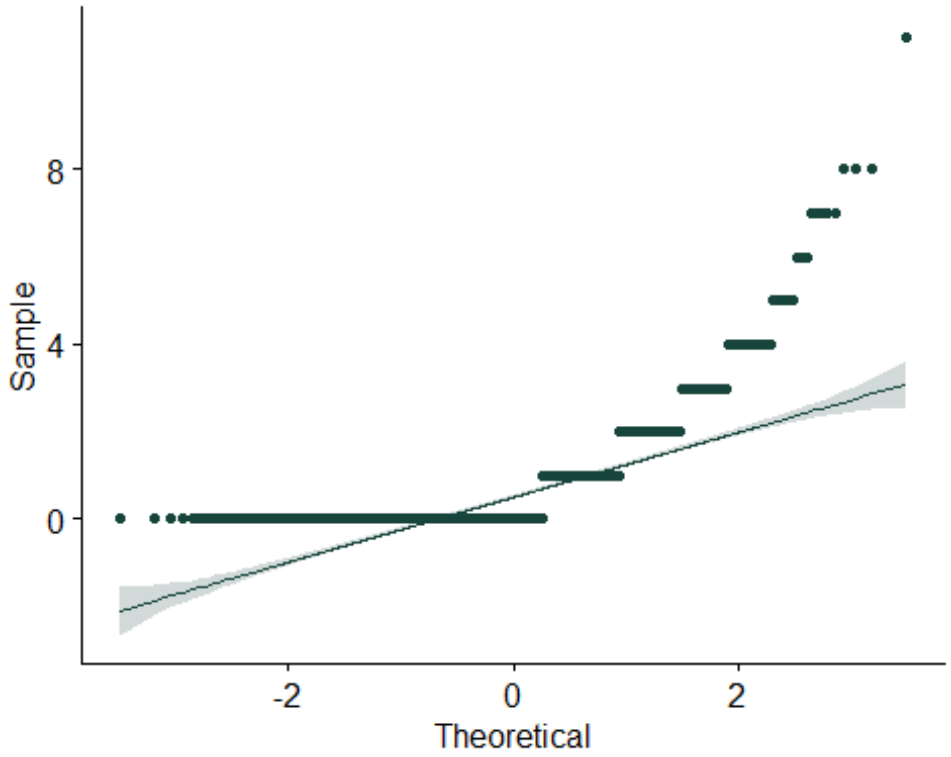
```
theme(text=element_text(family="Times", face="bold", size=12))+
scale_y_continuous(expand=c(0,0),limits = c(0,1400))
```




```
ggsave("GeesHistogram.png",width = 8,height = 8)
```

It doesn't look very normal from the histogram.

```
ggqqplot(geesonly$Daily_Catch,color = SpartanGreen)
```



Non-normal, since most dots are outside the CI. Finally, lets check with a Shapiro-Wilk Test; if p<0.05 it isn't a normal distribution.

```
shapiro.test(geesonly$Daily_Catch)

##
##  Shapiro-Wilk normality test
##
## data:  geesonly$Daily_Catch
## W = 0.65835, p-value < 2.2e-16
```

Non-normal distribution. However, depending on the sample size, we can use a parametric test anyway because of the Central Limit Theorem.

Statistical Tests

```
kruskal.test(Daily_Catch ~ Treatment, data = geesonly)

##
##  Kruskal-Wallis rank sum test
##
## data:  Daily_Catch by Treatment
## Kruskal-Wallis chi-squared = 26.674, df = 3, p-value = 6.892e-06

summary(aov(Daily_Catch~Treatment, data=geesonly))
```

```
##              Df Sum Sq Mean Sq F value   Pr(>F)
## Treatment     3   23.1   7.708   6.172 0.000357 ***
## Residuals   2083 2601.4   1.249
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairwise.wilcox.test(geesonly$Daily_Catch,as.factor(geesonly$Treatment),p.adjust="BH")
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test
##
## data:  geesonly$Daily_Catch and as.factor(geesonly$Treatment)
##
##           Food   Food+Sound None
## Food+Sound 0.0042 -          -
## None       0.0042 3.5e-06    -
## Sound      0.9425 0.2098     0.1307
##
## P value adjustment method: BH
```

Analysis on Distance to Speaker

```
kruskal.test(Daily_Catch ~ Distance, data = soundfoodgees)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  Daily_Catch by Distance
## Kruskal-Wallis chi-squared = 100.73, df = 96, p-value = 0.3505
```

```
kruskal.test(Daily_Catch ~ Distance, data = soundonlygees)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  Daily_Catch by Distance
## Kruskal-Wallis chi-squared = 45.769, df = 43, p-value = 0.3579
```

```
kruskal.test(Daily_Catch ~ Distance, data = soundgees)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  Daily_Catch by Distance
## Kruskal-Wallis chi-squared = 117.06, df = 96, p-value = 0.071
```

```
summary(aov(Daily_Catch~Distance, data=soundfoodgees))
```

```
##              Df Sum Sq Mean Sq F value Pr(>F)
## Distance      1    3.0   3.039   2.124  0.145
## Residuals   665  951.4   1.431
```

```
summary(aov(Daily_Catch~Distance, data=soundonlygees))
```

```
##              Df Sum Sq Mean Sq F value Pr(>F)
## Distance      1   0.43  0.4260   0.444  0.507
## Residuals    85  81.53  0.9592
```

```
summary(aov(Daily_Catch~Distance, data=soundgees))
```

```
##              Df Sum Sq Mean Sq F value Pr(>F)
## Distance      1    3.2   3.184   2.312  0.129
## Residuals   752 1035.6   1.377
```

No significance was found on distance to the speaker, using only Gee's Minnow traps, along any treatment with sound.

Analysis on Trap Type for *Food* Treatment

```
kruskal.test(Daily_Catch ~ as.factor(Trap_Type), data = FoodOnly)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  Daily_Catch by as.factor(Trap_Type)
## Kruskal-Wallis chi-squared = 71.915, df = 4, p-value = 8.943e-15
```

```
summary(aov(Daily_Catch~as.factor(Trap_Type), data=FoodOnly))
```

```
##                      Df Sum Sq Mean Sq F value   Pr(>F)
## as.factor(Trap_Type)    4   83.1  20.784   13.89 4.22e-11 ***
## Residuals            1326 1984.5   1.497
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

APPENDIX C: CHAPTER 4 SUPPLEMENTAL MATERIALS

GENERATED DESIGN CONCEPTS

The following are images of generated design concepts, transferred from engineering paper.

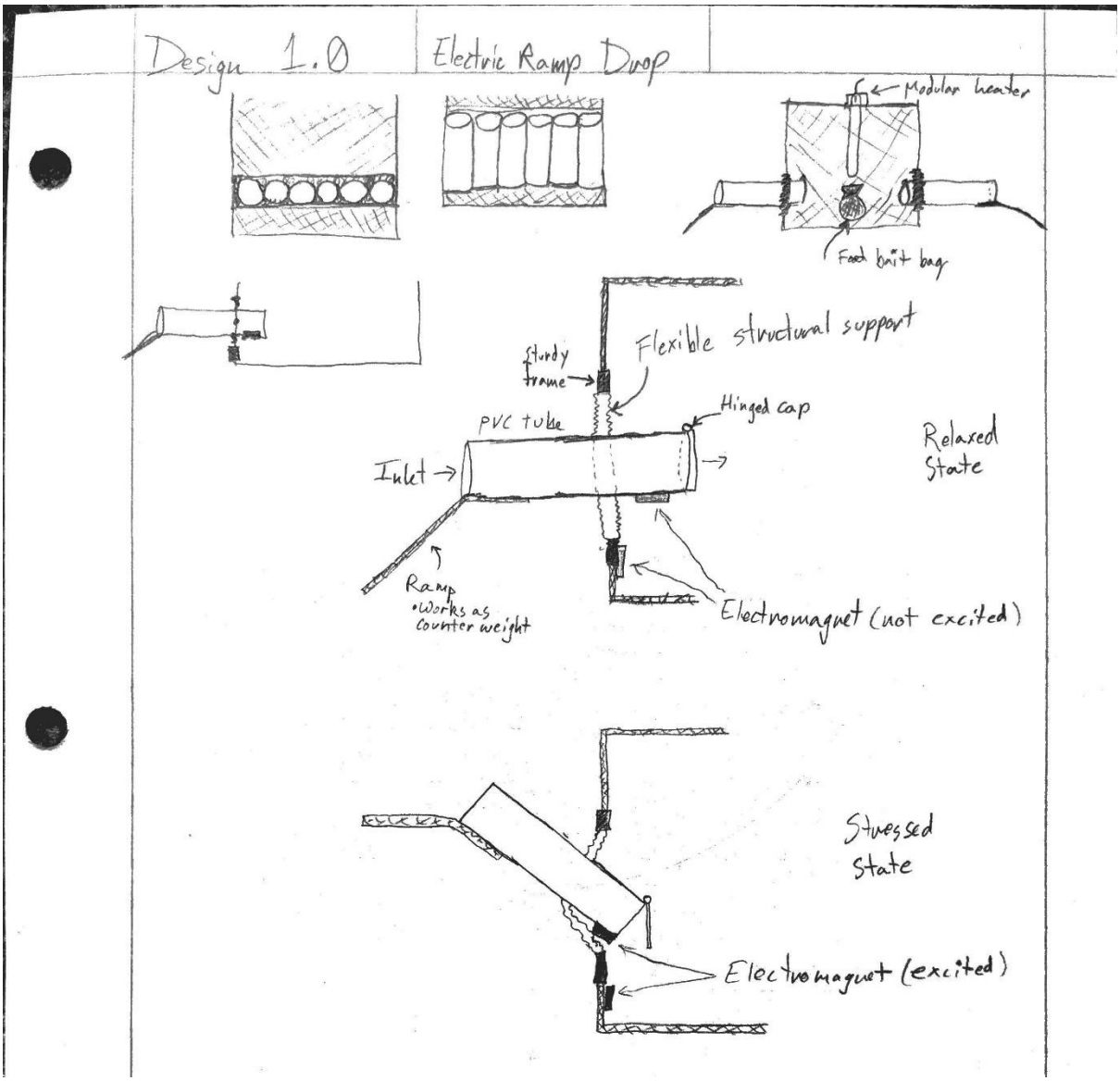


Figure C.1: PVC pipes are tipped upwards using electromagnets to empty crayfish into a cage.

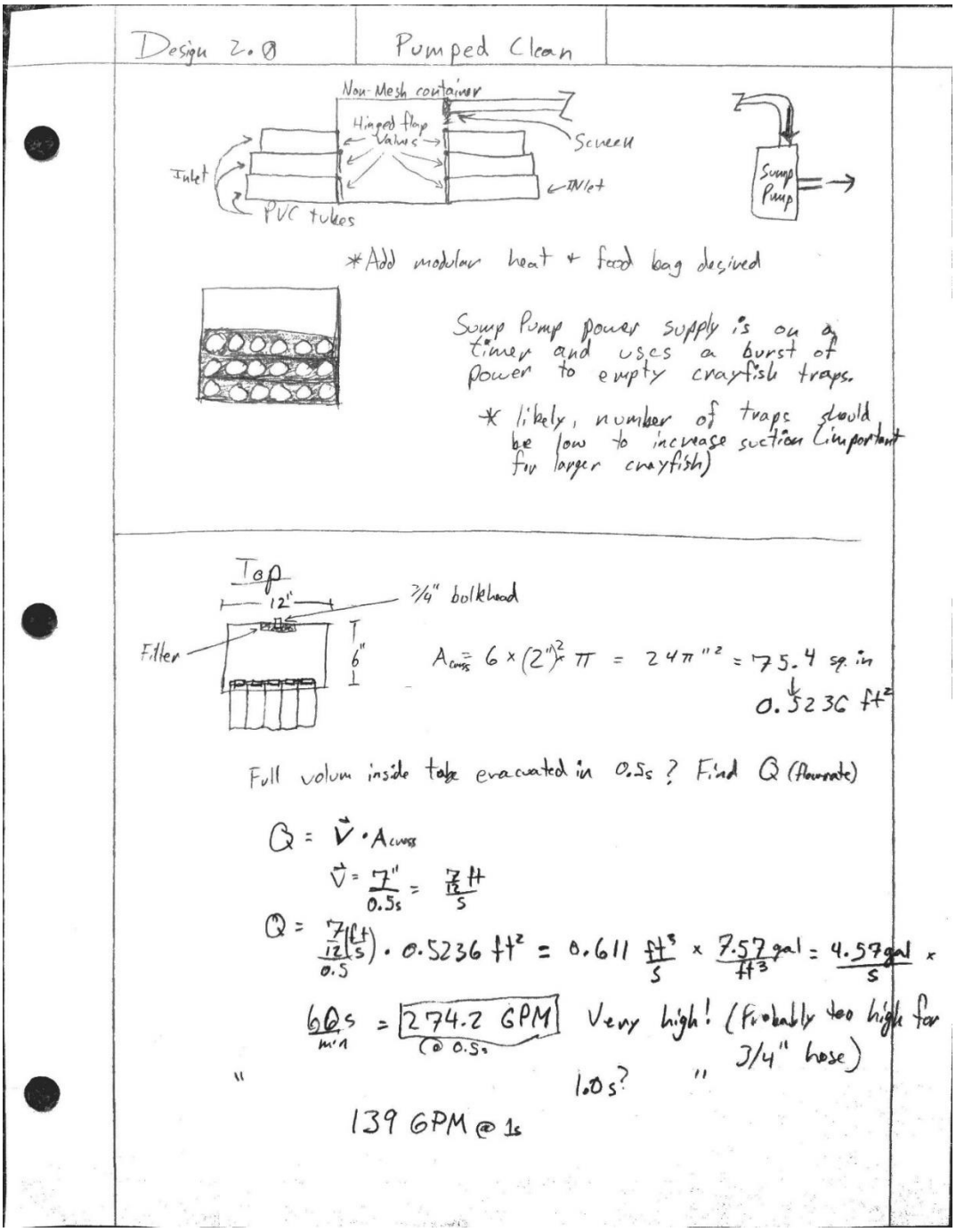


Figure C.2: Crayfish enter the refuge style traps before a pump intermittently empties traps into a cage through a flapper valve.

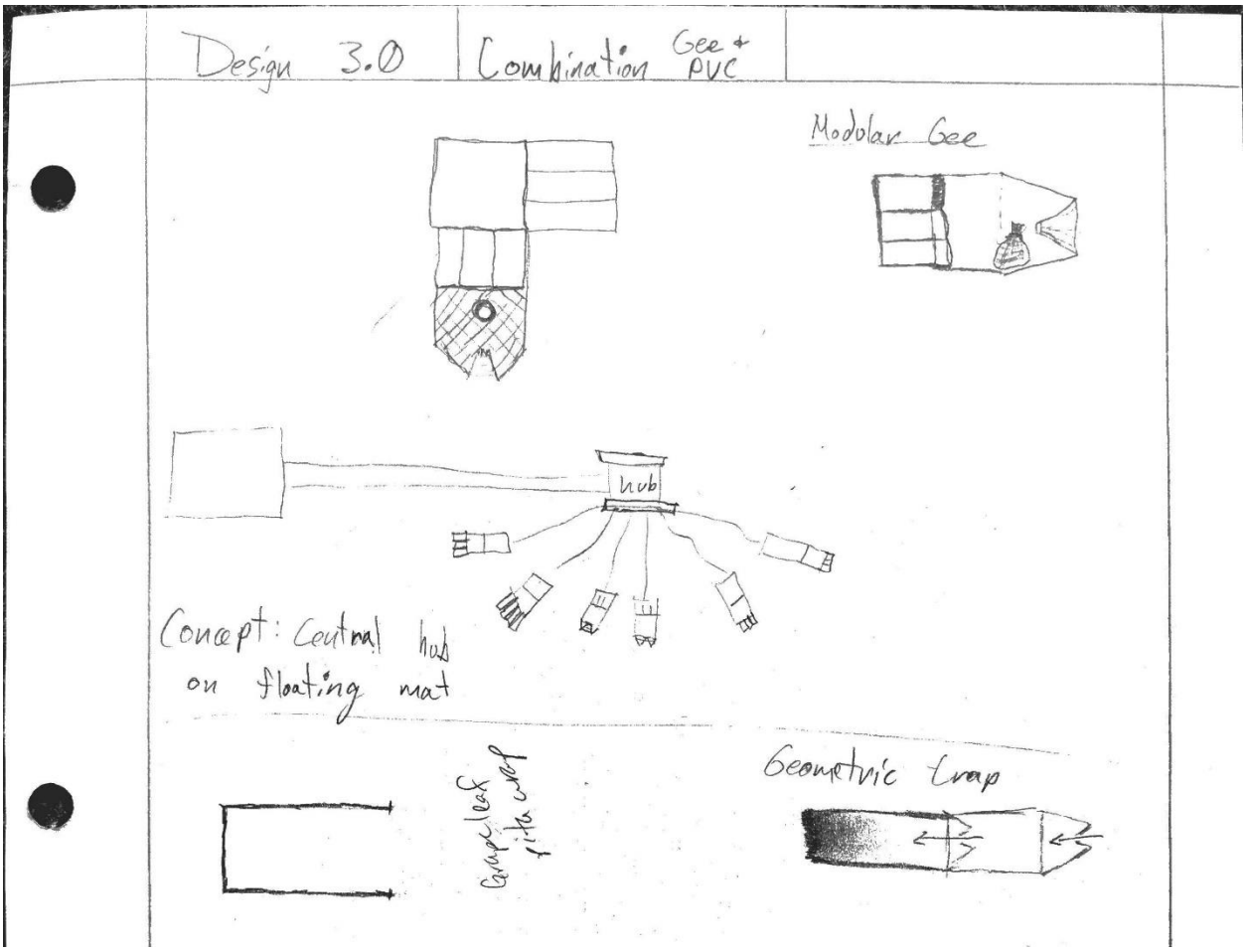


Figure C.3: The third design uses a combination of semi-cylindrical trap and refuge trap to direct crayfish toward a central floating cage.

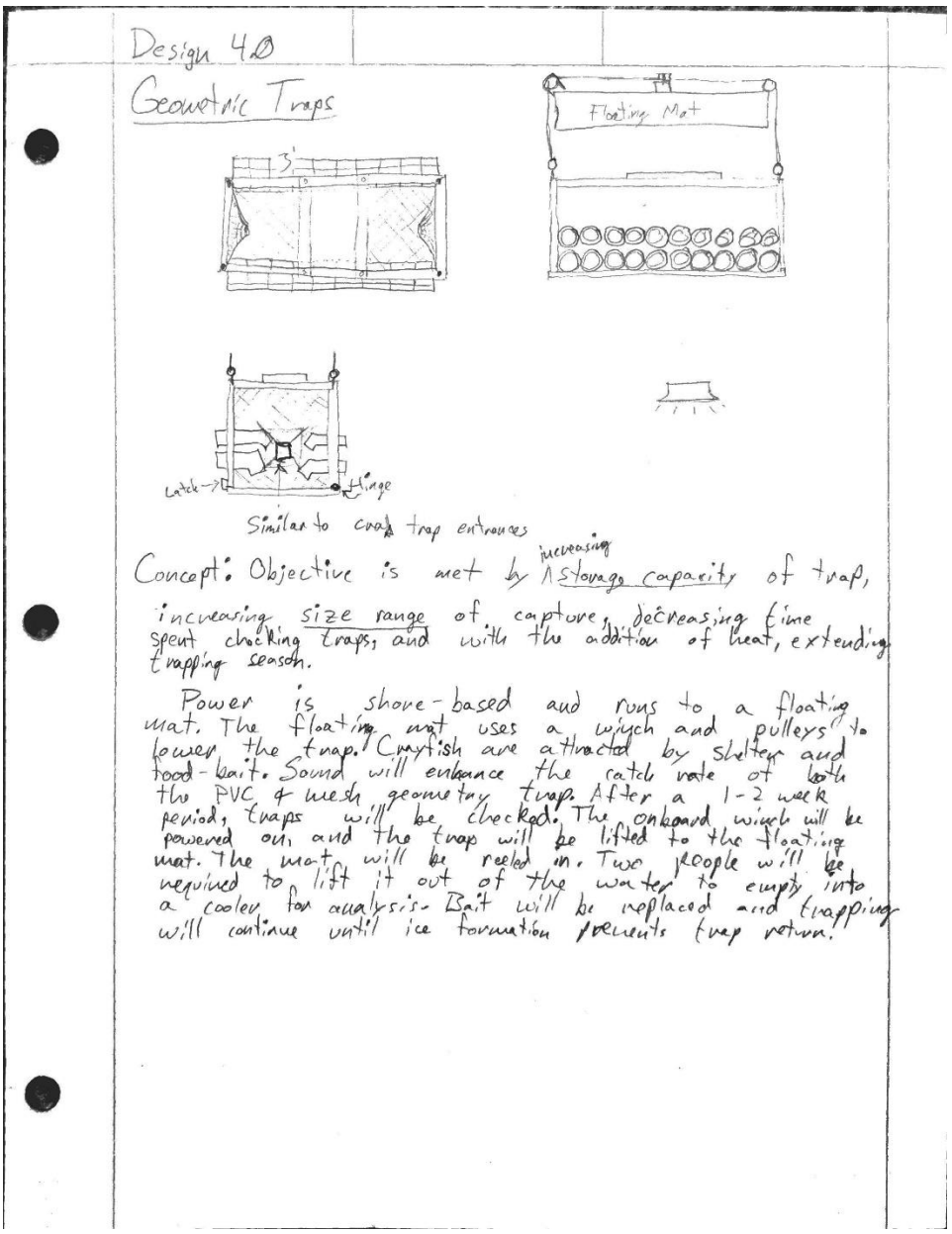


Figure C.4: This design uses refuge tubes with an angle at one end to act as a one-way valve for crayfish entry into a cage. An entrance similar to the Gee's Minnow Trap us used on each end.

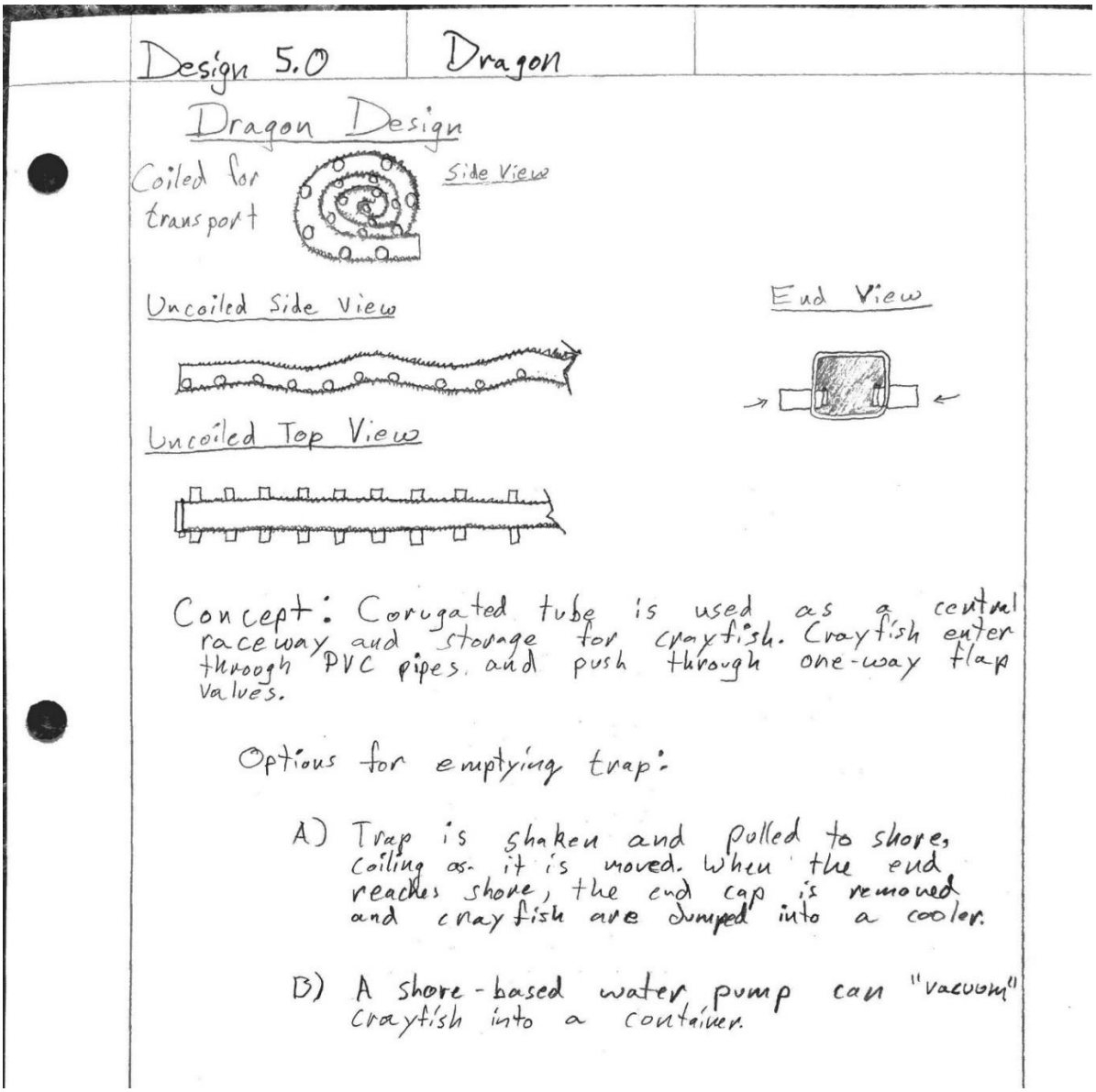


Figure C.5: The Dragon design is made of a corrugated plastic tube that coils up for storage and is uncoiled during deployment. Refuge tubes with flapper valves allow crayfish to enter into the corrugated tube.

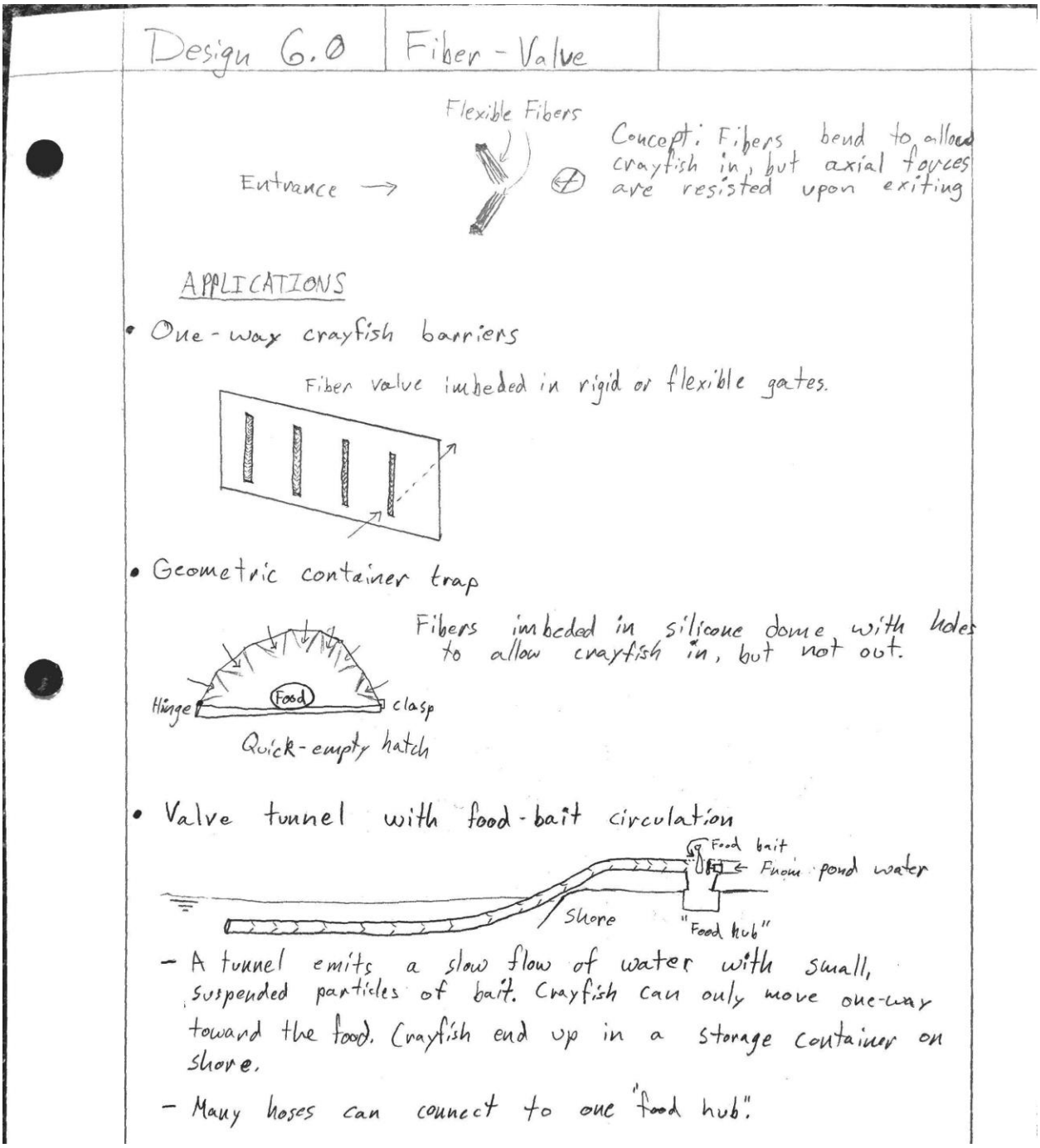


Figure C.6: Later termed the SoftValve, the concept is to use flexible bristles or fibers to allow a crayfish in one-way but not the opposing direction.

BLUEPRINTS FOR CURRENT DESIGN

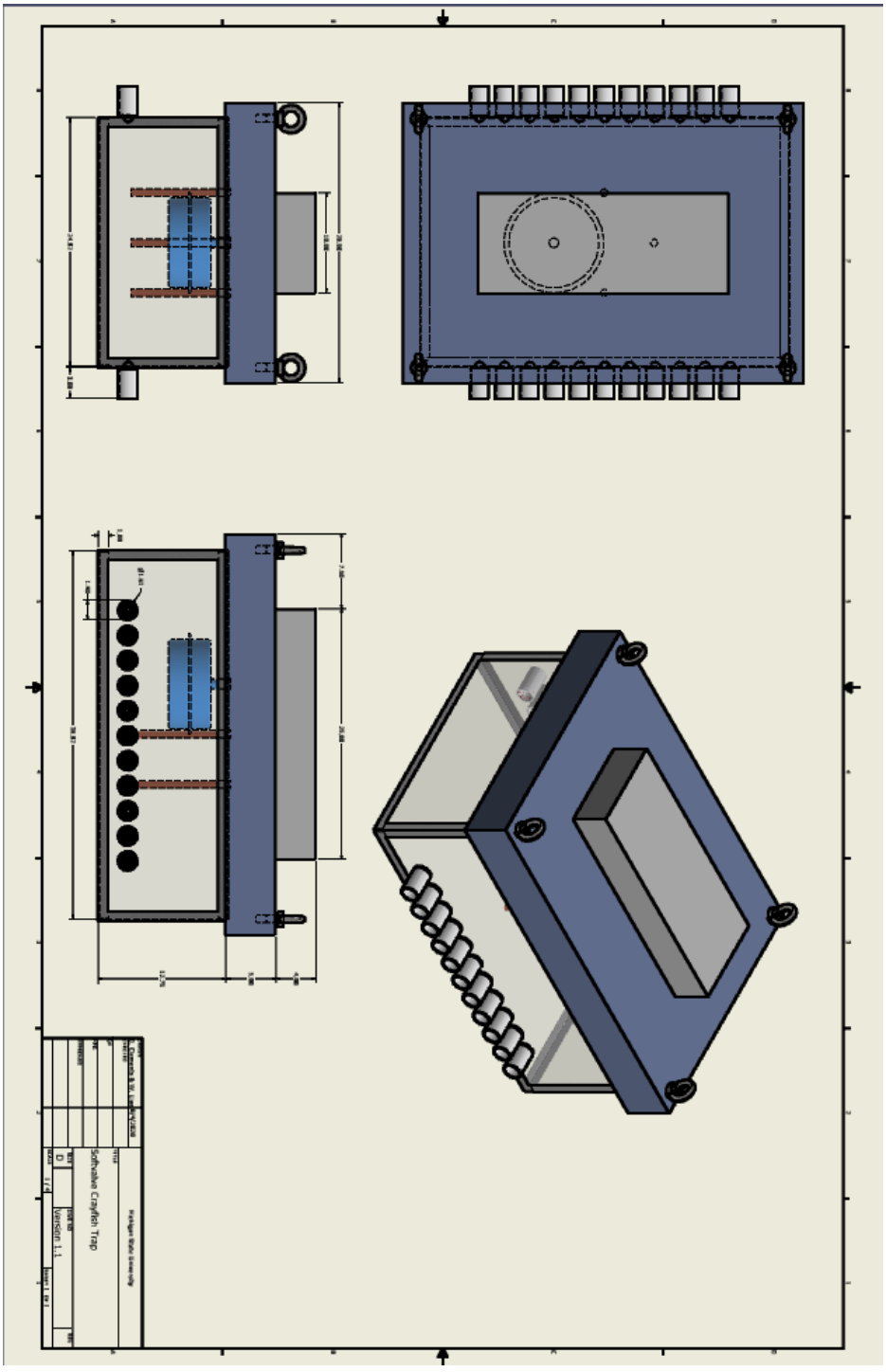


Figure C.7: Blue prints of the current design were created using Autodesk Inventor.

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REFERENCES

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