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Jennifer Nguyen

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THE CROSS-GENERATIONAL EFFECTS OF INVERTEBRATE PREDATORS (*CHAOBORUS SP.*) ON PREY (*DAPHNIA AMBIGUA)*

by

JENNIFER NHUY NGUYEN

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Thank you to Bobbie Brown for sending out the email that directed me towards Dr. Walsh for research experience. If not for you and Honors College, I would have not been granted the opportunity to research as well as gain experience in the field of ecological/evolutionary science.

Lastly, I want to give the biggest applause as well as thanks to my biggest supporters: my family and friends. My parents are the reason I am here today and without them, I don't know if I could have been able to gain the education that I have right now. With that said, I wouldn't be the person I am without my friends here by my side. My college friends aren't just friends… they are my forever friends. We will continue to grow together, experience life, and be there for each other for more years to come. Thank you to everyone who has changed my life for the better. I appreciate it.

April 24, 2015

ABSTRACT

THE CROSS GENERATIONAL EFFECTS OF INVERTEBRATE PREDATORS (*CHAOBORUS SP.*) ON PREY (*DAPHNIA AMBIGUA)*

Jennifer Nhuy Nguyen, BIOL

The University of Texas at Arlington, 2015

Faculty Mentor: Matthew Walsh

There is new evidence that environmental stressors can induce phenotypic changes that persist for several generations. These 'transgenerational' effects can be studied through the interplay between freshwater species of zooplankton and their predators. The transgenerational effects of invertebrate predator cues on the expression of life history traits in water fleas (*Daphnia ambigua*) were tested and analyzed. *Daphnia* were reared in the presence and absence of predator cues for X generations. An additional treatment received exposure to predators for the first generation only. The results show that predator cues caused increased reproductive outputs but no influence on the size of individuals across generations. By applying these findings beyond the scope of ecological properties and towards human communities, a better understanding of how

transgenerational effects can influence human-related diseases as well as host-pathogen interactions can be postulated.

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CHAPTER 1

INTRODUCTION

New exciting evidence is emerging that a variety of environmental stressors such as food limitation (Bashey 2006), shading (Galloway and Etterson 2007), and temperature (Steigenga and Fischer 2007; Salinas and Munch 2012) can induce physical changes that are passed on for several generations (Jablonka and Raz 2009). These 'transgenerational' responses are postulated to have far-reaching consequences for ecological communities (Agrawal 2001), and the rate and direction of evolutionary change (Bonduriansky et al. 2012). However, our understanding of the duration of cross-generational effects is limited.

The waterflea (*Daphnia sp.)* is a crustacean commonly found in lakes and ponds throughout North America and South America (Hebert and Grewe 1985). Although they are referred to as waterfleas, these organisms are not fleas at all. They are filter feeders that filter tiny food particles with hairlike projections found on their legs (Fleisher 1996). *Daphnia sp*. has well-established ecological roles in aquatic food webs as they are the dominant grazers on phytoplankton in lakes. They thus strongly influence rates of primary production and nutrient cycling (Carpenter et al. 1987, 1992; Elser et al. 1988).

Figure 1.1: *Daphnia ambigua*

The interplay between freshwater species of zooplankton and their predators have long served as a model to quantify these effects of predators on prey, and offer an excellent model system for studying transgenerational effects (Barry 1999). Notably, many species of water fleas (*Daphnia*) respond to the presence of predators by producing defense structures (head and tail spines) and altering life history traits such as age of maturity, size at maturity, and clutch size of an individual (development rate, no. of offspring) (Stibor 1992; Riessen 1999). These phenotypic changes are presumed, to be an adaptive response to enhance the probability of survival for the prey, or by shifting the preys life history traits to maximize fitness (Weider and Pijanowska 1993). Since predator influences strongly affect life history characteristics among individual *Daphnia*, it may be inferred that previous generations may affect later generations (Santangelo et al. 2010). This is important because *Daphnia* exhibit cyclical parthenogenesis whereby they alternate between bouts of asexual and sexual reproduction (Fleisher 1996). This characteristic is a crucial to the use of *Daphnia* in studying cross-generational effects because this means that any variations in cross-generation responses produced by asexual reproduction are unambiguously produced by plasticity (Walsh 2014). Variation in clonal responses to environmental stimuli cannot be induced from selection, recombination or genetic drift (Walsh 2014)

This study will test the influence of invertebrate predator cues on transgenerational responses using clones of *Daphnia ambigua* from lakes in Connecticut. *Chaoborus americanus* are tactile, gape-limited invertebrate predators of *Daphnia* (Havel and Dodson 1984). *Daphnia* are well known to respond to the presence of *Chaoborus sp.* by growing faster, delaying maturation, and producing fewer offspring (Riessen 1999). Here, it is important for *Daphnia* to attain growth as quickly as possible and attain a size larger than can be consumed by the gape-limited invertebrate predators. Given that predators strongly influence the expression of life history characteristics within a single generation, it follows logically that this predator may have the potential to induce life history shifts that span multiple generations (see also Santangelo et al. 2010). I tested this hypothesis by rearing *Daphnia* in a common environment for multiple generations and then exposing *Daphnia* to invertebrate predator cues and tracking the potential transgenerational effects of predator on prey for three experimental generations.

Figure 1.2: *Chaoborus americanus*

Since life history traits have a direct connection to rates of population growth, improved understanding of how *Daphnia* respond to environmental stressors across generations may improve our ability to predict the productivity of aquatic ecosystems. The implications of this project also extend beyond the ecological properties of populations and communities. Research indicates that transgenerational effects influence host-pathogen interactions, the formation of disease epidemics, and the transmission of prominent diseases in humans. Not only that, but phenotypic plasticity can increase the environmental tolerance of an organism and this might be an imperative factor for determining the temporal and spatial distribution of species (Lynch and Gabriel). Thus, a better understanding of cross-generational effects in *Daphnia* may significantly enhance research on a diverse array of complex interactions in the natural world.

CHAPTER 2

METHODOLOGY

2.1 Experimental Design

This experiment tested the influence of invertebrate predator cues on transgenerational plasticity using a single clone of *Daphnia*. The clone used in this experiment was originally collected from Dodge Pond in Connecticut. An Ekman grab was used to collect sediment in August 2009. This clone was hatched from a sexual resting egg (ephippia) in the Walsh lab at UTA. This clone was then maintained for several months with frequent replenishment of media and resources. Prior to the start of the experiment, 15 neonates were collected and reared individually in a 90 ml jar containing COMBO media and fed sufficient quantities of *Scenedesmus obliquus* (Concentration: >0.8 mg C L^{-1} day^{-1}) (Kilham et al. 1998). These individuals were reared under common temperature (18°C) and photoperiod regimes (Photoperiod: 14L: 10D).

Figure 2.1: Dodge Pond

The individuals were then reared for two generations under common laboratory settings similar to those stated earlier (temperature, photoperiod, food quantities). A generation refers to period of time between the birth of parents and the birth of the offspring. To begin the second laboratory generation, two newly born neonates from the second clutch (number of offspring borne) were transferred. All clones were transferred to fresh media (COMBO) every other day (Kilham et al. 1998). The density of individuals was reduced to one individual per container on day 3. These individuals experienced the same conditions (temperature, photoperiod, food quantities) and frequency of food/media replenishment (every day) as the previous generation.

The common garden experiment begins with third generation laboratory reared individuals; the experiment began by collecting 30 newly-born individuals (<12 hours old) and individually placing them into 90-ml jars containing COMBO media (Kilham et al. 1998). Each individual was randomly assigned to one of three treatments: (1) predator (PPP), (2) predator removal (PNN), and (3) no predator (NNN). For the 'predator' treatment, 10 individuals received conditioned water containing invertebrate predator kairomones changed every other day for the entire experiment. The 'predator removal' treatment received predator cues during the first experiment generation only (i.e., F_0 generation). The 'non-predator' treatment never received predator chemical cues. Each treatment was replicated 10 per generation and the experiment ran for a total of three generations (F_0, F_1, and F_2). The experimental conditions were the same as described above (temperature= 18°C, photoperiod = 14L: 10D). To make sure there was no contamination across treatment generations, glassware, pipettes, and dishes for predator and non-predator were separated and cleaned every day. Each treatment was replicated two more times per generation. Ninety jars were created and changed in the entirety of this experiment.

Figure 2.2: Jars are independently assigned into 1 of 3 different treatments: PPP, PNN, NNN.

To quantify life history traits, *Daphnia* were evaluated daily for maturation (defined as the release of the first clutch into the brood chamber). Maturation in *Daphnia* is typically defined by the release of the first clutch of offspring into the brood chamber. Upon maturation, all individuals were also photographed for estimates of size at maturation. All individuals were subsequently monitored daily for the production of clutches 2 and 3. To initiate the F_1 generation (and subsequent generations),newly born (<12 hours) individuals from the second clutch of each jar were collected and placed into a new jar containing fresh media (COMBO) and algae (and kairomones when appropriate) (Kilham et al. 1998). Timing of maturation and production of offspring were monitored using the same procedures as described above. These same protocols were used for the establishment and subsequent monitoring of the F_2 generation.

2.2 Invertebrate Predator Cue Collection

Invertebrate predators' chemical cues (kairomones) were isolated from frozen phantom midge larvae (*Chaoborus americanus*). These chemical cues were purchased for the experiment. The protocol for generating chemical cues from frozen *Chaoborus* is well established (Hebert and Grew 1985). In general, 1g of *Chaoborus* was boiled in 400ml of water for 10 minutes. This solution was then ultra-filtered through a series down to a pore size of 0.1µm. Over the course of the experiment each container of *Daphnia* in the predator treatments received 200µm (micrometer) of *Chaoborus* kairomones. Fresh kairomones were added to the predator jars every other day.

CHAPTER 3

RESULTS

3.1 Clutch Size

The results show that exposure to invertebrate predator cues significantly altered clutch size across generations (Fig. 3.1-3.2). Offspring from all clutches (C_1, C_2, and C_3) are averaged and compared to see the effect of offspring production across generations 1, 2, and 3 (Fig. 1-2). Overall, there was a significant effects of predator cues on clutch size ($F_{2, 79} = 11.76$, $p<0.001$) as *Daphnia* exposed to predators (PPP or PNN) produced fewer offspring than *Daphnia* reared in the absence of predators (NNN) (Fig. 3.1). NNN showed the largest average of offspring with approximately 26 total offspring produced across all three generations (Fig. 3.1). PNN has the second largest average of offspring produced out of the three treatments with approximately 20 total offspring produced total (Fig. 3.1). PPP had the lowest number of offspring produced out of the three treatments with approximately 15 total offspring produced.

These differences among predator treatments depended upon the duration of exposure to predators as it was also observed a significant predator x generation interaction for clutch size ($F_{4,79} = 2.65$, $p=0.039$) (Fig. 3.2). In generation 1, *Daphnia* reared in the absence of predators produced more offspring than the predator treatments (Fig. 3.2). Continued exposure to predator cues (i.e. PPP) led to progressive declines in reproductive outputs in generation 2 and 3 (Fig. 3.2). Conversely, the clutch sizes of

Daphnia exposed to predator cues for one generation only (i.e., PNN) increased in generation 2 and 3 to match those observed in the NNN treatments (Fig. 3.2).

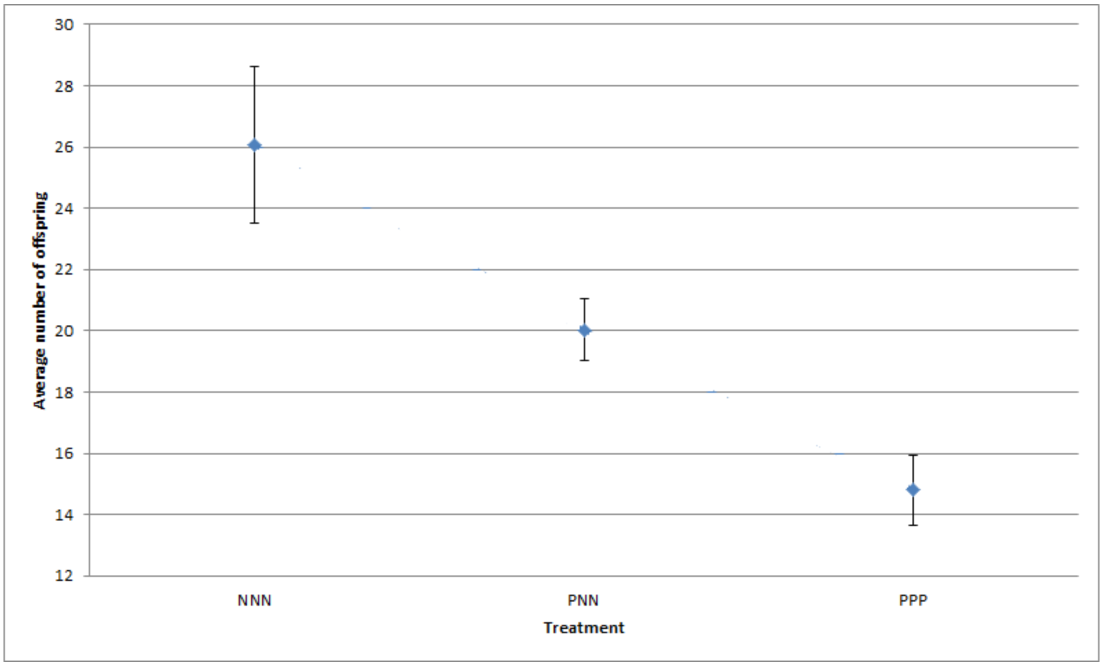


Figure 3.1: Clutch size (average number of offspring produced) across three different treatments: NNN, PNN, and PPP

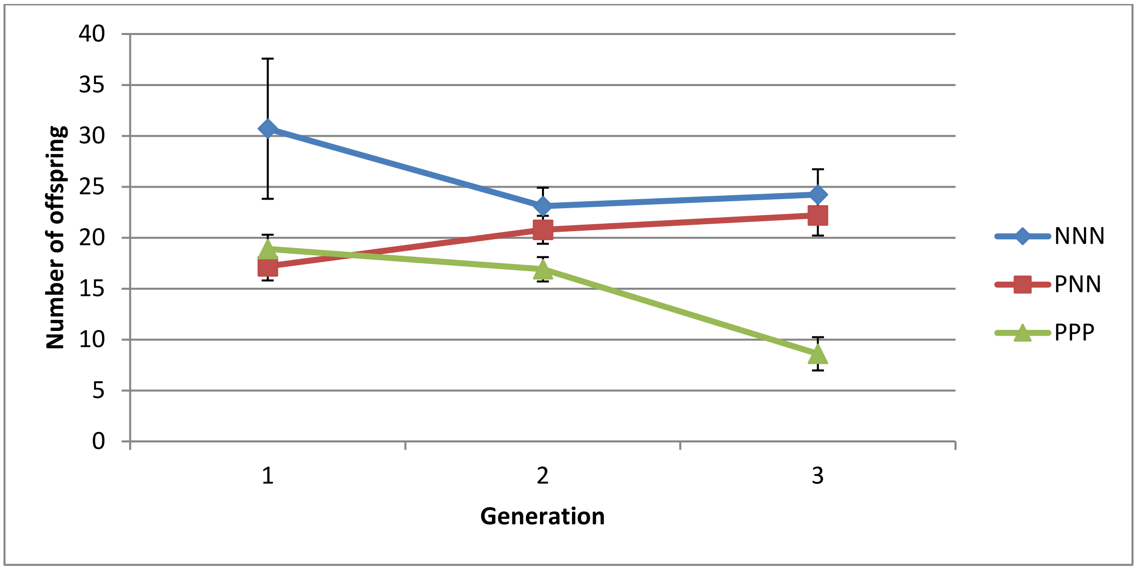


Figure 3.2: Clutch size (average number of offspring produced) across three generations: 1, 2, and 3

3.2 Size at Maturation

The results revealed non-significant ($p>0.05$) effects of predator cues on size at maturation. The overall predator treatment ($F_{2, 79} = 1.07$, $p=0.35$) and predator x generation $F_{4, 79} = 1.65$, $p=0.17$) terms were both non-significant.

3.3 Age at Maturation

Exposure to predator cues significantly altered the timing of maturation (Fig. 3.3-3.4). The higher the marginal mean of age, the longer it takes for the individual to mature. The results show that the overall effect of predators was significant ($F_{2, 79} = 5.74$, $p=0.005$) as *Daphnia* exposed to predator cues for three consecutive generations matured slower than the other two treatments (Fig. 3.3). PPP had an average age of maturation around 9.8 days (Fig. 3.3). PNN had an average age of maturation around 8 days (Fig. 3.3). NNN had an average age of maturation around 9.2 days (Fig. 3.3). A significant predator times treatment interaction ($F_{4, 79} = 3.66$, $p=0.009$) was observed. Here, small differences were observed among all treatments in generations 1 and 3. All treatments experienced similar times of maturation of approximately 6 to 8 days (Fig 3.4). However, the two predator treatments exhibited divergent responses to predator cues between generations 1 and 2 (Fig 3.4). PPP delayed maturation to a much greater extent than either PNN or NNN (Fig 3.4).

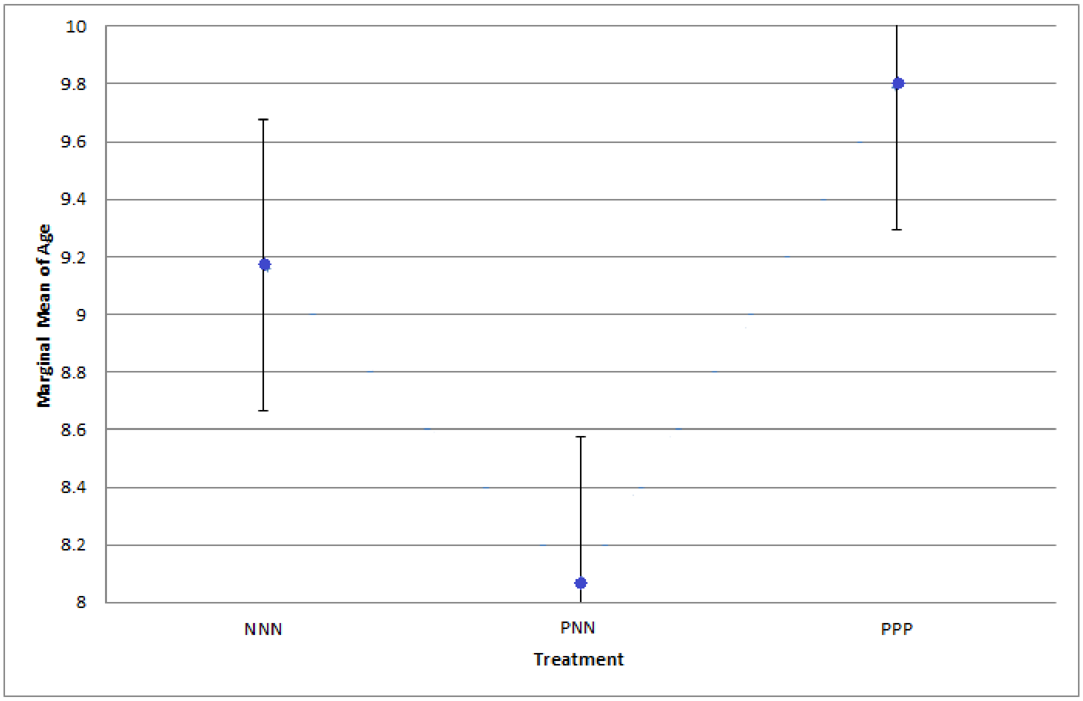


Figure 3.3: Averaged age of maturation in *Daphnia* across three different treatments: NNN, PNN, and PPP

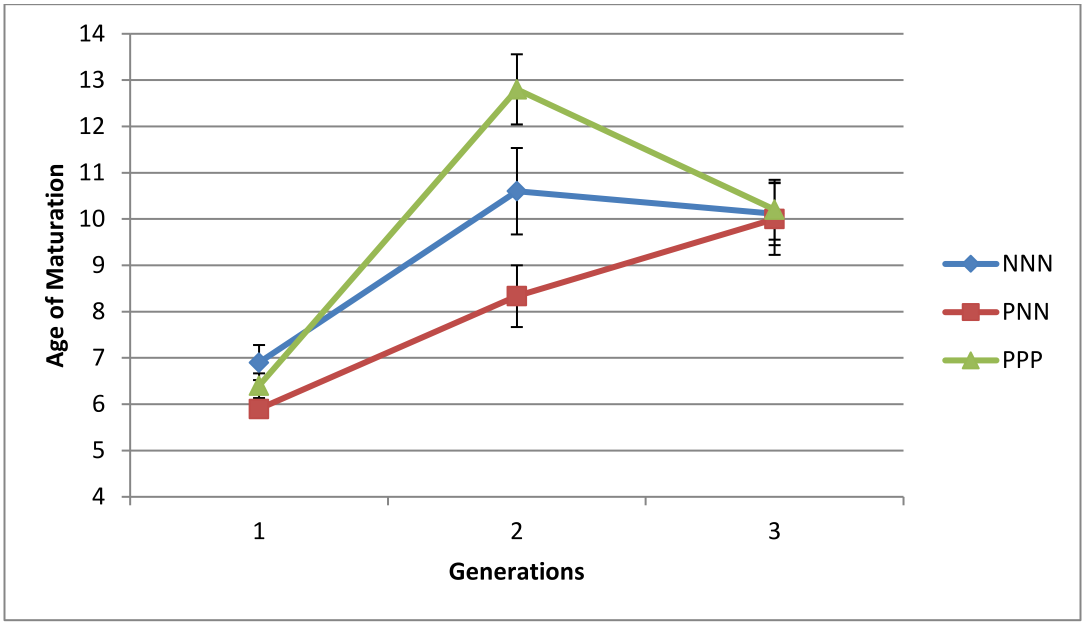


Figure 3.4: Average age of maturation in *Daphnia* across three different generations: 1, 2, and 3

CHAPTER 4

DISCUSSION

Chaoborus sp. is a gape-limited predator that consumes small size classes of *Daphnia*. Previous work has thus shown that *Daphnia* typically respond to the threat of predation by invertebrates by growing faster but reducing allocation to reproduction (Riessen 1999). It can be determined here that exposure to *Chaoborus* induces phenotypic responses in life history traits that persist for multiple generations. Predator-prey relationships cause drastic environmental stress on *Daphnia*. With that being said, when environmental conditions are lower than optimum, *Daphnia* will allocate their resources to improve their fitness.

This study has provided evidence of a transgenerational effect of invertebrate predator cues allocated to reproduction of *Daphnia.* Overall, *Daphnia* reared in the presence of predators in general produced fewer offspring than those not exposed to predator cues. Furthermore, *Daphnia* responded to continue exposure to predators (PPP) by decreasing of the size of their clutches. Conversely, *Daphnia* that experienced one generation of cue exposure initially produced smaller clutches than non-predator (NNN) treatments in generation 1. Yet allocation to reproduction quickly rebounded in generation 2 to match those of non-predator treatments. In contrast with allocation to reproduction, the results for size at maturation did not differ among treatments while those for age at maturation were equivocal. That is, a consistent association between predator exposure and the timing of maturation was of uncertain significance.

Since life history traits have a direct connection to rates of population growth, improved understanding of how *Daphnia* respond to environmental stressors across generations may improve our ability to predict the productivity of aquatic ecosystems. *Daphnia* are a key organism in the aquatic food chain. *Daphnia* take nutrients from algae and pass these nutrients to predators that prey on them in the environment. Not only are *Daphnia* consumed by *Chaoborus*, but they are also consumed by fish, lizards, and other aquatic insects. This makes them an important prey for many organisms found in the water. If *Daphnia* were removed from the ecosystem, disastrous effects may affect the freshwater food chain as well as all other associated studies. The implications of this project also extend beyond the ecological properties of populations and communities. Research indicates that transgenerational effects influence host-pathogen interactions, the formation of disease epidemics, and the transmission of prominent diseases in humans (Weider and Pijanowska 1993). Not only that, but phenotypic plasticity can increase the environmental tolerance of an organism and this might be an imperative factor for determining the temporal and spatial distribution of species (Lynch and Gabriel). Thus, a better understanding of the duration of cross-generational effects in *Daphnia* may significantly enhance research on a diverse array of complex interactions in the natural world.

Future implications to further this investigation on transgenerational effects should consist of changes in prey, predator, clone type, and lake sample. *Daphnia* was used in the experiment because they are model organisms for biological studies. *Daphnia* can still be used to further this study by using several other species of *Daphnia* and repeating the experiment. *Daphnia* vary in size, length, and living conditions. By manipulating other species, accuracy of this experiment can be determined. This applies changing the predator type, clone type, and lake sample as well.

APPENDIX A

ANOVA STATISTICS

Tests of Between-Subjects Effects

Dependent Variable: Age

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	417.266[a]	8	52.158	13.348	.000
Intercept	7157.342	1	7157.342	1831.715	.000
Generation	310.052	2	155.026	39.674	.000
Treatment	44.886	2	22.443	5.744	.005
Generation * Treatment	57.270	4	14.317	3.664	.009
Error	308.689	79	3.907		
Total	7890.000	88			
Corrected Total	725.955	87			

a. R Squared = .575 (Adjusted R Squared = .532)

Tests of Between-Subjects Effects

Dependent Variable:C1+C2+C3

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2936.478[a]	8	367.060	4.664	.000
Intercept	36154.800	1	36154.800	459.385	.000
Generation	227.114	2	113.557	1.443	.242
Treatment	1850.975	2	925.488	11.759	.000
Generation * Treatment	834.097	4	208.524	2.650	.039
Error	6217.511	79	78.703		
Total	45199.000	88			
Corrected Total	9153.989	87			

a. R Squared = .321 (Adjusted R Squared = .252)

Tests of Between-Subjects Effects

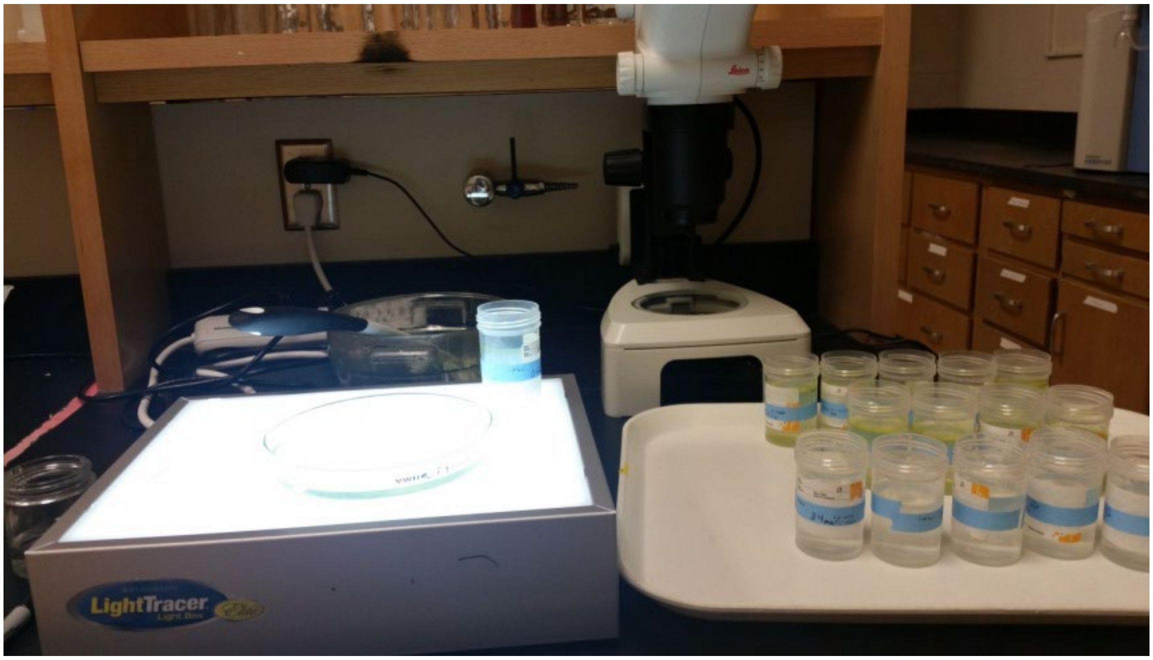
Dependent Variable: Area

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5.485[a]	8	.686	1.180	.321
Intercept	2526.313	1	2526.313	4349.237	.000
Generation	.623	2	.311	.536	.587
Treatment	1.242	2	.621	1.069	.348
Generation * Treatment	3.822	4	.956	1.645	.171
Error	45.888	79	.581		
Total	2578.206	88			
Corrected Total	51.373	87			

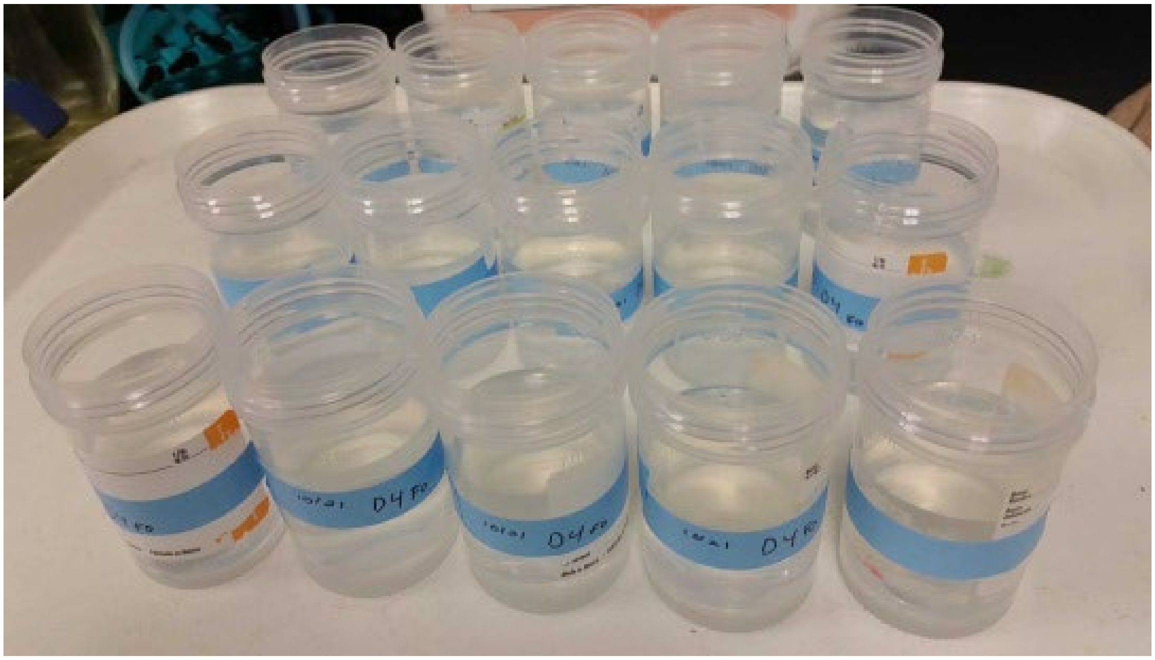
a. R Squared = .107 (Adjusted R Squared = .016)

APPENDIX B

EXPERIMENTAL DESIGN



Daphnia jars are labeled, changed, and data is recorded every other day.



Common laboratory experiment jars are created from D-6 clones.

Scenedesmus obliquus (Concentration: >0.8 mg C L^{-1} day^{-1}) is supplemented to COMBO food and is a constant factor through the entirety of this experiment (Kilham et al. 1998).

Daphnia is traced using Image#J to measure its area relative to a set scale.

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BIOGRAPHICAL INFORMATION

Jennifer is in her senior year at the University of Texas at Arlington where she is pursuing an Honors Bachelor of Science degree in Biology as well as minors in Psychology and Chemistry. Jennifer has been a member of the Honors College since her freshman year at UTA. She has been active in several organizations at UTA including UTA Volunteers and the Vietnamese Student Association. She came into Dr. Walsh's lab during her junior year and has been researching in the lab ever since. Under Dr. Walsh, she studies how predator-prey relationships can cause transgenerational responses on *Daphnia ambigua* as well as how certain food treatments can cause life history changes in this aquatic species. She has gained valuable research experience as well as an understanding of ecology and evolution to incorporate into her future studies. After graduation, she plans on attending veterinary school in order to pursue her dream of working as a wildlife veterinarian.