

THE INFLUENCE OF PREDATOR DETECTION ON LIFE HISTORY
STRATEGIES IN *CERIODAPHNIA RETICULATA* (CLADOCERA:
DAPHNIIDAE)

A Thesis Presented
to the
Graduate Faculty of Biology
Eastern New Mexico University

In Partial Fulfillment
of the Requirements
for the Degree
Master of Science

by
Irene M. Roselli
25 January 2008

Certificate of Acceptance

Final Copy

This Thesis Presented in Partial Fulfillment of the Requirements

for the Degree

Master of Science

by

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by the

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Commencement Date

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Abstract of a Thesis

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ABSTRACT

The energy used in a reproductive effort or growth of an individual can be evaluated as either an investment into a current reproductive event or potentially future reproductive event(s). These changes are reproductive tradeoffs, where more energy is allocated into one aspect of reproduction (e.g., egg size) while less energy is allocated into another aspect of reproduction (e.g., number of eggs per clutch) or growth.

I conducted an experiment investigating the influence of predator kairomones on life history characteristics of the water flea, *Ceriodaphnia reticulata*. My objectives were to evaluate changes in energy allocation in the presence of predator kairomones to population size (i.e., growth or decline), investment in body size and/or armament (spines), and egg investment (number of eggs in brood chamber). I hypothesized that the energy budget of an organism can be partially regulated by risk assessment.

Water fleas were allowed to grow in culture with predator kairomones. My experiment employed a basic two x two factorial design but added a control that was not originally considered as a level within either factor. Factors tested were kairomone concentration (two levels: high and low) and predator type (two levels: backswimmer and fish). Each treatment combination and the control were replicated 20 times. Measurements consisted of population counts, morphometric data, and egg counts. Two-factor ANOVAs were used whenever possible, otherwise a nonparametric single-factor ANOVA (Kruskal-Wallis test) was used to test for significant differences in means across all data sets.

The responses of all four treatments were interpreted in terms of reproductive strategies. Fish-high and fish-low treatments demonstrated a current investment strategy by increasing egg counts but not morphology. The backswimmer-high treatment demonstrated a

future investment strategy by changing body size but not shape (width: length). The backswimmer-low treatment demonstrated no response. All factors lacked the ability to influence any change in life history characteristics for these individuals. None of the four treatments showed a mixed strategy response. Furthermore, that differential responses were seen within predator type (e.g., high and low fish) demonstrates that the water fleas assess not only the presence of a predator but also the level of threat.

Understanding the exact delivery mode of kairomones, their viability in water, and how their presence leads to the modification of life history characteristics is important in the understanding of an organism that is important to the sustainability of a habitat. My study on the influence of predator detection on life history characteristics in *C. reticulata* is the first to look at these changes in the presence of two types of predators. I found that *C. reticulata* a) responds to fish kairomones by increasing egg counts without accompanied morphological changes, b) responds to high levels of backswimmer kairomones by neither increasing nor decreasing egg counts with accompanied morphological changes, and c) does not respond to low levels of backswimmer kairomones with either an increase or decrease in egg counts or morphology.

ACKNOWLEDGMENTS

I would like to take this opportunity to thank my advisor Dr. Marv Lutnesky for his outstanding efforts and patience during the course of this project. He helped make this project rigorous and successful. We have spent countless hours discussing testable questions, logistical problems, deadlines, and research opportunities. Without these valuable discussions I would have never reached my goal. My committee members, Dr. Gregory Keller, Dr. Darren Pollock, and Dr. Kenwyn Cradock have all played an integral role in the completion of this study. In their own unique ways, each has provided insight and assistance throughout the course of my project. Without their help, many parts of this project would be missing. In addition, Dr. Antonio Gennaro, an Eastern New Mexico University (ENMU) emeritus faculty member, has been a friend and mentor to me during my time at ENMU.

My parents, Vince and Kathy Roselli, have gone above and beyond the requirements of parents in order to raise a successful child. Without the support and confidence they have provided throughout my life, I would never have made it this far in my academic career. They have allowed me to pursue my dreams and challenge myself to do the best I can. My sister Annette and my brother Michael have been of great inspiration in their own unique ways. Each has provided me with support and confidence in my abilities to succeed. Furthermore, my best friend Amber Sharrar has been the best friend you could ask for since the beginning of my college career. Even though she is miles away, I have always been able to count on her to be supportive, a good listener, and give me advice when necessary.

I would like to thank Niki Harings for being a true friend and laboratory partner. Despite the intensity of our projects, we always managed to find a few hours to laugh, watch all three seasons of Grey's Anatomy, and spend several evenings sitting around a campfire.

Niki provided a listening ear and troubleshooting with my project. Without her presence in the laboratory, the numerous hours I spent in Roosevelt Hall 210 would have seemed endless. My two laboratory assistants, Stephanie Schmuck and Cassie Brooks, were extremely helpful in working out the logistical problems I encountered during my project. Their enthusiasm made it possible for me to complete my data collection. A New Mexico WRI Student Water Research Grant provided much needed funding for which I am very grateful. Without that group, this research would not have been possible.

Last but not least I would like to acknowledge my friend and companion Jonathan Dunbar. He has shown me the true meaning of support and patience. Despite my countless hours in the laboratory and library, he has always been there ready and willing to assist me in any way possible. His confidence in my abilities and our relationship has assisted me to accomplish more than I could have hoped. I owe so much to his compassionate personality and ability to plan amazing road trips, both of which have proved to be invaluable over the last eight months. I am looking forward to the times we still have yet to encounter together and many more great road trips!!

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INTRODUCTION

I start with a brief review of the hypotheses proposed for predation by vertebrates and invertebrates in aquatic habitats because many of these ideas are investigated during the experiment presented here. Next, I present hypotheses regarding predator kairomones, their occurrence in aquatic habitats, and the direct effects they have on prey species. Next, I present hypotheses regarding the occurrence of new defense mechanisms and adaptations in the presence of predatory kairomones. Finally, I present hypotheses on the occurrence of life history characteristics that are changed in the presence of predatory kairomones and the specific objectives of my study.

Predation

Predation can influence the abundance, size, composition, behavior, diversity, morphology, and reproductive output of prey species both directly and indirectly (Abjornsson, Bronmark & Hansson, 2002; Black, 1993; Dahl & Peckarsky, 2002; Dodson, 1970; Lehman & Campbell, 2007; Murdoch, Scott & Ebsworth, 1984; Rose, Warne & Lim, 2003). Predators play an important role in the ecology of zooplankton (e.g., planktonic cladocerans), especially concerning community structure, functional morphology, life history characteristics, and distribution (Dodson & Havel, 1988; Hanazato, 1990; Vijverberg & Vos, 2006). The impact of predation on a zooplankton community depends on the type of predators: vertebrate predators reduce populations of large-bodied prey species while invertebrate predators reduce populations of small-bodied prey species (Chang, Nagata & Hanazato, 2004; Gliwicz & Umana, 1994; Lane, 1979). Zooplankton communities exposed to a combination of vertebrate and invertebrate predators could potentially produce prey

species of an intermediate size, less vulnerable to predation (Lynch, 1980).

Aquatic habitats (i.e., lakes and ponds) maintain extensive species diversity, enabling them to provide unique examples of complex predator-prey systems. All predator-prey relationships are based on a balance between attack rates and defense strategies (O'Brien, 1979; Young & Riessen, 2005). The majority of predation occurring in aquatic habitats is through size-selective predation, where prey items are selected for consumption because predators are either gape-limited or size-dependent. Gape-limited predators can only consume prey items that will fit into their mouth and easily be swallowed, while size-dependent predators have morphological characteristics used to chew and breakdown food items that limit the size of potential prey (Scott & Murdoch, 1983). Prey species that allocate less energy into body size in the presence of predators actually increase their vulnerability to predation due to the occurrence of gape-limited predation which will increase mortality in small egg-carrying individuals (Hulsmann *et al.*, 2004).

Size-selective predation influences prey species populations by altering the size and age structure, yet the exact mechanism is not completely understood (Weider & Pijanowska, 1993). Field studies have shown that size-selective predation by fishes can decrease cladoceran population sizes and cause a shift in body size from large to small, as well as cause the first reproduction to occur at a smaller size (Brett, 1992; Vanni, 1987). Size-selective predation could possibly favor species with a more functional phenotypic response (Lampert, 1993). In an environment in which size-selective predation occurs on zooplankton by a vertebrate predator, it could be advantageous to undergo early maturation at a smaller body size (Rose, Warne & Lim, 2001a).

It is predictable from the optimal foraging theory that predators should forage and

consume prey items that will minimize the rate of energy expended (Macchiusi & Baker, 1991). From the predators' perspective, the consumption of egg-carrying individuals will be energetically more profitable, due to the added nutrients and energy the eggs provide (Hulsmann *et al.*, 2004). Physical consumption of a prey item does not completely determine the damaging effect predation has on a prey species because initial prey attacks can sometimes leave prey injured and eventually dead (Chang & Hanazato, 2003). The consequence of predation is based on the intensity and selectivity level at which it occurs (Dodson, 1970). It has been proposed that if the perception of predation risk is high, then prey should develop faster and invest less in growth (Ball & Baker, 1996).

Predator type

Vertebrate and invertebrate predators naturally coexist in many aquatic systems, yet the occurrence of vertebrate predators (i.e., fishes) often affects zooplankton communities indirectly by regulating the abundance of invertebrate predators (i.e., insect larvae) (Chang *et al.*, 2004). Compared to their vertebrate predators, zooplankton are small and usually hard to locate which makes prey location an important aspect for vertebrate predators (O'Brien, 1979). Aquatic community structures that involve the co-occurrence of planktivorous fishes and zooplankton species are common (Rose, Warne & Lim, 2001b). Most planktivorous fishes prefer to feed on *Daphnia* (Cladocera: Daphniidae) species which makes this predator-prey relationship evolutionarily old (Machacek, 1993). Smaller cladocerans (e.g., *Bosmina*) appear to be adapted to survive in lakes and ponds inhabited by vertebrate predators (Lynch, 1980). It has been hypothesized that in an environment where predation by fishes is prevalent, the prey population will consist of small-bodied individuals that produce even

smaller offspring (Boersma, 1995).

In sharp contrast to vertebrate predators, there are a variety of phyla and classes (e.g., Arthropoda and Insecta) to which invertebrate predators belong, and these may have different effects on zooplankton communities (Hanazato, 1990). In an environment that completely lacks fishes, the abundance of aquatic insects and larvae that occupy the littoral zone increases and this can have a damaging effect on zooplankton populations (Scott & Murdoch, 1983). Zooplankton are desirable prey items for invertebrate predators because predator and prey are similar in size and experience a high occurrence of predator-prey encounters (Young & Riessen, 2005). Invertebrate predators are having a large impact on the evolution of cladoceran life histories (Weider & Pijanowska, 1993). Predation on cladoceran species (e.g., *Daphnia*) by invertebrates is partly determined by the strength of the prey exoskeleton that determines if the prey is consumable (Caramujo & Boavida, 2000).

Most invertebrate predators seem to prey upon small-bodied zooplankton species due to the lack of intense morphological defense structures (Riessen & Young, 2005). However, there are exceptions to this common observation. For example, the backswimmer *Notonecta* sp. is a common aquatic invertebrate that preys upon species of *Daphnia* and *Ceriodaphnia*, showing preference for the larger bodied individuals of each species (Black, 1993; Murdoch & Scott, 1984). Murdoch and Scott (1984) have shown that larger *Notonecta* species have a higher survival rate (i.e., larger growth, faster development, and increased reproduction) when fed *Daphnia* compared to *Ceriodaphnia* due to the relative size of each prey species.

When predation by aquatic invertebrates has been investigated, several different morphological characteristics (i.e., body shape, carapace pigmentation, and mode of locomotion) beyond body size have been used to describe the adaptations prey species

undergo in these invertebrate-only habitats (Gliwicz & Umana, 1994). Adult zooplankton, when in the presence of an invertebrate predator that feeds on large instars, may be at an advantage to have a smaller, less detectable body size (Luning, 1992).

Predator kairomones

Chemical cues dispersed in aquatic habitats provide individuals with information on local environmental conditions that influence food availability, reproductive output, and survival (Turner, Bernot & Boes, 2000). Both vertebrate and invertebrate predators release water-borne chemicals to which prey have adapted, called kairomones, which can stimulate changes in body size and maturation time of zooplankton (Gliwicz, 1994; Hanazato, 1995; Rose *et al.*, 2003).

Kairomones are a good example of influential chemical cues because they are a direct byproduct of the predator (Sakwinska, 1993). They provide beneficial information to the receiver (i.e., location of predator and intensity of predation risk) and lack beneficial qualities for the releaser (Abjornsson *et al.*, 2002; Hanazato, Fueki & Yoshimoto, 2001). Kairomones can also inform the receiver of a releaser's diet (Dalesman *et al.*, 2006). The exact chemical composition of kairomones is unknown, but it has been determined that they are composed of low molecular weight, water soluble, non-volatile substances that can persist in environments fluctuating in temperature and pH (Rose *et al.*, 2001b).

Any changes a prey species undergoes due to the exposure of predator kairomones is called chemomorphosis (Dodson & Havel, 1988). In cladocerans, responses brought on by kairomones have been shown to be beneficial at the population level because they lead to faster growth rates (Hanazato, 1995).

Chemical cues can negatively impact the life history characteristics of prey species in such a way that a change could be seen in survival time, maturation time, and body size at maturation and even an increase in energy allocated to a single reproductive event (Rose *et al.*, 2001a; Vijverberg & Vos, 2006). Potentially, these kairomones could have a stronger impact on resource allocation during the early stages of development that could cause a greater overall reproductive effort (Weider & Pijanowska, 1993). Kairomone exposure in different concentration levels has been shown to produce variable changes in genetically identical organisms (Sakwinska, 1993).

Most *Daphnia* species are capable of modifying characteristics such as behavior, morphology, and life history traits in response to predatory kairomones (Hulsmann *et al.*, 2004). The presence of kairomones has led to dramatic life history characteristic changes such as lower growth rates, reduced maturation time and size, smaller offspring, and an increase in clutch size in several *Daphnia* species (Machacek, 1993; Hanazato *et al.*, 2001). In a laboratory study, *Daphnia pulex* produced several modifications in the physical presence of an invertebrate predator such as a smaller body size, a truncated development time, and no change to clutch size (Dodson & Havel, 1988). Furthermore, it has been shown that a greater proportion of energy has been allocated to reproduction than somatic growth in cladocerans (e.g., *Ceriodaphnia cf. dubia*) when in the presence of fish kairomones (Rose *et al.*, 2001a).

Defense mechanism adaptations

Predators usually attack and consume those prey species that have few antipredator defense mechanisms which complicates the process of prey selection (Chang *et al.*, 2004). The adaptation of different defense mechanisms (i.e., changes in morphology, behavior, and

life history) is common among zooplankton species that coexist with both vertebrate and invertebrate predators (Riessen, 1999). Some zooplankton populations can live in the presence of predators and avoid predation by undergoing adaptations that will compensate for the destabilizing effect of the predator (Murdoch *et al.*, 1984). However, when zooplankton populations are located in areas of mixed predators, adaptations that decrease vulnerability to one predator type may increase prey vulnerability to another predator type (Abjornsson, Hansson & Bronmark, 2004).

In order for prey species to remain present in areas containing predators, they must employ a method of predator detection so that an appropriate defense mechanism can be established (Machacek, 1993). The development and implementation of defense mechanisms are assumed to be energetically costly to an organism and can lead to a reduction in other life history characteristics such as reproduction and survival (Abjornsson *et al.*, 2002; Tollrian, 1995). Fluctuations in the number and size of offspring produced due to predation pressures are categorized as adaptive responses (Rose *et al.*, 2001b).

To evade a predator, prey species engage in several defense tactics such as vertical migration, small body size, and translucent carapaces (O'Brien, 1979). Defense mechanisms that are induced mainly by the presence of predators usually involve modifications to life history and morphology, all of which have been documented in several plant and animal species (Luning, 1992). An organism can reduce the energetic costs experienced with the adaptation of defense mechanisms by utilizing reliable cues that would warn the organism of the presence of a predator (Riessen, 1999). Prey species respond to costs and benefits at the individual level of antipredator responses, i.e., the responses are honed by selection (Relyea, 2001). Defense mechanisms are not changed or modified independently, but are part of a

suite of adaptations that occur under a particular set of environmental conditions (Riessen & Young, 2005).

An adaptation to a new antipredatory strategy comes with trade-offs, i.e., a change in a behavior or characteristic that increases one component of an organism's fitness while at the same time decreasing another component of fitness which occur when a new defense mechanism is created and implemented (Black, 1993; Losos, Schoener & Spiller, 2004). Four prerequisites have been hypothesized to occur during the evolution of antipredator defenses: i) prey species must be able to detect the presence and location of a predator without visual cues, ii) defense mechanism must be effective at avoiding predation, iii) the occurrence of predation pressure must be intense for at least a short period of time, and iv) the adaptations made to the defense mechanism must be costly (i.e., either reproductively or morphologically) (Dahl & Peckarsky, 2002). Adaptations that occur due to the exposure of cues can be classified as conditional switches (i.e., developmental changes between discrete morphologies in an individual) (Adler & Harvell, 1990).

Life history characteristics

The criteria an individual imposes on the decision regarding how much energy needs to be allocated to growth and reproduction are two main concepts of life history evolution (Stibor & Luning, 1994). The theory behind life history suggests that an increase in reproductive output comes at the cost of adult survival rates due to maturation at an earlier age (Reznick, Bryga & Endler, 1990). Life history characteristics such as size at first reproduction (SFR) are critically important in species with fast generation times (Lampert, 1993). Early maturation at a smaller body size would allow an individual to begin producing

offspring before it reached a body size desired by predators (Weider & Pijanowska, 1993).

Life-history shifts and changes are usually assumed to be the result of a genetic interruption or differentiation (Stibor, 1992). A small body size at metamorphosis means that less time was invested in reproduction and more time was invested in increasing developmental rates, all which could be caused by an increased exposure to predators (Ball & Baker, 1996).

Predator-induced changes in life history characteristics are adaptive because only negative impacts on the prey occur in the presence of a predator (Black, 1993). An effective life history adaptation is to reduce body size, produce more clutches, and smaller offspring during an early reproductive episode (Luning, 1992). For example, in *Chironomus tentans* (Diptera: Chironomidae) larvae it has been shown that changes in body size, development rate, and reproductive output are direct consequences of the alternation of behavioral patterns to avoid predation (Ball & Baker, 1996). Observed life history changes are based on these tradeoffs and are used to determine survival and fitness at the individual level (Crowl & Covich, 1990). It could be ecologically advantageous for an individual to increase the number of offspring produced under circumstances of high predation. This would ensure that the maximum number of offspring survive to reproductive age (Rose *et al.*, 2001b).

The overall allocation of nutrients, time, and energy into survival and reproduction is the ultimate mechanism governing the success of an individual (Lynch, 1980). In most animals, maturation rates (i.e., rate of progression toward reproductive maturity) have been shown to have a positive correlation with overall growth rates (i.e., rate of accumulation of body mass) (Ball & Baker, 1996). Hypothetically, the number of offspring produced should

reflect the highest potential fitness of the parents, while offspring survivorship will decrease as the clutch size increases due to offspring competition (Boersma, 1995).

The Order Cladocera (i.e., water fleas) contains egg-brooding species and is thought to have highly conservative resource allocation (i.e., energy spent is dependent on the size and age of an individual), thus making these species model organisms in predator-prey relationships (Stibor, 1992). An overall trend among cladocerans is that neonate size is dependent on temperature, food abundance, maternal resource allocation, and potentially predator presence (Lampert, 1993). All cladoceran species, regardless of habitat occupancy or predation pressure, exhibit iteroparity, a type of reproduction that is favored in situations where offspring survival is low or appears to be unpredictable (Lynch, 1980). This order provides some of the best examples of how natural selection can lead to the evolution of numerous adaptations in the presence of predators (Riessen & Young, 2005).

Objectives

The purpose of this study is to investigate the influence of predator kairomones on life history characteristics of the water flea, *Ceriodaphnia reticulata* (Jurine). The use of this model invertebrate species is advantageous for several reasons: i) they are easily maintained in a laboratory setting, ii) clutch sizes can be observed through the carapace, iii) their behavior is well characterized (Seely & Lutnesky, 1998), iv) several generations can be produced in a relatively short period of time (see below), and v) adaptations in defense mechanisms can be seen throughout the population after a few generations (see below).

My objectives are to evaluate potential changes in energy allocation due to the presence of both vertebrate and invertebrate predator kairomones to i) investment in

population growth, ii) investment in body size and/or armament (spines), and iii) investment in offspring (clutch sizes). I hypothesize that the energy budget of an organism can be partially regulated by risk assessment. For parental individuals, reallocation of energy can be shunted such that it benefits a current reproductive effort, future reproductive effort or both. Change in clutch or egg size might benefit current reproductive effort by increasing the probability of survival of the offspring. And, a change in adult morphology may benefit the potential for future reproduction by increasing the probability of adult survivorship, allowing for future reproductive events.

This study is the first to evaluate the changes in three life history characteristics in *C. reticulata*. The information from this study will be beneficial to the field of conservation biology because a variable scale of threat to a prey species may reveal its survivorship strategy. Such strategies that preserve offspring or adults for future reproduction, or both, may allow us to understand a species response in a disturbed environment.

METHODS

Study organism

The water flea, *C. reticulata* (Figure 1A), is a small-bodied planktonic cladoceran (Lynch, 1980). They are mostly found in freshwater ponds and lakes or shallow bodies of water, occupying the littoral macrophyte zone (Pichlova, 1997). *Ceriodaphnia* species are generally found during the summer season, usually residing in water of 12°C or warmer, but some individuals have been found during the autumn months in water below 10°C (Burgis, 1967). They are influential zooplankton because they aid in the reduction of pelagic algal accumulation through filter feeding, and they provide a significant source of food for planktivorous fishes (Lauridesen *et al.*, 1999).

The body of *C. reticulata* is enclosed in a non-segmented clear to light-yellow carapace (i.e., secreted shell) and typically lacks the presence of a rostrum and tail spine (Pennak, 1978). From a dorsal viewpoint the body is ovoid or disc-shaped but appears round when viewed laterally. Attached to each side of the head is a pair of antennae (i.e., first and second antennae), each differing in size and function. The first antennae are small and used in chemical detection and the second antennae are larger and used for locomotion (Seely & Lutnesky, 1998; Smith, 2001). Even though the small head and large compound eye are common diagnostic features, it is the presence of pecten (6-10 teeth) on the claw that distinguishes this particular species from other *Ceriodaphnia* species (Balcer, Korda & Dodson, 1984).

All thoracic appendages (i.e., legs) are enclosed in the carapace. The number of leg pairs usually ranges between five or six pairs, depending on the species. Each leg is covered with miniature spines used as filters. Feeding occurs when water is pumped into the carapace

opening, passed over the spiny legs (which traps mostly phytoplankton) and passed down to the mouth of *Ceriodaphnia* (Brooks, 1959). They are categorized as both herbivores and detritivores because both bacteria and detritus are main components of their daily food intake (Pichlova, 1997).

To investigate potential changes in life history characteristics, it is important to understand the life cycle and reproductive process of *Ceriodaphnia*. It has a four stage life cycle described as egg, juvenile, adolescent, and adult. The progression into each stage occurs through a molting of the carapace and an intermediate developmental stage (i.e., instar). Molting is when an individual produces a new, larger carapace underneath the existing external carapace and then pulls itself out of the old carapace. The molting process can happen several times during a lifespan under optimal conditions. Both the egg and juvenile stages undergo a single molting and instar. Each molting that occurs after adolescence is followed by the release of hatchlings from the brood chamber. The lifespan of *Ceriodaphnia* is species specific and influenced by temperature, water conditions, and population density. In the laboratory setting, water temperature averaged $26^{\circ} \pm 1^{\circ}$ C and *C. reticulata* had an average lifespan of 22 days (personal observation).

Reproduction occurs mainly through parthenogenesis, meaning that the population is female-biased, and all offspring produced are female. In parthenogenesis, *Ceriodaphnia* undergo asexual reproduction in which subitaneous eggs (i.e., eggs that develop and hatch without a break in development) are produced, characterized by a visible yolk sac and yellow pigmentation inside the brood chamber (Dodson & Frey, 2001). Eggs form inside the ovary and move into the brood chamber upon completion of the maturation phase (Pennak, 1978) and can be easily observed and counted with microscopy (Figure 1B). Inside the brood

A)



B)

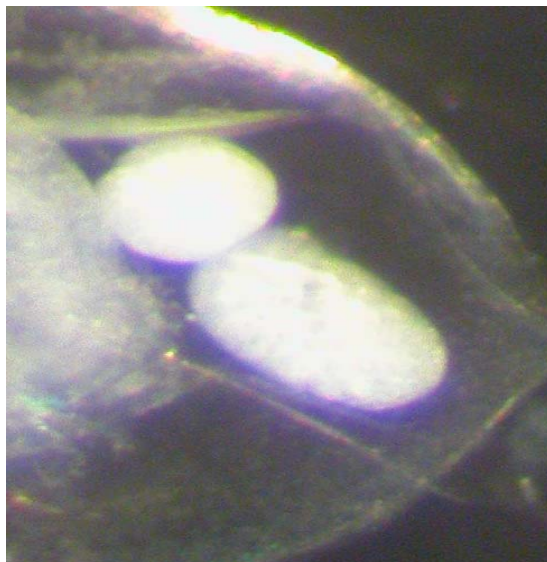


Figure 1. Image of the study organism *C. reticulata*. A) adult form and B) close up of an egg brood chamber.

chamber, eggs develop into neonates and are released following a molting of the female. The exact time that an egg spends developing inside the brood chamber depends primarily on the water temperature (Pennak, 1978).

Collection and maintenance of animals

Ceriodaphnia reticulata were collected in November 2006 from a fishless stock tank at the Melrose Migrant Trap (34°26'0.22"N, 103°48'38.25"W) outside Melrose, New Mexico (Figure 2). Zooplankton samples were collected haphazardly through vertical tows using a 0.150 cm mesh flow-through plankton net. Once collected, samples were placed in several 800 ml containers filled with sieved pond water (0.150 cm mesh) and transported back to the Freshwater Biology Laboratory (34°10'33.32"N, 103°20'48.76"W) at ENMU in Portales, New Mexico (Figure 2).

Water samples were allowed to settle overnight to allow zooplankton to separate out from the detritus collected. Organisms were separated and identified by adding serial 10 ml samples into a Petri dish and placing it under a 20x-dissecting microscope (Olympus SZ-ST). Individual adult (i.e., egg-bearing) *C. reticulata* were removed using a 10 ml pipette and placed into separate 250 ml containers, each with aged water (i.e., aerated tap water aged two days) to produce pure cloned cultures. Identification of *C. reticulata* followed Pennak (1978) and was confirmed by zooplankton specialist J.E. Havel (Missouri State University, personal communication).

After cloned cultures were stable, eight 800 ml containers were designated as stock cultures using aged water, and the clone cultures were distributed haphazardly between them. Cultures were maintained using a diet of daphnia food (Appendix A) and temperature was

held at $26^{\circ} \pm 1^{\circ}$ C using a thermostat. Once a week stock culture jars were cleaned and topped off using aged water.

Twenty green sunfish, *Lepomis cyanellus* (Rafinesque), were collected in June, July, and August 2007 from Green Acres Lake ($34^{\circ}25'3.93''\text{N}$, $103^{\circ}12'24.32''\text{W}$) in Clovis, New Mexico (Figure 2) and a private ranch ($34^{\circ}56'17.09''\text{N}$, $104^{\circ}40'55.15''\text{W}$) outside Santa Rosa, New Mexico (Figure 2). Green sunfish were transported in aerated 19 L buckets to the Freshwater Biology Laboratory at ENMU. The average standard length of the fishes was $9.40 \text{ cm} \pm 1.39 \text{ cm}$ and ranged from 6.28 cm to 11.80 cm. Green sunfish were separated equally into two 76 L stock aquaria and fed mealworms (3 per fish) daily. Water temperature was maintained at $26^{\circ} \pm 1^{\circ}$ C using a heater and tanks underwent a 10% partial water change and cleaning weekly.

Several hundred of a backswimmer species, *Notonecta* spp. (Heteroptera: Notonectidae) were collected in July, August, and September 2007 from Bitter Lake National Wildlife Refuge ($33^{\circ}27'24.79''\text{N}$, $104^{\circ}23'29.05''\text{W}$) outside Roswell, New Mexico (Figure 2). Backswimmers were transported in two 19 liter buckets (which were not the same buckets used for the transportation of the green sunfish) to the Freshwater Biology Laboratory at ENMU. Backswimmers remained in collecting buckets and original lake water until placed into a treatment.

Experimental design and procedures

For an organism exposed to a variety of predators, it would be useful for the organism to discriminate between predators and implement adaptive responses (Relyea, 2001). In my study, a clear discrimination in energy allocation is expected to occur in all fish kairomone



Figure 2. Map of collection sites in New Mexico. 1) Freshwater Biology Laboratory at ENMU (where all organisms were housed and experiments were conducted), 2) Melrose Migrant Trap (a fishless stock tank where *C. reticulata* were collected), 3) Green Acres Lake (a recreational park where green sunfish were collected), 4) Bitter Lake National Wildlife Refuge (where the stock of backswimmers were collected), and 5) Private Ranch (a pond where green sunfish were collected) (Google Earth, 2007).

treatments. This population presumably has not been exposed to vertebrate predators for many generations. This lack of exposure is predicted to lead to dramatic changes in several life history characteristics to be studied during this experiment. A clear discrimination in energy allocation is also expected to occur in all backswimmer kairomone treatments. This is predicted to occur because *Notonecta* is an abundant invertebrate predator found in the fishless stock tank where the experimental *C. reticulata* population was collected. Even though an association between this predator and prey species exists, life history changes should occur when individuals are exposed to different levels of predator density.

This experiment evaluated potential changes in energy allocation due to the presence of both vertebrate and invertebrate predator kairomones to i) investment in population growth, ii) investment in body size and/or armament (spines), and iii) investment in offspring (clutch sizes). The experiment employed a basic two x two factorial design (fish high, fish low, backswimmer high, backswimmer low) but added a control that was not originally considered as a level within either factor (see below). Each combination of factor and level was replicated 20 times, with each trial chosen randomly through time to avoid temporal biases during data collection. Factors consisted of kairomone concentration (two levels: high and low), and predator type (two levels: fish and backswimmer). The control trials contained no kairomones, and were also replicated 20 times.

Ceriodaphnia reticulata individuals used in experimental trials were newly hatched juveniles (i.e., hatched within 24 hours of being placed into treatment). Newly hatched juveniles were obtained from 40 egg-bearing females that were isolated in two 250 ml containers the day before the experiment began. The newly hatched juveniles were haphazardly placed into treatment. Newly hatched juveniles were used to eliminate biases

regarding an individual's maturity level, experience, or its ability to produce eggs.

Each treatment combination within the experiment ran for ten consecutive days. For each trial, feeding occurred twice during the experiment using the same food as used for stock cultures. Each trial container received 2 cc of daphnia food (Appendix A) on day one and day six, after the addition of water from the appropriate predator bath, or control water.

Each trial began by adding 10 haphazardly selected newly hatched *C. reticulata* to an 800 ml container containing 150 ml of aged water. Once hatchlings were in a trial container and a treatment was randomly assigned, then 30 ml of designated predator water and 2 cc of daphnia food was added to the container. For the next 9 days, 30 ml of predator water was added to the trial container between 1200 hrs. and 1300 hrs. daily. Trial containers were swirled by hand after predator water was added to re-suspend any food particles that had settled overnight. Even though trial containers were exposed to presumably higher than normal predator concentrations, it only occurred once in 24 hours. The once a day kairomone exposure was determined to be the best available delivery method to simulate a situation where both the natural predator populations and the longevity/viability of kairomones are unknown.

Measurements consisted of population counts, morphometric data (see below), and egg counts. Population counts began on day 11, roughly 24 hours after the last addition of predator water. Counts were accomplished by adding 10 ml samples of trial water serially into a Petri dish and placing it under a 20x-dissecting microscope. Every individual was counted, removed and separated out equally between 10 preservation cups (i.e., 1 individual added at a time into a cup). Each preservation cup contained 20 ml of cold club soda used as an anesthetizing solution (J.E. Havel, personal communication), and was used to produce a

representative sample of the population. All individuals remained in preservation cups for 2 minutes after counting concluded, to ensure all individuals were properly anesthetized. Total number of individuals per trial container was recorded.

After all individuals were anesthetized, each preservation cup was placed under a 20x-dissecting microscope to count individuals. A single individual was haphazardly pulled out from each cup (for a total of 10 individuals) using a 10 ml pipette and placed into a 20 ml container containing 70% isopropyl alcohol and labeled treatment average (see below). The ten individuals in this container were used to obtain an average body length measurement for the trial population. Remaining individuals were strained from preservation cups using 210 micron plankton netting and collectively placed into a 20 ml container containing 70% isopropyl alcohol.

Morphometric measurements (i.e., carapace length, carapace width, their ratio, presence of a tail spine, and its length) were taken for egg-bearing individuals in the treatment average container. The contents of the container were poured into a Petri dish and placed under a binocular dissecting microscope (Leica MZ 95 1.0x) containing an eyepiece micrometer (Leica 10x/21 B). Individuals were measured to the nearest 0.016 mm.

Carapace length (i.e., from the crown of the head to the posterior border of the carapace) and carapace depth (i.e., from the dorsal part to the ventral part of the carapace) were measured. For both measurements, an individual was aligned with the unit bars of the micrometer by positioning the head and tail in a vertical position on its right side. The presence of a spine was recorded categorically (i.e., present or absent). When a spine was present, spine length was determined by measuring total length including the spine and then subtracting carapace length.

Egg counts were conducted on measured individuals. All eggs within the brood chamber were counted. Some brood chambers contained developed hatchlings, which were also included in the counts.

Predator kairomone solutions

In two separate 76 liter containers aged water was added with a specific amount (see below) of haphazardly selected green sunfish. The low density treatment container was comprised of six fish and the high density treatment container was comprised of 12 fish. Fish remained in treatment containers for the duration of the experiment and then were placed back into a stock tank after data collection ended. Each treatment container underwent a 10% partial water change and cleaning every 5 days to ensure proper living conditions for the fish.

In two separate 19 L containers aged water was added with a specific amount (see below) of haphazardly selected backswimmers, both juveniles and adults. A combination of ages was used due to the similarity in body size. The low density treatment container was comprised of 25 backswimmers and the high density treatment container was comprised of 50 backswimmers. Backswimmers used in treatment containers were not fed during the experiment because their food (water fleas) would have been potentially added to experimental trials during the addition of predator water. Due to the high mortality rates (about 50% loss per day), dead animals were replaced daily to ensure kairomones were being produced by living individuals. Each treatment container underwent a 10% partial water change and cleaning every 5 days to ensure proper living conditions for the backswimmers.

In a 19 liter container, aged water was added to represent the control treatment. This

container lacked predators but underwent a 10% partial water change and cleaning every five days to ensure consistency among treatment containers.

Statistical analyses

Independent variables for this experiment include two factors: predator density (three levels) and predator type (two levels) (Table 1). Dependent variables include: population growth (number of individuals per treatment trial), body size (mean total carapace length and depth per treatment trial), armament length (total carapace length including spine), and egg investment (number of eggs in brood chamber).

Population count data sets were tested for normality using the D'Agostino's tests (Zar, 1974). Data sets significantly differed from normal, and transformations (e.g., log and arcsin) (Zar, 1999) did not significantly alter distributions. A nonparametric single-factor analysis of variance (ANOVA) (Kruskal-Wallis test with tied ranks) and nonparametric Tukey-type multiple comparisons with the Nemenyi test were used for each factor (Zar, 1999). In all analyses, the alpha level was set at 0.05.

Morphometric ratio data sets were tested for normality using the D'Agostino's tests (Zar, 1974). A single data set (fish high) significantly differed from normal and transformation (e.g., log and arcsin) was unsuccessful. A two-factor ANOVA (Table 2) was used on the four data sets that did not differ significantly from normal and a single-factor nonparametric ANOVA was used when including all five data sets. In all analyses, the alpha level was set at 0.05.

Total carapace length data sets were tested for normality using the D'Agostino's tests (Zar, 1974). Data sets did not differ significantly from normal and parametric analyses were

used. A two-factor ANOVA (including the control) (Table 2) was used to test each factor and the interaction between the two factors (Zar, 1999). In all analyses, the alpha level was set at 0.05.

Egg count data sets were tested for normality using the D'Agostino's tests (Zar, 1974). Data sets differed significantly from normal and were successfully transformed (using $(X_i + 0.5)^2$) for parametric analysis. A two-factor ANOVA was used for each factor (Zar, 1999). In all analyses, the alpha level was set at 0.05.

Table 1. Independent and dependent variables for data analysis.

Independent Variables	Description
Predator density:	Fish high (12 fish)
	Fish low (6 fish)
	Backswimmer high (50 backswimmers)
	Backswimmer low (25 backswimmers)
	Control (no predators)
Predator type:	Vertebrate predator (Green sunfish)
	Invertebrate predator (Backswimmer)
Dependent Variables	Description
Population growth:	Mean total population growth
Body size:	Mean total carapace length
	Mean total carapace depth
Armaments:	Total number of spines
	Mean total spine length
Egg investment:	Total number of eggs
	Mean total egg number

Table 2. Statistical analyses. Panel A represents the morphometric ratio data and Panel B represents both carapace length data and egg count data (C = control and Bksw = backswimmer).

A)

Predator Type				Predator Density			
Vertebrate		Invertebrate		Vertebrate		Invertebrate	
C	Fish	Bksw	Bksw	C	Low	High	Low

B)

Predator Type						Predator Density					
Vertebrate			Invertebrate			Vertebrate			Invertebrate		
C	Fish	Bksw	C	Fish	Bksw	C	Fish	Bksw	C	Fish	Bksw

RESULTS

Population counts

Treatment caused a highly significant difference in population counts (Kruskal-Wallis test, $n_{1-5} = 20$, $H_c = 45.36$, $p < 0.001$) (Table 3). Tukey-type multiple comparison showed that backswimmer high produced statistically significant differences in comparison with backswimmer low ($p < 0.001$) and fish high ($p < 0.001$). The control produced statistically significant differences in comparison with backswimmer low ($p < 0.001$) and fish high ($p < 0.001$). Fish low produced statistically significant differences in comparison with backswimmer low ($p < 0.001$) and fish high ($p < 0.005$). The ANOVA showed that fish high and backswimmer low treatments were significantly equivalent to each other (represented by letter a, Figure 3, and significantly lower than fish low, backswimmer high, and control), and backswimmer high, fish low, and control treatments which were significantly equivalent to each other (represented by letter b, Figure 3, and significantly lower than fish high and backswimmer low).

Morphology

The control treatment was used in the two-factor ANOVA in place of the fish high treatment because it could not be transformed for use in parametric analysis. Predator type did not influence morphology ($F_{1,76} = 0.015$, $P = 0.904$); neither did predator density ($F_{1,76} = 0.163$, $P = 0.688$) nor the interaction between the two ($F_{1,76} = 2.839$, $P = 0.096$) (Figure 4).

The two-factor ANOVA showed that predator type did not influence carapace length ($F_{1,114} = 0.479$, $P = 0.490$) but showed that predator density ($F_{2,114} = 6.420$, $P = 0.002$) and

the interaction between the two ($F_{2,114} = 3.677$, $P = 0.028$) influenced carapace length (Figure 5).

Egg counts

Two-factor ANOVA showed that predator type did not influence egg count ($F_{1,76} = 3.383$, $P = 0.070$) but showed that predator density ($F_{1,76} = 8.950$, $P = 0.004$) and the interaction between the two ($F_{1,76} = 5.097$, $P = 0.027$) influenced egg count (Figure 6).

Table 3. Statistic and probability values of the Tukey-type multiple comparisons for population counts. A Kruskal-Wallis test showed significant differences among means ($n_{1-5} = 20$, $H_c = 45.36$, $P \ll 0.001$). The ranking numbers represented the treatments included in analysis: 1) backswimmer high, 2) control, 3) fish low, 4) fish high, and 5) backswimmer low. The calculated means for each treatment were as follows: 1382.00 (backswimmer high), 1343.50 (control), 1250.50 (fish low), 604.50 (fish high), and 469.50 (backswimmer low).

Comparison	Rank Difference	Q	P value
1 vs. 5	912.50	7.03313	$P < 0.001$
1 vs. 4	777.50	5.99262	$P < 0.001$
1 vs. 3	131.50	1.01354	$P > 0.50$
1 vs. 2	38.50	0.29674	$P > 0.50$
2 vs. 5	874.00	6.73639	$P < 0.001$
2 vs. 4	739.00	5.69588	$P < 0.001$
2 vs. 3	93.00	0.71680	$P > 0.50$
3 vs. 5	781.00	6.01959	$P < 0.001$
3 vs. 4	646.00	4.97907	$P < 0.005$
4 vs. 5	135.00	1.04052	$P > 0.50$

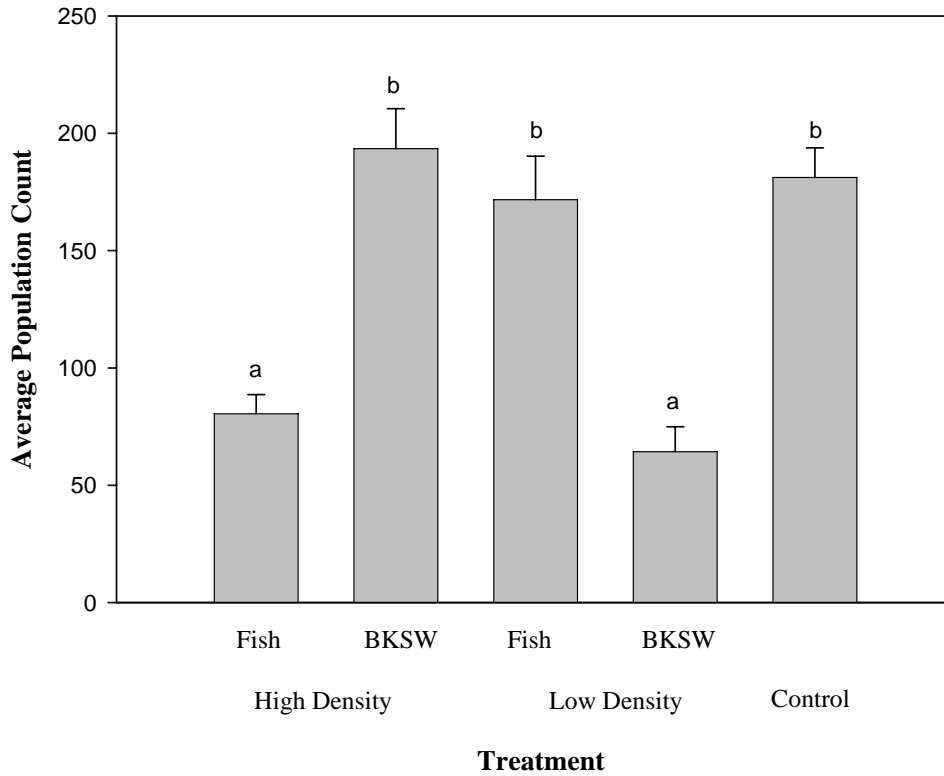


Figure 3. Average population count by treatment; a and b are significantly different (Kruskal-Wallis test, $N_{1-5} = 20$, $H_c = 45.36$, $p \ll 0.001$; Nonparametric multiple comparison tests, minimum p value of $P < 0.005$). The same lower case letters above error bars equal statistical equivalence; error bars equal one standard error of the mean. BKSW equals backswimmer.

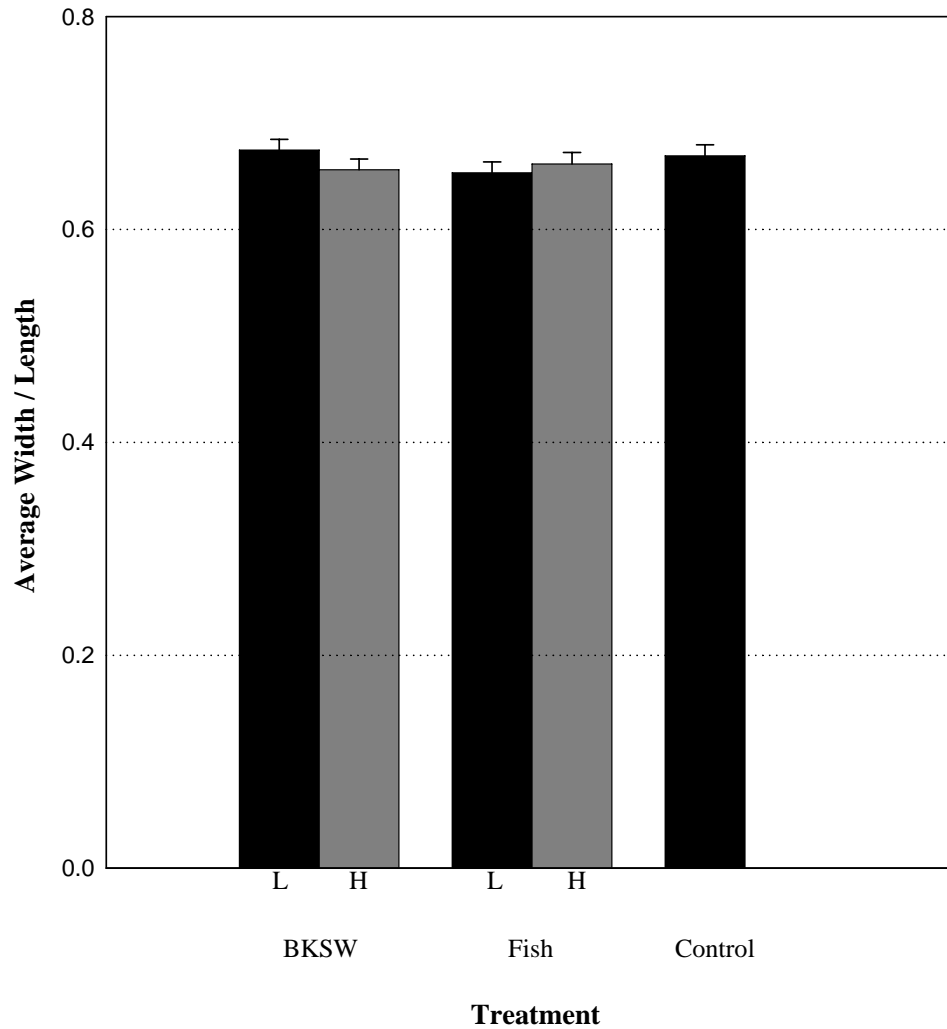


Figure 4. Average morphometric ratio by treatment. Predator kairomones did not significantly influence morphometric ratios (predator type: $F_{1,76} = 0.015$, $P = 0.904$, predator density: $F_{1,76} = 0.163$, $P = 0.688$, and the interaction: $F_{1,76} = 2.839$, $P = 0.096$). Error bars equal one standard error of the mean. BKSW equals backswimmer. L and H equal low and high relative predator density, respectively.

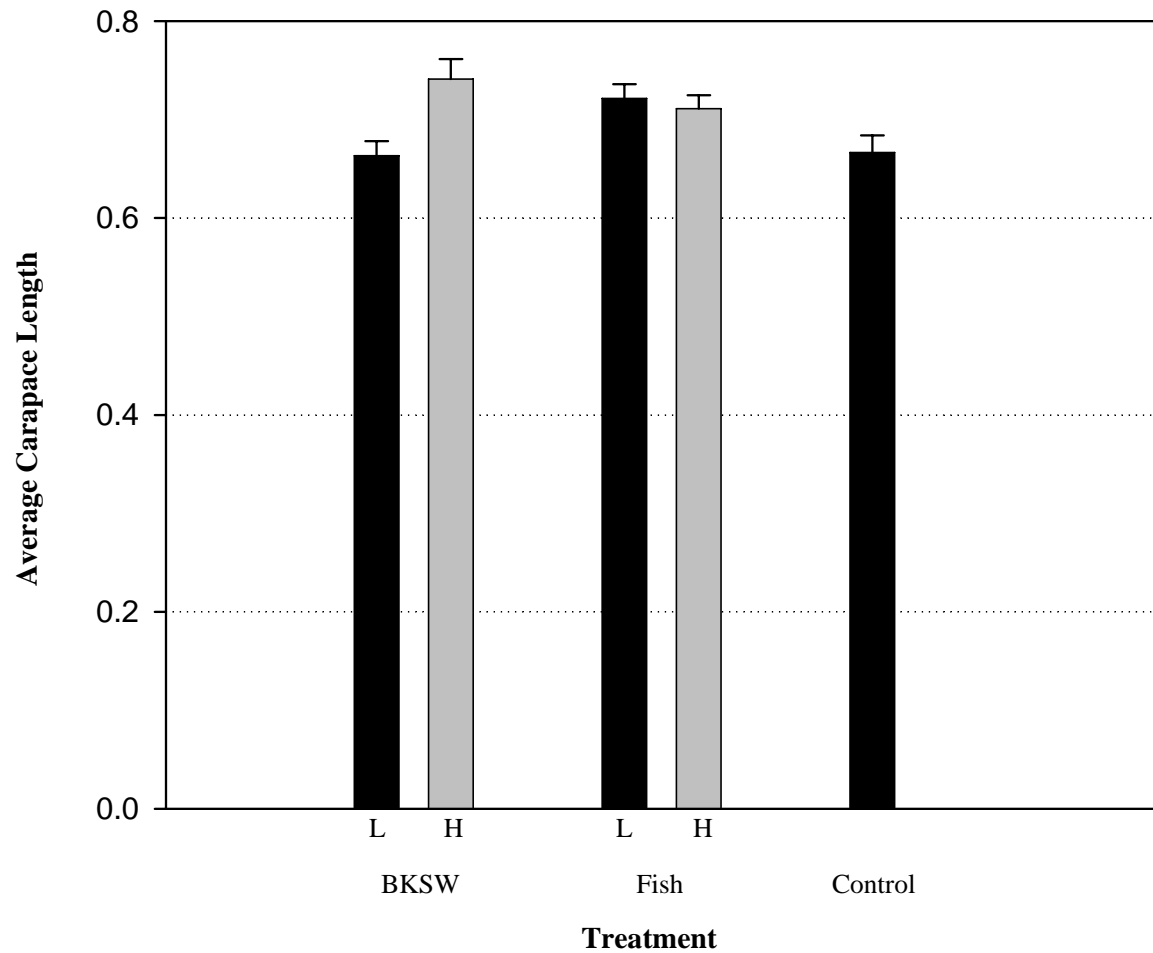


Figure 5. Average carapace length by treatment. Predator density significantly influenced average carapace length ($F_{2,114} = 6.420$, $P = 0.002$), but predator type did not ($F_{1,114} = 0.479$, $P = 0.490$), however, the interaction between predator type and density also significantly influenced average carapace length ($F_{2,114} = 3.677$, $P = 0.028$). Error bars equal one standard error of the mean. BKSE equals backswimmer. L and H equal low and high relative predator density, respectively.

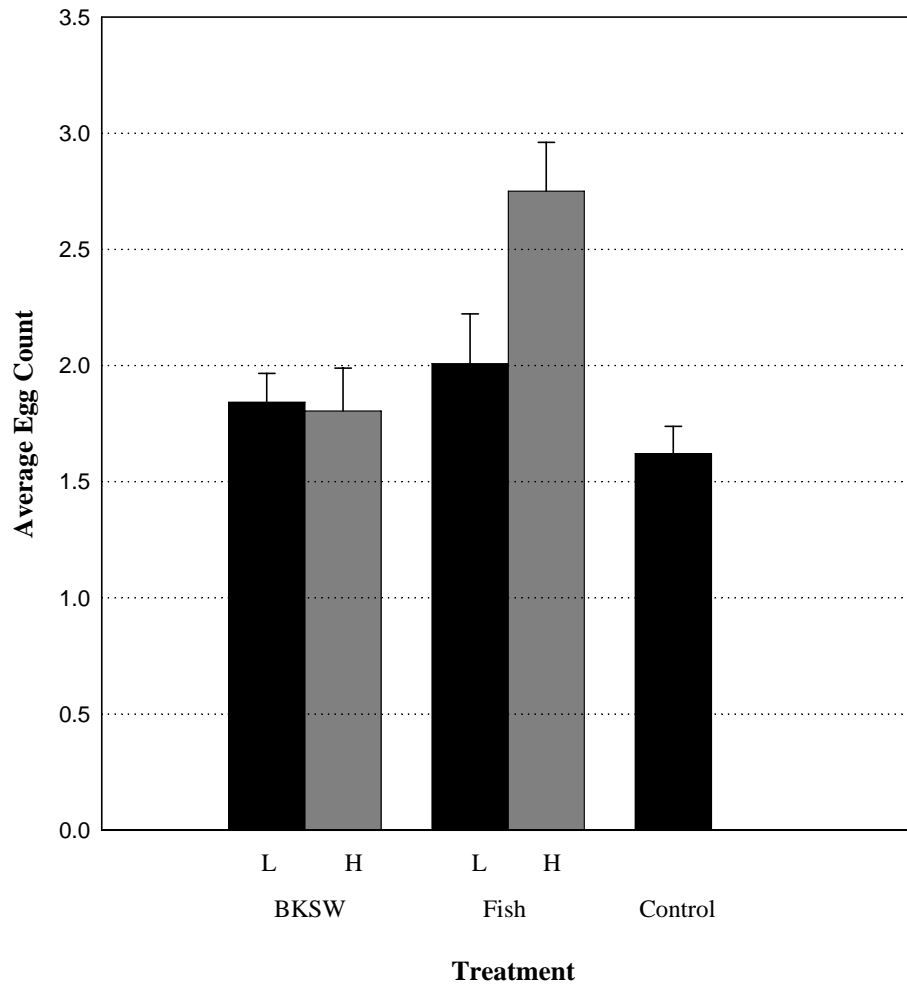


Figure 6. Average egg count by treatment. Predator density significantly influenced average egg count ($F_{1,76} = 8.950$, $P = 0.004$), but predator type did not ($F_{1,76} = 3.383$, $P = 0.070$), however, the interaction between predator type and density also significantly influenced average egg count ($F_{1,76} = 5.097$, $P = 0.027$). Error bars equal one standard error of the mean. BKSW equals backswimmer. L and H equal low and high relative predator density, respectively.

DISCUSSION

Evolutionarily, the energy used in a reproductive effort (size or number of offspring) or growth of an individual can be evaluated as either an investment into a current reproductive event or potentially future reproductive event(s). Both situations may affect the probability of survival of both the adults and the offspring (Korpimaki, Norrdahl & Valkama, 1994). These changes are reproductive tradeoffs, in which more energy is allocated into one aspect of reproduction (e.g., egg size) while less energy is allocated into another aspect of reproduction (e.g., number of eggs per clutch) or growth.

When energy is allocated into a current reproductive event, more investment will either go into the number of eggs produced or the size of individual eggs. The investment into the number of eggs produced may increase the output and survival of more offspring, while the investment into the size of individual eggs leads to fewer eggs produced but a higher probability that an individual offspring will survive (O'Donoghue, 1994). When energy is allocated into a future reproductive event, more investment goes into the growth of an individual. The investment into body size and/or armaments may increase the chances of survivorship and maximize the number of offspring produced during a lifetime (Norrdahl & Korpimaki, 1998).

Time (or size) to maturation is related to such tradeoffs because a small-bodied individual may invest more energy into the production of a larger body size before maturation and ensure a maximum number of offspring produced (Rose *et al.*, 2001b). This supports a future reproductive event because the maximum amount of offspring will be produced throughout the individual's lifespan. An increase in time between hatching and the first reproductive events may be an adaptation in the presence of predators because it may

lead to a larger body size and theoretically increase a future reproductive effort (Black, 1993).

The responses, or lack thereof, of all four treatments can be interpreted in terms of reproductive strategies (Appendix B). Fish high and fish low treatments demonstrated a current investment strategy (Appendix B). They responded with increased egg counts but did not change their morphology. The backswimmer high treatment demonstrated a future investment strategy (Appendix B). The change in morphology is based on carapace length and not the morphometric ratio (width/length), i.e., they responded with a change in size not shape. The backswimmer low treatment demonstrated no response (Appendix B). None of the factors influenced any changes in life history characteristics for these individuals. Interestingly, none of the four treatments responded with a mixed strategy, i.e., allocating energies favoring both current and future reproductive events, due to a lack of selection. Furthermore, that differential responses were seen within predator type (e.g., high and low fish) demonstrates that the water fleas assess not only the presence of a predator, but also the level of threat.

A slower population increase occurred in the fish high treatment but was not expected based on previous work that showed higher predator densities would lead to a larger adaptive prey response (Brett, 1992). Furthermore, he showed that it would be more advantageous for an individual exposed to large levels of fish predation to produce as many offspring as possible in the shortest amount of time before they reach a detectable body size. Despite previous findings, Brett (1992) was unable to show the same response in *C. reticulata*.

Predation of specific prey items by *Notonecta* spp. has been documented as being dependent upon such factors as predator density, type and abundance of prey species, and

size distribution of both predators and prey (Scott & Murdoch, 1983). *Daphnia* have shown signs of early reproduction with a smaller body size and a decrease in age at maturity in the presence of *Notonecta* kairomones (Riessen, 1999). Low levels of *Notonecta* kairomones caused a slower growth rate in *C. reticulata*. The differences between density levels shows that changes in energy allocation are occurring based on the perceived intensity of predation. Individuals in the low density treatment did not allocate their energies into either type of reproductive event. Those in the high density treatment allocated their energy into future reproductive events. *Notonecta* having an influence on life history characteristics implies that plasticity of response to levels of predation is maintained in natural populations that coexist throughout generations.

Changes in morphology may benefit the potential for future reproduction by increasing the probability of adult survivorship, as well as allow for several reproductive events to occur. Any change in morphology may be a byproduct of the tradeoff occurring between energy allocation into egg production and body size. When an individual invests most of their acquired energy and resources into body size, then they are forgoing the potential for more eggs per clutch (i.e., a current reproductive event). It makes evolutionary sense that individuals in the fish treatments would have allocated more energy into a current reproductive event than in body size because fish are size-selective predators and preferentially consumes large prey items (Brett, 1992; Dodson, 1970; Gliwicz, 1994; Lane, 1979). In contrast, it makes evolutionary sense that individuals in the backswimmer treatment would have allocated more energy into body size than in a current reproductive event because backswimmers can only consume prey items that fit into the grasp of their piercing mouthparts (Sakwinska, 1993).

A defense adaptation that has been shown to occur across taxa in the presence of predators is to increase the number of reproductive events and offspring from a given individual within the exposed population (Candolin, 1998). Individuals in a high risk area may be expected to produce more clutches per lifetime, while individuals in a low risk area may be expected to produce fewer clutches per lifetime. Unfortunately, differences in the number of clutches an individual *C. reticulata* produced in each treatment were not assessed due to experimental design.

The clonal species used for this study came from a stock tank that is unlikely to have ever contained fishes. This means that for an unknown number of generations (presumably hundreds), this species of *Ceriodaphnia* has only been exposed to invertebrate predation. However, all species found in this stock tank had to originate from an outside source because the habitat was manmade. The origin of *Ceriodaphnia* is unknown for this specific stock tank, but it is reasonable to assume that at one point in this population's history it was exposed to a type of vertebrate predation. Thus, defense mechanism adaptations may have occurred based on an evolutionary history involving fish predation. The responses of this population to fish kairomones suggest a genetic "memory" of previous fish exposure.

During this experiment two concentrations of predator kairomones were used to simulate an environment of high and low predation. Even though these levels were intended to represent a natural habitat, my predator densities may have been higher than what actually occurs in a natural predator-prey habitat. Any deviation from a normal situation would lead to physiological responses that may or may not be seen in nature. However, because changes were seen in the experimental population in a laboratory setting, it can be hypothesized to occur in nature.

Fishes are lacking in the stock tank environment from which the test population originated, but invertebrate species are present. I was unable to catalogue all invertebrates found in the stock tank, but casual observations showed that backswimmers were abundant. Based on these observations, I estimated that 50 backswimmers (2.5/L) would be a good approximation of a high predator level. Half of the high level was used for the low level. It seems plausible that an individual water flea would be exposed to kairomones of multiple backswimmers concurrently. I observed that backswimmers appear to spend most of their time near the surface in large groups. This type of behavior would cause a water flea to come into contact with large amounts of kairomones. Despite the opportunity to encounter a large concentration of kairomones, it is still unknown how long these remain viable in water. Thus, the daily exposure protocol of this experiment may have represented low exposure even in the high density treatment.

The lack of a significant change in body size may indicate that the predator densities were too low (or too high) to cause appropriate life history changes in the study population. If these density levels resembled a natural habitat, it is possible that a significant change in body size would have been observed in a few generations. In this study, generation times were undeterminable due to my experimental design. But, based on information regarding life history characteristics of *C. reticulata* and the length of this experiment, it can be assumed that at least two generations occurred.

The differential predator x density interaction shows that responses to density changes were not the same for the different predators. It can be assumed that the amount of kairomones a fish produces will not equal the amount of kairomones produced by a backswimmer. This assumption is based not only on the number of predators present in an

area, but also on the size of an individual. However, because the results showed some influence between the two factors means that in order to get an accurate assessment of the environment, an individual water flea needs to recognize not only the predator but how many predators are in the area.

Water fleas are constantly moving up and down in the water column. This continuous movement means that kairomone exposure may occur in limited amounts and vary in intensity. Based on this behavior displayed by *Ceriodaphnia*, the method of kairomone exposure used in this experiment should have provided enough information to cause defense mechanism changes to life history characteristics over a few generations. Note that individuals of *C. reticulata* have been shown to swim down the water column in the presence of fish kairomones (Seely & Lutnesky, 1998)

Understanding the exact delivery mode of kairomones, their viability in water, and how their presence leads to the modification of life history characteristics is important for the behavioral and morphological understanding in any organism. This study has demonstrated that a variety of strategies can be used by a prey species in the presence of predator kairomones. If the perceived level of predation is high enough, changes to body size, egg size, and number of eggs per clutch are observed at the individual level. Even though vertebrate and invertebrate predators release kairomones, each may have a distinct effect on prey species.

This experiment has provided a basis for further investigation into the effects of predatory kairomones on life history characteristics in *Ceriodaphnia*. Since this experiment only studied a population from a fishless habitat, the next step would be to make a comparison between a fishless pond and a pond that contains fishes. This comparison would

provide a more concrete foundation for specific defense mechanism adaptations that are being initiated due to perceived levels of predation. It would also provide information to the exact effects specific fish species have on life history characteristic in *Ceriodaphnia*.

To gain a more complete understanding of the exact life history characteristics that become modified, added, or lost in the presence of predation, data needs to be collected on i) time taken to reach first clutch, ii) number of instars to maturity, iii) body size at maturity, iv) number of eggs per clutch, v) individual egg size and weight, and vi) number of clutches during lifespan. All these variables influence an individual's survival, reproductive fitness, and overall contribution to the population.

Laboratory experiments that limit generation time (i.e., reproductive events) could potentially lead to an overestimate of the influence of predator kairomones on life-history traits in zooplankton (Sakwinska, 1993). However, because little is known about life history characteristics and predator avoidance tactics of *C. reticulata*, a laboratory study is a good starting point.

It has been suggested that a few clonal species are only a small representation of a natural population, which makes defense mechanism adaptations difficult to interpret (Luning, 1992). However, an advantage of using a clonal species is that all individuals are genetically identical and come from the same female. Having identical genetic backgrounds makes adaptation assumptions valid due to the lack of discrepancy between individuals within and between experimental trials. A clonal species also allows for the control of generation times, meaning that individuals from the same generation can be used in treatments. This control ensures that all organisms are at the same point in life and allows for

comparisons to be made regarding time to first reproduction, body size at maturation, number of clutches per offspring, and investment into a specific reproductive event.

My study on the influence of predator detection on life history characteristics in *C. retiulata* is the first to look at these changes in a population from a fishless environment in the presence of two types of predators. I found that *C. reticulata* i) responds to fish kairomones by increasing egg counts without accompanied morphological changes, and I interpret this as an energy allocation to a current reproductive event investment strategy; ii) responds to a high level of backswimmer kairomones by neither increasing nor decreasing egg counts with accompanied morphological changes, and I interpret this as an energy allocation to a future reproductive event investment strategy; and iii) does not respond to low levels of backswimmer kairomones with either an increase or decrease in egg counts or morphology, and I interpret this as a lack of energy allocation. My results were unexpected based on several studies that looked at the same life history changes in *Daphnia* species. I showed that closely related species (e.g., *Daphnia* and *Ceriodaphnia*) have very different adaptive responses in the presence of predators. *Ceriodaphnia reticulata* has very subtle life history changes in the presence of a vertebrate and invertebrate predator. I found that *C. reticulata* allocated energy differently than other cladoceran species in the presence of fish predators.

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APPENDIX A

Daphnia Food Recipe (modified from Norm Ruebsamen, personal communication):

3 tablets brewers yeast

25 frozen peas

4 spinach leaves

1 tablespoon sweet potato baby food

1 quart of water

Ingredients were blended until liquefied and kept refrigerated.

APPENDIX B

Categories used to interpret results.

Investment Strategy	Response
1. Current investment strategy:	Change in egg size or number without change in morphology
2. Future investment strategy:	No change in egg size or number with change in morphology
3. Mixed strategy:	Change in egg size or number with change in morphology
4. No strategy:	No change in egg size or number without change in morphology