

THE INTERPLAY OF ENVIRONMENTAL STRESSORS ON THE LIFE HISTORY  
TRAITS IN *DAPHNIA LUMHOLTZI*

by

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This thesis is dedicated to my sons, Clark and Pierce. You provided the inspiration  
necessary for me to complete this process.

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Abstract

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In natural environments, organisms face multiple stressors that threaten their persistence. Aquatic organisms respond to a change in environmental conditions by adaptively modifying the expression of traits (i.e., phenotypic plasticity). Yet, our understanding of the interactive effects of multiple simultaneous stressors on the fitness of organisms is currently limited. This is because previous research has typically focused on the influence of environmental stressors in isolation thus ignoring the impacts that multiple variables may have on an organismal fitness. Here, I assessed the interactive effects of predator cues (*Chaoborus*) and low food quality (*Cylindrospermopsis raciborskii*) on key life-history traits in the invasive water flea *Daphnia lumholtzi*. Increasing concentrations of sub-optimal cyanobacteria resulted in delayed maturation, and declines in growth, size at maturation, and reproductive outputs (and slower rate of population growth). Yet, such declines were much smaller in the presence of predator cues. I thus observed significant predator x resource interactions for growth, size at maturation, clutch size, and intrinsic rate of increase. The opposite pattern was observed for the size of defense traits; *Daphnia* reared in the absence of predator cues maintained larger spines when fed a very low quality of food. Such results indicate that *Daphnia* exposed to predator cues preferentially allocate energy towards life history traits at the

expense of defense characteristics when faced with severe food stress. Thus, a robust understanding of species responses to environmental stressors, such as global climate change, requires the robust manipulations of multiple key environmental variables.

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## Chapter 1

### Introduction

Organisms routinely experience spatial and temporal variation in a wide variety of environmental stressors such as temperature, acidity, food quality and quantity, predation, and competition (Altshuler et al. 2011; Coors and DeMeester 2008; Iwama et al. 1999; Jackson et al. 2001; Vinebrooke et al. 2004; Walls 1997). Such environmental variation, whether it occurs in predictable or unpredictable fashions, has the potential to negatively affect the fitness of individuals or populations. These biotic and abiotic stressors, in turn, have a significant influence on the abundance and diversity of organisms (Jackson et al. 2001; Walls 1997). As a result, organisms require mechanisms that enhance persistence in the face of severe environmental stressors (Altshuler et al. 2011; Bednarska et al. 2014; Black and Dodson 1990; Boone et al. 2007; Coors and Meester 2008; Dao et al. 2010; DeMott et al. 1991; Dodds 2002; Gonzalez and Tessier 1997; Herbert and Grewe 1985; Heugens et al. 2006; Nogueira et al. 2004; Pauwels et al. 2010; Relyea 2004; Scherer et al. 2013; Spitze 1992; Stibor 1992; Walls 1997; Yin et al. 2011).

Organisms typically respond to a change in environmental conditions by modifying the expression of traits. Such 'phenotypic plasticity' has been documented in many species of plants and animals in response to numerous environmental stressors (Bednarska et al. 2014; Black and Dodson 1990; Boone et al. 2007; Buskirk and Schmidt 2000; Coors and Meester 2008; Dao et al. 2010; DeMott et al. 1991; Gonzalez and Tessier 1997; Herbert and Grewe 1985; Heugens et al. 2006; Nogueira et al. 2004; Pauwels et al. 2010; Relyea 2004; Scherer et al. 2013; Spitze 1992; Stibor 1992; Walsh and Reznick 2009; Walls 1997; Yin et al. 2011). For example, Buskirk and Schmidt (2000) reported darker tail fin pigmentation and increases in head and tail size in two

species of larval newts exposed to predator cues compared to newts in predator-free ponds. Such morphological changes are presumed to represent an adaptive response that enhances survival in the presence of predators. Plastic changes in life history traits, such as development rate and reproductive effort in response to environmental stressors are particularly common (Bednarska et al. 2014; Dao et al. 2010; Boone et al. 2007; - Coors and Meester 2008; Gonzalez and Tessier 1997; Nogueira et al. 2004; Pauwels et al. 2010; Walsh and Reznick 2009; Walls 1997). However, organisms in nature are influenced by numerous biotic and abiotic stressors that occur simultaneously. This is important because phenotypic responses to environmental stressors are widely assumed to have costs and limitations (Dewitt 1998; Riessen 1999; Scheiner and Berrigan 1998; Walls 1997; Weber and Declerck 1997). Energy is limited and thus increased allocation towards one function is expected to correlate with decreased allocation towards some other component of fitness (Hansson et al. 2007; Loose and Dawidowicz 1994; Pauwels et al. 2010; Riessen 1999; Walls 1997). This is because all organisms possess a finite energy budget that is divided amongst competing functions such as growth, reproduction, and maintenance. As a result, the presence of multiple environmental stressors may alter the connection between a given environmental cue and resultant patterns of plasticity as organisms must prioritize trait responses in the face of multiple environmental stressors. For instance, multiple environmental stressors may result in synergistic (combined effect is greater than individual stressor), antagonistic (combined effect is less than individual stressor), and/or additive (effect of the combination of stressors equals the sum of the effect of the individual stressor) effects on the fitness of natural populations (Altshuler et al. 2011; Coors and De Meester 2008; Demott et al. 1991). Yet, research typically focuses on the connection between a single environmental stressor on a single class of traits (Black and Dodson 1990; Dao et al. 2010; Herbert and

Grewe 1985; Spitze 1992; Stibor 1992; Weider and Pijanowska 1993). Our understanding of how organisms cope with multiple simultaneous stressors is therefore limited.

### 1.1 *Daphnia*

*Daphnia* are small planktonic crustaceans that have a prominent influence on trophic structure and ecosystem function in aquatic environments. They provide a main source of nutrition for both vertebrate and invertebrate predators and are important consumers of phytoplankton (Abram 1980; Moss 2010, Schwarzenberger et al. 2013). *Daphnia* are also well known to respond phenotypically to a variety of environmental stressors by modifying many characteristics (Bednarska et al. 2014; Black and Dodson 1990; Dao et al. 2010; Herbert and Grewe 1985; Scherer et al. 2013; Spitze 1992; Stibor 1992; Weider and Pijanowska; Walls 1997). Below, I describe our current understanding of two key environmental variables that influence the fitness of *Daphnia*.

### 1.2 Predation

Predator-prey interactions are a predominant feature of natural systems (Abram 2001, Krohne 1998), constituting a major driver of natural selection (Abram 2001; Boersma et al. 1999; Yin et al. 2011). In particular, predator-induced mortality exerts a significant influence on the expression of traits in zooplankton (Black and Dodson 1990; Coors and Meester 2008; Dao et al. 2010; DeMott et al. 1991; Herbert and Grewe 1985; Heugens et al. 2006; Nogueira et al. 2004; Pauwels et al. 2009; Relyea 2004; Scherer et al. 2013; Spitze 1992; Stibor 1992; Walls 1997; Yin et al. 2011). Prominently, *Daphnia* produce elaborate morphological defenses and alter the expression of life history traits, such as age and size at maturation, when exposed to chemical cues emitted by predators (i.e., kairomones) (Black and Dodson 1990; Boersma et al. 1999; Riessen 2009; Hoverman et al. 2005; Tollrian and Dodson 1999; Walls 1997). For instance,

*Daphnia* typically respond to the presence of gape-limited invertebrate predator cues (Phantom midge larvae, *Chaoborus sp.*) by delaying maturation, producing smaller clutches of offspring, and increasing the size of defense characteristics (Boersma et al. 1998; Riessen 1999; Tollrian and Dodson 1999; Weder and Pijanowska 1993). This enhanced allocation to growth at the expense of reproduction is presumably an adaptive response to the presence of a gape-limited predator. Plastic responses to predators are not limited to life history and morphological responses; additional examples of predator-induced plasticity include changes in the behavior (i.e., diel vertical migration) and physiology of *Daphnia* (metabolic and respiration rate, immunity, stress protein production) (Burks et al. 2002; Cerenius and Söderhäll 2004; Pauwels et al. 2010; Söderhäll and Cerenius 1998).

### 1.3 Food Quality

Another significant environmental factor that influences the fitness and composition of natural populations is resource quality (Vanni and Lampert 1992). Aquatic environments typically contain a wide array of taxonomic groups of phytoplankton with varying degrees of nutritional qualities, ranging from high quality green algae (chlorophyta) to low quality, grazer-resistant blue-green algae (cyanobacteria). The abundances of these resources typically vary seasonally. In warm temperate climates (such as Texas), lakes and reservoirs experience seasonal variations in phytoplankton. Filamentous cyanobacteria dominate during summer when water temperatures are high and other phytoplankton, such as green algae; dominate during the other seasons (Grover and Chrzanowski 2006).

Increases in the duration and intensity of cyanobacteria blooms due to rising temperatures and eutrophication will likely occur in the near future (Wagner and Adrian, 2009). Generally, cyanobacteria exhibit highly efficient nutrient uptake and storage ability

resulting in optimal growth rates at relatively high temperature and increased nutrient input (Martin-Creuzburg and Elert 2009). They also appear to be more tolerant of increasing salinity and acidity than other phytoplankton species (Paerl and Huisman 2009). This increase in cyanobacteria populations is expected to cause shifts in abundance and composition of other algae species (chlorophytes), which may ultimately have implications for the ecology of zooplankton.

Cyanobacteria are a lower quality resource for *Daphnia* because they are nutritionally deficient, more difficult to eat, and can be toxic to *Daphnia* (Bednarska et al. 2014; Dao et al. 2010; Martin-Creuzburg et al. 2008; Martin-Creuzburg and Elert 2009; Schwarzenberg et al. 2010; Walls 1997). For instance, cyanobacteria are deficient in sterols, polyunsaturated fatty acids, and biochemical nutrients which, in turn, can reduce somatic growth and egg production in *Daphnia* (Martin-Creuzburg and Elert 2009). Cyanobacteria are also known to produce a wide array of secondary metabolites (i.e., protease inhibitors) or toxins (hepatotoxins, neurotoxins, etc.) that may negatively influence organismal fitness (Dao et al. 2010; Schwarzenberg et al. 2010; Walls 1997). Additional work has shown that exposure to cyanobacteria alters feeding rates and morphology; *Daphnia* increased their filtering apparatus by 12-15% when exposed to high biomass of filamentous cyanobacteria (Ghadouani and Pinel-Allol 2002). Several studies have indeed shown that cyanobacteria negatively impacts the growth and reproductive effort of *Daphnia* (Bednarska et al. 2014; Martin-Creuzburg and Elert 2009; Walsh et al. 2014). As a result, increasing amounts of cyanobacteria may challenge the ability of individuals or populations to adaptively respond to other sources of stress, such as predators.

#### 1.4 Study Species

*Daphnia* are well suited for assessing the impact of multiple stressors on life history traits. *Daphnia* sp. exhibit cyclical parthenogenesis, whereby they alternate between asexual and sexual stages of reproduction (Abram 1980; Moss 2010). The use of clonal organisms allows for the study of a single genotype in multiple environments. This coupled with rapid development and ease of cultivation make *Daphnia* an excellent organism to assess the ecological importance and evolutionary consequences of food quality and predation (Lass and Spaak 2003).

*Daphnia lumholtzi* is a large (up to 5.6mm in length including spines) cladoceran, native to Africa, Asia, and Australia. *Daphnia lumholtzi* was recently introduced in lakes in the United States as it first appeared in a lake in TX in 1990. They have since spread throughout the much of the southern United States and as far north as Lake Superior (Fey and Cottingham, 2011). A prominent feature of *D. lumholtzi* is their ability to produce very elaborate head and tail spines as anti-defense mechanisms in the presence of predators (Fig. 1; East et al. 1999; Dzialowski et al. 2003; Engel and Tollrian, 2009; Lennon et al. 2001; Fey and Cottingham, 2011). These large defense characteristics enhance their survival in the presence of predators when compared with native species and researchers have speculated that such traits have helped facilitate the rapid spread of this species (Engel and Tollrian 2010). In lakes in the United States, *D. lumholtzi* is usually prominent during the summer (Dzialowski et al. 2000; Work and Gophen 1999), which represents a period of time that coincides with increased densities of cyanobacteria (Pattinson et al. 2003) and often intense periods of planktivorous fish predation. This species will thus routinely experience stress associated with predators and food.

Here, I assessed the impacts of gape-limited invertebrate predator cues (*Chaoborus*) and declines in food quality (*Cylindrospermopsis raciborskii*) on key life-history traits in the water flea *D. lumholtzi*. Clones of *D. lumholtzi* from three reservoirs in Texas were reared in a common environment for multiple generations. Third generation lab born individuals were then reared in the presence and absence of predator cues while concurrently being exposed to increasing concentrations of low quality cyanobacteria. Each stressor, when applied alone, has been shown to induce stressor-specific adaptive responses in *Daphnia* (Black and Dodson 1990; Dao et al. 2010; DeMott et al. 1991; Hebert and Grewe 1985; Ghadouani and Pinel-Alloul 2002; Nogueira et al. 2004; Spitze 1992; Stibor 1992; Weider and Pijanowska 1993). Much less is known about the interactive effects of these stressors interact on life history parameters in *Daphnia* (Coors and Meester 2008). I specifically addressed the following questions:

**Question 1:** Is the production of anti-predator defenses correlated with concomitant decreases in allocation towards life history traits in *D. lumholtzi*?

**Hypothesis 1:** *Daphnia* exposed to *Chaoborus* predator cues will mature later, at a larger size, produce larger clutches and longer head and tail spines. An increased allocation to defense will occur at the expense of developmental rate (slower growth rate, delayed maturation). This will reduce their susceptibility for being consumed by this gape limited predator.

**Question 2:** How does the addition of low quality food, in the form of cyanobacteria, *Cylindrospermopsis raciborskii*, modify life history parameters in *D. lumholtzi*?

**Hypothesis 2:** Due to their filamentous morphology and nutritional inadequacies, *C. raciborskii* will cause delays in size and age of maturation, juvenile growth, and defense traits, as well as decreases in clutch size in *D. lumholtzi*.

**Question 3:** How does the interaction between predation and food quality (i.e., cyanobacteria) influence the expression of life history traits in *D. lumholtzi*?

**Hypothesis 3:** The addition of low quality food will limit the ability of *Daphnia* to adaptively respond to predator cues. As a result, the life history differences between predator and non-predator treatments will be reduced by increasing quantities of cyanobacteria.

## Chapter 2

### Methods

This work crossed a predator cue exposure treatment with a food quality treatment that consisted of increasing amounts of cyanobacteria (at the expense of green algae). Below I discuss the preparation of each environmental stressor and then the experimental design.

#### 2.1 Kairomone Collection

To generate media conditioned with *Chaoborus* kairomones, standard protocols were used (Herbert and Grewe 1985). Predator cues were prepared by boiling 200 hundred grams (wet wt) of commercially obtained *Chaoborus punctipennis* in 200mL of water for 3-min. The liquid was first passed through a 100um sieve to remove any larvae and coarse material. Next the extract was filtered through a series of (2.7um, 1.6um, 1.2um, 0.8um, 0.45um, 0.2um) Whatman glass microfiber filters to remove any impurities. Approximately 350mL of filtrate was recovered, which was then frozen in 10mL aliquots at -20°C. Samples were defrosted daily over the course of the experiment and any unused predator cues were discarded after 4 hrs. *Daphnia* in the predator treatments received 0.12mL of *C. punctipennis* extract per 60mL of COMBO media (Kilham et al. 1998) every other day. *Daphnia* in non-predator treatments received 0.12mL of nanopure water per 60mL of COMBO.

#### 2.2 Algal Species

*Cylindrospermopsis raciborskii* is a filamentous, heterocystous cyanobacteria that is native to freshwater habitats in tropical and subtropical environments but has recently invaded water systems throughout the world (Padisak 1997; Yilmaz et al. 2008). *Cylindrospermopsis raciborskii* is notable for its role in the Palm Island Mystery Disease

in Australia in November of 1979. Nearly 150 people, mostly children, became ill with symptoms such as gastroenteritis, diarrhea, and kidney malfunction. Initially unripen mangoes were blamed until a linkage between the outbreak and *C. raciborskii* in the water supply was confirmed to be the cause (Hawkins et al. 1997; Yilmaz et al. 2008).

This experiment used food treatments that crossed *C. raciborskii* with the green algae *Scenedesmus obliquus*. *Cylindrospermopsis raciborskii* was purchased from the Culture Collection at the University of Texas (LB 2897). This strain is a straight trichome morphotype and was originally obtained from Lake Lemon in Indiana on 8/18/05 (Fig. 2). *Scenedesmus obliquus* was supplied by and maintained in the Walsh lab (Fig. 3, Walsh et al. 2014). Stock cultures of each species were maintained in 1.5L of algae COMBO and kept at room temperature with pH = 7.8. They were continuously aerated and kept under fluorescent lights (12/12h light: dark cycle) (Fig.4). New cultures of *C. raciborskii* were started by transferring 5,000 cells/ml into fresh COMBO. Cell concentration was estimated using a Gridded Sedgewick Rafter cell (cell size: 50mm x 20mm x 1; cell volume: 1.0mL). Cultures were allowed to grow for approximately 2 weeks before being utilized in this experiment. This work used cultures of *C. raciborskii* from the late-exponential phase (Walsh et al. 2014). To quantify resource availability in this experiment (see below for descriptions of the food treatments), carbon contents were measured using a Perkin Elmer Series II CHNS/O Analyzer. Linear regressions between absorbance and carbon content were strong (absorbance value of 664:  $r^2 = 0.9827$ ; absorbance value at 750:  $r^2 = 0.9786$  for *S. obliquus*, and 664:  $r^2 = 0.9758$ ; absorbance value at 750:  $r^2 = 0.9768$  for *C. raciborskii*).

### 2.3 Study Lakes

This study used clones of *D. lumholtzi* from 3 freshwater reservoirs in Texas (Lake Arlington, Joe Pool Lake, and Grapevine Lake). Created in 1957, Lake Arlington

(32.72°N, 97.2°W) provides drinking water for the city of Arlington in addition to being a popular recreation and fishing spot (TSHA, 2013). Located on Trinity Creek in the Trinity basin, Lake Arlington has a surface area of 885ha and a maximum depth of 20m (Simek and Chrzanowski 1992). Joe Pool Lake (32.35°N, 97.0°W) was impounded in 1986 and provides drinking water to the City of Midlothian. Located in the southern region of the Dallas-Fort Worth metroplex, Joe Pool Lake has a surface area of 3620 ha and a maximum depth of depth 23m (Chrzanowski and Grover 2008). The third reservoir, Grapevine Lake (32.9722°N, 97.0567°W), is located 20mi northeast of Dallas. Impounded in 1952 through the damming of Denton Creek, it provides drinking water, flood control, and recreational usage (USACE 2013). It has a surface area of 2704 ha and a maximum depth of 20m (TPWD.gov 2014).

#### 2.4 Establishment of Study Organisms

Laboratory populations of *D. lumholtzi* were established by hatching resting eggs (ephippia) from lake sediment that were collected with an Ekman grab during the fall 2012 and spring/summer 2013. This work used 2 clones from Joe Pool, 2 clones from Grapevine, and 1 clone from Arlington (Fig. 5). Each clone was kept in batch cultures under common conditions (Photoperiod 16L: 8D; 28°C) for 3 months prior to the start of the experiment. During this period of time, media (COMBO) was replaced every other day and the clones were fed a non-limiting supply of green algae (*Scenedesmus obliquus*). To standardize conditions prior to the common garden experiment, I reared all clones under common conditions for two generations. To do this, I pulled adult females from each stock culture and immediately collected offspring upon the release of the next clutch. These females were reared until maturation. Offspring were collected from the subsequent and later clutches from these females and reared in replicate in the presence and absence of predator cues. Fifteen parental clones were reared per treatment

(predator vs. non-predator), with the exception of the Lake Arlington clone which was replicated twenty times due to their small clutch sizes. Parents of our experimental animals were reared in the presence of predator's cues because previous research has shown that *Daphnia* are sensitive to predator cues during embryonic development (Agrawal et al. 1999). Offspring from the third clutch were then collected and used in the common garden experiment. Throughout all phases of laboratory rearing, animals were reared individually in 90mL jars containing 60mL of COMBO media. Each individual was fed >1.0 mg C/l of *S. obliquus*. Clones were transferred to jars containing new media and algae every other day and raised under the same photoperiod and temperature regimes (Photoperiod 16L: 8D; 28°C) (Fig. 6).

## 2.5 Experimental Design

The common garden experiment utilized a full factorial design and included manipulations of predator cues presence (presence vs. absence) and four concentrations of cyanobacteria (100% *S. obliquus*/0% *C. raciborskii*, 66% *S. obliquus*/33% *C. raciborskii*, 33% *S. obliquus*/66% *C. raciborskii*, and 0% *S. obliquus*/100% *C. raciborskii*) (Fig. 7). There were thus eight unique treatments in this experiment (predator present/0% cyanobacteria, predator present/33% cyanobacteria, predator present/66% cyanobacteria, and predator present/100% cyanobacteria; predator absent/0% cyanobacteria, predator absent/33% cyanobacteria, predator absent/66% cyanobacteria, and predator absent/100% cyanobacteria). Regressions between carbon content and absorbance in a spectrophotometer (at 664 and 750nm) were used to create the four resource treatments (Walsh et al. 2014). *Cylindrospermopsis raciborskii* was added to the treatments containing cyanobacteria such that the total algal carbon concentration was kept constant across all treatments. The experiment began by collecting 64 newly-born individuals (~12 hours old) per clone from the third and subsequent clutches of the

second generation laboratory reared *Daphnia*. All individuals were transferred into 90mL jars containing COMBO medium and randomly assigned to one of the eight treatments. Each clone was replicated 20X per treatment (one clone per jar) (5 clones x 8 treatments x 20 replicates= 800 jars total). COMBO, algae, and predator cues were changed every other day throughout the experiment.

## 2.6 Dependent Variables

The dependent variables measured in this study included (1) growth rate, (2) age at maturation, (3) size at maturation, (4) clutch size (no. of offspring produced in clutches 1-3), (5) defense traits (length of head and tail spines), and (6) intrinsic rate of increase ( $r$ ). Growth rate was measured by photographing each individual on day 1 and day 5. This measurement was then converted to growth rate via:  $[(\text{length on day 5} - \text{length on day 1})/\text{no. of days}]$ . Age at maturation is defined as the release of the first clutch into the brood chamber. All animals were monitored for maturation ~3 daily. Upon attaining maturation, the size of the first clutch was recorded and all individuals were photographed for estimates of size at maturation. All individuals were subsequently monitored daily for the production of clutches 2-3. Defense traits, head and tail spine length, were determined by photographing all individuals on day 5. Estimates of age at maturation and number of offspring (C1-C3) were combined and used to calculate the intrinsic rate of increase ( $r$ ) for each treatment. Intrinsic rate of increase ( $r$ ) is a commonly used index of fitness that is a composite of several life history traits (Mackenzie et al. 2001).  $r$  was calculated  $r = \ln(R_0/G)$ , where  $R_0$  is the net reproductive rate (fecundity) and  $G$  is generation time (average age at time of maturation for each clone in each treatment).

## 2.7 Statistical Analysis

Dependent variables were analyzed using general linear models (SPSS v. 21). Predator treatment (presence vs. absence), resource treatment (0, 33, 67, 100% *C. raciborski*), and the predator x resource interaction were entered as fixed effects. Clonal lineages and all clone x treatment interactions were entered as random effects. The full model included all two-way interactions as well as the three way interaction between predator, resource, and clone. Satterthwaite approximations were used to estimate denominator degrees of freedom. Data for all parameters was tested for homogeneity and normality. Age at maturation was logarithmic-transformed and clutch size was square root-transformed to improve homoscedasticity and fits with normality. For the analyses of the defense traits, head and tail spine length were combined into a single variable with size at maturation included as a covariate. This corrected for differences in size amongst clones (Fig 6).

## 2.8 Missing Values and Outliers

Pre-reproductive mortality (i.e. from onset of treatment to maturation) was very low (<1%). Mortality/ephippial production between maturation and the production of clutch 3 were slightly higher but consistent across all five clones [Mortality rate (%): Arlington = 1.5%, Grapevine #1 = 2.5%, Grapevine #2 = 2.6%, Joe Pool #1 = 1.3%, Joe Pool #2 = 2.7%]. For the analysis of age at maturation, clutch size, and defense traits extreme values were removed to improve fits with normality. 0-2 extreme values were removed per clone. Removal of these extreme values did not alter trends or significance of results.

## Chapter 3

### Results

The expression of life history traits in *D. lumholtzi* depended strongly upon the interactive effects of predator cues and resource quality. The 'predator x resource' interaction was significant ( $p < 0.05$ ) for several traits including growth rate, size at maturation, clutch size, and intrinsic rate of increase ( $r$ ) (but not for age at maturation and head and tail spines). Significant variation in clonal responses to the combined effects of predator cues and food quality was also observed (i.e., significant clone x predator and clone x predator x resource interactions were observed for several traits). Below I first describe the patterns for the main effects and then detail the nature of these interactions.

#### 3.1 Effects of Predation

There was non-significant ( $p > 0.05$ ) influence of predator cues on all measured variables (Table 1).

#### 3.2 Effects of Food Quality

Increasing amounts of *C. raciborskii* significantly influenced the expression of all traits (Table 1; Fig. 8). Across an increasing gradient of cyanobacteria, *D. lumholtzi* experienced a 13% delay in maturation, a 25% decrease in growth rate, 28% decrease in size at maturation, and a 29% reduction in clutch size. *Daphnia lumholtzi* also experienced a 26% decrease in intrinsic rate of increase ( $r$ ) when fed an increasing concentration of *C. raciborskii*.

#### 3.3 Predator x Food Quality Interaction

There was a significant interaction ( $p < 0.05$ ) between predator treatment and food quality for all traits save age at maturation and head and tail spine length (Table 1; Fig. 8). Here, the differences between predator and non-predator treatments depended strongly on increasing concentrations of cyanobacteria. Differences between predator

and non-predator treatments were generally small in the '0%', '33%' and '66%' *C. raciborskii* treatments. However, the differences between predator and non-predator treatments were much stronger in the '100%' *C. raciborskii* treatment; *D. lumholtzi* exposed to predator cues grew faster, matured at a larger size, produced more offspring, and exhibited a higher rate of intrinsic increase in the '100%' *C. raciborskii* treatment. The opposite trend was observed for defense traits; *Daphnia* reared in the presence of predator cues produced smaller spines compared with the no predator treatments in the 100% *C. raciborskii* treatment (predator x food interaction  $p = 0.13$ ).

### 3.4 Clonal Variation

#### 3.4.1 Clone x Predator Interactions

A significant predator x clone interaction was observed for age at maturation, clutch size, spine length, and intrinsic rate of increase ( $r$ ) (Table 1; Fig. 9). These interactions indicate that responses to predator cues varied across clonal lineages. For example, three clones responded to the presence of predator cues by maturing earlier than the non-predator treatments while two other clones responded in the opposite fashion. Such divergent responses amongst clones were also apparent for clutch size, spine length, and intrinsic rate of increase ( $r$ ) (Fig. 9).

#### 3.4.2 Clone x Predator x Resource Interactions

The expression of life history traits varied as a function of clonal lineage, predator treatment, and food quality (i.e., significant clone x predator x food interaction) for age at maturation and head and tail spines (Table 2; Fig 10). Similar to the 'clone x predator' interaction, clone-specific responses to predator cues were further complicated by exposure to increasing concentrations of *C. raciborskii*. For instance, increasing concentrations of cyanobacteria typically resulted in slower rates of development. Yet, the magnitude of such an effect on each clone differed across predator and resource

treatments. Some clones exhibited stronger delays in the timing of maturation across the increasing gradient of cyanobacteria in the presence of predator cues. While other clones yielded the opposite response; stronger delays in maturation in the absence of predator cues (Fig. 10).

## Chapter 4

### Discussion

These results show that the expression of life history traits in *D. lumholtzi* depends upon the combined effects of predation and food quality. Increasing concentrations of sub-optimal cyanobacteria resulted in delayed maturation, reductions in growth, a smaller size at maturation, and the production of smaller clutches (and slower rate of population growth). However, the life history traits of *Daphnia* that were reared in the presence of predator cues were less negatively impacted by this low quality food source. For example, *Daphnia* exhibited a 29% decrease in growth in response to an increasing concentration of cyanobacteria when reared in the absence of predator cues. This negative impact of low quality food on the growth of *Daphnia* was reduced to 15% in the presence of predator cues. These significant ‘food x predator’ interactions for age and size at maturation, clutch size, and intrinsic rate of increase ( $r$ ) show that *Daphnia* maintain higher fitness in the face of increasing quantities of poor food in the presence of predators. However, such allocation to life history traits came at the expense of investment in defense traits. Head and tail spine length increased in response to increasing quantities of cyanobacteria and decreased in the treatments reared in the presence of predators. These results argue that *Daphnia* preferentially divert energy to life history traits at the expense of defense characteristics when faced with simultaneous increases in stress associated with predators and food quality.

In isolation, predation and food quality have been shown to significantly influence the expression of traits in *Daphnia*. Reductions in survivorship, growth, and fecundity have been well documented in *Daphnia* species in response to predation and cyanobacteria (Black and Dodson 1990; Dao et al. 2010; Hebert and Grewe 1985; Nogueira et al. 2004; DeMott et al. 1991; Spitze 1992; Stibor 1992). A growing body of

literature has begun to consider the simultaneous influence of multiple stressors on the fitness of a variety organisms including zooplankton (Boone et al. 2007; Coors and Meester 2008; Heugens et al. 2006; Pauwels et al. 2009; Relyea 2001; Scherer et al. 2013; Walls 1997; Yin et al. 2011). This includes a similar study that explored the synergistic effects of food stress (manipulation of resource levels) and predator exposure on *Daphnia* (Pauwels et al. 2009). Pauwels et al. (2009) also found that the differences between predator and non-predator treatments were much stronger under stressful conditions (i.e., low resource levels). Thus, as observed in the present study, *Daphnia* preferentially allocated more energy towards life history traits when exposed to more stressful conditions. Additional studies have begun to explore the stress-induced effects of pesticides. For example, Coors and Meester (2008) found that pesticide exposure accelerated the infection caused by the parasite, *Pasteuria ramosa*, in *Daphnia*. Infection from *P. ramosa* did not significantly impact *Daphnia*'s survival until coupled with pesticide exposure, thus illustrating the synergistic effects of these stressors. Scherer et al. (2013) investigated the effects that temperature and predation had on fungicide exposure in *Daphnia*. They discovered low temperatures coupled with exposure to predator cues masked the negative impacts that a fungicide had on *Daphnia*'s fitness (see also Qin et al. 2011). While the specific stressors and organisms vary across studies, all of this work illustrates that interactions among multiple stressors are complex and often unpredictable. As a result, the results of the current experimental work in conjunction with this previous research collectively argues that multiple environmental cues need to be considered to accurately understand the fitness of natural populations given that organisms are exposed to various environmental stressors.

#### 4.1 Fitness Consequences of Cyanobacteria: What is the Mechanism?

Previous studies have demonstrated significant fitness reductions in *Daphnia* that are fed cyanobacteria ( Bednarska 2014; Dao et al. 2010; Hairston et al. 2001; Martin-Creuzburg and Elert 2009; Nogueira et al. 2004; Schwarzenberg et al. 2010; Walls 1997). Similar to this work, the current set of results also revealed that increasing quantities of cyanobacteria negatively impacted the expression of all life history traits. This influence of food type on *Daphnia* traits was particularly strong in the '100% *C. raciborskii*' treatment. For instance, growth and reproductive output was reduced by 25% and 29% when compared to the control treatment (predator-free and 100% *S. obliquus*), respectively. Supplementation with the high quality green algae, *S. obliquus*, in the 33% and 67% *C. raciborskii* treatments largely mitigated the negative fitness consequences of cyanobacteria (Fig 8).

A variety of mechanisms may explain the negative impact of *C. raciborskii* on the traits of *Daphnia*. First, *C. raciborskii* can be toxic to *Daphnia*. Certain strains of *C. raciborskii* have been shown to produce hepatotoxins (cylindrospermopsin) and neurotoxins (saxitoxins and anatoxin-a). However, the presence of toxins and level of toxicity varies greatly among strains of *C. raciborskii* and it remains unclear if any strains present in the United States are toxic (Graham et al. 2010; Yilmaz et al. 2007). While the toxicity of the strain of *C. raciborskii* used in the present study is unknown, we did not observe any symptoms associated with toxin production (i.e., increased mortality rate, acute paralysis) (Bednarska et al. 2014; Ferrao-Filho et al. 2007). The production of toxins per se thus appears unlikely to explain the fitness consequences of *C. raciborskii*. Second, *C. raciborskii* is characterized by lower nutritional content than green algae. This is due to the absence of essential lipids and sterols which are vital components of the diet of zooplankton (Martin-Creuzburg and Elert 2009). Finally, *C. raciborskii* are

filamentous, which can cause mechanical interference during food gathering thus resulting in decreased feeding efficiency and increased energetic costs (Bednarska et al. 2014). It is unlikely that *C. raciborskii*'s filaments were inedible as there was no significant difference in mortality among treatments. While the exact mechanism(s) is unclear, poor manageability combined with low nutritional content are likely responsible for the observed declines in fitness in response to increasing amounts of cyanobacteria.

#### 4.2 Clonal Variation

*Chaoborus* are gape-limited ambush predators (Abram 1980). *Daphnia* thus frequently respond to the presence of *Chaoborus* by accelerating growth and increase investment in defense traits at the expense of reproductive characteristics (i.e., delayed maturation and smaller clutches) in the presence of *Chaoborus* (Black and Dodson 1990; Hebert and Grewe 1985; Riessen 1999). As a result, a surprising aspect of this study was the non-significant influence of predator cues on the expression of life-history traits. Overall, the influence of predator cues on life history traits was minor because the clones used in this study differed strongly in the direction and magnitude of their responses to predators. I observed a significant clone x predator interaction for most measured life history traits (growth rate, size at maturation, clutch size, and intrinsic rate of increase ( $r$ ) (see Table 1) and a significant clone x predator x food interaction for age at maturation and spine length. For instance, two clones matured faster in the presence of predator cues, while the other clones exhibited the opposite response to predator cues (i.e., delayed maturation) (Fig. 9). Increasing concentrations of cyanobacteria further complicated the clone-specific responses to predator cues (see Fig. 10). Clone-specific responses to environmental stressors have been observed in several other studies (Pauwels et al. 2010; Riessen 1999; Walls 1997; Weider and Pijanowski 1990). Yet, the majority of studies exploring phenotypic plasticity continue to draw conclusions based

upon a single clone (Black and Dodson 1990; Stibor 1992). It is becoming increasingly clear that any conclusions regarding plastic responses to environmental cues will depend strongly upon the amount of genetic variation that is included in any given study.

#### 4.3 Climate Change

It is widely accepted that global temperatures are increasing at an accelerated rate (Paerl and Hulsman 2009; Winder and Schindler 2004; Woodward et al. 2010). With climate change, the frequency and duration of harmful algal blooms (HAB) are expected to increase, displacing chlorophytes and diatoms (Paerl and Hulsman 2009). This shift in phytoplankton assemblage has the potential to significantly alter zooplankton populations and the community structure of freshwater habitats. Harmful algae, such as species of cyanobacteria, have clear negative impacts on zooplankton and thus have the potential to exert strong selection on zooplankton communities (Hairston and Hairston 1993; Hairston et al. 2001). Yet, my results, using a modest number of clones, revealed significant genetic variation in responses to cyanobacteria (food x predator x clone interactions). These results potentially foreshadow that natural populations of *Daphnia* harbor the necessary genetic variation to rapidly respond to expected changes in the phytoplankton community that is correlated with global climate change. Incorporating climate change into studies concerning stressors and their influence on organisms is imperative.

#### 4.4 Conclusions

The simultaneous effects of two common environmental stressors (predators and food quality) on the expression of life history traits in the invasive species of zooplankton, *Daphnia lumholtzi* were examined in this study. The expression of traits was found to depend strongly upon the combined effects of predator exposure and food quality (Fig. 8). *Daphnia* exposed to predator cues maintained faster rates of growth and higher

reproductive outputs than *Daphnia* from non-predator treatments when faced with severe declines in resource quality. Yet the opposite pattern was observed for defense characteristics. Understanding multiple stressors is challenging because their combined effect cannot be predicted based on the outcomes of single-stressor studies (Black and Dodson 1990; Boone et al. 2007; Dao et al. 2010; DeMott et al. 1991; Hebert and Grewe 1985; Nogueira et al. 2004; Spitze 1992; Stibor 1992). Given that organisms routinely experience many sources of environmentally-driven stress, further insight into these complex responses to multiple stressors is needed to better understand and predict species responses to changes in ecological conditions including global climate change.

Appendix A  
Figures



Figure 1. *Daphnia lumholtzi* photographed by Deirdre Leonard-Whittington at Matt Walsh's laboratory (UTA) in 2013.

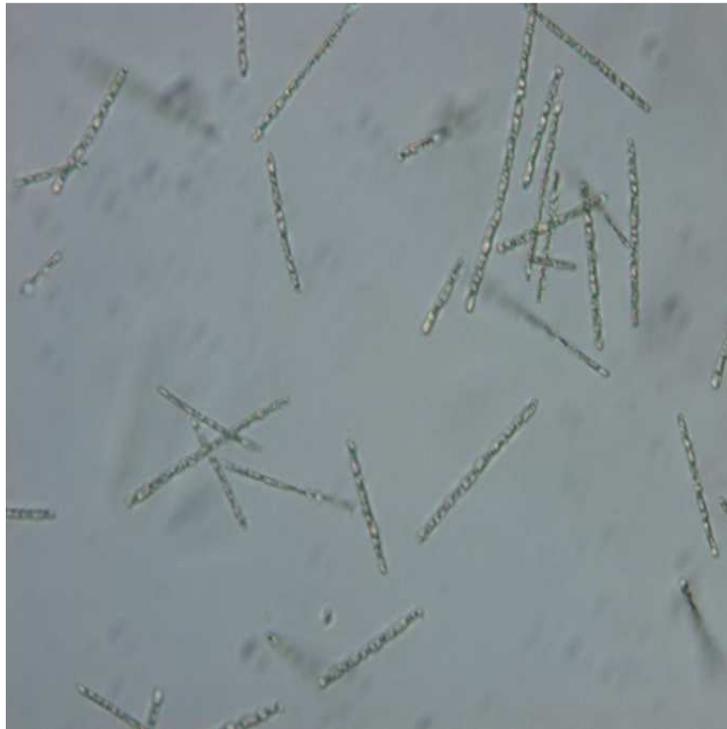


Figure 2. *Cylindrospermopsis raciborskii* photographed by Deirdre Leonard-Whittington at Sophia Passy's laboratory (UTA) in 2014.

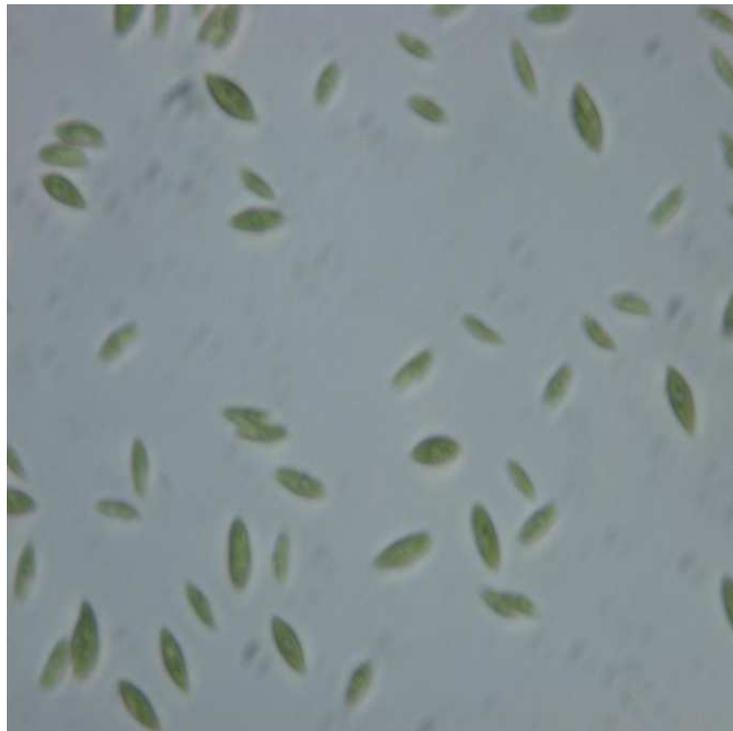


Figure 3. *Scenedesmus Obliquus* photographed by Deirdre Leonard-Whittington  
in Sophia Passey's laboratory (UTA) in 2014.



Figure 4. Stock cultures of *Cylindrospermopsis raciborskii* (red label) and *Scenedesmus obliquus* (green label) in Dr. Matthew Walsh's lab (UTA). Photographed by Deirdre Leonard-Whittington in November 2013.

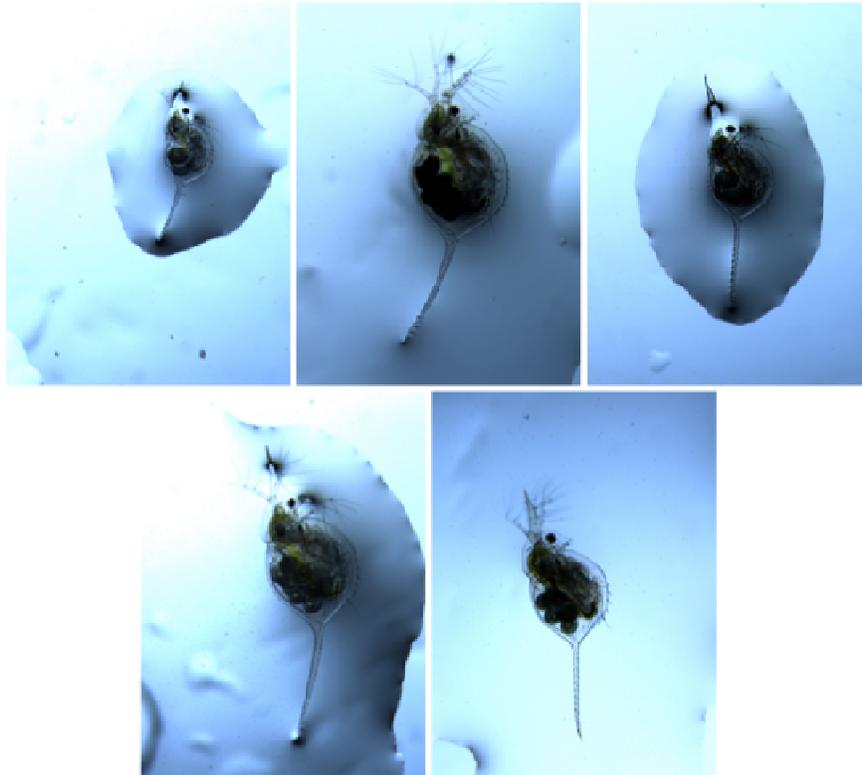


Figure 5. Experimental clones of *Daphnia lumholtzi* photographed by Deirdre Leonard-Whittington at Matt Walsh's laboratory (UTA) in 2014. All photographs were taken within 12hrs. of maturation. a, clone 1-Arlington; b, clone 2- Grapevine; c, clone 3- Grapevine; d, clone 4- Joe Pool; e, clone 5- Joe Pool lakes.



Figure 6. Laboratory conditions of *Daphnia lumholtzi* clones.

(Photoperiod 16L: 8D; 28°C).

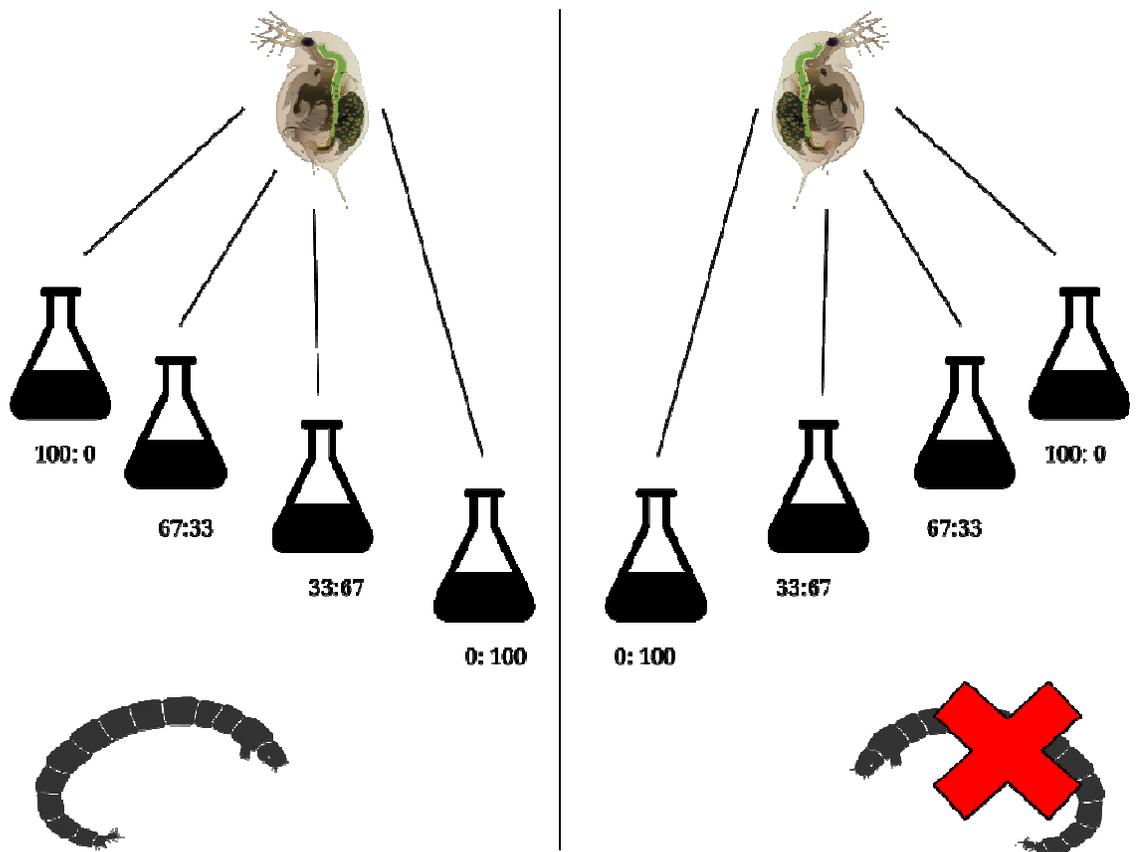


Figure 7. Full factorial design of the common garden experiment that included manipulations of predator cues presence (presence vs. absence) and four concentrations of cyanobacteria (100% *S. obliquus*/0% *C. raciborskii*, 67% *S. obliquus*/33% *C. raciborskii*, 33% *S. obliquus*/67% *C. raciborskii*, and 0% *S. obliquus*/100% *C. raciborskii*. Total algal carbon concentration was kept constant across all treatments. Temperature: 28°C, Photoperiod 16L:8D.

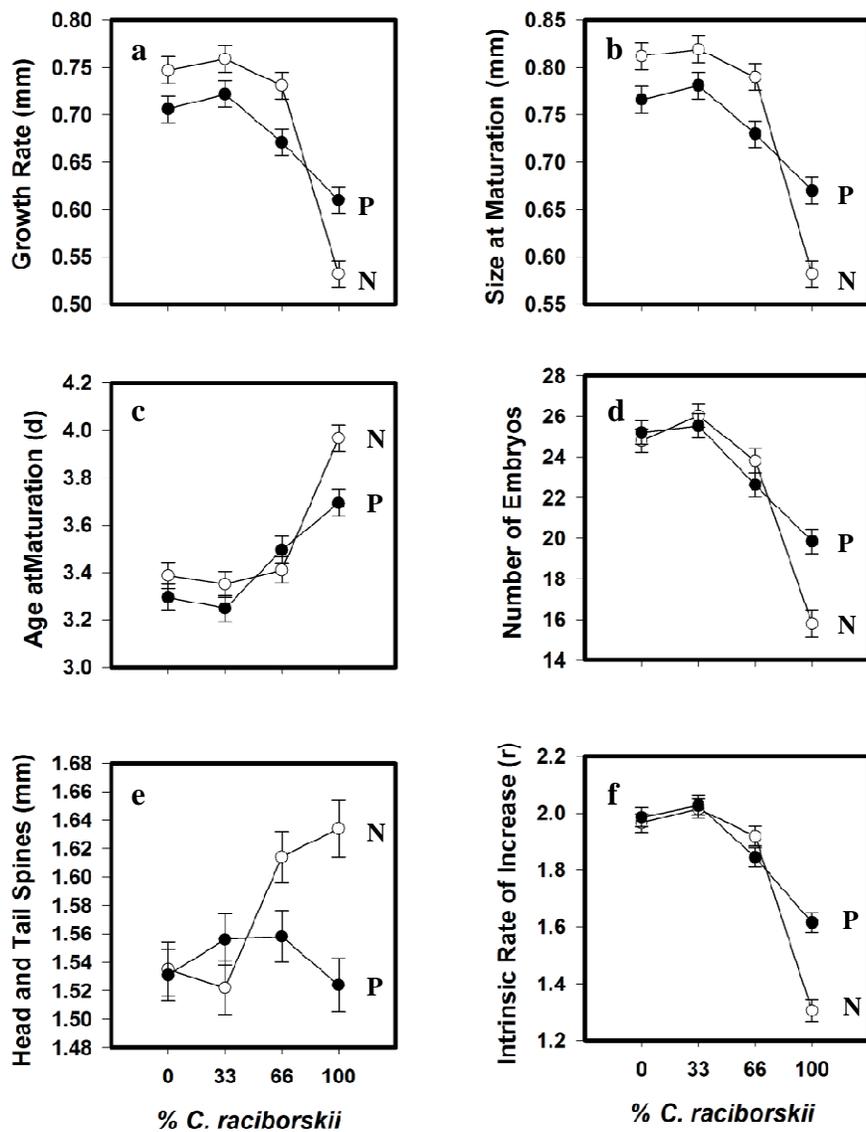


Figure 8. Life-history differences among resource treatments in the presence and absence of predator cues. (a) Growth rate, (b) size at maturation, (c) age at maturation, (d) total number of embryos produced in clutch 1-3, (e) defense traits (length of head spine + tail spine), and (f) intrinsic rate of increase ( $r$ ). Error bars = +1 SE. P, predator; N, non-predator. *Daphnia* maintained higher fitness in the face of increasing quantities of poor food in the presence of predators.

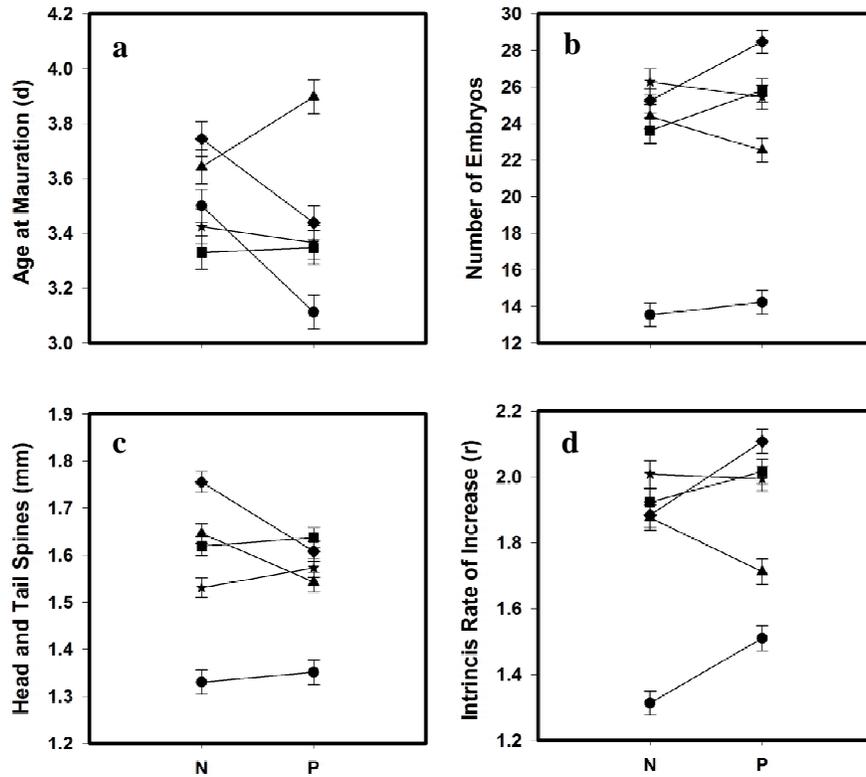


Figure 9. Life-history differences among clones in the presence and absence of predator cues. Significant ( $p < 0.05$ ) clone  $\times$  predator treatment interactions were observed for (a) age at maturation, (b) number of embryos, (c) head and tail spines, (d) intrinsic rate of increase ( $r$ ). Error bars =  $\pm 1$  SE. ●, clone 1- Arlington; ▲, clone 2- Grapevine; ■, clone 3- Grapevine; ◆, clone 4- Joe Pool; \*, clone 5- Joe Pool lake. P, predator; N, non-predator.

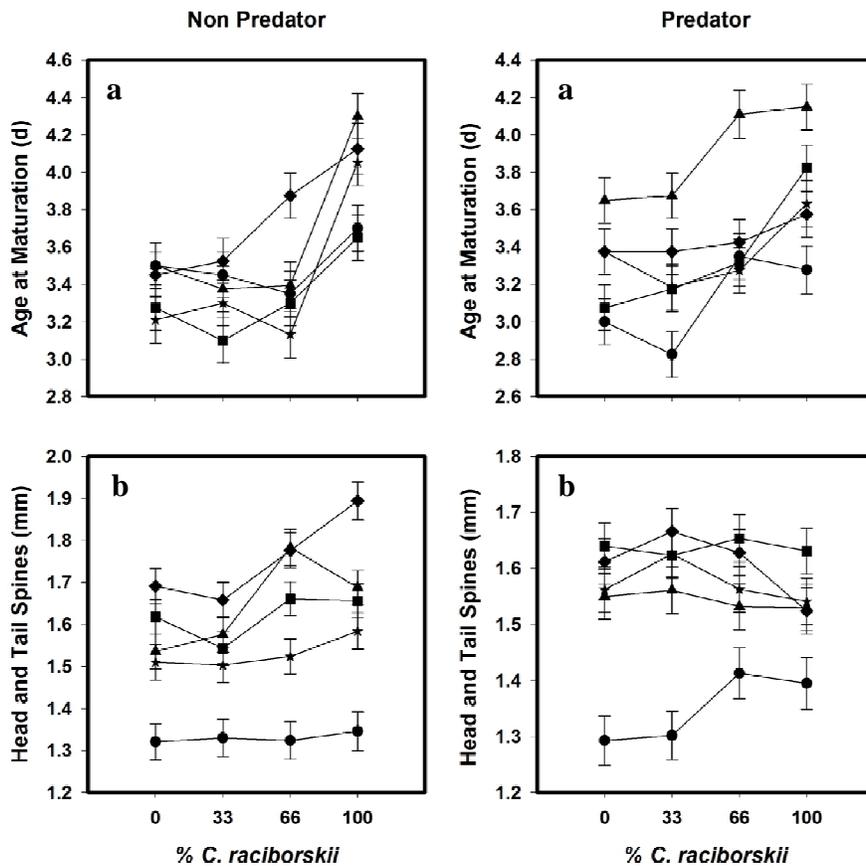


Figure 10. Trait differences among clones as a function of predator and food treatments.

Significant ( $p < 0.05$ ) clone x predator x resource treatment interactions were observed

for (a) age at maturation and (b) defense traits. Error bars =  $\pm 1$  SE. . ●, clone 1 -

Arlington; ▲, clone 2 - Grapevine; ■, clone 3 - Grapevine; ◆, clone 4 - Joe Pool; \*, clone 5

- Joe Pool lakes.

Appendix B

Tables

Table 1. Analysis of life-history and defense traits. Linear mixed models were used with predator treatment and food resource entered as fixed effects and clone entered as a random effect. The denominator degrees of freedom are displayed after each F value.

Factor	Trait d.f.	Growth Rate	Age at Maturation	Size at Maturation	Number of Embryos	Head and Tail Spines	Intrinsic Rate of Increase (r)
		F (d.f)	F (d.f.)	F (d.f)	F (d.f)	F (d.f)	F (d.f)
Fixed effects:							
Predator	1	2.151 <sup>NS</sup> (4.0)	0.767 <sup>NS</sup> (4.0)	1.424 <sup>NS</sup> (4.0)	0.654 <sup>NS</sup> (4.0)	0.791 <sup>NS</sup> (4.002)	0.859 <sup>NS</sup> (4.001)
Resource	3	26.962 <sup>***</sup> (12.001)	32.825 <sup>***</sup> (12.008)	27.656 <sup>***</sup> (12.001)	15.737 <sup>***</sup> (12.010)	6.221 <sup>**</sup> (14.849)	25.653 <sup>***</sup> (12.013)
Predator x Resource	3	7.698 <sup>**</sup> (12.002)	1.560 <sup>NS</sup> (12.005)	8.651 <sup>**</sup> (12.002)	6.400 <sup>**</sup> (12.037)	2.396 <sup>NS</sup> (12.127)	9.522 <sup>**</sup> (12.046)
Random effects:							
Clone	4	129.620 <sup>***</sup> (4.888)	2.371 <sup>NS</sup> (3.228)	120.880 <sup>***</sup> (5.252)	16.526 <sup>***</sup> (8.620)	5.850 <sup>NS</sup> (2.606)	7.544 <sup>*</sup> (6.584)
Predator x Clone	4	0.842 <sup>NS</sup> (12.003)	4.699 <sup>*</sup> (12.006)	1.091 <sup>NS</sup> (12.003)	3.976 <sup>*</sup> (12.081)	3.640 <sup>*</sup> (12.005)	7.696 <sup>**</sup> (12.095)
Resource x Clone	12	1.743 <sup>NS</sup> (12)	0.564 <sup>NS</sup> (12)	1.727 <sup>NS</sup> (12)	3.713 <sup>*</sup> (12)	0.260 <sup>NS</sup> (12.014)	3.557 <sup>*</sup> (12)
Predator x Resource x Clone	12	1.354 <sup>NS</sup> (750)	2.057 <sup>*</sup> (745)	1.395 <sup>NS</sup> (750)	1.390 <sup>NS</sup> (664)	2.359 <sup>**</sup> (746)	1.143 <sup>NS</sup> (665)

<sup>\*</sup>  $p < 0.05$ , <sup>\*\*</sup>  $p < 0.01$ , <sup>\*\*\*</sup>  $p < 0.001$ , <sup>NS</sup>  $p > 0.05$

Table 2. Clone means ( $\pm 1$  s.e.) for life history traits and head and tail spines across each predator and resource treatment.

Predator Resource	NP				P			
	0%	33%	66%	100%	0%	33%	66%	100%
<b>Growth Rate (mm)</b>								
Clone 1	0.400 (0.030)	0.429 (0.031)	0.352 (0.030)	0.289 (0.030)	0.357 (0.030)	0.411 (0.030)	0.381 (0.030)	0.320 (0.031)
Clone 2	0.850 (0.030)	0.892 (0.030)	0.864 (0.031)	0.585 (0.030)	0.853 (0.030)	0.881 (0.030)	0.742 (0.030)	0.657 (0.030)
Clone 3	0.760 (0.030)	0.793 (0.030)	0.767 (0.030)	0.631 (0.030)	0.769 (0.030)	0.729 (0.030)	0.706 (0.031)	0.676 (0.030)
Clone 4	0.914 (0.030)	0.864 (0.030)	0.843 (0.030)	0.581 (0.032)	0.833 (0.030)	0.842 (0.030)	0.812 (0.030)	0.748 (0.030)
Clone 5	0.811 (0.031)	0.817 (0.030)	0.827 (0.031)	0.575 (0.032)	0.717 (0.030)	0.764 (0.030)	0.711 (0.030)	0.648 (0.030)
<b>Size at Maturation (mm)</b>								
Clone 1	0.438 (0.031)	0.468 (0.032)	0.389 (0.031)	0.323 (0.031)	0.398 (0.031)	0.451 (0.031)	0.422 (0.031)	0.359 (0.032)
Clone 2	0.923 (0.031)	0.955 (0.031)	0.927 (0.032)	0.637 (0.031)	0.913 (0.031)	0.938 (0.031)	0.803 (0.031)	0.732 (0.031)
Clone 3	0.822 (0.031)	0.860 (0.031)	0.833 (0.031)	0.686 (0.031)	0.835 (0.031)	0.793 (0.031)	0.768 (0.032)	0.742 (0.031)
Clone 4	0.994 (0.031)	0.932 (0.031)	0.897 (0.031)	0.637 (0.032)	0.905 (0.031)	0.917 (0.031)	0.880 (0.031)	0.810 (0.031)
Clone 5	0.881 (0.032)	0.882 (0.031)	0.906 (0.032)	0.629 (0.032)	0.778 (0.031)	0.808 (0.031)	0.775 (0.031)	0.704 (0.031)

\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , <sup>NS</sup>  $p > 0.05$

Table 2. Continued

Predator Resource	NP				P			
	0%	33%	66%	100%	0%	33%	66%	100%
<b>Age at Maturation (d)</b>								
Clone 1	3.5 (0.122)	3.45 (0.122)	3.35 (0.122)	3.7 (0.122)	3.0 (0.122)	2.825 (0.122)	3.35 (0.122)	3.278 (0.129)
Clone 2	3.5 (0.122)	3.375 (0.122)	3.395 (0.125)	4.3 (0.122)	3.65 (0.122)	3.675 (0.122)	4.111 (0.129)	4.150 (0.122)
Clone 3	3.275 (0.122)	3.1 (0.122)	3.3 (0.122)	3.65 (0.122)	3.075 (0.122)	3.175 (0.122)	3.316 (0.125)	3.825 (0.122)
Clone 4	3.45 (0.122)	3.525 (0.122)	3.875 (0.122)	4.125 (0.137)	3.375 (0.122)	3.375 (0.122)	3.425 (0.122)	3.575 (0.122)
Clone 5	3.211 (0.125)	3.3 (0.122)	3.132 (0.125)	4.053 (0.125)	3.375 (0.122)	3.184 (0.125)	3.275 (0.122)	3.632 (0.125)
<b>Number of Embryos</b>								
Clone 1	15.25 (1.242)	14.95 (1.242)	13.85 (1.242)	10.118 (1.347)	12.85 (1.242)	14.75 (1.242)	15.529 (1.347)	13.8 (1.434)
Clone 2	26.75 (1.242)	30.0 (1.242)	24.333 (1.309)	16.429 (1.484)	26.5 (1.309)	27.263 (1.274)	20.187 (1.388)	16.211 (1.274)
Clone 3	25.882 (1.347)	28.167 (1.309)	25.313 (1.388)	15.077 (1.54)	27.882 (1.347)	26.588 (1.347)	25.632 (1.274)	23.158 (1.274)
Clone 4	26.789 (1.274)	26.5 (1.309)	25.706 (1.347)	22.0 (1.484)	30.722 (1.309)	28.737 (1.274)	27.15 (1.242)	27.25 (1.242)
Clone 5	29.353 (1.347)	30.579 (1.274)	29.857 (1.484)	15.333 (1.603)	28.056 (1.309)	30.316 (1.274)	24.611 (1.309)	18.813 (1.388)

\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , <sup>NS</sup>  $p > 0.05$

Table 2. Continued

Predator Resource	NP				P			
	0	33	66	100	0	33	66	100
<b>Head and Tail Spines (mm)</b>								
Clone 1	1.321 (0.043)	1.33 (0.044)	1.324 (0.044)	1.346 (0.046)	1.293 (0.044)	1.302 (0.043)	1.413 (0.045)	1.395 (0.046)
Clone 2	1.536 (0.041)	1.576 (0.042)	1.783 (0.042)	1.688 (0.042)	1.550 (0.041)	1.561 (0.042)	1.532 (0.041)	1.530 (0.041)
Clone 3	1.618 (0.041)	1.543 (0.041)	1.661 (0.041)	1.656 (0.041)	1.64 (0.041)	1.623 (0.041)	1.654 (0.042)	1.631 (0.041)
Clone 4	1.691 (0.42)	1.658 (0.042)	1.776 (0.041)	1.894 (0.045)	1.612 (0.041)	1.666 (0.041)	1.628 (0.041)	1.524 (0.041)
Clone 5	1.510 (0.042)	1.503 (0.41)	1.524 (0.042)	1.584 (0.043)	1.562 (0.041)	1.626 (0.041)	1.563 (0.041)	1.541 (0.041)
<b>Intrinsic Rate of Increase (r)</b>								
Clone 1	1.453 (0.072)	1.437 (0.072)	1.389 (0.072)	0.972 (0.078)	1.457 (0.072)	1.666 (0.072)	1.509 (0.078)	1.408 (0.083)
Clone 2	2.031 (0.072)	2.182 (0.072)	2.033 (0.078)	1.259 (0.086)	1.954 (0.076)	2.011 (0.074)	1.569 (0.080)	1.312 (0.074)
Clone 3	2.084 (0.078)	2.209 (0.076)	2.033 (0.08)	1.367 (0.089)	2.185 (0.078)	2.087 (0.078)	2.088 (0.076)	1.703 (0.074)
Clone 4	2.035 (0.074)	2.023 (0.076)	1.892 (0.078)	1.586 (0.086)	2.233 (0.076)	2.125 (0.074)	2.063 (0.072)	2.007 (0.072)
Clone 5	2.220 (0.078)	2.228 (0.074)	2.242 (0.086)	1.341 (0.093)	2.097 (0.076)	2.244 (0.074)	1.992 (0.076)	1.642 (0.080)

\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , <sup>NS</sup>  $p > 0.05$

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### Biographical Information

Deirdre Leonard-Whittington was born in Oklahoma City, Oklahoma and moved to Texas at the age of 8. She received her Bachelor's degree in Biology in 2011 and a Masters in Biology in 2014 from the University of Texas at Arlington. During that time, Deirdre worked as a veterinary technician and a graduate teaching assistant. Deirdre and her husband, Ron, welcomed their first son, Clark, in January 2012 and their second son, Pierce, in July 2014. Following graduation, Deirdre intends to pursue a career in environmental science; protecting human health and the environment.