

**THE EFFECT OF COPPER ON KAIROMONE-MEDIATED  
RESPONSES BY WILD *DAPHNIA PULICARIA* CLONES FROM  
LAKES ALONG A COPPER GRADIENT**

by

Colleen Mary Inglis

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## Abstract

Kairomones are infochemicals that benefit a heterospecific receiver. *Chaoborus spp.* release a kairomone from their gut when feeding on *Daphnia spp.* *Daphnia* respond to kairomone by shifting life history parameters or producing neonates with induced morphological defenses, increasing their chance of survival. When laboratory-cultured *Daphnia* are exposed to environmentally-relevant metal concentrations, a kairomone response is not induced, increasing predation vulnerability.

Currently *Daphnia* live in metal-contaminated lakes in Sudbury, ON. It is possible the extant population is tolerant of relatively high copper (Cu) concentrations and can still induce a kairomone response. In comparison, it is hypothesized clones found in lakes isolated from anthropogenic sources of metal-contamination would be less tolerant as they have not been exposed to high Cu concentrations.

The purpose of this study was to examine how multiple clones of *D. pulicaria* obtained from lakes along a Cu gradient respond to kairomone in the absence and presence of copper. Several different clones from Ontario lakes located in the Canadian Shield were exposed to environmentally-relevant Cu concentrations and *Chaoborus* kairomone. Neonates were collected and measured to assess predator-induced defenses.

Results indicate that kairomone-mediated responses and Cu-tolerance vary among *D. pulicaria* clones. Clones from the Sudbury area were able to induce a response to kairomone when exposed to Cu, indicating a Cu-tolerance. However, this was not true for all Sudbury clones. In contrast, most clones from clean lakes did not respond to kairomone when exposed to Cu; while some clones exhibited a Cu-tolerance. Clones that were not tolerant of Cu were affected at concentrations much lower than those predicted by the Biotic Ligand Model (BLM)

that induce acute toxicity. Predictions generated by the BLM can be conservative making them useful when creating water quality criteria; however, my results indicate these predictions can also be under-protective. Chemosensory cues mediate vital life processes that are essential for survival. Populations may be devastated if metals interfere with chemosensory cues. Overall, the results of my study suggest that genetic variation is important for population establishment and maintenance, specifically when exposed to multiple stressors, and that directional selection may result in stress tolerance.

## Co-Authorship

This thesis conforms to the manuscript format as outlined by the School of Graduate Studies and Research. The manuscript that is a direct result of this thesis and its coauthors are:

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# Table of Contents

Abstract .....	ii
Co-Authorship .....	iv
Acknowledgements.....	v
Table of Contents.....	vii
List of Abbreviations .....	ix
List of Figures .....	x
List of Tables .....	xii
Chapter 1 Introduction .....	1
Chapter 2 Literature Review .....	5
2.1 Chemical Cues and Chemosensation .....	5
2.2 Chemically Mediated Predator-Prey Interactions .....	6
2.3 Inducible Antipredator Defense Strategies in <i>Daphnia</i> spp.....	7
2.4 The Cost of Inducible Defenses .....	9
2.5 Factors Affecting Kairomone-Mediated Responses and Response Variation .....	11
2.6 Contaminant Effect on Chemosensory Function .....	12
2.7 Chronic Metal Exposure and Adaptive Metal-Tolerance .....	15
2.8 Research Overview .....	17
Chapter 3 Materials and Methods .....	18
3.1 Experimental Approach .....	18
Experiment 1.....	18
Experiment 2.....	18
3.2 General Methods .....	19
Study Lakes .....	19
Collection and Maintenance of Animals .....	20
FLAMES Medium Preparation.....	20
Stimulus Preparation.....	21
Metal Solution Preparation.....	21
3.3 Experimental Design.....	22
Experiment 1: Evaluating kairomone response in various <i>D. pulicaria</i> clones.....	22
Experiment 2: Effect of Cu on <i>D. pulicaria</i> clones from lakes along a Cu gradient.....	23

3.4 Data Analysis.....	23
Chapter 4 Results .....	30
4.1 Experiment 1: Evaluating kairomone response in various <i>D. pulicaria</i> clones .....	30
4.2 Experiment 2: Effect of Cu on <i>D. pulicaria</i> clones from lakes along a Cu gradient .....	31
Chapter 5 Discussion .....	47
5.1 Kairomone-Mediated Inducible Response.....	47
5.2 Costs and Trade-offs of Inducible Defenses .....	50
5.3 Effect of Cu on Kairomone Mediated Responses .....	51
5.4 Effect of Natural Environment on Cu-Tolerance.....	53
5.5 Implications of Research.....	56
5.6 Conclusion .....	57
Chapter 6 Conclusions and Summary .....	59
Summary .....	64
References.....	65
Appendix A FLAMES Medium Preparation .....	78
Appendix B Preparation of Sugar-Buffered Formalin .....	81
Appendix C Supplementary Experimental Data .....	82

## List of Abbreviations

ANOVA: Analysis of Variance

BLM: Biotic Ligand Model

CCC: Criterion for Continuous Concentration

CMC: Criterion for Maximum Concentration

DESC: Dorset Environmental Science Centre

df: Degrees of Freedom

DOC: Dissolved Organic Carbon

FAV: Final Acute Value

F1 and F2: Filial Generations 1 and 2

FLAMES: Field Laboratory for the Assessment of Multiple Ecological Stressors

HA: Humic Acid

HSD: Honestly Significant Difference

ICP-AES: Inductively Coupled Plasma Atomic Emission Spectroscopy

LUCAS: Lakehead University Center for Analytical Services

M1: Maternal Generation

*n*: Number of replicates

*p*: Significance Level

S: Sulfur

SD: Standard Deviation

SEM: Standard Error of the Mean

WQC: Water Quality Criteria

## List of Figures

- Figure 1: Map of Ontario indicating the general locations of the study lakes that were used in the experiment. .... 26
- Figure 2: Diagram of *D. pulicaria* illustrating how total body length was measured..... 29
- Figure 3: Comparison of neonate body length when exposed to the control stimulus or kairomone. Bars represent mean +SEM, asterisks indicate significant differences and numbers on bars represent *n*. .... 35
- Figure 4: Comparison of time to first reproduction in days from all clones exposed to either the control or kairomone. Bars represent mean +SEM, asterisks indicate significant differences between stimuli, and numbers on bars represent *n*. .... 37
- Figure 5: Comparison of the size of the first brood (number of neonates) released by all clones exposed to either the control stimulus or kairomone. Bars represent means +SEM and difference and asterisks indicate significant differences between stimuli. Numbers on bars represent *n*..... 39
- Figure 6: Comparison of the average brood size (number of neonates) produced by all clones exposed to the control stimulus or kairomone. Bars represent means +SEM. Numbers on bars represent *n* and asterisks denote significant differences between the stimuli. .... 41
- Figure 7: A significant interaction between Cu and kairomone was observed for the following clones. Measured responses were not affected by Cu within the control stimulus; however, Cu did inhibit kairomone-mediated responses in these three clones. Mean responses within the control and kairomone stimulus are shown at each Cu concentration  $\pm$ SEM. Asterisks denote the lowest Cu concentration at which kairomone response was lost compared to the kairomone response at 0  $\mu$ g/L Cu (post hoc Tukey HSD). .... 43
- Figure 8: Comparison of the relative kairomone response for all clones when exposed to 0  $\mu$ g/L Cu and 10  $\mu$ g/L Cu. The bars represent the mean kairomone response as a percentage, where

kairomone response at 0  $\mu\text{g/L}$  Cu is considered 100% of the induced response. Significant differences are denoted by asterisks. .... 44

## List of Tables

Table 1: Characteristics of lakes from which clones originated, including corresponding clone names. Data were obtained from the DESC in 2007, except for data from Round Lake, which were collected by C. Inglis in 2008.....	27
Table 2: The mean measured Cu concentrations ( $\pm$ SD) for all treatments used in the experiment relative to the nominal Cu concentrations that were intended. Actual values vary from nominal values as there were trace levels of Cu present in FLAMES medium. ....	28
Table 3: Summary of Student's t-test (or Welch's t-test) evaluating differences in total neonate length when clones were exposed to <i>Chaoborus</i> kairomone or the control stimulus. ....	34
Table 4: Summary of Student's t-test evaluating differences in time to first reproduction when clones were exposed to <i>Chaoborus</i> kairomone or the control stimulus.....	36
Table 5: Summary of Student's t-test (or Welch's t-test) evaluating the difference in the size of first brood produced by clones when exposed to <i>Chaoborus</i> kairomone compared to those exposed to the control stimulus.....	38
Table 6: Summary of Student's t-test (or Welch's t-test) evaluating the average brood size that was released by each clone when exposed to <i>Chaoborus</i> kairomone compared to the control stimulus.....	40
Table 7: Summary of two-way ANOVAs used to assess the multiple effects of stimulus, Cu, and the interaction between those two variables on the kairomone response induced by each clone. Asterisks denote differences that are significant.....	42
Table 8: Water chemistry data from each study lake used in the BLM to generate acute toxicity predictions for <i>D. pulicaria</i> . Data provided by the DESC, except for Round Lake, which was collected by C. Inglis in 2008. ....	45

Table 9: Summary of Cu acute toxicity predictions for <i>D. pulicaria</i> generated by the BLM compared to the Cu concentrations that inhibited kairomone responses. ....	46
Table 10: Required compounds for preparation of FLAMES medium (Celis-Salgado et al 2008). ....	78
Table 11: Requirements for the preparation of chemical stock solutions for the mineral trace elements solution as seen in Table 10 (Celis-Salgado et al. 2008). ....	79
Table 12: Ingredients required for the preparation of the vitamin solution as seen in Table 10 (Lynch et al 1986). ....	80
Table 13: Morphometric data for all clones when exposed to each treatment. ....	82
Table 14: Life historical data for all clones exposed to each treatment. ....	99

# Chapter 1

## Introduction

The ability to detect chemical cues via chemosensation mediates many different ecological functions that are essential for survival (Brönmar and Hansson 2000). Chemical communication that occurs between two species is facilitated by chemical cues known as kairomones. Kairomones are released by one species and detected by another species that will subsequently induce a response (Whittaker and Feeny 1971; Dicke and Grostal 2001). Many predator-prey interactions are mediated by kairomones and consequently this form of interspecific communication is important for both predator and prey species (Krueger and Dodson 1981; Lima and Dill 1990; Kats and Dill 1998; Wisenden 2000). An example of this type of kairomone-mediated predator-prey relationship exists between the common waterflea, *Daphnia* spp. (prey) and the phantom midge larva, *Chaoborus* spp. (predator). *Chaoborus* release a kairomone from its digestive tract to the surrounding water when actively feeding on *Daphnia* (Krueger and Dodson 1981). *Daphnia* detect this kairomone via chemosensation and respond with antipredator defenses that increase their probability of survival (Kats and Dill 1998).

Kairomone-induced responses in *Daphnia* may exist as behavioural, morphological, or life historical changes. The type of response that is induced is not only species specific, but also varies between clones of a single species and may reflect current environmental conditions (Parejko and Dodson 1991; Spitze 1992; Tollrian 1995; De Meester 1996; Lass and Spaak 2003; Boeing et al. 2006a). Recently it has been determined that relatively high metal concentrations typically seen in industrially-contaminated lakes, interfere with the induction of kairomone-mediated morphological responses (Hunter and Pyle 2004; Mirza and Pyle 2009). When *Daphnia pulex* are exposed to *Chaoborus* kairomone they typically produce neonates with

neckteeth, which are small protrusions found on the back of the head (Spitze 1992; Tollrian 1993). Neckteeth serve to enlarge the neonate beyond the gape limit of the predator and reduce predation (Swift and Fedorenko 1975; Pastorok 1981; Tollrian 1995). Hunter and Pyle (2004) found when *D. pulex* were exposed to environmentally-relevant concentrations of Cu (5 µg/L) necktooth induction was inhibited. Several studies have reported impaired chemosensation in other aquatic species when exposed to low concentrations of Cu (Beyers and Farmer 2001; McPherson et al. 2004; Carreau and Pyle 2005; Mirza et al. 2009), including additional accounts of impaired kairomone-induced defenses in *Daphnia* (Mirza and Pyle 2009). If *Daphnia* are not able to induce an appropriate kairomone-mediated response they will be more susceptible to predation. Havel and Dodson (1984) found that the induction of neckteeth in response to kairomone increased neonate survival by 64% relative to those that did not induce neckteeth. This indicates how important inducible antipredator defenses are for survival.

The protection conferred by kairomone-mediated responses is vital for the maintenance of healthy *Daphnia* populations. This is ecologically important because *Daphnia* hold an essential position in aquatic food webs. As primary consumers, *Daphnia* provide a food source for many secondary consumers, while controlling populations of primary producers (Tsui and Wang 2007). A decrease in *Daphnia* populations as a result of increased predation could have a top-down and bottom-up effect on the entire food web (Carpenter et al. 2001).

For several decades *Daphnia* have been used as a model species in toxicity testing because they are easily cultured in the laboratory, they have a relatively short lifecycle, they are cyclic parthenogens, and they hold an important trophic position in aquatic food webs (Lampert 2006). However, most research that has examined the effects of contaminants on *Daphnia* uses only a single clone from which to draw conclusions (Gillis et al. 2005; Guan and Wang 2006; Barata et al. 2007; Deleebeeck et al. 2008; Wu et al. 2008; Vandenbrouch et al. 2009). Results

from these studies are extrapolated to wild *Daphnia* populations and used to establish water quality criteria. These results only reflect a single clone and do not consider differences in the measured response due to clonal variation. Furthermore, wild *Daphnia* populations chronically exposed to metals for multiple generations may respond differently to laboratory metal exposures relative to laboratory-reared clones. It is possible that when animals are chronically exposed to a metal they may develop a tolerance as a result of adaptive phenotypic plasticity or genetic adaptation (Klerks and Weise 1987; Lata et al. 2007).

In the Sudbury, ON area, approximately 7000 lakes in a 17 000 km<sup>2</sup> area experienced Cu-contamination as a result of intense mining activity since the late 1800s (Keller et al. 1992; Gunn et al. 1995). Although smelter emission reduction efforts have been in place since the 1970s, Cu concentrations are still high relative to lakes that have not been affected by industrial activity (Dillon et al. 1986; Keller and Piblado 1986; Pyle et al. 2005; Keller 2009). Regardless of Cu-contamination many aquatic organisms have re-established in these lakes (Gunn and Keller 1990; Keller and Yan 1991; Keller et al. 2002). It is possible that aquatic organisms are able to persist in these lakes because they have developed a tolerance to increased Cu concentrations. Lopes et al. (2006) found populations of *Daphnia longispina* that originated from a Cu-contaminated environment were more tolerant to Cu stress, in comparison to a reference population from a non-contaminated site. This tolerance remained even after the animals were cultured in clean water for an extended period of time, indicating that metal-tolerance was the result of genetic adaptation as opposed to phenotypic plasticity (Lopes et al. 2006).

If *Daphnia* living in a Cu-contaminated environment have developed a tolerance to Cu it is possible that they may still respond to kairomone, even though previous literature using only single, laboratory-reared clones has shown that metals inhibit chemosensory responses. Furthermore, work by Hunter and Pyle (2004) and Mirza and Pyle (2009) established response

inhibition in *Daphnia* at Cu concentrations much lower than those that cause acute toxicity. This is important because most water quality guidelines are based on acute toxicity testing and even though low Cu concentrations may not induce mortality, they do interfere with ecological functions (e.g., predator avoidance) that are essential for survival. Therefore, it is imperative to study wild *Daphnia* populations along a gradient of Cu-contamination and assess a more appropriate endpoint in order to establish ecologically relevant water quality criteria.

The objectives of this research were to (1) determine how different clones of *D. pulicaria* originating from various lakes along a Cu-gradient, respond to *Chaoborus* kairomone under clean laboratory conditions, (2) determine how Cu affects the ability of these clones to induce kairomone-mediated responses, and (3) determine if Cu effects are reflective of the clones' historical exposure to Cu.

## Chapter 2

### Literature Review

#### 2.1 Chemical Cues and Chemosensation

Chemosensation is a mode of communication that mediates many intra- and interspecific interactions that are critical for survival (Brönmar and Hansson 2000). Infochemicals, which are semiochemicals (chemical cues) such as pheromones or kairomones, can provide information that can be used during the mate selection process (Svensson 1996; Fisher and Rosenthal 2006; Whitlock 2006), kin recognition (Olsén et al. 2002; Mann et al. 2003; Mateo 2003; Green et al. 2008), the location of migration routes (Dittman and Quinn 1996; Barbin et al. 1998), habitat suitability assessments (Tamburri et al. 1996; Welch et al. 1997), foraging opportunities (Roth et al. 1999; Valentinčič et al. 2000; Fink et al. 2006; Krueter et al. 2008), and predator identification (Krueger and Dodson 1981; Lima and Dill 1990; Kats and Dill 1998; Wisenden 2000). Chemical communication is particularly important for aquatic organisms as other sensory modalities are not always reliable (Brönmar and Hansson 2000). For example, vision may be reduced due to increased water turbidity. Furthermore, water is considered the universal solvent, which allows most chemical cues to rapidly dissolve and diffuse throughout the environment (Weissburg 2000). As a result, aquatic animals have developed refined and sophisticated chemical communication systems.

Due to their complexity, chemical cues can be divided into two general categories: pheromones and allelochemicals (Nordlund and Lewis 1976). Pheromones are chemical cues that mediate intraspecific interactions (Karlson and Lüscher 1959), while allelochemicals are involved in interspecific interactions (Whittaker and Feeny 1971). Allelochemicals can be further divided into three groups: 1) synomones, which are adaptively favourable to both the species producing

the cue (sender) and the species detecting the cue (receiver), 2) allomones, which benefit the sender, and 3) kairomones, which benefit the receiver (Whittaker and Feeny 1971; Dicke and Grostal 2001). These different types of chemical cues provide aquatic organisms with relevant information required to mediate most ecological activities and subsequent survival. Kairomones in particular, are evolutionarily intricate because the sender obtains no adaptive benefit and could ultimately experience reduced fitness (Otte 1974). For example, kairomones emitted by a predator species can directly impact the survival of entire prey populations (Lass and Spaak 2003).

## **2.2 Chemically Mediated Predator-Prey Interactions**

The survival of many different prey species is dependent on the detection of kairomones that have been emitted by potential predators. Failure to detect predator kairomones can lead to death, directly affecting the fitness of the receiving organism and entire populations (Lima and Dill 1990; Dicke and Grostal 2001). The chemical structure of kairomones allows them to readily dissolve, dissipate and persist in the water column for extended periods of time, which make them effective chemical cues (Tollrian and von Elert 1994). Aquatic organisms have such well developed chemosensory systems that many are able to distinguish between kairomones produced by predators that are an imminent threat and those that are not (Laforsch et al. 2006). For example, snails (*Theba pisana*) that are exposed to excreta from beetles (*Carabus carabus*) that had been fed conspecifics will respond more strongly compared to snails exposed to excreta from beetles fed liver (Lefcort et al. 2006). Predator-derived kairomones can indicate the presence of the predator because they can be the product of the predator's diet and emitted via excreta (Dicke and Grostal 2001; Slusarczyk and Rygielska 2004; Wilder and Rypstra 2004; Lefcort et al. 2006).

Once the predator kairomone has been detected, prey species can respond accordingly to increase their probability of survival (Kats and Dill 1998).

Responses typically elicited by kairomones can include alterations in life historical parameters (Sih and Moore 1993; Repka et al. 1994; Ball and Baker 1996) and the induction of physiological (Rehnberg and Schreck 1987; Stoks and McPeck 2003b; Pauwels et al. 2005), behavioural (Chivers et al. 1996; Berendonk 1999; Hazlett 1999; von Elert and Pohnert 2000), or morphological defenses (McCollum and Leimberger 1997; Engel and Tollrian 2009). Kairomones are only beneficial to a prey species if members of that species appropriately respond to the cue once it has been detected. If a response is not elicited the prey species will be more susceptible to predation (Havel and Dodson 1984; Mirza and Pyle 2009).

A frequently studied example of this type of kairomone-mediated predator-prey interaction exists between the common waterflea, *Daphnia* spp. (prey) and the phantom midge larva, *Chaoborus* spp. (predator). This interaction has been used as a model system in research because both *Chaoborus* and *Daphnia* are sympatric, they are easily collected from the wild and maintained in the laboratory, and *Daphnia* reproduce quickly. When actively feeding on *Daphnia*, *Chaoborus* will release a kairomone from its digestive tract to the surrounding water (Krueger and Dodson 1981). Once detected, *Daphnia* may induce several different types of responses that are effective defense strategies to prevent predation by *Chaoborus* (Kats and Dill 1998; Boeing et al. 2006a).

### **2.3 Inducible Antipredator Defense Strategies in *Daphnia* spp.**

The type of antipredator response induced is reflective of the species of *Daphnia*, the current environmental conditions, and the presence or absence of additional predator species. Depending on the type of response that is induced, *Daphnia* may be protected directly by

preventing predation, or indirectly by increasing population growth. This response may include a change in behaviour, morphology, or life history parameters.

Behavioural responses to *Chaoborus* kairomone has been well documented in several previous studies. For example, Pijanowksa and Kowalczewki (1997) found that *Daphnia magna* Straus clones decreased swimming activity when exposed to *Chaoborus* kairomone. This type of response is effective because *Chaoborus* rely on mechanical stimuli in order to locate prey (Lewis 1977; Giguere and Dill 1979). Meanwhile, Boeing et al. (2006a) found that *D. pulex* responded to *Chaoborus* kairomone by migrating vertically upward to the water surface during the day. This type of response may be induced because *Chaoborus* occupy the lower, darker levels of the water column during the day to avoid fish predators (Luecke 1986; Voss and Mumm 1999). By relocating to an area in the water column where *Chaoborus* are not present, *Daphnia* are able to avoid predation (Dodson 1988).

In a more indirect attempt to defend against predation, *Daphnia* may alter life history parameters (Dodson 1988b). A common response seen in *Daphnia* that have been exposed to *Chaoborus* kairomone is an increase in age at maturity and a delay in reproduction (Havel 1985; Dodson 1988; Hanazato 1995; Tollrian 1995; Riessen 1999). Altering life history parameters can act to reduce the impact of predation on a population because allocating more energetic resources to growth allows an individual to quickly attain a size-refuge from predators before they start allocating energy to reproduction. Although this strategy will help to increase the likelihood of survival of the reproducing individual, it does not always ensure offspring success.

As a more direct way to reduce predation many *Daphnia* species reproduce and release neonates with induced morphological defenses when exposed to *Chaoborus* kairomone (Kruegar and Dodson 1981). Morphological defenses can include increases in body and caudal spine length, as well as the formation or elongation of helmets and neckteeth (Kruegar and Dodson

1981; Hebert and Grew 1985; Harvell 1990; Hanazato 1991; Tollrian 1990; Laforsche et al. 2006). Morphological defenses help enlarge the neonate beyond the gape limit of the predator, which reduces handling efficiency making consumption much more difficult (Swift and Fedorenko 1975; Havel and Dodson 1984; Tollrian 2004) and has been shown to increase survival by as much as 64% (Mirza and Pyle 2009). An increase in daphniid body surface area may also increase the encounter rate between predator and prey; however, capture success is decreased as this increase in surface area may lead to increased swimming speed and escape ability (Tollrian 1993).

These different antipredator defenses are only induced when kairomone is detected and disappear once the threat of predation has been removed. Inducible responses or traits are prime examples of phenotypic plasticity (Agrawal 2001). The plastic nature of these traits allows them to only be induced when needed. This infers a potential cost to inducing an antipredator defense because the trait is not expressed throughout the entire span of an individual's life.

## **2.4 The Cost of Inducible Defenses**

Phenotypic plasticity allows an individual to accommodate a changing environment by inducing traits that are optimal for that new environment (Price et al. 2003). However, a plastic trait is usually only expressed when it is necessary to do so, indicating that there must be an associated cost of inducing the trait (Harvell 1990). The potential cost of inducing an antipredator defense may require an energetic investment, which could result in reduced reproductive output (Riessen 1991). Therefore, there is a trade-off between avoiding predation by inducing an antipredator defense, and decreased fecundity that may lower the fitness of an individual (Riessen 1991).

Currently there is much debate in the literature regarding the costs of inducing kairomone-mediated antipredator defenses. For example, Spitze (1992) and Tollrian (1995)

found no clear association between induced morphological defenses and shifts in life historical traits. Tollrian (1995) also found that even when food availability was low there was no reduction in necktooth expression, suggesting little energetic investment is required to induce a morphological defense. However, Black (1993) and Lüning (1995) indicate that daphniids respond to invertebrate predators by investing energy in neonate growth at the cost of reproduction, which results in later maturation and smaller brood sizes.

Due to the variability in previous findings it is difficult to assess whether or not the cost of inducible defenses are direct or indirect. Some earlier studies suggest that inducible antipredator defenses may indirectly incur a cost because they do not cost much to produce. For example, *Daphnia galeata* induces the formation of an elongated helmet and caudal spine in the presence of a predator. The amount of energy required to produce these defenses was approximately 1/7<sup>th</sup> of that required to produce a resting egg; however, this increase in body size caused the species to sink faster (Jacobs 1967 as reviewed in Dodson 1974). It appears that possessing defenses that increase overall body size (and consequently weight) of the daphniid may incur indirect energetic costs that are required to maintain its position in the water column (Jacobs 1967 as seen in Dodson 1974). This hypothesis was further supported by Stenson (1987), who suggested that *Holopedium gibberum* that have acquired a larger capsule experience an increase in drag that hinders mobility, which could be accompanied by a decrease in food uptake. In addition to an increase in weight, morphological defenses may alter the shape of the daphniid in such a way that it affects the hydrodynamic properties of the animal, which may also increase the amount of energy required for swimming (Tollrian 1995).

Changes in life history that are coupled with morphological defenses are likely a result of the indirect costs of producing morphological defenses, but it has been shown that life history and morphological changes are not always coupled and can occur independently of one another. This

supports the idea that these defenses do not incur large costs. It is difficult to fully assess the cost of inducible defenses due to inconsistencies in the literature; however, if an inducible defense response ensures survival, it may be worth the potential cost.

## **2.5 Factors Affecting Kairomone-Mediated Responses and Response Variation**

Several factors dictate whether or not *Daphnia* respond to *Chaoborus* kairomone and what type of response is elicited. Water quality (e.g., low versus high pH), variation in food quality and abundance, and genotype may determine how an individual responds. For example, Tollrian and von Elert (1994) determined that under low pH conditions *Chaoborus* kairomone is less water soluble relative to when the water is more alkali. Therefore, pH may affect the efficacy of the kairomone altering its availability. This may limit or enhance the opportunity for *Daphnia* to detect the cue. Also, when exposed to kairomone under restricted feeding regimes, *Daphnia* have a greater propensity to induce morphological defenses in comparison to when food is readily available (Parejko and Dodson 1991). These results seem counterintuitive because a decrease in food availability translates into a decrease in energetic resources, which may affect the ability to induce antipredator defenses if they are energetically costly. However, under low food conditions *Daphnia* development is much slower, causing them to be smaller and more susceptible to predation for a longer period of time (Riessen 1992). Therefore, it is more advantageous to induce morphological defenses under low food conditions because it takes neonates longer to outgrow the gape limit of *Chaoborus*.

Kairomone response not only relies on external environmental factors, but is reflective of the individual itself. Previous studies have determined that different species of *Daphnia* will induce different responses to kairomone, but the type of response can also vary within a single species. This indicates that predator-defense mechanisms are not only species specific, but also genotype dependent (Spitze 1992; Dodson 1993; Larsson and Dodson 1993; De Meester 1996;

Boersma et al. 1999; Lass and Spaak 2003). For example, Spitze (1992) found that kairomone-induced morphological defenses and changes in life history were highly variable among different genotypes of *D. pulex*. Boeing et al. (2006a) also found that *D. pulex* neonate size may increase or decrease when exposed to *Chaoborus* kairomone depending on the clone. This provides evidence that kairomone-mediated inducible antipredator defenses in *Daphnia* are naturally variable among clones. Therefore, it is likely that not all individual genotypes will respond and subsequently be selected for under suboptimal natural (e.g., severe weather fluctuations, increased predation pressure, inter- or intraspecific competition) or anthropogenically altered (e.g., land development, foreign species introductions, industrial activity) conditions.

Typically in the past most toxicological research that has been done on *Daphnia* used only a single clone (e.g. Gillis et al. 2005; Guan and Wang 2006; Barata et al. 2007; Deleebeeck et al. 2008; Wu et al. 2008; Vandenbrouch et al. 2009). An individual daphniid is easily used to culture large populations in the laboratory because they reproduce parthenogenetically. Although using a single clone may be convenient, it poses problems when attempting to extrapolate results to the population level. Since *Daphnia* are commonly used to evaluate the effects of aquatic contaminants multiple clones should be used to obtain realistic and ecologically relevant results that will reflect the response of entire populations. Recently, the importance of using multiple clones in research has been recognized and as a result more toxicological studies are no longer utilizing results obtained from a single clone (Picado et al. 2007; Coors et al. 2009; Haap and Köhler 2009).

## **2.6 Contaminant Effect on Chemosensory Function**

Industrial activity will typically result in the deposition of foreign substances or chemicals into the environment. Consequently, this input of pollutants, which include pesticides, surfactants, and metals, has led to severe contamination of once pristine environments (Lüring

and Scheffer 2007). Intense mining activity in the Sudbury, ON, area has been ongoing since the late 1800s, which has led to acidification and severe metal contamination of approximately 7,000 lakes over a 17,000 km<sup>2</sup> industrial zone of influence (Keller et al. 1992; Gunn et al. 1995). The release of metals into the aquatic environment can have detrimental effects on organisms inhabiting these areas (Gorham and Gordon 1960). Metal contamination of aquatic environments can cause physiological stress and impair behavioural and ecological activities of local biota (Atchison et al. 1987; Fleeger et al. 2003; Scott and Sloman 2004). Metals can be hazardous to aquatic organisms because they are highly persistent, can be toxic, and have a tendency to bioaccumulate (Atchison et al. 1988).

Due to the nature of chemosensory epithelium it is not surprising that metals can inhibit chemosensory function (Lüring and Scheffer 2007; G.G. Pyle, personal communication). Chemosensation relies on the binding of molecules to chemosensory epithelium, and it is this high capacity to bind molecules that make it susceptible to metal contamination. Metals can interfere with many physiological, behavioural, and ecological activities that are mediated by chemical cues. For example, Lefcort et al. (2000) determined that snails (*Physella columbiana*) exposed to metals failed to respond to predator kairomone. Metals have also been shown to decrease the chemosensory ability of fishes. When exposed to low copper concentrations (<66 µg/L), Colorado pikeminnow (*Ptycholcheilus lucius*) were unable to effectively elicit an antipredator response to conspecific alarm cue, due to olfactory impairment (Beyers and Farmer 2001). Similar effects were seen when yellow perch (*Perca flavescens*) and fathead minnows (*Pimephales promelas*) were exposed to environmentally relevant concentrations of metals (Carreau and Pyle 2005; Mirza et al. 2009).

In the past environmental regulations were based on research that used acute toxicity or mortality as an endpoint. However, recently more research has focused on the subtle, sublethal

effects of contaminants, which are the endpoints now being used when creating legislation. It is important to consider the sublethal effects of contaminants because it has been shown that concentrations much lower than those that cause acute toxicity can interfere with an organism's ability to respond to chemical cues. Hunter and Pyle (2004) examined the effects of low, environmentally relevant concentrations of copper (Cu) and nickel (Ni) (5-25 µg/L and 40-200 µg/L, respectively) on *Daphnia pulex* chemosensation. They determined that *D. pulex* were unable to induce neckteeth in response to *Chaoborus* kairomone even at the lowest metal concentration. If *Daphnia* cannot induce antipredator defenses due to metal-induced chemosensory impairment, populations will have difficulty sustaining themselves because they will be more susceptible to predation. *Daphnia* represent an important link within aquatic ecosystems because they are primary consumers, who help control primary producer populations, while providing a food source for secondary consumers (Tsui and Wang 2007). A decline in *Daphnia* populations could cause a shift in species composition and potentially have a top-down and/or bottom-up effect on the food web, which could be detrimental to the entire ecosystem (Carpenter et al. 2001).

Research that examines metal-induced chemosensory impairment has implications for areas like Sudbury that have lakes with elevated concentrations of metals as a result of industrial activity. Over the last several decades remediation efforts have been put in place in an attempt to recover lakes in this area. This has been done by reducing sulphur dioxide and trace metal emissions from smelters, which has resulted in improved water quality for many of the lakes (Dillon et al. 1986; Keller and Pitblado 1986). Although there have been substantial improvements in water quality, metal concentrations still remain high in several lakes relative to concentrations found in lakes that have not experienced industrial-related metal contamination. The metal concentrations found in Sudbury lakes are well below the lethal response for 50% of

the population (LC50; 3-19 µg/L Cu, data obtained from the DESC 2008) for many different species, but these concentrations are still high enough to cause chemosensory impairment (Hunter and Pyle 2004; McPherson et al. 2004; Pyle et al. 2005; Pyle and Mirza 2007).

Since the 1970s reductions in smelter emissions has led to the re-establishment of planktonic rotifers (Mac Issac et al. 1986), zooplankton (i.e. *Holopedium* spp., *Daphnia* spp., copepods.; Keller and Yan 1991; Yan et al. 1996; Keller et al. 2002) zoobenthos (i.e. leeches, crayfish; Gunn and Keller 1990), and fish (i.e. lake trout; Gun and Keller 1990) in many Sudbury lakes. If metal concentrations in these lakes are still relatively high it is possible that most organisms would be unable to persist in these areas as a result of chemosensory impairment; however, this is not the case.

## **2.7 Chronic Metal Exposure and Adaptive Metal-Tolerance**

Considering many zooplankton and fish populations have re-established and currently persist in Sudbury area lakes, it is possible that these organisms have adapted to function under chronic metal stress and can still effectively rely on chemosensation. Adaptive metal-tolerance has been documented in several species over a broad range of taxa (Klerks and Weis 1987). For example adapted metal resistance in bacteria is quite common. Timoney et al. (1978) and Timoney and Port (1982) found that *Bacillus* spp. and *Vibrio* spp. collected from metal-laden dredge spoils and sewage sludge dumpsites had a higher incidence of mercury resistance in comparison to the same bacteria species from a non-contaminated reference site. Similarly, metal-tolerance has been documented in many Mollusc species. Burrowing bivalves (*Macoma balthica*) from a Cu-contaminated site had higher tolerance to Cu relative to those from references sites when exposed to Cu (Luoma et al. 1983). Similarly, marine gastropods (*Cerithium rupestre*) from a mercury contaminated site had a higher rate of survival when exposed to mercury in the laboratory, compared to animals from a reference site (Baker et al. 1985). Increased metal-

tolerance exhibited by an organism may be the result of acclimation or genetic adaptation (Latta et al. 2007).

Several authors have determined that increased metal-tolerance is the result of a change in genotype. For example, selenium usually has an inhibitory effect on the growth of *Chlorella vulgaris*; however, when *C. vulgaris* is chronically exposed to selenium, growth is no longer inhibited allowing algal growth to proliferate (Shrift 1954). This metal-tolerance remained after culturing the algae in selenium-free medium, indicating that metal-tolerance in this case was not due to acclimation, but actually an adaptive outcome (Shrift 1954). This is important because acclimation indicates that phenotypic plasticity has allowed an organism to shift to a new fitness optimum in order to meet the requirements of the changed environment (Latta et al. 2007). If a population is not able to acclimate to the new environment genetic adaptation will occur (Latta et al. 2007). Adaptive metal-tolerance has also been documented for *Daphnia* species. Lopes et al. (2006) determined that populations of *D. longispina* from a metal-contaminated environment were more tolerant of metal stress, in comparison to a reference population from a pristine environment. It was found that the metal-tolerance acquired by *D. longispina* remained even after the animals were cultured in clean water for an extended period of time, indicating that metal-tolerance was genetically based rather than the result of acclimation. It is important to make the distinction between adaptation and acclimation, because an adaptive trait indicates a new fixed genotype within the population, whereas acclimation is caused by a change in phenotype. Although *Daphnia* are known to have very plastic phenotypes, the selection pressure exerted by a stressor may be strong enough to cause a change in genotype.

There is mounting evidence to suggest metal-tolerance is an adaptable trait. This means it may be possible for aquatic organisms, living in metal-contaminated environments to tolerate these typically unfavourable conditions. Although, metal-tolerance in *Daphnia* has never been

examined from a chemosensory perspective, it is possible that these animals still possess fully functional chemosensory systems as a result of chronic metal exposure. *Daphnia* populations that currently inhabit metal-contaminated lakes in the Sudbury area, have likely adapted a metal-tolerance in order to persist under these conditions. This adaptation would permit *Daphnia* to have functional chemosensory abilities, allowing them to detect the presence of predator kairomone, even when exposed to metals.

## **2.8 Research Overview**

Previous research that has examined the effects of metals on kairomone-induced morphological defenses has been restricted to laboratory-reared *Daphnia* cultures. These results are difficult to extrapolate to wild populations due to the highly variable nature of inducible defenses. In addition, wild *Daphnia* may not be affected by metals in the same way as laboratory-reared cultures because wild populations that originate from metal-contaminated lakes may be more tolerant of metals as a result of chronic exposure. Therefore, it would be more relevant to examine the effects of metals on kairomone-mediated antipredator defenses using natural populations from lakes along a metal-contamination gradient to determine what role prior exposure to metals may have on chemosensory function. Analysis of wild *Daphnia* from metal-contaminated lakes will provide ecologically relevant information, which may help to create more accurate environmental risk assessments.

## Chapter 3

### Materials and Methods

#### 3.1 Experimental Approach

##### *Experiment 1*

Previous studies have revealed high levels of inter-clonal variation in response to predators (Boersma et al. 1998; Boeing et al. 2006a; Boeing et al. 2006b). Therefore, I conducted an experiment to determine how different clones, originating from lakes along a Cu-gradient, respond to *Chaoborus* kairomone. I hypothesized that all *D. pulicaria* clones, regardless of what lake they originated from, would be able to respond to *Chaoborus* kairomone, but that response would vary among clones. When mother daphniids are exposed to kairomone they alter life-history parameters or produce neonates with induced morphological defenses. To test this, I exposed adult *D. pulicaria* and their daughters to two stimuli: *Chaoborus* kairomone or a control stimulus.

##### *Experiment 2*

Hunter and Pyle (2004) previously determined that Cu inhibited necktooth production in a laboratory-reared *Daphnia pulex* clone. This provided evidence that *D. pulex* cannot effectively respond to kairomone when living in a Cu-contaminated environment. However, it is unknown whether or not these results are applicable to *Daphnia* clones that are found in the wild, specifically clones that originate from lakes with elevated Cu concentrations. Currently, many *Daphnia* species are found in lakes with relatively high Cu concentrations. Some of these lakes have Cu concentrations as high, or higher than those known to cause inhibition of kairomone-mediated antipredator responses.

In Experiment 2, I assessed the effect of increasing Cu concentrations on kairomone response in several different clones based on the initial response elicited in Experiment 1. Experiment 2 set out to determine if the Cu concentration of the original lakes from which the clones were obtained influenced whether or not kairomone induced an antipredator response in the presence of Cu.

I hypothesized that clones originating from lakes with elevated Cu concentrations would either (i) have adapted a metal-tolerance and are able to respond to kairomone regardless of Cu concentration, or (ii) be unable to use chemical communication to detect predators. I tested this by exposing adult *D. pulicaria* and their daughters to the same stimuli as in Experiment 1, but with the addition of environmentally-relevant concentrations of Cu. Granddaughters produced by the exposed adult were used to control for maternal effects and were assessed for a kairomone response by examining the presence of morphological defenses. In addition, alterations in life history parameters of the daughter *Daphnia* were monitored throughout the study to determine whether or not Cu had any effect on the induction of kairomone-mediated antipredator defenses.

### **3.2 General Methods**

#### ***Study Lakes***

All *D. pulicaria* clones were collected from Canadian Shield Lakes in Ontario (Figure 1). Lakes were selected based on a Cu gradient (Table 1). Four lakes used in the study are located in the Sudbury, ON area and have historically been metal contaminated as result of mining activity and were considered as metal-contaminated lakes throughout the study. These include Ramsey, Kelly, Simon, and Joe lakes. All of these lakes, except for Joe, currently have Cu concentrations > 10 µg/L. Three of the remaining lakes, Blue Chalk, Glen, and Crown lakes, are in the Dorset, ON area and are relatively isolated from industrial activity. Round Lake is also isolated from industrial activity, but is located in the Chaffey's Locks, ON area. These four lakes were

considered as clean lakes throughout the study because they have never experienced anthropogenically-induced metal contamination and currently have Cu concentrations  $< 5 \mu\text{g/L}$ , except Crown Lake, which has a Cu concentration of  $7.2 \mu\text{g/L}$ . The current Provincial Water Quality Objective for the Protection of Aquatic Life is  $5 \mu\text{g/L}$  Cu (Ministry of the Environment and Energy 1994).

### ***Collection and Maintenance of Animals***

I collected *Daphnia pulicaria* clones from Joe and Round lakes during the summer of 2007 with a vertical plankton tow net (80  $\mu$  mesh). The remaining clones from Blue Chalk, Crown, Glen, Kelly, Ramsey and Simon Lake were obtained from the DESC (Dorset Environmental Science Center, Dorset, ON) where they have been cultured since 2006. *Daphnia* were then cultured in the laboratory and maintained in FLAMES culture medium (Celis-Salgado et al. 2008) at  $21^\circ\text{C}$  under a 16:8 light:dark photoperiod and fed *Chlamydomonas* spp. (3 mL/week; cell density  $2.8 \times 10^6$  cells/mL). I cultured the clones under these conditions to ensure that responses seen were the result of genotypic differences and not acclimation or maternal effects. Clones were provided with an abbreviated name (Table 1).

Fourth instar *Chaoborus americanus* larvae were collected from a fishless pond in North Bay, ON. *Chaoborus* were maintained as larvae in FLAMES culture medium at  $8^\circ\text{C}$  to delay pupation and fed weekly with live brine shrimp larvae, *Artemia salina*, prior to experimental use.

### ***FLAMES Medium Preparation***

FLAMES is an artificial culture medium that I used to culture and maintain zooplankton in the laboratory (Celis-Salgado et al. 2008). FLAMES was developed to provide an artificial culture medium that had water chemistry that was representative of lakes found in the Canadian Shield, which is where all the clones used in this study were obtained. See Appendix A for

details on FLAMES preparation. This medium was also used to prepare all stimuli used during experimentation.

### ***Stimulus Preparation***

I used two stimuli, control (FLAMES culture medium) and *Chaoborus* kairomone to evaluate the presence and type of antipredator response induced by *D. pulicaria* clones. The kairomone was made from FLAMES culture medium that had been conditioned by *Chaoborus* feeding on *D. pulicaria* neonates. *Chaoborus* were fed *Daphnia* neonates at least one week prior to experimentation. This was to ensure *Artemia* were no longer present in the digestive tract and the kairomone produced was the result of a *Daphnia* diet. To make the kairomone stimulus I placed ten *Chaoborus* into 1 L of FLAMES culture medium and fed each one three neonates daily. To ensure kairomone concentration did not fluctuate throughout the experiment, 50% water changes were done daily on the *Chaoborus* holding water. Both stimuli were stored in the refrigerator at 8°C in 1 L glass jars until they were used the next day. Stimuli were warmed to 21°C before being presented to *Daphnia* to reduced thermal shock to experimental animals.

### ***Metal Solution Preparation***

I prepared a Cu stock solution ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , Fisher Scientific, Ottawa, ON) by dissolving the Cu salt in double deionized water. To yield the required Cu concentrations for the exposure solutions, I added appropriate aliquots of the stock solution to each stimulus. Nominal Cu concentrations tested were 0, 5, 10, 15, 25  $\mu\text{g/L}$ . Water samples were taken throughout the study to obtain actual Cu concentrations (Table 2). Prior to analysis, samples were run through a 0.45 $\mu\text{m}$  mixed cellulose ester filter (Milipore MF membrane sterile syringe filter, Millipore, Carrigtwohil, Co. Cork, Ireland) into a 20 mL high-density polyethylene scintillation vial (Fisher Scientific, Ottawa, Ontario) and acidified to 1% with trace metal grade concentrated nitric acid ( $\text{HNO}_3$ , Fisher Scientific, Ottawa, Ontario). Actual Cu concentrations were measured using

inductively coupled plasma atomic emission spectroscopy (ICP-AES, model: Varian Vista Pro ICAP Radial with a Cetac Autosampler) at the Lakehead University Center for Analytical Services (LUCAS; Table 2). LUCAS is a member of the Canadian Association for Laboratory Accreditation Inc (CALA) and implements the use of blanks and control standards (National Institute of Standards and Technology, Gaithersburg, MD) to ensure quality assurance and quality control.

### **3.3 Experimental Design**

#### ***Experiment 1: Evaluating kairomone response in various *D. pulicaria* clones***

Ten replicate exposures of each treatment were conducted in 20 mL glass test tubes at 21°C under a 16:8 light:dark photoperiod. Individual *Daphnia* were tested in 10 mL of the appropriate stimulus [pH median: 6.02 (range: 5.54-6.36); hardness:  $11.77 \pm 3.17$  mg/L as CaCO<sub>3</sub>; alkalinity:  $6.12 \pm 2.47$  mg/L as CaCO<sub>3</sub>]. I placed a single gravid, female *D. pulicaria* into a test tube and exposed her to one of two stimuli: 1) control [no kairomone], or 2) kairomone). *Daphnia* were fed 0.2 mL aliquots of *Chlamydomonas* (cell density:  $7 \times 10^5$  cell/0.2 mL aliquot) daily. All stimuli were made up and mixed with food prior to being added to test tubes. I examined test tubes every 24 hours for neonates and replaced all experimental solutions to avoid bacterial degradation of the kairomone. If neonates had been released by the adult, they were removed, while the adult was left in the original test tube.

To eliminate any maternal effects, and to ensure that the responses that were seen were reflective of the genotype, I used the F2 generation to evaluate kairomone response. The first brood of neonates was discarded to ensure that mothers (M1) had adequate exposure time to the stimulus. Once a female had released her second brood (F1), she was discarded and one of her neonates was then randomly selected to continue exposure to the stimulus. Once again I discarded the first brood of neonates and collected and preserved the second brood of neonates

(F2) in 5.5% sugar-buffered formalin (Haney and Hall 1974, Appendix B) for later examination of inducible morphological defenses. The entire trial up to second brood release by the F2 generation lasted approximately 80-100 days.

To evaluate the induction of morphological defenses, I measured the total body length of neonates from the F2 generation that were preserved using a micrometer and Ultra-Cal Mark III 6" digital callipers (Fred V. Fowler Co. Inc., Newton Massachusetts, United States) with a Leica MZ 12.5 dissecting microscope (Leica Microsystems, Richmond Hill, Ontario, Canada). The total body length measurement included the length from top of the head to the end of the caudal spine (Figure 2). I also recorded several life-historical parameters throughout the experiment, which included: 1) the time to first reproduction, 2) mean size of the first brood produced, and 3) average brood size (based on three broods).

### ***Experiment 2: Effect of Cu on D. pulicaria clones from lakes along a Cu gradient***

Similar to the first experiment, all exposures were conducted in 20 mL glass test tubes and mothers were exposed to 10 mL of stimuli: either kairomone or the control stimulus. In addition, I exposed *Daphnia* to one of five nominal concentrations of Cu: 0, 5, 10, 15 and 25 µg/L. Each combination of stimulus and metal was replicated ten times, creating a 5 x 2 x 10 x 8 factorial design. All exposures were maintained at the same temperature and daphniids were provided the same amount of food as in Experiment 1. Exposures were then executed and neonates were collected and analyzed for antipredator defenses as described in Experiment 1.

### **3.4 Data Analysis**

I conducted all statistical analyses using JMP (Version 7.0, SAS 2007). Sample size was determined by the number of mothers exposed to chemical stimuli and not the number of offspring produced. Differences in sample sizes were due to natural mortalities throughout the experiment.

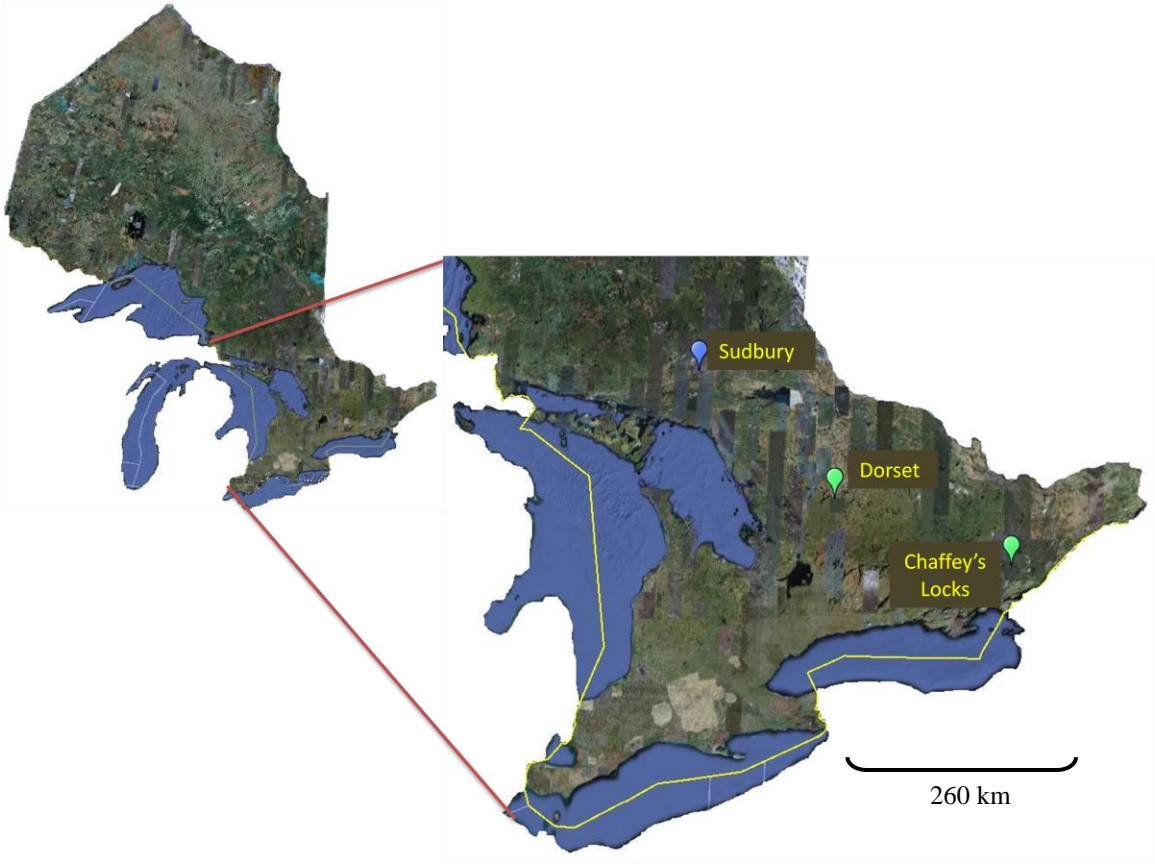
For Experiment 1, all morphological and life historical variables were analyzed to assess whether kairomone induced a response. The distribution of the data collected on neonate body length was determined to be normal by using the Shapiro-Wilk test for normality and homogeneity of variances was confirmed using Bartlett's test of homogeneity of variances. I used a Student's t-test assuming equal variances to determine if neonates were larger when exposed to kairomone relative to the control stimulus. The variance of the total length data for the RO clone was not homogenous; therefore I used a Welch's t-test to analyze this data set. I applied a sequential Bonferroni adjustment to the significance level (alpha) of the analysis to account for the increased probability of Type I errors associated with multiple tests (Hochberg 1988).

Life history data was  $\log_{10}(x + 1)$  transformed to reclaim homogeneity of variance. Although this transformation did not reclaim normality there were still improvements to the distribution of this data and therefore I used parametric analyses. I analyzed differences in life history parameters in clones exposed to kairomone relative to the control stimulus using a Student's t-test test assuming equal variance. A Welch's t-test was used for the KE clone when analyzing first brood size and for the RO clone when analyzing average brood size because the variance of this data was not homogenous. A sequential Bonferroni adjustment was applied to the significance level to reduce the chance of committing Type I errors.

To determine if Cu influenced the ability of *Daphnia* to respond to predators I conducted a two-way ANOVA on clones that exhibited a significant kairomone effect under 0  $\mu\text{g/L}$  Cu. This examined the main effects of the stimulus (control or kairomone), Cu (0, 5, 10, 15, 25  $\mu\text{g/L}$  Cu), and stimulus x Cu interactions on kairomone-induced antipredator defenses in *D. pulicaria*. Main effects were identified using a post hoc t-test. Interactions were further analyzed using a contrast analysis to identify any significant trends in either stimulus as Cu concentration

increased and a Tukey Honestly Significant Difference test was used to determine differences between Cu concentrations.

In addition, I used the values from Table 8 and the Biotic Ligand Model (BLM; HydroQual, Inc, Windows Interface, Version 2.2.3) to determine what concentration of Cu may cause acute toxicity to these clones when living in their natural habitat. The BLM is a model developed to predict the effects of water chemistry on metal speciation and subsequent bioavailability. This model has implications for generating relevant water quality guidelines because it considers site-specific water chemistry and could be used on a lake-by-lake basis (Paquin et al. 2000). It is important to consider the effects of water chemistry on metal speciation because metals can be taken out of solution via precipitation, complexation, and/or adsorption rendering the metal biologically unavailable (Flemming and Trevors 1989). If Cu is not biologically available, aquatic organisms will remain unaffected even if measured concentrations are relatively high. The risk of water quality guidelines being either over-or under-protective is reduced when predictions are generated by a model like the BLM because it considers site-specific water chemistry (Paquin et al. 2000). All of the study lakes have very different water chemistry, which determines Cu toxicity because water chemistry dictates how Cu speciates.



**Figure 1:** Map of Ontario indicating the general locations of the study lakes that were used in the experiment.

**Table 1:** Characteristics of lakes from which clones originated, including corresponding clone names. Data were obtained from the DESC in 2007, except for data from Round Lake, which were collected by C. Inglis in 2008.

Lake	Clone Name	Location	pH	Cu <sup>a</sup>	DOC <sup>b</sup>	Ca <sup>c</sup>	Mg <sup>c</sup>	Alkalinity <sup>d</sup>
Blue Chalk	BC	45°11' N, 78°56' W	6.59	0.5	1.5	2.3	0.6	7.2
Glen	GL	45°08' N, 78°30' W	7.35	0.2	3.6	23.0	0.0	62.8
Round	RO	44°32' N, 76°24' W	8.06	1.0	4.4	24.2	4.5	177.5
Joe	JO	46°44' N, 81°01' W	6.55	3.1	1.9	2.5	0.7	4.2
Crown	CR	45°26' N, 78°40' W	6.28	7.2	2.8	4.0	1.1	3.3
Ramsey	RA	46°28' N, 80°56' W	7.30	10.2	3.9	10.8	4.3	34.2
Simon	SI	46°23' N, 81°11' W	7.49	10.5	4.6	162.0	14.4	37.2
Kelly	KE	46°26' N, 81°03' W	7.13	18.3	4.9	214.0	14.3	39.0

<sup>a</sup>µg/L

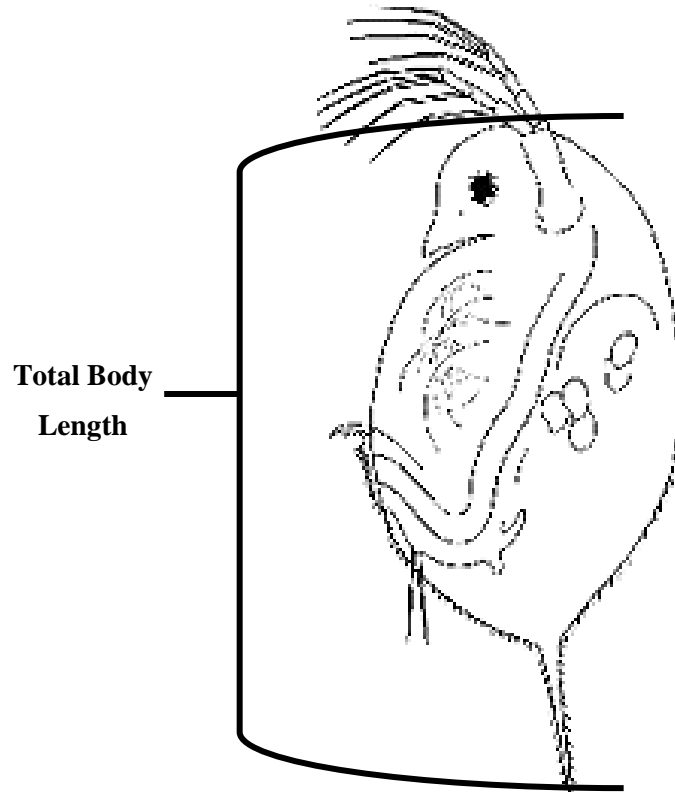
<sup>b</sup>mg C/L

<sup>c</sup>mg/L

<sup>d</sup>mg/L CaCO<sub>3</sub>

**Table 2:** The mean measured Cu concentrations ( $\pm$ SD) for all treatments used in the experiment relative to the nominal Cu concentrations that were intended. Actual values vary from nominal values as there were trace levels of Cu present in FLAMES medium.

Stimulus	Nominal Cu ( $\mu\text{g/L}$ )	<i>n</i>	Mean Actual Cu ( $\mu\text{g/L}$ )
Control	0	10	1.72 ( $\pm$ 1.32)
	5	14	6.08 ( $\pm$ 2.42)
	10	13	9.55 ( $\pm$ 0.97)
	15	13	14.99 ( $\pm$ 2.41)
	25	12	26.05 ( $\pm$ 4.65)
Kairomone	0	9	1.16 ( $\pm$ 0.78)
	5	14	6.57 ( $\pm$ 1.63)
	10	14	10.37 ( $\pm$ 2.25)
	15	14	16.23 ( $\pm$ 3.15)
	25	14	29.15 ( $\pm$ 6.72)



**Figure 2:** Diagram of *D. pulicaria* illustrating how total body length was measured.

## Chapter 4

### Results

#### 4.1 Experiment 1: Evaluating kairomone response in various *D. pulicaria* clones

The clones used in this study responded to kairomone by inducing various types of antipredator defenses. Some clones responded to kairomone by producing neonates with morphological defenses, while others altered life historical parameters. The GL, RO, and JO clones responded to kairomone by significantly increasing neonate body length relative to the control stimulus (Table 3; Figure 3). Neonates produced by the GL clone that were exposed to kairomone were 5% longer compared to those exposed to the control stimulus. The JO clone produced neonates with bodies that were 17% longer when exposed to kairomone relative to the control stimulus and the RO clone produced neonates that were 16% longer compared to the control stimulus.

After examining life history parameters, I found that the BC clone responded to kairomone by significantly increasing the number of days required before the first brood was released by 19% in comparison to those exposed to the control stimulus (Table 4, Figure 4). I also found that the SI clone was the only clone to increase the size of the first brood in response to kairomone (Table 5, Figure 5). The first brood produced by SI was 33% larger when exposed to kairomone compared to those exposed to the control stimulus. In contrast, the KE clone responded by significantly decreasing the number of neonates released in the first brood by 31% (Table 5, Figure 5). Both the CR and JO clones responded to kairomone by significantly increasing the average number of neonates released per brood (Table 6; Figure 6). The average brood size of the CR clone increased by 21%, while the JO clone increased average brood size by

42%. The JO clone was the only clone that responded to kairomone using two different strategies: increasing neonate total body length and the average size of their broods.

#### **4.2 Experiment 2: Effect of Cu on *D. pulicaria* clones from lakes along a Cu gradient**

Two of the three clones obtained from the Sudbury area maintained their ability to respond to kairomone when additionally exposed to Cu (Table 7). There was a significant effect of Cu on neonate length of the JO clone when exposed to kairomone and the control stimulus; however, neonate length was never different at any Cu concentration compared to the 0  $\mu\text{g/L}$  in either stimulus. Furthermore, the average brood size produced by the JO clone was always larger when exposed to kairomone regardless of Cu concentration. Similar results were seen in the KE clone, as the size of the first brood was significantly smaller when exposed to kairomone relative to the control stimulus regardless of Cu concentration (Table 7). These results indicate that neither clones, JO or KE were affected by Cu because kairomone response did not change when additionally exposed to Cu.

Contradictory to my expectations, one clone from the Sudbury area, the SI clone, was unable to respond to kairomone when exposed to Cu. I found there was no change in the size of the first brood produced by the SI clone as Cu increased when exposed to the control stimulus; however, there was a significant decrease in the size of the first brood as Cu increased when exposed to kairomone (Figure 7). For this clone kairomone response was inhibited at 5  $\mu\text{g/L}$ , which was the lowest concentration tested (post hoc Tukey HSD,  $p > 0.05$ ).

The effects of Cu on the clones obtained from clean lakes were also variable. When exposed to Cu both the GL and RO clones could no longer respond to kairomone. As Cu concentration increased there was no significant change in total length in the neonates produced by the GL and RO clones in the control stimulus; however, both clones exhibited a significant decrease in total neonate length when exposed to kairomone as Cu concentrations increased,

indicated by the Cu x stimulus interaction (Table 7, Figure 7). The lowest Cu concentration that inhibited kairomone response was 5 µg/L for the GL clone and 10 µg/L for the RO clone (post hoc Tukey HSD). I found the CR clone was affected by both the kairomone and Cu concentration (Table 7). When exposed to kairomone the CR clone produced larger broods on average relative to those exposed to the control stimulus averaged across all Cu concentrations. When examining the effects of Cu, I found the average brood size produced by the CR clone decreased at higher Cu concentrations in both the control and kairomone treatments. This indicates an overall physiological effect of Cu on this particular clone. Surprisingly, the BC clone, which originated from a clean lake, was unaffected by Cu. The release of the first brood produced by the BC clone was delayed when exposed to kairomone compared to the control stimulus, but this effect was not altered by Cu concentration.

In summary, two of the three clones obtained from the Sudbury area that induced an antipredator defense were able to respond to kairomone regardless of Cu exposure (Figure 8). In comparison, three of the four clones obtained from clean lakes that are relatively isolated from anthropogenic metal-contamination were significantly affected by Cu and could no longer induce a response to kairomone when living in a Cu contaminated environment (Figure 8).

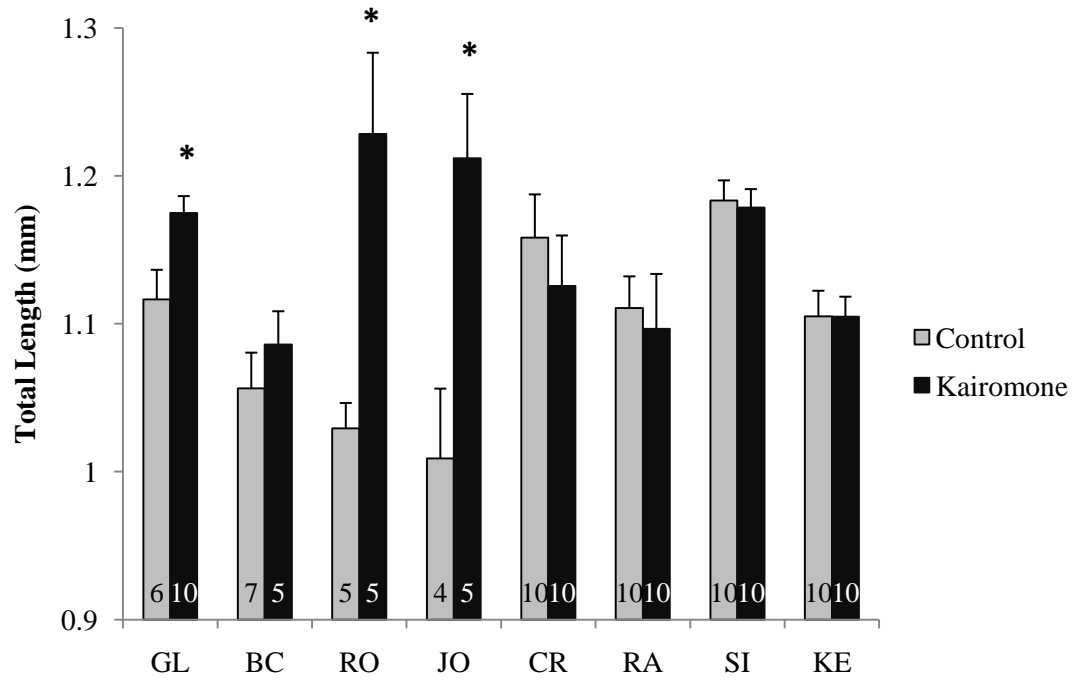
I found some discrepancies between the BLM predictions of acute toxicity and the Cu concentrations that impaired phenotypic responses to kairomone for three clones: RO, GL, and SI (Table 9). The BLM predicted that the Cu concentration the RO clone could be exposed to continuously without inducing acute toxicity, also known as, criterion of continuous concentration (CCC) was 45% higher than the Cu concentration that inhibited kairomone response. Similar results were seen in the predictions generated for the SI clone, where the CCC was 62% higher than the Cu concentration that inhibited kairomone response during this experiment. The CCC predicted for the GL clone was actually lower than the concentration that inhibited kairomone response, indicating that in some instances predictions generated by the

BLM may be robust enough to ensure that concentrations do not exceed those that inhibit the induction of kairomone responses.

**Table 3:** Summary of Student's t-test (or Welch's t-test) evaluating differences in total neonate length when clones were exposed to *Chaoborus* kairomone or the control stimulus.

Clone	Body Length		
	df	t	p
GL	14	2.750	0.020*
BC	10	0.862	0.409
RO	4.8	3.452	0.020*
JO	7	3.154	0.016*
CR	18	-0.728	0.476
RA	18	-0.330	0.746
SI	18	-0.257	0.800
KE	18	-0.017	0.987

\*denotes significance

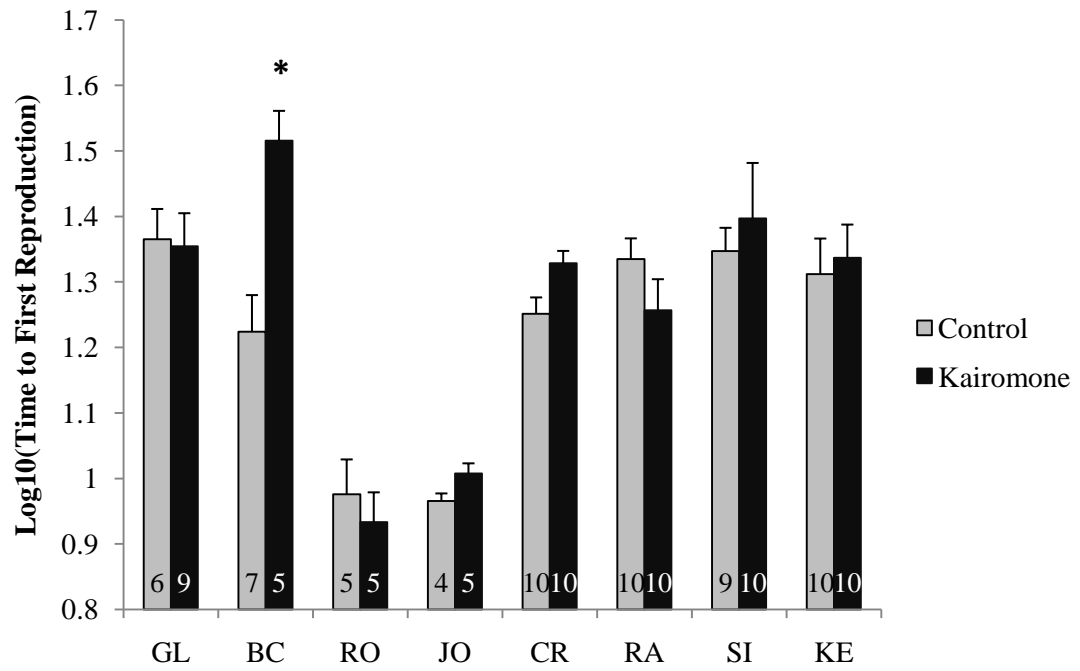


**Figure 3:** Comparison of neonate body length when exposed to the control stimulus or kairomone. Bars represent mean +SEM, asterisks indicate significant differences and numbers on bars represent *n*.

**Table 4:** Summary of Student's t-test evaluating differences in time to first reproduction when clones were exposed to *Chaoborus* kairomone or the control stimulus.

Clone	Time to First Reproduction		
	df	t	p
GL	13	-0.123	0.904
BC	10	3.577	0.005*
RO	8	-0.548	0.599
JO	7	1.993	0.043
CR	18	2.438	0.025
RA	18	-1.337	0.198
SI	17	0.561	0.582
KE	18	0.348	0.732

\*denotes significance

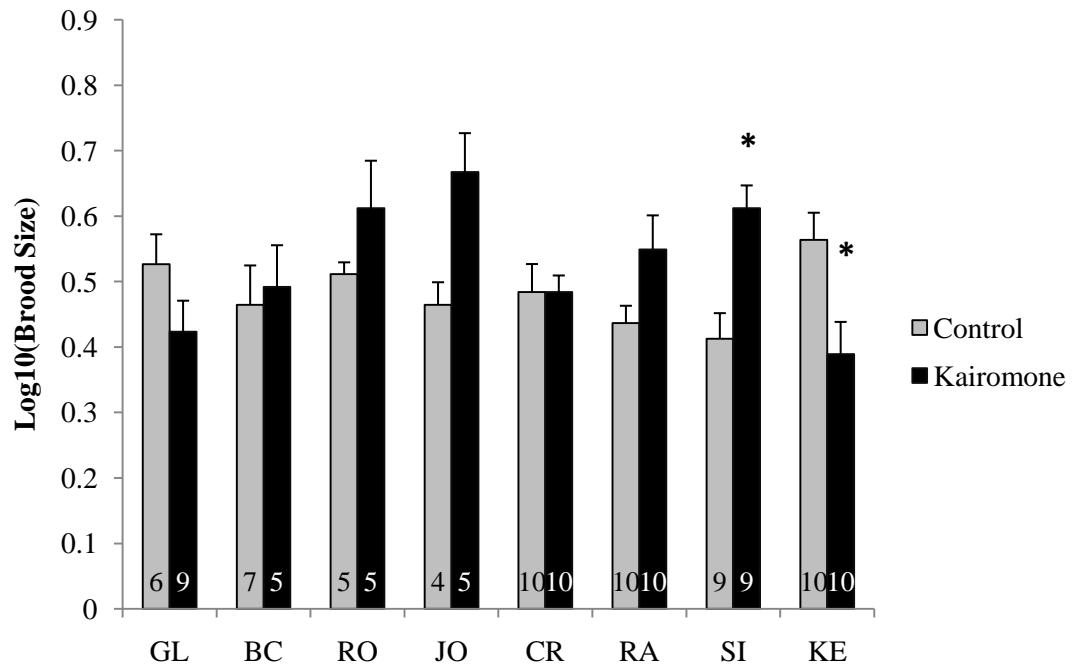


**Figure 4:** Comparison of time to first reproduction in days from all clones exposed to either the control or kairomone. Bars represent mean +SEM, asterisks indicate significant differences between stimuli, and numbers on bars represent *n*.

**Table 5:** Summary of Student's t-test (or Welch's t-test) evaluating the difference in the size of first brood produced by clones when exposed to *Chaoborus* kairomone compared to those exposed to the control stimulus.

Clone	Size of First Brood		
	df	t	p
GL	13	-1.424	0.178
BC	10	0.248	0.809
RO	8	1.225	0.255
JO	7	1.437	0.194
CR	18	-1.5 x 10 <sup>-15</sup>	1.000
RA	18	1.769	0.094
SI	17	4.060	0.001*
KE	12.9	-2.583	0.023*

\*denotes significance

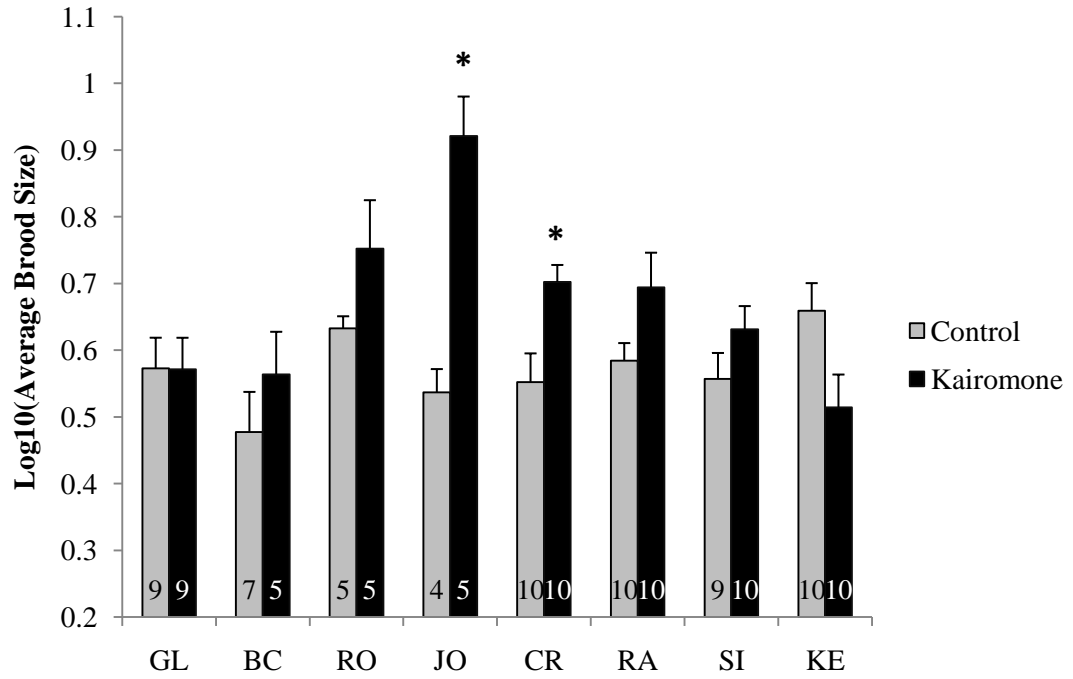


**Figure 5:** Comparison of the size of the first brood (number of neonates) released by all clones exposed to either the control stimulus or kairomone. Bars represent means +SEM and difference and asterisks indicate significant differences between stimuli. Numbers on bars represent *n*.

**Table 6:** Summary of Student's t-test (or Welch's t-test) evaluating the average brood size that was released by each clone when exposed to *Chaoborus* kairomone compared to the control stimulus.

Clone	Average Brood Size		
	df	t	p
GL	13	-0.021	0.984
BC	10	1.015	0.334
RO	4.31	1.302	0.258
JO	7	4.732	0.002*
CR	18	2.851	0.011*
RA	18	1.924	0.070
SI	17	1.308	0.208
KE	18	-2.135	0.468

\*denotes significance

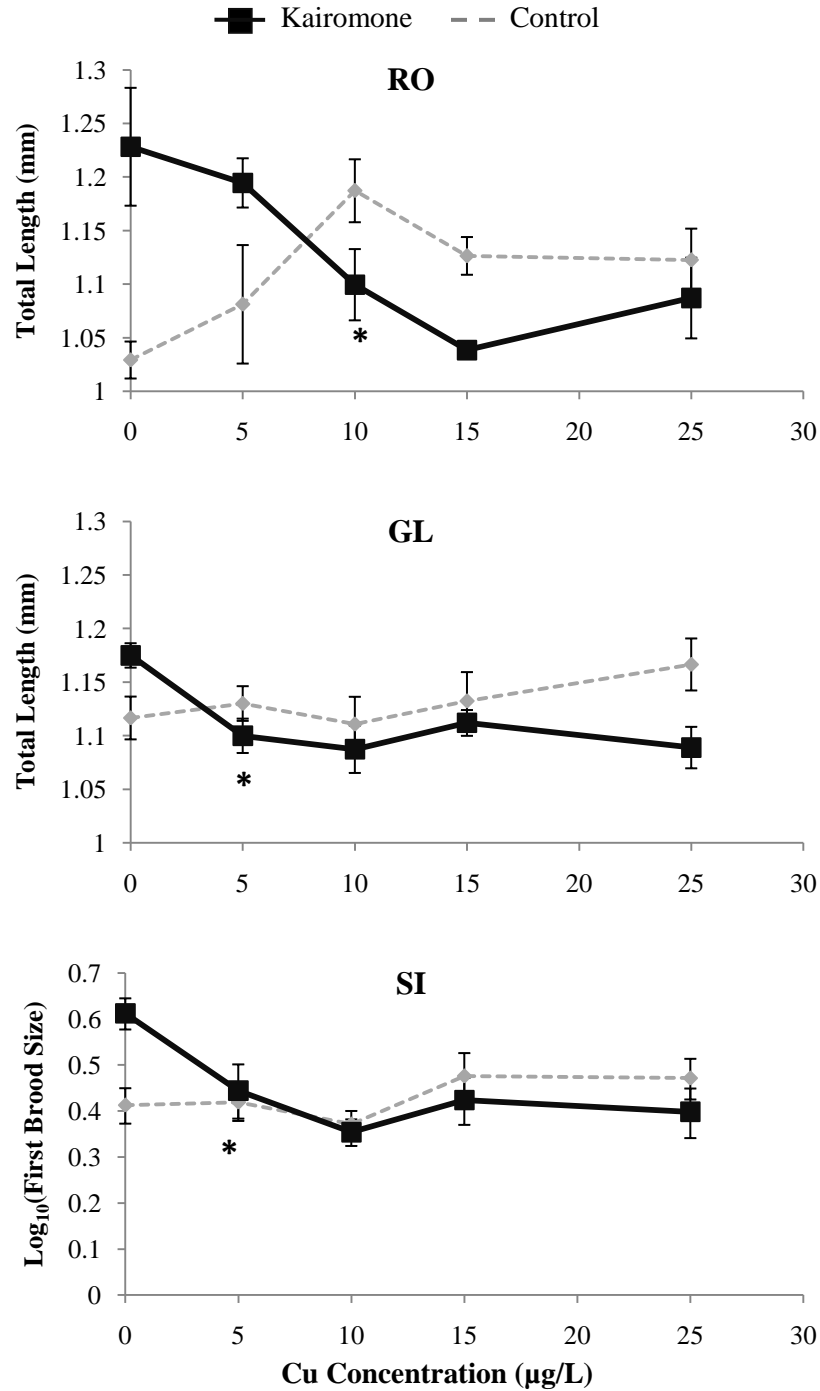


**Figure 6:** Comparison of the average brood size (number of neonates) produced by all clones exposed to the control stimulus or kairomone. Bars represent means +SEM. Numbers on bars represent *n* and asterisks denote significant differences between the stimuli.

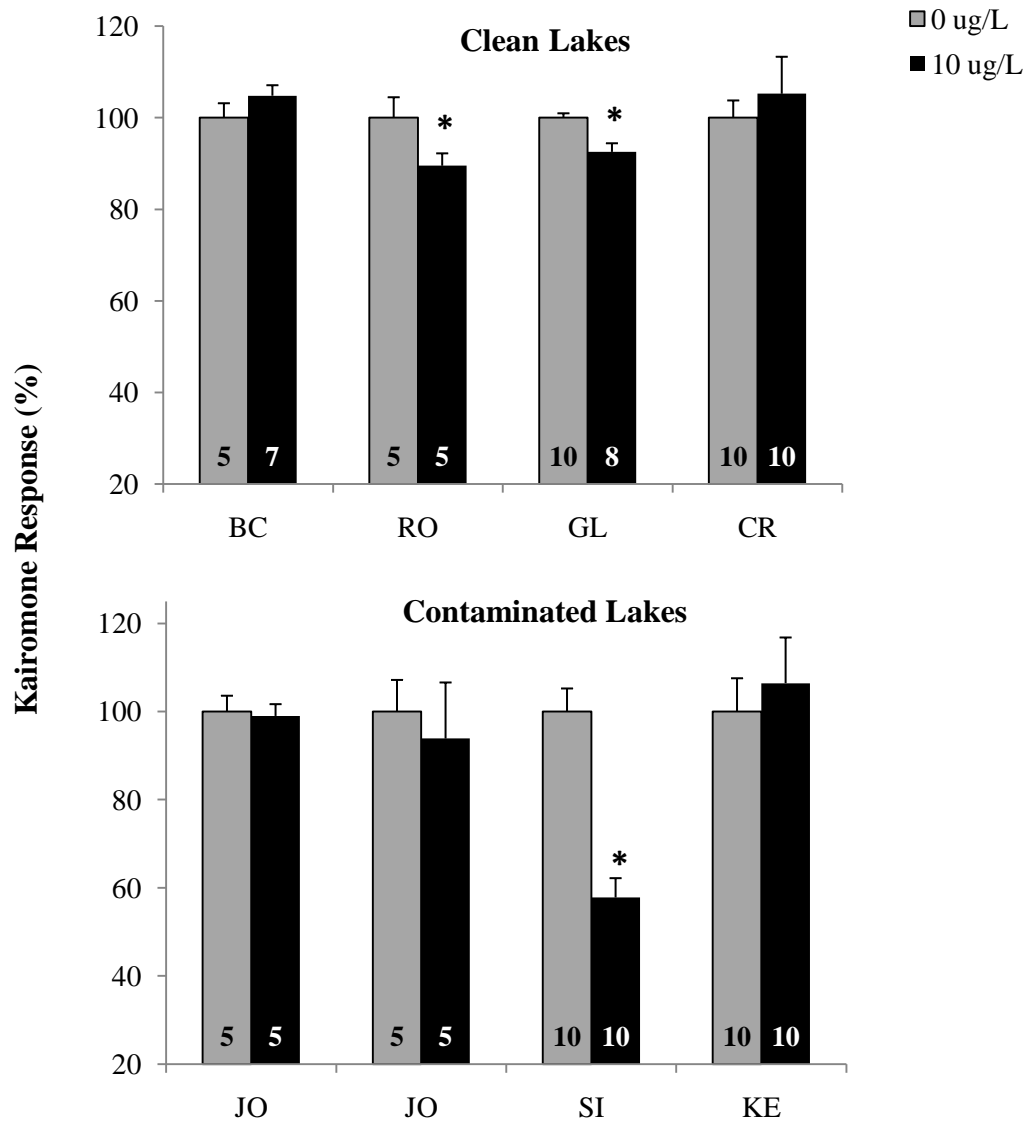
**Table 7:** Summary of two-way ANOVAs used to assess the multiple effects of stimulus, Cu, and the interaction between those two variables on the kairomone response induced by each clone. Asterisks denote differences that are significant.

Clone	Response	Source	df	F ratio	p
GL	Total Body Length	Stimulus	1	2.127	0.149
		Cu	4	1.372	0.252
		Cu x Stimulus	4	2.718	0.036*
		Error	76		
JO	Total Body Length	Stimulus	1	16.917	0.0002*
		Cu	4	2.622	0.050
		Cu x Stimulus	4	3.040	0.029*
		Error	38		
JO	Average Brood Size	Stimulus	1	48.163	<0.0001*
		Cu	4	0.847	0.505
		Cu x Stimulus	4	0.529	0.7152
		Error	38		
RO	Total Body Length	Stimulus	1	0.831	0.368
		Cu	4	1.022	0.408
		Cu x Stimulus	4	6.958	0.0003*
		Error	38		
BC	Time to First Reproduction	Stimulus	1	6.348	0.016*
		Cu	4	0.704	0.593
		Cu x Stimulus	4	2.641	0.047
		Error	43		
KE	Size of First Brood	Stimulus	1	6.116	0.016*
		Cu	4	1.382	0.248
		Cu x Stimulus	4	1.112	0.357
		Error	77		
SI	Size of First Brood	Stimulus	1	0.382	0.538
		Cu	4	3.313	0.014*
		Cu x Stimulus	4	3.339	0.014*
		Error	87		
CR	Average Brood Size	Stimulus	1	11.625	0.001*
		Cu	4	4.154	0.004*
		Cu x Stimulus	4	0.247	0.911
		Error	88		

\*denotes significance



**Figure 7:** A significant interaction between Cu and kairomone was observed for the following clones. Measured responses were not affected by Cu within the control stimulus; however, Cu did inhibit kairomone-mediated responses in these three clones. Mean responses within the control and kairomone stimulus are shown at each Cu concentration  $\pm$ SEM. Asterisks denote the lowest Cu concentration at which kairomone response was lost compared to the kairomone response at 0  $\mu\text{g/L}$  Cu (post hoc Tukey HSD).



**Figure 8:** Comparison of the relative kairomone response for all clones when exposed to 0  $\mu\text{g/L}$  Cu and 10  $\mu\text{g/L}$  Cu. The bars represent the mean kairomone response as a percentage  $\pm$ SEM, where kairomone response at 0  $\mu\text{g/L}$  Cu is considered 100% of the induced response. Significant differences in kairomone response by individual clones are denoted by asterisks.

**Table 8:** Water chemistry data from each study lake used in the BLM to generate acute toxicity predictions for *D. pulicaria*. Data provided by the DESC, except for Round Lake, which was collected by C. Inglis in 2008.

Lake	Temperature <sup>c</sup>	pH	Cu <sup>a</sup>	DOC <sup>f</sup>	HA <sup>d</sup>	Ca <sup>b</sup>	Mg <sup>b</sup>	Na <sup>b</sup>	K <sup>b</sup>	SO <sub>4</sub> <sup>b</sup>	Cl <sup>b</sup>	Alkalinity <sup>e</sup>	S <sup>b</sup>
Blue Chalk	20	6.59	0.5	1.5	10	2.3	0.6	0.8	0.4	4.8	0.4	7.2	1.0E-14
Glen	20	7.35	0.2	3.6	10	23.0	0.0	0.1	6.8	28.0	1.9	62.8	1.0E-14
Round	20	8.06	1.0	4.4	10	24.2	4.5	1.3	0.7	10.0	5.0	177.5	2.5E+00
Joe	20	6.55	3.1	1.9	10	2.5	0.7	1.0	0.4	7.7	0.9	4.2	1.0E-14
Crown	20	6.28	7.2	2.8	10	4.0	1.1	4.8	0.6	9.5	9.2	3.3	1.0E-14
Ramsey	20	7.30	10.2	3.9	10	10.8	4.3	51.2	1.3	17.0	148.0	34.2	1.0E-14
Simon	20	7.49	10.5	4.6	10	162.0	14.4	93.0	14.1	197.0	46.0	37.2	1.0E-14
Kelly	20	7.13	18.3	4.9	10	214.0	14.3	109.0	16.9	500.0	98.2	39.0	1.0E-14

<sup>a</sup>µg/L

<sup>b</sup>mg/L

<sup>c</sup>°C

<sup>d</sup>%

<sup>e</sup>mg/L CaCO<sub>3</sub>

<sup>f</sup>mg C/L

**Table 9:** Summary of Cu acute toxicity predictions for *D. pulicaria* generated by the BLM compared to the Cu concentrations that inhibited kairomone responses.

Lake	Lake Predictions (µg/L Cu)			Lake Concentration (µg/L Cu)	Response Inhibition Concentration (µg/L Cu)
	FAV	CMC	CCC		
Glen	20.0	10.0	6.2	0.2	9.7
Blue Chalk	2.7	1.3	0.8	0.5	n/a
Round	62.4	31.2	19.4	1.0	10.6
Joe	3.1	1.5	1.0	3.1	n/a
Crown	2.5	1.2	0.8	7.2	n/a
Ramsey	29.4	14.7	9.1	10.2	n/a
Simon	55.9	28.0	17.4	10.5	6.6
Kelly	37.6	18.8	11.7	18.3	n/a

FAV: Final Acute Value = the concentration that will cause acute toxicity

CMC: Criterion for Maximum Concentrations = the maximum concentration the animal can be exposed to without causing acute toxicity

CCC: Criterion for Continuous Concentration = the concentration that the animal can continuously be exposed to without causing acute toxicity

## Chapter 5

### Discussion

#### 5.1 Kairomone-Mediated Inducible Response

The *D. pulicaria* clones used in my study induced variable responses to *Chaoborus* kairomone, which included producing neonates with longer bodies, increasing the time to first reproduction, altering the size of the first brood, and increasing the average brood size. These findings are supported by many other studies that show *Daphnia* clones vary in their ability to detect predators, as well as the type and strength of the induced response (Spitze 1992; De Meester 1993; Reede and Ringelberg 1995; De Meester 1996).

*Daphnia* may induce antipredator responses that are behavioural or morphological defenses to reduce predation, or life history shifts that will allow high population growth to counteract the effects of increased predation (Zaret and Suffern 1976; Lampert 1993). Three of the eight clones used in my study responded to *Chaoborus* kairomone by producing neonates with longer bodies. An increase in body length makes it more difficult for *Chaoborus* to ingest neonates because a larger body will likely exceed the predators gape limit (Swift and Fedorenko 1975). Mirza and Pyle (2009) found that *D. pulex* produced neonates that were 4-7% longer when exposed to *Chaoborus* kairomone. This small increase in body length translated into a 46-80% increase in survival when actually confronted with the predator in comparison to those with no previous exposure who did not induce any morphological defense to *Chaoborus* (Mirza and Pyle 2009).

One of the eight clones I used in these experiments responded by increasing the time to first reproduction. *Daphnia* may delay reproduction by diverting energetic resources to increased growth rather than reproduction to reach a larger body size at maturity (Black 1993; Tollrian 1995; Riessen 1999). This type of response has been reported in several previous studies that

have examined shifts in life history in *Daphnia* as an antipredator response mechanism (Riessen and Sprules 1990; Stibor 1992; Weider and Pijanowska 1993; Stibor and Lüning 1994; Repka and Pehlajamaa 1996).

Two clones used in my study responded to kairomone by altering the size of the first brood that was released. One clone increased the size of the first brood, while the other decreased the size of the first brood. An increase in the first brood size is a response not typically induced by *Daphnia* when threatened by an invertebrate predator (Weber and Declerck 1997). Most studies report larger brood sizes in response to vertebrate predators, like fish (Reede 1997), while exposure to invertebrate predators will usually result in the production of fewer eggs (Black 1993; Stibor and Lüning 1994; Lüning 1995). Fewer eggs are likely produced in the presence of an invertebrate predator due to the cost of inducing morphological defenses. It may be more effective for daphniids to invest in egg production in the presence of a vertebrate predator because morphological defenses will not be effective if the neonates do not exceed the predator's gape. However, a larger brood may be an alternative defense against invertebrate predators, like *Chaoborus*, if an individual does not respond by inducing morphological defenses. I monitored brood size throughout the entire kairomone exposure and found that two additional clones responded by increasing the average size of their broods relative to those exposed to the control stimulus. Although the first brood produced by these clones was not significantly larger when exposed to kairomone, the average brood size was. Increased reproductive output may increase the likelihood that the *Daphnia* population will be maintained in the presence of a predator. Mirza and Pyle (2009) also found that the *D. pulex* clone used in their study produced 20-48% more neonates when exposed to *Chaoborus* kairomone. Therefore, it is not surprising that I saw an increase in the brood size of several clones because these neonates were not equipped with any morphological defenses. However, it is unexpected that one clone did respond to kairomone by decreasing the size of the first brood because neonates produced by this clone were also not

equipped with morphological defenses. Similar results have been reported where a decrease in brood size was not always coupled with the induction of a morphological defense (Lüning 1994). It is possible that a reduction in the size of the first brood may be the result of general stress that may be caused by the presence of kairomone. Predator-associated stress may affect filtering and foraging capabilities, which in turn would reduce the overall acquisition of energetic resources, limiting the reproductive output of this particular clone (Ketola and Vuorinen 1989).

One clone in this study did not exhibit any significant antipredator response to kairomone. Caution must be taken when categorizing a genotype as being responsive or non-responsive because it is possible that a response may have been induced, but was not a trait that was specifically examined. In a study by Boersma et al. (1998), multiple clones were used to evaluate the type of response(s) induced when exposed to predator kairomone. They determined that eight out of sixteen clones of *D. magna* responded to predator kairomone by changing phototactic behaviour, five of those clones showed a change in size at maturity, while four out of the original sixteen clones exhibited no response at all (Boersma et al. 1998). These results along with those from my study indicate the importance of measuring multiple responses and that it is insufficient to measure only one trait due to the variable nature of these traits among different genotypes. The one clone in my study that did not respond by altering any of the traits I recorded, may have responded by inducing another type of antipredator defense that was not examined, such as reversed diel vertical migration, reduce swimming activity or even an increase neonate carapace thickness (Weber and Declerck 1997; Laforsch et al. 2004; Boeing et al. 2006a and b). Alternatively, this clone may not have responded to *Chaoborus* kairomone because it was unfamiliar with this chemical cue. Several studies have reported that only clones that originate from lakes containing *Chaoborus* will respond to *Chaoborus* kairomone (De Meester 1996; Boersma et al. 1998; Repka and Walls 1998). All of the other clones used in this study originated from lakes that also contain *Chaoborus* populations (Kirk 1993; Wissel et al. 2003; Arnott et al.

2006). The clone that did not exhibit any measured response in this study was obtained from Ramsey Lake where it is unknown if a stable *Chaoborus* populations presently exist. Therefore, it is difficult to determine whether no response was induced as a result of predatory naivety or because not all possible responses were measured.

It is interesting to note that the ‘non-responsive’ clone was obtained from a Sudbury lake, which contains relatively high concentrations of metals. When Mirza et al. (2009) exposed wild yellow perch from Ramsey Lake to conspecific chemical alarm cue under clean laboratory conditions they found the typical behavioural response normally associated with this stimulus was not elicited. They determined that wild yellow perch chronically exposed to elevated concentrations of metals cannot effectively respond to chemical stimuli. Similarly, this particular *Daphnia* clone may no longer effectively respond to predator kairomone as a result of chronic exposure to elevated metal concentrations in Ramsey Lake. Even though this clone has been cultured for several generations under controlled laboratory conditions, no measured chemosensory response was induced. Chemical cues provide aquatic organisms with vital information pertaining to food acquisition, mate location, kin recognition, and predator avoidance. If aquatic organisms that are chronically exposed to metals cannot use this form of communication, the population and community structure of that ecosystem may be severely impaired. Further research is required to assess whether or not this clone is able to respond to chemosensory cues, by measuring all possible response traits.

## **5.2 Costs and Trade-offs of Inducible Defenses**

The variability in antipredator defenses induced by clones used in this study demonstrates that it is possible to employ several different strategies to reduce predation from a single type of predator. The responses induced by these clones are most likely reflective of the original habitat from which they were obtained and the selective pressures that acted on them prior to being

cultured in the laboratory. Even though a different response may be induced by a single predator it is possible that all responsive clones would have similar fitness. For example, De Meester and others (1995) examined two clones of *D. hyaline* X *galeata* that responded to fish kairomones with different size at first maturity and vertical migration behaviours and found that each clone had similar population growth rates. Although the ability to respond to predator kairomone directly influences an individual's fitness, it is possible to maintain genetic variation in a given environment if these responses result in similar fitness.

The induction of an antipredator response may be at the cost or tradeoff of another trait because antipredator defenses in *Daphnia* are only induced when a predator threat is detected (Harvell 1990; Parejko and Dodson 1991; Loose and Dawidowicz 1994; Stoks et al. 1999; Persons et al. 2002; Wilder and Rypstra 2004). Results from my study both support and contradict this hypothesis. For example, GL responded by producing significantly larger neonates, which resulted in a slightly smaller brood size. In contrast, JO responded by significantly increasing neonate body length, but also showed a significant increase in average brood size. The results obtained from JO suggest that the induction of morphological defenses is not overly costly because this clone also produced larger broods. These contradictions to the cost hypothesis are in accordance with previously mentioned studies that found that morphological and life history changes are sometimes coupled (Harvell 1990; Black 1993; Lüning 1995), but also occur independently (Spitze 1992; Tollrian 1995).

### **5.3 Effect of Cu on Kairomone Mediated Responses**

Previous literature suggests that Cu interferes with the ability of *Daphnia* spp. to use chemosensation, as they can no longer effectively induce morphological defenses when exposed to kairomone in a Cu-contaminated environment (Hunter and Pyle 2004; Mirza and Pyle 2009). The results from my study both support and contradict these findings. For most of the clones,

except CR, Cu had no effect on the measured responses when exposed to the control stimulus (Figure 7). This indicates that generally Cu induces no obvious physiological stress on *Daphnia* in respect to the responses that I measured throughout the experiment. When the clones were exposed to kairomone, I found that four of the seven clones that induced a response to kairomone were no longer able to induce a response when exposed to Cu. Because Cu had no effect on clones when exposed to the control stimulus it is probable that Cu affected the chemosensory capabilities of some clones (except for the CR clone as Cu had an effect on individuals exposed to the kairomone and control stimulus). This supports previous literature suggesting that Cu impairs the ability of aquatic organisms to effectively use chemosensation (McPherson et al 2004; Hunter and Pyle 2004; Carreau and Pyle 2005; Pyle et al. 2005; Mirza and Pyle 2009; Mirza et al. 2009).

The mechanism behind metal-induced chemosensory impairment has not yet been elucidated; however, Cu likely interferes with the signal transduction cascade that is usually triggered when kairomone is detected (Hunter 2006; Mirza et al. 2009). Ultimately, this would prevent the signal from being properly propagated to a higher processing centre. Further investigation using a more mechanistic approach is required to confidently support this hypothesis. Although most evidence supports the idea that metals interfere with chemosensation, it has also been proposed that response inhibition can be caused by an increased energetic investment in detoxification or a reduction in resource allocation, limiting energetic investment in antipredator defenses (Baird et al. 1990; Rainbow 1997). However, there is much debate as to whether or not inducible antipredator defenses incur a substantial energetic cost. Overall, it is difficult to assess how Cu is interfering with the ability of *Daphnia* to effectively respond to kairomone without further research.

The average brood size produced by the CR clone was reduced by Cu in both the kairomone and control stimulus, indicating that Cu had an overall physiological effect. This may

be the result of increased metabolic rate when the clone is under toxic stress, which in turn increases the utilization of carbohydrate reserves (Knops et al. 2001; Sancho et al. 2009). Energetic resources may be reallocated from reproduction to meet the demands of the increased metabolic cost when under toxic stress (Beyers et al. 1999).

Kairomone-mediated responses were not affected when exposed to Cu in four of the clones used in my study. These clones were able to effectively maintain a response to kairomone even at relatively high Cu concentrations, indicating that chemosensory capabilities were not compromised. It may be that these clones have developed a tolerance to Cu after years of exposure as there have been other accounts of aquatic organisms becoming more tolerant of contaminants (Shrift 1954; Luoma et al. 1983; Baker et al. 1985; Derry and Arnott 2007).

#### **5.4 Effect of Natural Environment on Cu-Tolerance**

All clones used in this study were obtained from Ontario Shield Lakes along a Cu-gradient. The lakes that were selected ranged from those that have extremely low concentrations of Cu to those that have elevated Cu concentrations as a result of industrial effluent (Table 1). Metal-tolerance has been documented for several other species including *Daphnia* (Shrift 1954; Luoma et al. 1983; Baker et al. 1985; Lopes et al. 2006). I hypothesized that clones originating from environments with high concentrations of Cu, would have adapted a Cu-tolerance and still effectively respond to *Chaoborus* kairomone when subsequently exposed. In comparison, those clones that had never been naturally exposed to influxes of Cu would no longer be able to induce a response to kairomone when exposed to Cu. I found evidence of metal-tolerance in three of the seven clones I tested (Figure 7, Figure 8). Two of the clones, JO and KE originated from lakes in the Sudbury area that have experienced high influxes of Cu since the onset of mining in the late 1800s (Gunn et al. 1988). I expected that these clones would maintain their response to kairomone regardless of the addition of Cu to the water because they have been chronically

exposed to higher concentrations of Cu in their natural habitats. Although, Joe Lake currently has relatively low concentrations of Cu ( $\sim 3 \mu\text{g/L}$ ), it was considered to be severely metal-contaminated ( $\sim 22 \mu\text{g/L}$  Cu) before emission reductions and chemical neutralization attempts were put in place approximately 25 years ago (Gunn et al. 1988). It is likely that the tolerance exhibited by the JO clone is reflective of the previous chemical conditions of Joe Lake. When Cu concentrations were higher in the past strong forces would have favoured metal-tolerant genotypes. If there is no strong selection against Cu-tolerance it is likely that these clones would be prevalent in Joe Lake today even though Cu-contamination is no longer a serious issue.

The other tolerant clone, BC originated from Blue Chalk Lake, which is relatively isolated from anthropogenic influence and considered to be a clean lake compared to those located in the Sudbury area. It was unexpected that BC would be able to respond to kairomone when exposed to Cu because this clone had no prior exposure to Cu. The nature of the response induced by this clone (delayed reproduction) make it difficult to establish whether or not these results support the notion that this clone exhibits a tolerance to Cu because delaying reproduction does not require any sort of energetic investment. If increased Cu concentrations require energetic resources to be diverted to detoxification it is possible that delayed reproduction may be the result of this reallocation and not necessarily an induced response to kairomone. However, it is also possible that this clone is naturally more plastic and able to tolerate a wide range of Cu even though this clone has had no previous exposure to relatively high concentrations of Cu.

Metal-tolerance has been documented for several invertebrate species (Klerks and Weis 1987; Posthuma and Van Straalen 1993), which may be the result of increased metallothionein production. Metallothionein is a metal-binding protein that aids in the elimination of metals from the body (Shaw et al. 2007). Several studies have found there is a positive correlation between metal concentrations in the environment and constitutive metallothionein gene expression in animals living in metal-contaminated environments (Timmermans et al. 2005; Janssens et al.

2009). It is likely that increased metal-tolerance is the result of higher basal expression levels of the metallothionein gene, which allows an animal to maintain cellular homeostasis (Roelofs et al. 2009). Furthermore, it has been shown that if metallothionein gene expression is increased, we typically see an increase in the production of the metallothionein protein itself, illustrating that overexpression of this gene could infer increased detoxification capabilities and metal-tolerance (Gonzales et al. 2006).

The clone from Simon Lake in Sudbury was unable to respond to kairomone when exposed to Cu, despite current Cu concentrations of  $>10 \mu\text{g/L}$ . Although, this clone is naturally exposed to high concentrations of Cu, it cannot effectively induce antipredator defenses when exposed to Cu. More importantly, response was inhibited at concentrations lower than those that the clone is naturally exposed to. It would appear that chronic Cu exposure does not always increase the likelihood of having a higher tolerance to Cu. It is possible that the *Daphnia* clone currently living in Simon Lake recolonized when Cu concentrations in this lake were at its lowest or that Cu is not a strong selective force when considering chemosensory function, which may be the result of low *Chaoborus* predation. Further research is required to evaluate the importance of chemical cues for this particular clone when living in the natural environment. Most importantly, these results may have implications for remediation efforts in the Sudbury area, as current Cu concentrations in these lakes are high enough to inhibit chemosensory responses of clones naturally exposed to elevated Cu concentrations.

Results from my study indicate that most clones from the Sudbury area were more tolerant of metals compared to those clones from clean lakes; however, there was considerable variation seen between genotypes (Figure 7). Chronic, natural Cu exposure did not necessarily confer Cu-tolerance for all clones. This variation provides evidence to emphasize the importance of examining natural population when creating risk assessments. Although clones may be exposed to similar conditions, it is difficult to make generalizations due to genetic variability.

## 5.5 Implications of Research

Taking the water chemistry data from the lakes used in this study I was able to determine what Cu concentrations should cause acute toxicity for *D. pulicaria* on a site-specific basis (Table 8, Table 9). Comparing the results obtained from the three clones that could not induce a response to kairomone when exposed to Cu, it is apparent that Cu concentrations much lower than those predicted to cause acute toxicity can potentially inhibit kairomone mediated-responses (Table 9). *Daphnia* that are unable to appropriately respond to a predator threat are more likely to be predated upon. Therefore, relatively low levels of Cu that does not directly cause mortality can inhibit kairomone-mediate responses, which can be detrimental to entire *Daphnia* populations if predation is increased. In addition to the present study, previous scientific literature has found that odour-induced responses are inhibited in many other aquatic organisms when exposed to low concentrations of Cu (Hunter and Pyle 2004; McPherson et al. 2005; Carreau and Pyle 2005; Pyle et al. 2005; Pyle and Mirza 2007; Mirza and Pyle 2009; Mirza et al. 2009). The Cu concentrations that inhibited kairomone-mediated responses in this study are lower than what the BLM predicts will cause toxicity. Although these concentrations do not cause increased mortality they do interfere with a response that mediates important ecological functions and ultimately survival. For those clones that were unaffected by the low Cu concentrations used in this study the BLM provided conservative predictions to ensure toxicity, including sublethal effects were avoided. This indicates the BLM may prove to be a useful tool when creating WQC; however, not all predictions can be reliable as a result of genetic variability and differences in metal-tolerances.

Currently the BLM is being considered for use as a regulatory tool by the United States, Canada, Europe and Australia. By using this tool, many countries may implement water quality criteria (WQC) that that are not stringent enough to protect aquatic animals from low metal concentrations that interfere with chemosensory responses. It has been proposed by Pyle and

Wood (2008) that it may be more appropriate to create a model that is based on chemosensory epithelium as opposed to the gill or whole body binding sites. Chemosensory epithelium may be a more favourable tissue to use in this model because chemosensation seems to be impaired by metal concentrations lower than those that cause toxicity. This may provide more ecologically relevant endpoints, as it establishes when chemosensory-mediated life processes are inhibited as opposed to acute toxicity, which would be useful when making environmental risk assessments. Furthermore, the current BLM is a gill-based model, which makes it difficult to assess exactly how metals will bind to this tissue because this tissue is involved in several physiological functions like ionoregulation, respiration, and waste removal (Playle 1998). The gill-based BLM also only assumes waterborne exposure of metals, but it has been shown that dietary sources of metals have a large influence on BLM predictions (Szebedinszky et al. 2001). Using chemosensory epithelium in a predictive model is ideal as the only function of this tissue is to bind dissolved molecules and there is little influence by dietary metals. The cbBLM would also be more favourable than the current BLM because the mechanisms involved in chemosensation are phylogenetically conserved among several species (Krieger and Breer 1999; Firestein 2001) and would thereby be more applicable to a wide array of organisms. Of course development of this model is still underway and extensive research must be done to confirm the validity of the model in the laboratory and field before it can be proposed as a regulatory tool to establish WQC.

## **5.6 Conclusion**

Results from this study suggest that the effect of metals on chemically-mediated responses may be detrimental to aquatic organisms that have low metal-tolerance. Most clones originating from lakes in the Sudbury area used in my study demonstrated an increased tolerance to Cu in respect to kairomone-mediated responses. This variation in metal-tolerance suggests that Cu contamination of an aquatic ecosystem and predation may be strong selective forces that

favour those individuals who can defend against predation, while living in a Cu-contaminated environment. It is likely that both of these stressors vary spatially and temporally, which may explain why this trend was not seen in all clones obtained from Sudbury lakes. The majority of the clean lake clones were less tolerant of Cu. However; these populations demonstrated much variation with regards to Cu tolerance. Therefore, it is possible that sudden influxes of Cu into a lake may devastate entire populations if they are no longer able to respond to chemosensory cues and protect against predation. It is also possible that these stressors may allow individuals with a higher degree of Cu-tolerance to persist and maintain a steady population. Regardless, these selective forces may reduce the gene pool, which can limit the ability of a population to respond and persist if new stressors are introduced. It is important, however, to recognize that populations do have the ability to adapt to the introduction of contaminants and other stressors, as a result of genetic variation. It is essential that we monitor aquatic ecosystems on a lake-by-lake basis to fully understand the impacts contaminants may have on aquatic biota because there can be a high degree of genetic variation within and between populations.

## Chapter 6

### Conclusions and Summary

This study is the first to examine the effect of Cu on kairomone-mediated antipredator defenses in multiple clones of *D. pulicaria*. I found that *D. pulicaria* clones responded to *Chaoborus* kairomone by inducing several different antipredator defenses. Although, the animals used in my study were all the same species, kairomone-mediated responses were genotype dependent. These results illustrate why it is essential to use multiple genotypes to make scientifically sound conclusions when performing *Daphnia* research. Behavioural, morphological, physiological, and life history responses are not identical for all genotypes that comprise a population. It is difficult to assess whether or not a response induced by a single clone is reflected by the population as a whole. For example, the clones used in this study responded to *Chaoborus* kairomone by producing longer neonates, altering brood size, or delaying time to first reproduction, while some clones did not show any measured response at all.

I hypothesized that the historical environmental conditions of the lakes from which the clones originated would reflect how these clones responded to different stressors: predation and Cu contamination. Of the eight clones used in my study, only one did not respond to *Chaoborus* kairomone. Previous research has found that *Daphnia* that do not naturally co-exist with *Chaoborus* will not always induce an antipredator defense when exposed to *Chaoborus* kairomone because they are naive to this type of threat (Boersma et al. 1998; Repka and Walls 1998; De Meester 2006). The single clone that did not respond originated from a lake where it is unknown if a current population of *Chaoborus* spp exists, illustrating that predator familiarity can be essential for the induction of antipredator defenses. However, it is also possible that a response to kairomone was induced by this clone, but the type of response induced was not measured. For example, *Daphnia* may shift diel vertical migration or increase carapace thickness

in response to kairomone (Weber and Declerck 1997; Laforsch et al. 2004; Boeing et al. 2006a and b). Further investigation of potential predator-induced responses for this clone is required before making any conclusions regarding why no response was seen.

When clones that responded to kairomone were exposed to Cu, those from the Sudbury area were considered to be more tolerant to relatively high Cu concentrations, as they could induce a response to kairomone when additionally exposed to Cu. However, there was a considerable amount of genotypic variation when comparing clones obtained from lakes in the Sudbury area and those from clean-lakes. Three of the seven clones that responded to kairomone were unaffected by Cu exposure. Two of those clones originated from Joe and Kelly Lakes located in the Sudbury area. This provides evidence that these clones have developed a tolerance to Cu from a chemosensory perspective, which is most likely the result of chronic Cu exposure in the natural environment. Although the current Cu concentration of Joe Lake is lower than most other lakes in the Sudbury area as result of intense remediation efforts, approximately 25 years ago the Cu concentration of this lake was 22 µg/L (Gunn et al. 1988). During that time selective forces would have favoured a genotype that was more tolerant of higher Cu concentrations. As time progressed and Cu concentrations decreased, if there was no strong selective force against Cu-tolerance, than it is probable that this trait would still be prevalent in clones currently living in Joe Lake.

The remaining clone that induced a response to kairomone when exposed to Cu did not originate from a metal-contaminated lake in the Sudbury area. When exposed to Cu the BC clone still responded to kairomone by delaying reproduction. Because this clone had no previous exposure to relatively high concentrations of Cu it was not expected that it would induce a response to kairomone when exposed to Cu. It is possible that this clone is more plastic and able to naturally tolerate higher Cu concentrations with no prior exposure to the contaminant. However, Mirza and Pyle (2009) found that when laboratory-cultured *D. pulex* were exposed to

kairomone, reproduction was delayed and this response was unaffected by Cu. This indicates that there is a lot of variation across populations; however, because delaying reproduction does not incur a direct energetic cost it is difficult to assess whether or not reproduction was delayed as a result of kairomone detection or diversion of energetic resources to detoxification mechanisms (Rainbow 1997).

Adaptive metal-tolerance has been recorded for many species including *Daphnia* (Shrift 1954; Luoma et al. 1983; Baker et al. 1985; Lopes et al. 2006). Although the results from the BC clone are somewhat inconclusive, the two clones from the Sudbury area that were able to respond to kairomone in the presence of Cu indicate that natural, chronic Cu exposure may infer a higher tolerance to Cu.

The remaining clones used in this study could not induce a response to kairomone at relatively low concentrations of Cu which is in accordance with previous research (Hunter and Pyle 2004; Mirza and Pyle 2009). If *Daphnia* cannot respond to predator kairomone, it is more likely they will be predated upon. This has implications for entire *Daphnia* populations that are exposed to influxes of Cu contamination. Increased predation may influence the sustainability of *Daphnia* populations, which could potentially have a bottom-up and/or top-down effect on the entire aquatic ecosystem (Carpenter et al. 2001). Two of the clones that could not induce a response when exposed to Cu originated from lakes that had extremely low concentrations of Cu (< 1 µg/L). This is in agreement with my original hypothesis that clones from lakes with low Cu concentrations will be more sensitive to Cu and no longer induce a response to kairomone when living in a Cu-contaminated environment. However, not all clones that were less tolerant of Cu originated from clean lakes. The SI clone originated from a lake in the Sudbury area and has a Cu concentration of 10 µg/L, but could not induce a response to kairomone when additionally exposed to Cu. I expected this clone would be more tolerant to Cu and induce a response to kairomone regardless of the Cu exposure because it has naturally been exposed to relatively high

concentrations. If animals that are naturally exposed to elevated concentrations of Cu cannot induce chemically-mediated responses at low concentrations, it is likely they no longer rely on chemosensation to mediate many ecological functions; otherwise these populations would be completely devastated due to increased predation (Hunter 2006).

The results from this study exemplify how sensitivity to a contaminant can vary between clones of the same species and further supports the use of multiple clones when studying contaminant effects on aquatic organisms (Figure 7). Often contaminants will affect laboratory cultured animals differently than those that experience natural contaminant exposure. Although some clones from the Sudbury area were more tolerant of Cu than those from clean lakes, Cu tolerance was not characteristic of all clones from the Sudbury area. This indicates the importance of using natural populations when performing risk assessment studies because not all genotypes will respond similarly even though they are naturally found living under similar conditions making it difficult to extrapolate results to the field.

Metal contamination is a serious problem in many lakes located within the vicinity of industrial activity, which is why it is important to assess the WQC for aquatic organisms that may be affected by metal-contamination. It is difficult to make general guidelines that are suited for all lentic bodies of water because water chemistry plays an important role in determining the toxicity of heavy metals (Paquin et al. 2000). Currently provincial water quality guidelines (PWQG) for Ontario do not consider site-specific water chemistry of individual lakes, which make it difficult to gauge whether or not these guidelines are over- or under-protective. The BLM is a predictive model that will determine the acute toxicity of a certain metal for an organism by considering site-specific water chemistry. Several countries have considered adopting this model to use when creating WQC; however, several factors confound the validity of this model because it is based on the gill. It is difficult to assess how a metal will bind to the gill and its potential for toxicity because this ligand is involved in several other physiological

functions. Furthermore, the BLM predicts acute toxicity, while the results of my study have determined that metals, Cu specifically, can interfere with essential ecological activities at extremely low concentrations that do not necessarily induce mortality (Table 9). Low Cu concentrations that impair chemosensory reliability may be detrimental to populations because chemosensation mediates many vital life processes. It may be more ecologically relevant to use a model that bases predictions on the binding capacity of chemosensory epithelium because chemically-induced responses are impaired at such low Cu concentrations (Pyle and Wood 2008). This type of model would also be useful for several different species because there is a high degree of phylogenetic conservation in chemosensory systems (Krieger and Breer 1999; Firestein 2001). Using a chemosensory-based BLM will provide predictions and environmental risk assessments that have the highest degree of ecological relevance.

Overall, this study has demonstrated that there is a lot of within species variation in regards to induced antipredator responses and contaminant effects. When only a certain genotype is examined it is difficult to extrapolate results that reflect an entire species. Contributions of this study will lead toward a better understanding of metal-tolerance in animals exposed to anthropogenic contamination. Most of the clones from Sudbury lakes exhibited an increased tolerance to Cu in respect to chemosensory responses, while most clones from clean lakes did not. This illustrates that Cu contamination and predation are both strong selective forces that will favour individuals that are able to respond to chemosensory cues when exposed to increased concentrations of Cu. Some clones that originated from clean lakes did demonstrate a higher tolerance to Cu compared to others, which suggests genetic variation may be a key contributor to the maintenance of populations when stressors are introduced. Although genetic variation may be responsible for the maintenance of certain populations, the introduction of stressors will likely reduce variation in the gene pool. A reduction in the gene pool can limit the ability of a population to maintain itself if another stressor is introduced. This emphasizes the importance of

monitoring individual lakes to gain a complete understanding of how contaminants affect aquatic organisms and what measures can be taken to prevent the devastation of entire ecosystems.

### *Summary*

1. Kairomone-mediated inducible responses vary among genotypes.
2. Cu interferes with the ability of some clones to effectively respond to kairomone.
3. Generally, Cu interference seems to be related directly to chemosensation, as *Daphnia* were unaffected when exposed to the control stimulus and Cu.
4. Most clones chronically and naturally exposed to higher concentrations of Cu were able to respond to kairomone when additionally exposed to Cu. This may infer Cu-tolerance for these clones; however, not all clones naturally exposed to increased Cu concentrations were more tolerant of Cu.
5. Variations in Cu tolerance seen in all clones used in my study demonstrate that genetic variation plays an important role in the establishment and maintenance of populations.
6. Predictions provided by BLM were over- and under-protective for some clones in regards to chemosensory-induced responses. Creating a predictive model using chemosensory epithelium as the biotic ligand may provide more ecological-relevant predictions.
7. My research suggests that genetic variability, adaptation, and tolerance may be responsible for the ability of aquatic organisms to persist in ecosystems that are exposed to anthropogenic stressors and the importance of monitoring individual lakes.

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## Appendix A

### FLAMES Medium Preparation

**Table 10:** Required compounds for preparation of FLAMES medium (Celis-Salgado et al 2008).

Compound	Stock Solution <sup>a</sup>	Add aliquot in mL, make up to 1 L	Final Element Concentration in FLAMES <sup>b</sup>
Calcium sulphate dehydrate ℓ	0.547	20	Ca: 2.531 SO <sub>4</sub> : 6.081
Ferric chloride hexahydrate†	0.082	1	Fe III: 0.017 Cl: 0.032
Boric acid†	0.715	1	B: 0.125
Sodim metasilicate nonahydrate	4.573	1	Na: 0.740 Si: 0.452
Potassium chloride†	0.795	1	K: 0.370 Cl: 0.335
Magnesium sulphate heptahydrate†	7.6	1	Mg: 0.749 SO <sub>4</sub> : 2.962
Monobasic potassium phosphate‡	0.044	1	K: 0.0126 P: 0.010
Sodium nitrate†	0.082	1	Na: 0.0222 NO <sub>3</sub> -N: 0.060 Na <sub>2</sub> EDTA.2H <sub>2</sub> O:
Disodium EDTA dihydrate‡	1	1	1
Biotin (from Lynch et al 1986)	0.1 See Table	1	Biotin: 0.100
Vitamin solution	I.2	0.03	
Mineral Trace Elements Solution	See Table I.3	1	

<sup>a</sup> g/L

<sup>b</sup> mg/L

†Fisher Scientific Company, Ottawa, ON, Canada

‡Sigma-Aldrich, St. Louis, MO, United States

ℓAcros Organics, Morris Plains, NJ, United States

**Table 11:** Requirements for the preparation of chemical stock solutions for the mineral trace elements solution as seen in Table 10 (Celis-Salgado et al. 2008).

Compound	Compound Concentration in Element Stock Solution <sup>a</sup>	Element Concentration in FLAMES medium <sup>b</sup>
CuSO <sub>4</sub> ·5H <sub>2</sub> O†	0.109	Cu: 0.2842 SO <sub>4</sub> : 0.4297
ZnSO <sub>4</sub> ·7H <sub>2</sub> O†	1.840	Zn: 4.185 SO <sub>4</sub> : 6.1481
LiCl†	0.114	Li: 0.186 Cl: 0.953
RbCl‡	0.120	Rb: 0.848 Cl: 0.352
SrCl <sub>2</sub> ·6H <sub>2</sub> O†	3.500	Sr: 17.371 Cl: 14.057
NaBr‡	0.400	Na: 0.893 Br: 3.106
KI‡	0.330	K: 0.777 I: 2.522
Na <sub>2</sub> SeO <sub>3</sub> ‡	0.220	Na: 0.267 Se: 1005
NH <sub>4</sub> VO <sub>3</sub> ‡	0.0073	NH <sub>4</sub> : 0.011 V: 0.032
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O‡	0.0034	Co: 0.010 NO <sub>3</sub> : 0.021
NaMoO <sub>4</sub> ·2H <sub>2</sub> O†	1.600	Na: 1.679 Mo: 7.017
NiSO <sub>4</sub> ·6H <sub>2</sub> O‡	0.083	Ni: 0.185 SO <sub>4</sub> : 0.303
CrCl <sub>3</sub> ·6H <sub>2</sub> O†	0.0196	Cr: 0.038 Cl: 0.078
MnCl <sub>2</sub> ·4H <sub>2</sub> O†	1.538	Mn: 4.300 Cl: 5.500

<sup>a</sup>g/100 mL

<sup>b</sup>µg/L

†Fisher Scientific Company, Ottawa, ON, Canada

‡Sigma-Aldrich, St. Louise, MO, United States

**Table 12:** Ingredients required for the preparation of the vitamin solution as seen in Table 10 (Lynch et al 1986).

<b>Ingredient</b>	<b>Amount (g)</b>
Calcium pantothenate†	7.00
Vitamin B <sub>12</sub> ℓ	0.0003
Thiamin‡	0.60
Riboflavin‡	0.40
Nicotinamide†	1.30
Folic Acid‡	3.30
Putrescine‡	0.30
Choline‡	5.00

†Fisher Scientific, Ottawa, ON, Canada

‡Sigma-Aldrich, St. Louis, MO, United States

ℓJamieson Natural Sources, Jamieson Laboratory, Toronto, ON, Canada

## **Appendix B**

### **Preparation of Sugar-Buffered Formalin**

Formalin is acidic and buffered to reduce any acid degradation that may occur to the sample. Typically, this procedure is used for field collection and storage of zooplankton samples and the recipe yields an 11% formalin solution. In this study formalin was used for preservation of laboratory samples, so the 11% solution was diluted in half with water to achieve a 5.5% formalin solution.

1. Add 359.92 g of white granular sugar to 5 L of distilled water.
2. Using a dropper add 43 drops of 0.5N NaOH to buffer.
3. Add 620 mL formaldehyde (distributed from the Ministry of the Environment at 37% strength) and stir solution. This creates an 11% solution and must then be stored in a fume hood until used.
4. Dilute 11% solution 50:50 with distilled water. The solution now yields 5.5%

## Appendix C

### Supplementary Experimental Data

**Table 13:** Morphometric data for all clones when exposed to each treatment.

Clone	Stimulus	Concentration ( $\mu\text{g/L}$ of Cu)	Total Length (mm)
BC	DW	0	1.0303
BC	DW	0	1.0632
BC	DW	0	1.1109
BC	DW	0	1.1106
BC	DW	0	0.9461
BC	DW	0	1.0146
BC	DW	0	1.1189
BC	DW	5	1.0313
BC	DW	5	1.0556
BC	DW	10	1.0984
BC	DW	10	1.0916
BC	DW	10	1.1650
BC	DW	10	1.0398
BC	DW	10	1.0440
BC	DW	10	1.1308
BC	DW	10	1.0116
BC	DW	10	1.1016
BC	DW	15	1.1206
BC	DW	15	1.1553
BC	DW	15	1.1388
BC	DW	15	1.1391
BC	DW	15	1.1780
BC	DW	15	1.1075
BC	DW	15	1.2056
BC	DW	15	1.0721
BC	DW	15	1.0718
BC	DW	25	1.1350
BC	DW	25	1.1540
BC	DW	25	1.1590
BC	DW	25	1.0686
BC	K(+)	0	1.0826
BC	K(+)	0	1.0672
BC	K(+)	0	1.0343
BC	K(+)	0	1.0766

**Table 13 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L}</math> of Cu)</b>	<b>Total Length (mm)</b>
BC	K(+)	0	1.1694
BC	K(+)	5	1.0784
BC	K(+)	5	1.2016
BC	K(+)	5	1.1043
BC	K(+)	5	1.1314
BC	K(+)	5	1.1416
BC	K(+)	5	1.1718
BC	K(+)	5	1.0960
BC	K(+)	10	1.2363
BC	K(+)	10	1.1106
BC	K(+)	10	1.1875
BC	K(+)	10	1.1997
BC	K(+)	10	0.9870
BC	K(+)	10	0.9816
BC	K(+)	10	1.1056
BC	K(+)	15	1.1714
BC	K(+)	15	1.0318
BC	K(+)	15	1.1735
BC	K(+)	25	1.0275
GL	DW	0	1.1464
GL	DW	0	1.1867
GL	DW	0	1.1125
GL	DW	0	1.0408
GL	DW	0	1.1163
GL	DW	0	1.0964
GL	DW	5	1.1176
GL	DW	5	1.1336
GL	DW	5	1.1112
GL	DW	5	1.1490
GL	DW	5	1.1106
GL	DW	5	1.0992
GL	DW	5	1.1091
GL	DW	5	1.0985
GL	DW	5	1.1028
GL	DW	5	1.2687
GL	DW	10	1.0322
GL	DW	10	1.2049
GL	DW	10	1.0869

**Table 13 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L}</math> of Cu)</b>	<b>Total Length (mm)</b>
GL	DW	10	1.1259
GL	DW	10	1.0366
GL	DW	10	1.1728
GL	DW	10	1.1190
GL	DW	10	1.0051
GL	DW	10	1.2136
GL	DW	15	1.0899
GL	DW	15	1.1242
GL	DW	15	1.1833
GL	DW	15	1.2023
GL	DW	15	1.0988
GL	DW	15	1.0192
GL	DW	15	1.0854
GL	DW	15	1.2560
GL	DW	25	1.1096
GL	DW	25	1.2594
GL	DW	25	1.1668
GL	DW	25	1.0552
GL	DW	25	1.0448
GL	DW	25	1.2268
GL	DW	25	1.1670
GL	DW	25	1.2486
GL	DW	25	1.1602
GL	DW	25	1.2267
GL	K(+)	0	1.1865
GL	K(+)	0	1.1847
GL	K(+)	0	1.1891
GL	K(+)	0	1.1782
GL	K(+)	0	1.1317
GL	K(+)	0	1.1399
GL	K(+)	0	1.1702
GL	K(+)	0	1.2131
GL	K(+)	0	1.2356
GL	K(+)	0	1.1202
GL	K(+)	5	1.1148
GL	K(+)	5	1.1573
GL	K(+)	5	1.0224
GL	K(+)	5	1.1289

**Table 13 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L}</math> of Cu)</b>	<b>Total Length (mm)</b>
GL	K(+)	5	1.1636
GL	K(+)	5	1.1082
GL	K(+)	5	1.0672
GL	K(+)	5	1.0466
GL	K(+)	5	1.0899
GL	K(+)	10	1.1407
GL	K(+)	10	1.1382
GL	K(+)	10	1.0324
GL	K(+)	10	1.0308
GL	K(+)	10	1.0426
GL	K(+)	10	1.0887
GL	K(+)	10	1.0336
GL	K(+)	10	1.1916
GL	K(+)	15	1.1499
GL	K(+)	15	1.1367
GL	K(+)	15	1.0650
GL	K(+)	15	1.0780
GL	K(+)	15	1.0733
GL	K(+)	15	1.1212
GL	K(+)	15	1.1103
GL	K(+)	15	1.1701
GL	K(+)	15	1.1028
GL	K(+)	25	1.0499
GL	K(+)	25	1.0505
GL	K(+)	25	1.0826
GL	K(+)	25	1.1712
GL	K(+)	25	1.1234
GL	K(+)	25	1.1183
GL	K(+)	25	1.0265
JO	DW	0	0.9415
JO	DW	0	0.9397
JO	DW	0	1.1411
JO	DW	0	1.0133
JO	DW	5	1.0479
JO	DW	5	1.2069
JO	DW	5	1.1008
JO	DW	5	1.0477
JO	DW	10	1.1740

**Table 13 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L}</math> of Cu)</b>	<b>Total Length (mm)</b>
JO	DW	10	1.0295
JO	DW	10	1.1800
JO	DW	10	1.1345
JO	DW	10	1.2677
JO	DW	15	1.0065
JO	DW	15	0.9912
JO	DW	15	1.0404
JO	DW	15	0.9138
JO	DW	15	1.1324
JO	DW	25	1.1800
JO	DW	25	1.1130
JO	DW	25	1.2172
JO	DW	25	1.1545
JO	DW	25	1.1869
JO	K(+)	0	1.2300
JO	K(+)	0	1.0580
JO	K(+)	0	1.3256
JO	K(+)	0	1.2393
JO	K(+)	0	1.2072
JO	K(+)	5	1.1439
JO	K(+)	5	1.1431
JO	K(+)	5	1.1224
JO	K(+)	5	1.1498
JO	K(+)	5	1.1551
JO	K(+)	10	1.2468
JO	K(+)	10	1.2210
JO	K(+)	10	1.0952
JO	K(+)	10	1.2763
JO	K(+)	10	1.1598
JO	K(+)	15	1.2151
JO	K(+)	15	1.1884
JO	K(+)	15	1.0544
JO	K(+)	15	1.1941
JO	K(+)	15	1.1834
JO	K(+)	25	1.1545
JO	K(+)	25	1.2722
JO	K(+)	25	1.1134
JO	K(+)	25	1.2491

**Table 13 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L}</math> of Cu)</b>	<b>Total Length (mm)</b>
JO	K(+)	25	1.0768
RA	DW	0	1.0873
RA	DW	0	1.1515
RA	DW	0	0.9977
RA	DW	0	1.1131
RA	DW	0	1.2537
RA	DW	0	1.1228
RA	DW	0	1.0689
RA	DW	0	1.1510
RA	DW	0	1.0675
RA	DW	0	1.0935
RA	DW	5	1.1261
RA	DW	5	1.0658
RA	DW	5	1.0730
RA	DW	5	1.1030
RA	DW	5	1.0620
RA	DW	5	1.1809
RA	DW	5	1.1171
RA	DW	5	1.0788
RA	DW	5	1.0984
RA	DW	5	1.0289
RA	DW	10	1.0969
RA	DW	10	1.1285
RA	DW	10	1.0424
RA	DW	10	1.1026
RA	DW	10	0.9857
RA	DW	10	1.1954
RA	DW	10	1.0883
RA	DW	10	1.1044
RA	DW	10	1.2117
RA	DW	10	1.1911
RA	DW	15	1.2552
RA	DW	15	1.1010
RA	DW	15	1.1517
RA	DW	15	1.1795
RA	DW	15	1.0649
RA	DW	15	1.0308
RA	DW	15	1.1092

**Table 13 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L}</math> of Cu)</b>	<b>Total Length (mm)</b>
RA	DW	15	1.0694
RA	DW	25	1.1907
RA	DW	25	1.1476
RA	DW	25	1.2022
RA	DW	25	1.0553
RA	DW	25	1.0525
RA	DW	25	1.0993
RA	DW	25	1.1554
RA	DW	25	1.2646
RA	DW	25	1.1416
RA	DW	25	1.1112
RA	K(+)	0	1.1694
RA	K(+)	0	1.0933
RA	K(+)	0	1.1789
RA	K(+)	0	1.1903
RA	K(+)	0	0.8719
RA	K(+)	0	1.0173
RA	K(+)	0	1.1272
RA	K(+)	0	0.9798
RA	K(+)	0	1.0658
RA	K(+)	0	1.2721
RA	K(+)	5	1.0759
RA	K(+)	5	1.3000
RA	K(+)	5	1.1350
RA	K(+)	5	1.1586
RA	K(+)	5	1.0716
RA	K(+)	5	1.1179
RA	K(+)	5	1.1498
RA	K(+)	5	1.0728
RA	K(+)	5	1.1257
RA	K(+)	5	1.0834
RA	K(+)	10	1.0896
RA	K(+)	10	1.1208
RA	K(+)	10	1.2054
RA	K(+)	10	1.0550
RA	K(+)	10	1.0678
RA	K(+)	10	1.1402
RA	K(+)	10	1.1809

**Table 13 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L}</math> of Cu)</b>	<b>Total Length (mm)</b>
RA	K(+)	10	1.1290
RA	K(+)	10	1.0274
RA	K(+)	10	1.0513
RA	K(+)	15	1.1982
RA	K(+)	15	1.1169
RA	K(+)	15	1.1112
RA	K(+)	15	1.1333
RA	K(+)	15	1.0740
RA	K(+)	15	1.1331
RA	K(+)	15	1.2161
RA	K(+)	15	1.2154
RA	K(+)	15	1.2258
RA	K(+)	25	1.0734
RA	K(+)	25	1.2520
RA	K(+)	25	1.1457
RA	K(+)	25	1.2548
RA	K(+)	25	1.0835
RA	K(+)	25	1.1025
RA	K(+)	25	1.0341
RA	K(+)	25	0.9700
RA	K(+)	25	1.1132
RA	K(+)	25	1.0352
RO	DW	0	1.0520
RO	DW	0	0.9620
RO	DW	0	1.0562
RO	DW	0	1.0335
RO	DW	0	1.0424
RO	DW	5	1.2869
RO	DW	5	0.9567
RO	DW	5	1.0315
RO	DW	5	1.0507
RO	DW	5	1.0802
RO	DW	10	1.2459
RO	DW	10	1.1562
RO	DW	10	1.1484
RO	DW	10	1.2679
RO	DW	10	1.1176
RO	DW	15	1.1093

**Table 13 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L}</math> of Cu)</b>	<b>Total Length (mm)</b>
RO	DW	15	1.0880
RO	DW	15	1.1292
RO	DW	15	1.1917
RO	DW	15	1.1136
RO	DW	25	1.1697
RO	DW	25	1.0874
RO	DW	25	1.1748
RO	DW	25	1.0584
RO	K(+)	0	1.1163
RO	K(+)	0	1.3519
RO	K(+)	0	1.2595
RO	K(+)	0	1.0827
RO	K(+)	0	1.3309
RO	K(+)	5	1.1532
RO	K(+)	5	1.2294
RO	K(+)	5	1.1316
RO	K(+)	5	1.2546
RO	K(+)	5	1.2039
RO	K(+)	10	1.1267
RO	K(+)	10	1.1022
RO	K(+)	10	1.0670
RO	K(+)	10	1.2016
RO	K(+)	10	1.0000
RO	K(+)	15	1.0557
RO	K(+)	15	1.0289
RO	K(+)	15	1.0453
RO	K(+)	15	1.0238
RO	K(+)	25	1.1841
RO	K(+)	25	1.1736
RO	K(+)	25	1.0207
RO	K(+)	25	1.0182
RO	K(+)	25	1.0388
KE	DW	0	1.1599
KE	DW	0	1.0385
KE	DW	0	1.1912
KE	DW	0	1.0885
KE	DW	0	1.0916
KE	DW	0	1.0951

**Table 13 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L}</math> of Cu)</b>	<b>Total Length (mm)</b>
KE	DW	0	1.0430
KE	DW	0	1.0763
KE	DW	0	1.0833
KE	DW	0	1.1836
KE	DW	5	1.1789
KE	DW	5	1.0986
KE	DW	5	1.0850
KE	DW	5	1.1050
KE	DW	5	1.1342
KE	DW	5	1.0617
KE	DW	5	1.0193
KE	DW	5	1.0671
KE	DW	5	1.0713
KE	DW	5	0.9626
KE	DW	10	1.0967
KE	DW	10	1.2139
KE	DW	10	1.0765
KE	DW	10	1.0485
KE	DW	10	0.9854
KE	DW	10	1.0665
KE	DW	10	1.0917
KE	DW	10	1.1740
KE	DW	10	1.1460
KE	DW	15	1.1463
KE	DW	15	0.9828
KE	DW	15	0.9863
KE	DW	15	1.0578
KE	DW	15	1.0233
KE	DW	15	1.0944
KE	DW	15	1.0476
KE	DW	15	1.1422
KE	DW	25	1.0129
KE	DW	25	1.1181
KE	DW	25	1.1378
KE	DW	25	1.1349
KE	DW	25	0.9772
KE	K(+)	0	1.0941
KE	K(+)	0	1.0785

**Table 13 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L}</math> of Cu)</b>	<b>Total Length (mm)</b>
KE	K(+)	0	1.1633
KE	K(+)	0	1.1040
KE	K(+)	0	1.0638
KE	K(+)	0	1.1247
KE	K(+)	0	1.1763
KE	K(+)	0	1.1218
KE	K(+)	0	1.0385
KE	K(+)	0	1.0824
KE	K(+)	5	1.0876
KE	K(+)	5	1.0450
KE	K(+)	5	1.0045
KE	K(+)	5	1.0509
KE	K(+)	5	1.0472
KE	K(+)	5	1.0938
KE	K(+)	5	1.0882
KE	K(+)	5	1.0449
KE	K(+)	5	1.2134
KE	K(+)	5	0.9413
KE	K(+)	10	1.1906
KE	K(+)	10	1.0239
KE	K(+)	10	1.0459
KE	K(+)	10	1.0643
KE	K(+)	10	1.0843
KE	K(+)	10	1.1368
KE	K(+)	10	1.0589
KE	K(+)	10	1.0725
KE	K(+)	10	0.9594
KE	K(+)	10	1.1053
KE	K(+)	15	1.0955
KE	K(+)	15	1.0344
KE	K(+)	15	1.0140
KE	K(+)	15	1.1091
KE	K(+)	15	0.9923
KE	K(+)	15	1.1074
KE	K(+)	15	1.0336
KE	K(+)	15	1.0128
KE	K(+)	25	1.0201
KE	K(+)	25	1.0753

**Table 13 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L}</math> of Cu)</b>	<b>Total Length (mm)</b>
KE	K(+)	25	1.0772
KE	K(+)	25	1.0836
KE	K(+)	25	1.0568
KE	K(+)	25	1.0054
KE	K(+)	25	1.0432
KE	K(+)	25	0.9274
CR	DW	0	1.1973
CR	DW	0	1.1413
CR	DW	0	0.9549
CR	DW	0	1.2783
CR	DW	0	1.2127
CR	DW	0	1.2435
CR	DW	0	1.1047
CR	DW	0	1.2096
CR	DW	0	1.1350
CR	DW	0	1.1057
CR	DW	5	1.0312
CR	DW	5	1.1533
CR	DW	5	1.2450
CR	DW	5	1.2131
CR	DW	5	1.0580
CR	DW	5	1.1381
CR	DW	5	1.0448
CR	DW	5	1.2981
CR	DW	5	1.0537
CR	DW	5	0.9736
CR	DW	10	1.0813
CR	DW	10	1.0958
CR	DW	10	0.9633
CR	DW	10	1.2276
CR	DW	10	1.1002
CR	DW	10	1.0710
CR	DW	10	1.0965
CR	DW	10	1.1007
CR	DW	10	1.1366
CR	DW	10	1.0651
CR	DW	15	1.0760
CR	DW	15	1.2970

**Table 13 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L}</math> of Cu)</b>	<b>Total Length (mm)</b>
CR	DW	15	1.0588
CR	DW	15	1.1528
CR	DW	15	1.1574
CR	DW	15	1.1058
CR	DW	15	1.2231
CR	DW	15	1.0978
CR	DW	15	1.1125
CR	DW	15	1.2479
CR	DW	25	1.0468
CR	DW	25	1.1544
CR	DW	25	1.0187
CR	DW	25	1.1165
CR	DW	25	1.0247
CR	DW	25	0.9794
CR	DW	25	1.1277
CR	DW	25	1.1772
CR	DW	25	1.0968
CR	DW	25	1.0131
CR	K(+)	0	1.0640
CR	K(+)	0	1.0750
CR	K(+)	0	1.1427
CR	K(+)	0	1.2287
CR	K(+)	0	1.0970
CR	K(+)	0	1.1128
CR	K(+)	0	1.1918
CR	K(+)	0	1.2188
CR	K(+)	0	1.2435
CR	K(+)	0	0.8824
CR	K(+)	5	1.1119
CR	K(+)	5	1.1210
CR	K(+)	5	1.0575
CR	K(+)	5	1.2987
CR	K(+)	5	1.2809
CR	K(+)	5	1.2544
CR	K(+)	5	1.0608
CR	K(+)	5	1.0304
CR	K(+)	5	1.1697
CR	K(+)	5	1.0753

**Table 13 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L}</math> of Cu)</b>	<b>Total Length (mm)</b>
CR	K(+)	10	1.2036
CR	K(+)	10	1.0956
CR	K(+)	10	1.0450
CR	K(+)	10	1.0600
CR	K(+)	10	1.0047
CR	K(+)	10	1.1139
CR	K(+)	10	1.1039
CR	K(+)	10	1.0842
CR	K(+)	10	1.0592
CR	K(+)	10	0.9633
CR	K(+)	15	1.1243
CR	K(+)	15	1.0195
CR	K(+)	15	1.0684
CR	K(+)	15	0.9897
CR	K(+)	15	1.1081
CR	K(+)	15	1.1278
CR	K(+)	15	1.1586
CR	K(+)	15	0.9498
CR	K(+)	15	1.1513
CR	K(+)	25	1.1147
CR	K(+)	25	1.0305
CR	K(+)	25	1.0314
CR	K(+)	25	1.0770
CR	K(+)	25	1.2540
CR	K(+)	25	1.0638
CR	K(+)	25	0.8405
CR	K(+)	25	1.0373
CR	K(+)	25	1.0096
SI	DW	0	1.1992
SI	DW	0	1.2116
SI	DW	0	1.1479
SI	DW	0	1.2052
SI	DW	0	1.1062
SI	DW	0	1.2241
SI	DW	0	1.1725
SI	DW	0	1.1870
SI	DW	0	1.1356
SI	DW	0	1.2447

**Table 13 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L}</math> of Cu)</b>	<b>Total Length (mm)</b>
SI	DW	5	1.1602
SI	DW	5	1.1528
SI	DW	5	1.1512
SI	DW	5	1.2661
SI	DW	5	1.1279
SI	DW	5	1.2715
SI	DW	5	1.1304
SI	DW	5	1.1175
SI	DW	5	1.0799
SI	DW	5	1.1760
SI	DW	10	1.1500
SI	DW	10	1.2059
SI	DW	10	1.1815
SI	DW	10	1.1242
SI	DW	10	1.2739
SI	DW	10	1.1110
SI	DW	10	1.2329
SI	DW	10	1.1177
SI	DW	10	1.1548
SI	DW	10	1.1616
SI	DW	15	1.1801
SI	DW	15	1.1524
SI	DW	15	1.1576
SI	DW	15	1.2374
SI	DW	15	1.2236
SI	DW	15	1.2008
SI	DW	15	1.1074
SI	DW	15	1.0998
SI	DW	15	1.0824
SI	DW	15	1.1576
SI	DW	25	1.0569
SI	DW	25	1.0803
SI	DW	25	1.0624
SI	DW	25	1.1520
SI	DW	25	1.1207
SI	DW	25	1.0495
SI	DW	25	1.1411
SI	DW	25	1.2201

**Table 13 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L}</math> of Cu)</b>	<b>Total Length (mm)</b>
SI	DW	25	1.2152
SI	DW	25	1.1198
SI	K(+)	0	1.1537
SI	K(+)	0	1.2271
SI	K(+)	0	1.1982
SI	K(+)	0	1.1940
SI	K(+)	0	1.2313
SI	K(+)	0	1.2129
SI	K(+)	0	1.1657
SI	K(+)	0	1.1357
SI	K(+)	0	1.1217
SI	K(+)	0	1.1464
SI	K(+)	5	1.1859
SI	K(+)	5	1.1128
SI	K(+)	5	1.0460
SI	K(+)	5	1.2468
SI	K(+)	5	1.1794
SI	K(+)	5	1.1776
SI	K(+)	5	1.1463
SI	K(+)	5	1.1254
SI	K(+)	5	1.0520
SI	K(+)	5	1.1036
SI	K(+)	10	1.1198
SI	K(+)	10	1.2722
SI	K(+)	10	1.1549
SI	K(+)	10	1.1817
SI	K(+)	10	1.2115
SI	K(+)	10	1.2019
SI	K(+)	10	1.1171
SI	K(+)	10	1.2009
SI	K(+)	10	1.1322
SI	K(+)	10	1.1474
SI	K(+)	15	1.2143
SI	K(+)	15	1.0672
SI	K(+)	15	1.1565
SI	K(+)	15	1.1679
SI	K(+)	15	1.1094
SI	K(+)	15	1.0833

**Table 13 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L}</math> of Cu)</b>	<b>Total Length (mm)</b>
SI	K(+)	15	1.2715
SI	K(+)	15	1.2024
SI	K(+)	15	1.2543
SI	K(+)	15	1.0306
SI	K(+)	25	1.1570
SI	K(+)	25	1.1239
SI	K(+)	25	1.1895
SI	K(+)	25	1.1472
SI	K(+)	25	1.1703
SI	K(+)	25	1.1922
SI	K(+)	25	1.1608
SI	K(+)	25	1.0708

DW = Control

K(+) = Kairomone

**Table 14:** Life historical data for all clones exposed to each treatment.

Clone	Stimulus	Concentration ( $\mu\text{g/L Cu}$ )	Log10(First Brood Size)	Log10(Time to First Reproduction)	Log10(Average Brood Size)
BC	DW	0	1.4624	0.3010	0.3979
BC	DW	0	1.1139	0.3010	0.3979
BC	DW	0	1.2553	0.3010	0.3979
BC	DW	0	1.3222	0.3010	0.3680
BC	DW	0	1.0000	0.6021	0.4260
BC	DW	0	1.3010	0.6021	0.6021
BC	DW	0	1.1139	0.8451	0.7533
BC	DW	5	1.1461	0.3010	0.3010
BC	DW	5	1.1761	0.4771	0.6767
BC	DW	10	1.3010	0.4771	0.4393
BC	DW	10	1.2041	0.4771	0.6232
BC	DW	10	1.2788	0.3010	0.3010
BC	DW	10	1.2553	0.6990	0.6532
BC	DW	10	0.9542	0.6021	0.6990
BC	DW	10	1.2041	0.3010	0.5528
BC	DW	10	1.1761	0.4771	0.4771
BC	DW	10	1.6021	0.6021	0.6021
BC	DW	15	1.2788	0.4771	0.5441
BC	DW	15	1.1761	0.4771	0.5229
BC	DW	15	1.2304	0.6990	0.5229
BC	DW	15	1.3010	0.3010	0.5441
BC	DW	15	1.3010	0.4771	0.6368
BC	DW	15	1.6335	0.3010	0.5441
BC	DW	15	1.2553	0.3010	0.4771
BC	DW	15	1.8633	0.3010	0.3010
BC	DW	15	1.1139	0.4771	0.3979
BC	DW	25	1.3010	0.6990	0.7782
BC	DW	25	1.4771	0.4771	0.4771
BC	DW	25	1.2788	0.3010	0.4771
BC	DW	25	1.5051	0.4771	0.5441
BC	K(+)	0	1.5798	0.5391	0.6212
BC	K(+)	0	1.5185	0.4771	0.4771
BC	K(+)	0	1.6532	0.3010	0.3979
BC	K(+)	0	1.4472	0.5391	0.7782
BC	K(+)	0	1.3802	0.6021	0.5441

**Table 14 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L Cu}</math>)</b>	<b>Log10(First Brood Size)</b>	<b>Log10(Time to First Reproduction)</b>	<b>Log10(Average Brood Size)</b>
BC	K(+)	5	1.5563	0.3010	0.3010
BC	K(+)	5	1.1461	0.4771	0.5643
BC	K(+)	5	1.2041	0.6021	0.6532
BC	K(+)	5	1.4314	0.3010	0.4771
BC	K(+)	5	1.3979	0.3010	0.3979
BC	K(+)	5	1.5441	0.3010	0.3979
BC	K(+)	5	1.7709	0.4771	0.4771
BC	K(+)	10	1.7482	0.6021	0.6021
BC	K(+)	10	1.6532	0.3010	0.3010
BC	K(+)	10	1.6021	0.6990	0.6021
BC	K(+)	10	1.5051	0.7782	0.8451
BC	K(+)	10	1.6021	0.6990	0.6021
BC	K(+)	10	1.5051	0.7782	0.8451
BC	K(+)	10	1.5051	0.4771	0.4771
BC	K(+)	15	1.3010	0.3010	0.3010
BC	K(+)	15	1.3802	0.4771	0.4771
BC	K(+)	15	1.3424	0.6990	0.6990
BC	K(+)	25	1.2304	0.3010	0.3010
GL	DW	0	1.1461	0.3010	0.5119
GL	DW	0	1.3424	0.4771	0.5315
GL	DW	0	1.3222	0.6021	0.6021
GL	DW	0	1.3979	0.6021	0.7404
GL	DW	0	1.5185	0.6990	0.6532
GL	DW	0	1.4624	0.4771	0.3979
GL	DW	5	1.5682	0.4771	0.6021
GL	DW	5	1.5051	0.6021	0.7782
GL	DW	5	1.5911	0.6021	0.6990
GL	DW	5	1.4472	0.4771	0.4472
GL	DW	5	1.1461	0.4771	0.5441
GL	DW	5	1.2041	0.4771	0.4771
GL	DW	5	1.6232	0.4771	0.4771
GL	DW	5	1.3617	0.3010	0.6690
GL	DW	5	1.4914	0.4771	0.4771
GL	DW	5	1.4771	0.6021	0.5229
GL	DW	10	1.4624	0.6021	0.5441
GL	DW	10	1.1139	0.4771	0.4771
GL	DW	10	1.5185	0.3010	0.3010
GL	DW	10	1.3222	0.4771	0.3979

**Table 14 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L Cu}</math>)</b>	<b>Log10(First Brood Size)</b>	<b>Log10(Time to First Reproduction)</b>	<b>Log10(Average Brood Size)</b>
GL	DW	10	1.4624	0.7782	0.7782
GL	DW	10	1.4914	0.6990	0.7533
GL	DW	10	1.3802	0.4771	0.4771
GL	DW	10	1.7076	0.3010	0.4260
GL	DW	10	1.3424	0.4771	0.5441
GL	DW	15	1.5185	0.6021	0.5441
GL	DW	15	1.5441	0.4771	0.6021
GL	DW	15	1.3424	0.4771	0.6021
GL	DW	15	1.4914	0.6021	0.4260
GL	DW	15	1.3222	0.3010	0.4771
GL	DW	15	1.5798	0.3010	0.6021
GL	DW	15	1.1461	0.3010	0.4771
GL	DW	15	1.1761	0.3010	0.4260
GL	DW	25	1.3222	0.6990	0.6767
GL	DW	25	1.2788	0.6990	0.6628
GL	DW	25	1.4314	0.7782	0.6368
GL	DW	25	1.6232	0.4771	0.5229
GL	DW	25	1.4472	0.6021	0.6021
GL	DW	25	1.4150	0.6021	0.7404
GL	DW	25	1.4472	0.6021	0.7404
GL	DW	25	1.4914	0.4771	0.4771
GL	DW	25	1.6232	0.6021	0.5441
GL	DW	25	1.4472	0.3010	0.3680
GL	K(+)	0	1.2553	0.3010	0.4771
GL	K(+)	0	1.0792	0.3010	0.3979
GL	K(+)	0	1.1139	0.4771	0.4771
GL	K(+)	0	1.4472	0.4771	0.7270
GL	K(+)	0	1.5051	0.3010	0.5441
GL	K(+)	0	1.5682	0.4771	0.3979
GL	K(+)	0	1.3424	0.3010	0.6021
GL	K(+)	0	1.5185	0.6990	0.7404
GL	K(+)	0	1.3617	0.4771	0.7782
GL	K(+)	5	1.4624	0.6021	0.6021
GL	K(+)	5	1.1139	0.8451	0.8129
GL	K(+)	5	1.3802	0.9542	0.9542
GL	K(+)	5	1.3010	0.4771	0.5229
GL	K(+)	5	1.6021	0.4771	0.6021
GL	K(+)	5	1.2553	0.4771	0.4771

**Table 14 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L Cu}</math>)</b>	<b>Log10(First Brood Size)</b>	<b>Log10(Time to First Reproduction)</b>	<b>Log10(Average Brood Size)</b>
GL	K(+)	5	1.3222	0.3010	0.3010
GL	K(+)	5	1.2553	0.4771	0.6990
GL	K(+)	5	1.4314	0.6990	0.7782
GL	K(+)	5	1.0000	0.4771	0.7202
GL	K(+)	10	1.4914	0.4771	0.6021
GL	K(+)	10	1.2553	0.6021	0.6990
GL	K(+)	10	1.4771	0.7782	0.6990
GL	K(+)	10	1.5051	0.8451	0.6532
GL	K(+)	10	1.7782	0.4771	0.5441
GL	K(+)	10	1.2553	0.6990	0.6021
GL	K(+)	10	1.3424	0.6990	0.6021
GL	K(+)	15	1.2553	0.3010	0.3979
GL	K(+)	15	1.4314	0.6990	0.7533
GL	K(+)	15	1.5051	0.3010	0.3680
GL	K(+)	15	1.3010	0.4771	0.6368
GL	K(+)	15	1.5563	0.3010	0.4260
GL	K(+)	15	1.3222	0.6021	0.6532
GL	K(+)	15	1.4314	0.4771	0.3979
GL	K(+)	15	1.4472	0.6990	0.7270
GL	K(+)	15	1.5185	0.6021	0.6021
GL	K(+)	25	1.3222	0.6021	0.6532
GL	K(+)	25	1.5563	0.3010	0.3680
GL	K(+)	25	1.3979	0.4771	0.5441
GL	K(+)	25	1.7160	0.4771	0.4771
GL	K(+)	25	1.7324	0.4771	0.6021
GL	K(+)	25	1.3979	0.3010	0.6021
GL	K(+)	25	1.1761	0.3010	0.4771
JO	DW	0	0.9542	0.6021	0.5740
JO	DW	0	0.9542	0.3010	0.6198
JO	DW	0	0.9542	0.4771	0.4771
JO	DW	0	1.0000	0.4771	0.4771
JO	DW	5	1.0414	0.6021	0.4771
JO	DW	5	0.9542	0.3010	0.4771
JO	DW	5	1.2788	0.6021	0.6532
JO	DW	5	1.1761	0.3010	0.4393
JO	DW	10	1.1461	0.8451	0.7404
JO	DW	10	0.9031	0.4771	0.7404
JO	DW	10	1.1139	0.3010	0.3979

**Table 14 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L Cu}</math>)</b>	<b>Log10(First Brood Size)</b>	<b>Log10(Time to First Reproduction)</b>	<b>Log10(Average Brood Size)</b>
JO	DW	10	1.3617	0.6990	0.6990
JO	DW	10	0.9031	0.4771	0.3979
JO	DW	15	1.0792	0.3010	0.6021
JO	DW	15	1.0000	0.3010	0.4337
JO	DW	15	1.0414	0.3010	0.5441
JO	DW	15	0.9542	0.4771	0.4771
JO	DW	15	0.8451	0.3010	0.3010
JO	DW	25	1.3010	0.9542	0.9542
JO	DW	25	1.0792	0.6021	0.6368
JO	DW	25	1.0000	0.4771	0.4771
JO	DW	25	1.2041	0.4771	0.6021
JO	DW	25	1.2788	0.6990	0.7404
JO	K(+)	0	1.0000	0.3010	0.9208
JO	K(+)	0	1.0000	0.9542	0.9294
JO	K(+)	0	0.9542	0.8451	0.6990
JO	K(+)	0	1.0414	0.6990	0.9420
JO	K(+)	0	1.0414	0.5391	1.1139
JO	K(+)	5	0.9031	0.6021	0.8451
JO	K(+)	5	0.9542	0.6021	0.6990
JO	K(+)	5	1.2553	1.2788	1.2175
JO	K(+)	5	1.2041	0.7782	0.7404
JO	K(+)	5	0.9542	0.8451	1.0911
JO	K(+)	10	0.8451	0.6990	0.9379
JO	K(+)	10	1.0414	1.2041	1.1461
JO	K(+)	10	0.9542	0.9542	0.7404
JO	K(+)	10	1.0000	0.6021	1.0212
JO	K(+)	10	1.0792	0.3010	0.4771
JO	K(+)	15	1.3222	0.4771	0.8129
JO	K(+)	15	0.9031	0.6021	1.0280
JO	K(+)	15	0.8451	0.8451	0.9542
JO	K(+)	15	1.0000	0.6990	0.6990
JO	K(+)	15	1.1461	0.7782	0.7782
JO	K(+)	25	0.9542	0.3010	0.8129
JO	K(+)	25	0.9542	0.9031	1.0414
JO	K(+)	25	1.2304	1.1461	0.9165
JO	K(+)	25	1.2788	1.0414	1.0414
JO	K(+)	25	0.8451	0.4771	0.7677
RA	DW	0	1.2788	0.4771	0.7324

**Table 14 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L Cu}</math>)</b>	<b>Log10(First Brood Size)</b>	<b>Log10(Time to First Reproduction)</b>	<b>Log10(Average Brood Size)</b>
RA	DW	0	1.2788	0.6021	0.6021
RA	DW	0	1.2553	0.3010	0.6021
RA	DW	0	1.4472	0.4771	0.5229
RA	DW	0	1.2788	0.4771	0.5798
RA	DW	0	1.4314	0.3010	0.3979
RA	DW	0	1.3222	0.4771	0.6532
RA	DW	0	1.4472	0.3010	0.6021
RA	DW	0	1.4624	0.4771	0.5229
RA	DW	0	1.1461	0.4771	0.6284
RA	DW	5	1.0414	0.6990	0.6990
RA	DW	5	1.2788	0.3010	0.4771
RA	DW	5	1.0414	0.6021	0.7270
RA	DW	5	1.2304	0.3010	0.4150
RA	DW	5	1.4771	0.9031	0.8751
RA	DW	5	1.5315	0.6021	0.4771
RA	DW	5	1.5441	0.6021	0.5441
RA	DW	5	1.4150	0.6021	0.5643
RA	DW	5	1.3802	0.4771	0.5229
RA	DW	5	1.4314	0.4771	0.5441
RA	DW	10	1.4914	0.8451	0.8129
RA	DW	10	1.3010	0.3010	0.5119
RA	DW	10	1.2553	0.6021	0.6990
RA	DW	10	1.3979	0.4771	0.5441
RA	DW	10	1.2553	0.3010	0.4771
RA	DW	10	1.3802	0.3010	0.3358
RA	DW	10	1.4150	0.3010	0.6990
RA	DW	10	1.3802	0.3010	0.5740
RA	DW	10	1.4914	0.4771	0.4771
RA	DW	10	1.1761	0.3010	0.6021
RA	DW	15	1.5185	0.3010	0.4260
RA	DW	15	0.9031	0.7782	0.7404
RA	DW	15	1.4472	0.3010	0.4771
RA	DW	15	1.3802	0.4771	0.4260
RA	DW	15	1.5051	0.3010	0.3979
RA	DW	15	1.3979	0.4771	0.5441
RA	DW	15	1.7243	0.6021	0.6532
RA	DW	15	1.5315	0.4771	0.5441
RA	DW	25	1.2788	0.6990	0.6021

**Table 14 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L Cu}</math>)</b>	<b>Log10(First Brood Size)</b>	<b>Log10(Time to First Reproduction)</b>	<b>Log10(Average Brood Size)</b>
RA	DW	25	1.4914	0.3010	0.3010
RA	DW	25	1.1139	0.6021	0.7959
RA	DW	25	1.6335	0.3010	0.6368
RA	DW	25	1.3222	0.3010	0.3522
RA	DW	25	1.3979	0.3010	0.5798
RA	DW	25	1.3617	0.6021	0.7901
RA	DW	25	1.2304	0.3010	0.3979
RA	DW	25	1.3222	0.4771	0.3979
RA	K(+)	0	1.1461	0.3010	0.6021
RA	K(+)	0	1.2788	0.4771	0.5229
RA	K(+)	0	1.5185	0.7782	0.5643
RA	K(+)	0	1.1461	0.7782	0.7202
RA	K(+)	0	1.1139	0.6990	0.8129
RA	K(+)	0	1.3424	0.4771	0.6021
RA	K(+)	0	1.1461	0.6021	0.5441
RA	K(+)	0	1.4150	0.6021	1.0000
RA	K(+)	0	1.0792	0.3010	0.7160
RA	K(+)	0	1.3802	0.4771	0.8573
RA	K(+)	5	1.1139	0.6021	0.8451
RA	K(+)	5	1.4624	0.6990	0.6990
RA	K(+)	5	1.5051	0.6021	0.6532
RA	K(+)	5	1.4314	0.3010	0.5643
RA	K(+)	5	1.4314	0.4771	0.7782
RA	K(+)	5	1.3222	0.4771	0.6021
RA	K(+)	5	1.3222	0.3010	0.6284
RA	K(+)	5	1.3617	0.4771	0.6021
RA	K(+)	5	1.3010	0.3010	0.6990
RA	K(+)	5	1.1139	0.3010	0.6021
RA	K(+)	10	1.4314	0.9031	0.8129
RA	K(+)	10	1.4624	0.3010	0.6021
RA	K(+)	10	1.6532	0.6990	0.6532
RA	K(+)	10	1.6902	0.4771	0.6021
RA	K(+)	10	1.2041	0.3010	0.3979
RA	K(+)	10	1.4472	0.4771	0.3979
RA	K(+)	10	1.5911	0.4771	0.4771
RA	K(+)	10	1.4914	0.4771	0.6435
RA	K(+)	10	1.3010	0.3010	0.5643
RA	K(+)	10	1.5563	0.6990	0.6021

**Table 14 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L Cu}</math>)</b>	<b>Log10(First Brood Size)</b>	<b>Log10(Time to First Reproduction)</b>	<b>Log10(Average Brood Size)</b>
RA	K(+)	15	1.3979	0.3010	0.7404
RA	K(+)	15	1.4771	0.3010	0.5229
RA	K(+)	15	1.5682	0.4771	0.7270
RA	K(+)	15	1.1461	0.3010	0.6021
RA	K(+)	15	1.5051	0.3010	0.4771
RA	K(+)	15	1.5441	0.3010	0.4771
RA	K(+)	15	1.5682	0.3010	0.4771
RA	K(+)	15	1.4624	0.3010	0.6232
RA	K(+)	15	1.3802	0.4771	0.7782
RA	K(+)	25	1.5185	0.6021	0.5119
RA	K(+)	25	1.4472	0.3010	0.6532
RA	K(+)	25	1.4914	0.8451	0.8751
RA	K(+)	25	1.6812	0.4771	0.7533
RA	K(+)	25	1.6812	0.3010	0.8451
RA	K(+)	25	1.6628	0.6990	0.7404
RA	K(+)	25	1.4914	0.3010	0.3979
RA	K(+)	25	1.5682	0.5391	1.0000
RA	K(+)	25	1.3424	0.3010	0.6021
RA	K(+)	25	1.8261	0.4771	0.4771
RO	DW	0	0.9542	0.6021	0.6021
RO	DW	0	1.0000	0.4771	0.6021
RO	DW	0	0.7782	0.3010	0.6232
RO	DW	0	1.1461	0.4771	0.6368
RO	DW	0	1.0000	0.6990	0.6990
RO	DW	5	1.0792	0.6990	0.6990
RO	DW	5	0.9542	0.4771	0.5229
RO	DW	5	0.8451	0.3010	0.6232
RO	DW	5	1.1761	0.3010	0.5836
RO	DW	5	1.3010	0.6990	0.6532
RO	DW	10	0.9542	0.6021	0.8016
RO	DW	10	1.1139	0.3010	0.5229
RO	DW	10	0.9031	0.6990	0.6990
RO	DW	10	0.9542	0.6021	0.6021
RO	DW	10	1.0000	0.4771	0.6767
RO	DW	15	1.2788	0.6990	0.6990
RO	DW	15	0.9031	0.8451	0.8081
RO	DW	15	1.1139	0.7782	0.7782
RO	DW	15	0.9542	0.6990	0.6767

**Table 14 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L Cu}</math>)</b>	<b>Log10(First Brood Size)</b>	<b>Log10(Time to First Reproduction)</b>	<b>Log10(Average Brood Size)</b>
RO	DW	15	0.9542	0.7782	0.7782
RO	DW	25	1.0000	0.3010	0.4771
RO	DW	25	0.9542	0.4771	0.7404
RO	DW	25	1.0000	0.6021	0.6021
RO	DW	25	0.9542	0.6021	0.7533
RO	K(+)	0	1.0414	0.6021	0.9345
RO	K(+)	0	1.0414	0.7782	0.9031
RO	K(+)	0	0.9031	0.4771	0.8451
RO	K(+)	0	0.9031	0.6021	0.6021
RO	K(+)	0	0.7782	0.6021	0.4771
RO	K(+)	5	0.9031	0.9031	0.8751
RO	K(+)	5	0.9031	0.9031	0.9823
RO	K(+)	5	0.9031	1.0000	0.9542
RO	K(+)	5	0.9542	0.6990	0.8873
RO	K(+)	5	0.9542	0.9542	1.0512
RO	K(+)	10	0.7782	0.3010	0.6812
RO	K(+)	10	1.0000	0.6990	0.6021
RO	K(+)	10	1.0000	0.6021	0.7270
RO	K(+)	10	1.2553	0.6990	0.6532
RO	K(+)	10	0.9542	0.8451	0.8016
RO	K(+)	15	1.1761	0.6021	0.6021
RO	K(+)	15	1.0000	0.3010	0.6284
RO	K(+)	15	1.0414	0.3010	0.5441
RO	K(+)	15	1.0414	0.3010	0.5051
RO	K(+)	25	1.3945	0.5391	0.6212
RO	K(+)	25	0.9031	0.4771	0.9379
RO	K(+)	25	1.0414	0.7782	0.9208
RO	K(+)	25	0.8451	0.3010	0.7202
RO	K(+)	25	0.9542	0.4771	0.6532
KE	DW	0	1.0414	0.6021	0.6021
KE	DW	0	1.1761	0.4771	0.6368
KE	DW	0	1.2553	0.4771	0.6368
KE	DW	0	1.3617	0.6021	0.6990
KE	DW	0	1.3010	0.3010	0.5229
KE	DW	0	1.2788	0.3010	0.6021
KE	DW	0	1.4314	0.8451	0.9031
KE	DW	0	1.6532	0.4771	0.4771
KE	DW	0	1.3424	0.7782	0.6990

**Table 14 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L Cu}</math>)</b>	<b>Log10(First Brood Size)</b>	<b>Log10(Time to First Reproduction)</b>	<b>Log10(Average Brood Size)</b>
KE	DW	0	1.2788	0.7782	0.8129
KE	DW	5	1.1461	0.9542	0.7959
KE	DW	5	1.2553	0.6021	0.7270
KE	DW	5	1.5441	0.6021	0.6532
KE	DW	5	1.5051	0.3010	0.4771
KE	DW	5	1.2304	0.3010	0.6284
KE	DW	5	1.3222	0.4771	0.6021
KE	DW	5	1.6021	0.3010	0.6021
KE	DW	5	1.3222	0.3010	0.3979
KE	DW	5	1.1461	0.6021	0.7202
KE	DW	5	1.1139	0.4771	0.5798
KE	DW	10	1.3979	0.6990	0.7404
KE	DW	10	1.3010	0.3010	0.7782
KE	DW	10	1.3802	0.4771	0.5119
KE	DW	10	1.0414	0.6990	0.7782
KE	DW	10	1.5911	0.3010	0.3522
KE	DW	10	1.3222	0.4771	0.4393
KE	DW	10	1.2553	0.3010	0.5798
KE	DW	10	1.3222	0.7782	0.7782
KE	DW	10	1.3010	0.3010	0.6284
KE	DW	15	1.3802	0.6990	0.6532
KE	DW	15	1.2041	0.6021	0.7404
KE	DW	15	1.2304	0.4771	0.5441
KE	DW	15	1.4914	0.3010	0.3010
KE	DW	15	1.2553	0.4771	0.4771
KE	DW	15	1.3010	0.4771	0.5229
KE	DW	15	1.6128	0.4771	0.4771
KE	DW	15	1.3010	0.3010	0.6021
KE	DW	25	1.6628	0.3010	0.3979
KE	DW	25	1.4472	0.3010	0.4771
KE	DW	25	1.1461	0.4771	0.5643
KE	DW	25	1.1139	0.3010	0.5229
KE	DW	25	1.3617	0.3010	0.4771
KE	K(+)	0	1.3802	0.3010	0.3010
KE	K(+)	0	1.2041	0.4771	0.6690
KE	K(+)	0	1.4472	0.4771	0.5119
KE	K(+)	0	1.2553	0.4771	0.7202
KE	K(+)	0	1.0792	0.3010	0.7202

**Table 14 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L Cu}</math>)</b>	<b>Log10(First Brood Size)</b>	<b>Log10(Time to First Reproduction)</b>	<b>Log10(Average Brood Size)</b>
KE	K(+)	0	1.2788	0.4771	0.6628
KE	K(+)	0	1.2553	0.3010	0.4771
KE	K(+)	0	1.6435	0.4771	0.4771
KE	K(+)	0	1.4624	0.3010	0.3010
KE	K(+)	0	1.3617	0.3010	0.3010
KE	K(+)	5	1.6128	0.3010	0.5441
KE	K(+)	5	1.6721	0.4771	0.3979
KE	K(+)	5	1.5911	0.4771	0.5229
KE	K(+)	5	1.5185	0.3010	0.4260
KE	K(+)	5	1.5563	0.3010	0.3010
KE	K(+)	5	1.4914	0.3010	0.5229
KE	K(+)	5	1.3222	0.3010	0.3680
KE	K(+)	5	1.4624	0.4771	0.4771
KE	K(+)	5	1.5315	0.3010	0.4771
KE	K(+)	5	1.5563	0.3010	0.5229
KE	K(+)	10	1.6232	0.6021	0.7404
KE	K(+)	10	1.5911	0.6021	0.6021
KE	K(+)	10	1.5441	0.3010	0.5643
KE	K(+)	10	1.6812	0.3010	0.3010
KE	K(+)	10	1.3222	0.4771	0.5740
KE	K(+)	10	1.2788	0.3010	0.4771
KE	K(+)	10	1.4472	0.3010	0.4393
KE	K(+)	10	1.6902	0.4771	0.5441
KE	K(+)	10	1.6721	0.4771	0.3979
KE	K(+)	10	1.7324	0.3010	0.3522
KE	K(+)	15	1.3617	0.3010	0.3979
KE	K(+)	15	1.4914	0.3010	0.4771
KE	K(+)	15	1.6232	0.3010	0.4771
KE	K(+)	15	1.5441	0.4771	0.4260
KE	K(+)	15	1.8261	0.6021	0.5229
KE	K(+)	15	1.6435	0.3010	0.3010
KE	K(+)	15	1.5682	0.6021	0.6021
KE	K(+)	15	1.6021	0.4771	0.3979
KE	K(+)	25	1.5911	0.3010	0.5643
KE	K(+)	25	1.5441	0.6021	0.3010
KE	K(+)	25	1.3802	0.3010	0.3010
KE	K(+)	25	1.6532	0.3010	0.3979
KE	K(+)	25	1.3979	0.3010	0.4393

**Table 14 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L Cu}</math>)</b>	<b>Log10(First Brood Size)</b>	<b>Log10(Time to First Reproduction)</b>	<b>Log10(Average Brood Size)</b>
KE	K(+)	25	1.6335	0.4771	0.3979
KE	K(+)	25	1.6628	0.3010	0.3010
CR	DW	0	1.1761	0.4771	0.4771
CR	DW	0	1.1761	0.3010	0.4393
CR	DW	0	1.2553	0.6021	0.7782
CR	DW	0	1.2041	0.6990	0.6532
CR	DW	0	1.2041	0.4771	0.4771
CR	DW	0	1.1761	0.3010	0.3010
CR	DW	0	1.3424	0.6021	0.6021
CR	DW	0	1.3010	0.6021	0.7160
CR	DW	0	1.3979	0.3010	0.4771
CR	DW	0	1.2788	0.4771	0.6021
CR	DW	5	1.0414	0.4771	0.5119
CR	DW	5	1.3617	0.4771	0.5119
CR	DW	5	1.2304	0.3010	0.5798
CR	DW	5	1.3802	0.3010	0.5441
CR	DW	5	1.1461	0.4771	0.6284
CR	DW	5	1.3979	0.6021	0.6368
CR	DW	5	1.2304	0.3010	0.5643
CR	DW	5	1.0414	0.4771	0.4771
CR	DW	5	1.4314	0.4771	0.6990
CR	DW	5	1.5798	0.6021	0.6021
CR	DW	10	1.0414	0.4771	0.6990
CR	DW	10	1.6335	0.6990	0.7404
CR	DW	10	1.1761	0.6021	0.4771
CR	DW	10	1.2788	0.4771	0.5643
CR	DW	10	1.6232	0.6021	0.7782
CR	DW	10	1.6335	0.8451	0.8451
CR	DW	10	1.7634	0.3010	0.3979
CR	DW	10	1.5798	0.6990	0.7782
CR	DW	10	1.3617	0.4771	0.6368
CR	DW	10	1.3617	0.6990	0.8451
CR	DW	15	1.2788	0.3010	0.5229
CR	DW	15	1.3222	0.3010	0.4260
CR	DW	15	1.5563	0.7782	0.6990
CR	DW	15	1.2041	0.4771	0.4771
CR	DW	15	1.2788	0.3010	0.3979
CR	DW	15	1.4914	0.7782	0.6021

**Table 14 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L Cu}</math>)</b>	<b>Log10(First Brood Size)</b>	<b>Log10(Time to First Reproduction)</b>	<b>Log10(Average Brood Size)</b>
CR	DW	15	1.2553	0.4771	0.4771
CR	DW	15	1.3617	0.4771	0.4771
CR	DW	15	1.4472	0.3010	0.4771
CR	DW	15	1.2304	0.3010	0.4260
CR	DW	25	1.3010	0.3010	0.4393
CR	DW	25	1.3424	0.3010	0.3802
CR	DW	25	1.3979	0.3010	0.4393
CR	DW	25	1.2041	0.4771	0.5229
CR	DW	25	1.3424	0.3010	0.3680
CR	DW	25	1.7634	0.8451	0.8451
CR	DW	25	1.3424	0.3010	0.3010
CR	DW	25	1.3424	0.3010	0.3424
CR	DW	25	1.2788	0.8451	0.7782
CR	DW	25	1.5315	0.4771	0.4771
CR	K(+)	0	1.2041	0.4771	0.6021
CR	K(+)	0	1.3979	0.6021	0.6990
CR	K(+)	0	1.3617	0.4771	0.7324
CR	K(+)	0	1.4150	0.6990	0.6368
CR	K(+)	0	1.3617	0.3010	0.5740
CR	K(+)	0	1.2788	0.6021	0.7404
CR	K(+)	0	1.3010	0.7782	0.6532
CR	K(+)	0	1.3617	0.3010	0.7597
CR	K(+)	0	1.3010	0.3010	0.8129
CR	K(+)	0	1.3010	0.3010	0.8129
CR	K(+)	5	1.5185	0.6021	0.6021
CR	K(+)	5	1.2041	0.6021	0.7160
CR	K(+)	5	1.2788	0.6990	0.7270
CR	K(+)	5	1.4624	0.3010	0.6690
CR	K(+)	5	1.3424	0.3010	0.6368
CR	K(+)	5	1.4624	0.4771	0.4771
CR	K(+)	5	1.3979	0.3010	0.7634
CR	K(+)	5	1.3802	0.7782	0.8451
CR	K(+)	5	1.3010	0.6021	0.6021
CR	K(+)	5	1.0792	0.3010	0.6767
CR	K(+)	10	1.3979	1.1139	0.9777
CR	K(+)	10	1.4914	0.4771	0.6532
CR	K(+)	10	1.3222	0.6021	0.4771
CR	K(+)	10	1.5563	0.3010	0.7533

**Table 14 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L Cu}</math>)</b>	<b>Log10(First Brood Size)</b>	<b>Log10(Time to First Reproduction)</b>	<b>Log10(Average Brood Size)</b>
CR	K(+)	10	1.6435	0.9031	0.9294
CR	K(+)	10	1.2553	0.6021	0.7959
CR	K(+)	10	1.3222	0.7782	0.7634
CR	K(+)	10	1.2553	0.3010	0.4260
CR	K(+)	10	1.2041	0.6990	0.8751
CR	K(+)	10	1.7709	0.8451	0.7404
CR	K(+)	15	1.5563	0.6990	0.8451
CR	K(+)	15	1.6335	0.6021	0.4771
CR	K(+)	15	1.3222	0.3010	0.3010
CR	K(+)	15	1.3010	0.3010	0.4393
CR	K(+)	15	1.4472	0.6990	0.7404
CR	K(+)	15	1.7482	0.6021	0.6021
CR	K(+)	15	1.5563	0.6990	0.8451
CR	K(+)	15	1.5185	0.6021	0.6990
CR	K(+)	15	1.2553	0.3010	0.3010
CR	K(+)	25	1.6232	1.0000	1.0000
CR	K(+)	25	1.5911	0.4771	0.4260
CR	K(+)	25	1.4472	0.6021	0.6690
CR	K(+)	25	1.3802	0.6021	0.5441
CR	K(+)	25	1.2041	0.4771	0.6021
CR	K(+)	25	1.3617	0.3010	0.6690
CR	K(+)	25	1.4624	0.6021	0.5441
CR	K(+)	25	1.3617	0.5391	0.6990
CR	K(+)	25	1.7482	0.3010	0.3010
SI	DW	0	1.3802	0.4771	0.4771
SI	DW	0	1.2553	0.3010	0.6284
SI	DW	0	1.0792	0.6021	0.7404
SI	DW	0	1.3010	0.4771	0.6532
SI	DW	0	1.4771	0.4771	0.4771
SI	DW	0	1.4150	0.4771	0.6284
SI	DW	0	1.3802	0.3010	0.6284
SI	DW	0	1.3424	0.3010	0.3522
SI	DW	0	1.4914	0.3010	0.4260
SI	DW	5	1.4150	0.4771	0.4472
SI	DW	5	1.4472	0.3010	0.4472
SI	DW	5	1.4314	0.3010	0.3522
SI	DW	5	1.6128	0.3010	0.3680
SI	DW	5	1.4314	0.4771	0.5229

**Table 14 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L Cu}</math>)</b>	<b>Log10(First Brood Size)</b>	<b>Log10(Time to First Reproduction)</b>	<b>Log10(Average Brood Size)</b>
SI	DW	5	1.5682	0.4771	0.5441
SI	DW	5	1.2041	0.4771	0.4260
SI	DW	5	1.4914	0.6021	0.6532
SI	DW	5	1.5185	0.3010	0.3979
SI	DW	5	1.5315	0.4771	0.4771
SI	DW	10	1.6128	0.3010	0.4771
SI	DW	10	1.5798	0.3010	0.5119
SI	DW	10	1.4472	0.3010	0.4771
SI	DW	10	1.3424	0.3010	0.5643
SI	DW	10	1.2041	0.3010	0.3010
SI	DW	10	1.6128	0.4771	0.4771
SI	DW	10	1.5315	0.4771	0.4771
SI	DW	10	1.0792	0.4771	0.6021
SI	DW	10	1.5051	0.4771	0.4771
SI	DW	10	1.4624	0.3010	0.4393
SI	DW	15	1.2041	0.3010	0.5441
SI	DW	15	1.6628	0.6021	0.6368
SI	DW	15	1.5911	0.6990	0.6990
SI	DW	15	1.7924	0.6990	0.5229
SI	DW	15	1.3802	0.6021	0.6021
SI	DW	15	1.4624	0.3010	0.4393
SI	DW	15	1.4314	0.3010	0.3802
SI	DW	15	1.6628	0.4771	0.5441
SI	DW	15	1.6435	0.4771	0.5441
SI	DW	15	1.6435	0.3010	0.4771
SI	DW	25	1.3979	0.6990	0.6021
SI	DW	25	1.4914	0.4771	0.4260
SI	DW	25	1.4771	0.3010	0.4771
SI	DW	25	1.3617	0.4771	0.6021
SI	DW	25	1.4771	0.3010	0.4771
SI	DW	25	1.4314	0.4771	0.6021
SI	DW	25	1.6021	0.6021	0.5643
SI	DW	25	1.7559	0.3010	0.3979
SI	DW	25	1.3617	0.4771	0.3680
SI	DW	25	1.4914	0.6021	0.5229
SI	K(+)	0	1.2304	0.6021	0.7404
SI	K(+)	0	1.3010	0.6021	0.6532
SI	K(+)	0	1.4624	0.7782	0.7404

**Table 14 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L}</math> Cu)</b>	<b>Log10(First Brood Size)</b>	<b>Log10(Time to First Reproduction)</b>	<b>Log10(Average Brood Size)</b>
SI	K(+)	0	1.3222	0.4771	0.4314
SI	K(+)	0	1.0792	0.7782	0.7782
SI	K(+)	0	1.9031	0.6021	0.5441
SI	K(+)	0	1.5798	0.6021	0.6532
SI	K(+)	0	1.4150	0.6021	0.7324
SI	K(+)	0	1.1461	0.6021	0.5643
SI	K(+)	0	1.5315	0.4771	0.4771
SI	K(+)	5	1.3424	0.4771	0.7482
SI	K(+)	5	1.2041	0.3010	0.3979
SI	K(+)	5	1.5315	0.6021	0.6532
SI	K(+)	5	1.2304	0.3010	0.7160
SI	K(+)	5	1.5051	0.3010	0.6532
SI	K(+)	5	1.3010	0.6021	0.6021
SI	K(+)	5	1.3010	0.3010	0.3424
SI	K(+)	5	1.6335	0.7782	0.7782
SI	K(+)	5	1.7782	0.4771	0.6990
SI	K(+)	5	1.6435	0.3010	0.5441
SI	K(+)	10	1.5051	0.3010	0.3010
SI	K(+)	10	1.4150	0.4771	0.4771
SI	K(+)	10	1.2553	0.3010	0.3979
SI	K(+)	10	1.6021	0.3010	0.3680
SI	K(+)	10	1.4914	0.4771	0.5229
SI	K(+)	10	1.3617	0.3010	0.3979
SI	K(+)	10	0.6990	0.3010	0.5315
SI	K(+)	10	1.3617	0.3010	0.4472
SI	K(+)	10	1.5441	0.4771	0.4260
SI	K(+)	10	1.4771	0.3010	0.5643
SI	K(+)	15	1.5315	0.6021	0.6021
SI	K(+)	15	1.4150	0.3010	0.4393
SI	K(+)	15	1.3979	0.4771	0.5643
SI	K(+)	15	1.5563	0.6990	0.6990
SI	K(+)	15	1.1761	0.3010	0.5119
SI	K(+)	15	1.7076	0.3010	0.5441
SI	K(+)	15	1.4771	0.4771	0.5441
SI	K(+)	15	1.5441	0.3010	0.4771
SI	K(+)	15	1.5682	0.3010	0.4771
SI	K(+)	15	1.5682	0.4771	0.5441
SI	K(+)	25	1.3979	0.3010	0.3979

**Table 14 Continued...**

<b>Clone</b>	<b>Stimulus</b>	<b>Concentration (<math>\mu\text{g/L Cu}</math>)</b>	<b>Log10(First Brood Size)</b>	<b>Log10(Time to First Reproduction)</b>	<b>Log10(Average Brood Size)</b>
SI	K(+)	25	1.2788	0.3010	0.4393
SI	K(+)	25	1.4771	0.6021	0.4771
SI	K(+)	25	1.4771	0.4771	0.6021
SI	K(+)	25	1.8195	0.3010	0.3979
SI	K(+)	25	1.5911	0.3010	0.3680
SI	K(+)	25	1.4150	0.3010	0.3010
SI	K(+)	25	1.4771	0.6021	0.5229

DW = Control

K(+) = Kairomone