

**FLEEING PREDATION: THE EFFECT OF COPPER EXPOSURE
ON INDUCIBLE ANTIPREDATOR DEFENSES IN *DAPHNIA*
PULICARIA CLONES FROM A HISTORICALLY METAL
CONTAMINATED LAKE**

by

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Abstract

Antipredator defenses are ubiquitous in aquatic ecosystems. In the widely studied *Chaoborus-Daphnia* predator-prey system, *Daphnia* elicit a variety of phenotypically plastic responses to *Chaoborus* including: morphological, life history, and behavioral responses. While these inducible defenses benefit the prey, metal contaminants have been shown to interfere with chemosensory functions, thereby inhibiting antipredator defenses and decreasing survivorship. However, in lakes with a history of metal contamination, such as Kelly Lake in Sudbury, Ontario, there is evidence to suggest that *Daphnia* may have adapted to high, ambient copper concentrations. Using seven distinct *Daphnia* clones that were hatched from resting eggs from Kelly Lake, we examined morphological and life history traits when clones were exposed to either a nominal concentration of copper, kairomone, or a combination of both. As expected, clones displayed a variety of inducible responses in both kairomone-control and kairomone-copper treatments, which was attributed to genetic variability. Expected trade-offs in life history traits were not always observed, suggesting that inducible traits may be coupled. Furthermore, in contradiction to life history theory, one clone exhibited both increased somatic growth and increased reproductive output, indicating that clones likely adopted adaptive strategies to stressors rather than eliciting trade-offs in traditional traits. Our results indicate that environmentally relevant copper concentrations do not inhibit the induction of antipredator defenses in *Daphnia* from Kelly Lake, and we conclude that Kelly Lake *Daphnia* have developed an adaptive tolerance to copper. Adaptation to copper contamination may have implications for resilience in natural Kelly Lake populations.

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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Chapter 1

General Introduction

The importance of predator-prey dynamics on aquatic community composition is irrefutable. Two widely studied model organisms, the invertebrate predator *Chaoborus* spp. and the zooplankter *Daphnia* spp. were employed in this study to elucidate the impact of metal contamination on inducible anti-predator defenses. The *Chaoborus-Daphnia* model system is frequently incorporated in the study of inducible defenses, as *Daphnia* express phenotypically plastic responses to *Chaoborus* predation (see section 1). While these responses reduce prey mortality, metals have been observed to interfere with chemosensory pathways, thereby inhibiting the induction of antipredator responses, leading to decreased survivorship and fitness in *Daphnia* species (Hunter and Pyle 2004, Pyle and Mirza 2007, Mirza and Pyle 2009). *Daphnia* are a keystone herbivore in lake systems. As such, they represent an important trophic link between phytoplankton and predators, being regulated by both bottom-up and top-down processes. If metals impede the ability of daphniids to respond to predation, community composition could be drastically altered; however, if *Daphnia* acquire metal resistance ecosystem functioning may be maintained. Numerous studies have elucidated the ability of *Daphnia* to acclimate or adapt to metal contaminants (e.g., Muysen et al. 2002, Lopes et al. 2004) resulting in increased survivorship in tolerant individuals. Former ecotoxicological studies have been limited in their analysis of the role of metal contaminants on *Daphnia* antipredator defenses because they have used single isolates, making it difficult to a) account for genetic variability in responses and b) extrapolate to the population level (De Schampelaere et al. 2011). Incorporating the use of multiple *Daphnia* genotypes in metal stress studies will advance our knowledge of metal tolerance mechanisms and their ecotoxicological implications on natural populations.

Chapter 2

Literature Review

2.1 Antipredator defenses and chemosensation

Antipredator defenses are ubiquitous in aquatic systems. While former studies have focused strictly on non-visual signaling in terrestrial systems, the role of chemical cues in benthic and planktonic communities has become a popular emergent topic in the literature (Dodson et al. 1994). Predator defenses are induced via chemoreception, i.e. the ability of the prey to detect a non-visual cue (kairomone) that is exuded by the predator during feeding (Stabel et al. 2003). This kairomone is characterized as a non-olefinichydroxy-carboxylic acid of low molecular weight (Tollrian and Elert 1994) and is classified as a subset of allelochemicals in that it conveys a non-visual communication signal between species, which is typically advantageous to the signal receiver, i.e. the prey (Dodson et al. 1994). Upon the release of this chemical substance by the predator, a variety of phenotypic changes may be induced in the prey (Laforsch et al. 2006). Most commonly, these phenotypic changes include morphological, life history, and behavioral alterations.

Daphnia is a model organism that displays a high degree of phenotypic plasticity in their antipredator responses. Due to their ability to be easily cultured in the lab, their quick generation time, and because they are an important trophic link in many aquatic ecosystems,, *Daphnia* are often employed in predation experiments. The induction of antipredator responses in *Daphnia* is typically contingent upon the following: 1) the type of predator and 2) the cost of the defense (Weber and Declerck 1997, Spitze 1991) .

2.2 Predator regime and inducible defenses

Inducible defenses (named for their ability to be turned on and off in the presence and absence of a predator) are often regulated by specific predator regimes (Weber and Declerck 1997). For example, invertebrate predators, which are typically gape-limited, non-visual hunters, prefer smaller sized individuals, as these prey are more easily ingested. Therefore, one predator-avoidance mechanism in *Daphnia* is to increase body size at maturation thereby avoiding size-selective predation (Boeing et al. 2006). Alternatively, *Daphnia* may decrease their body size in the presence of planktonic fish, which are non-gape limited, visual hunters, thereby reducing the probability of visual detection (Boeing et al. 2006). Additional inducible morphological traits include: tail spines, neck teeth/ neck spines, and helmets (Hebert and Grewe 1985). By eliciting morphological responses, the prey are able to either avoid ingestion by the predator or increase predator handling time, which increases the probability of escape by the prey (Hebert and Grewe 1985). If prey are unable to reduce predation by eliciting morphological traits, then they may increase population growth to compensate for predation pressures (Boeing et al. 2006).

One way to ensure population growth is to induce life history alterations, which are also dependent on the specific predator regime. Vertebrate predators typically induce increases in reproductive investment, i.e. larger clutch sizes with smaller bodied neonates, while invertebrate predators elicit changes in growth investments, i.e. smaller clutch sizes with larger bodied neonates (Weber and Declerck 1997). In either case, the inducible trait is a mechanism to ensure neonate survival and consequently stimulate population growth.

A final survival mechanism is predator avoidance via inducible behavioral traits. Zooplankton are known to practice diurnal vertical migration, staying in shallower water at night and deeper waters during the day. This behavior is mediated by surrounding predation pressures, as the detection of exudates from

visual predators or from predators occupying surface waters induces a more negatively phototactic behavior, and consequently a larger occupation of deeper waters by *Daphnia* (De Meester 1993). Conversely, predators residing in deeper waters, e.g. *Chaoborus*, induce a more positively phototactic behavior (upward migration) in daphniids (Nesbitt et al. 1996). The induction of migratory behavior in *Daphnia* aids in reducing the spatial overlap between the predator and prey so as to minimize the encounter rate with the predator (Nesbitt et al. 1996). Thus, inducible behavioral traits are yet another strategy to ensure survivorship.

2.3 Costs and benefits of inducible defenses

The phenomena of inducible defenses is that they can be turned on and off in the presence and absence of predators, but at what cost (if any) to the prey? According to Spitze (1991) predator defenses must incur a cost, otherwise they would be constitutive rather than inducible. In conjunction with this logic, classical postulates dictate that the induction of one response may incur a cost to the prey and thus, prey must be selective in which responses to induce (Boersma et al. 1998). However, Tollrian (1995) argues that direct physiological costs, e.g. energy requirements for the production or maintenance of morphological defenses, are usually minimal and not necessarily correlated with inducible defenses, but rather, indirect costs have a more profound effect on survivorship and population growth. Thus, costs are typically quantified through trade-offs, or an allocation of resources to one beneficial response that is typically linked to a concomitant detrimental response (Stearns 1989). For example, investments in somatic growth typically lead to reductions in reproductive output. A study by Boeing et al. (2005) was the first to quantify the costs of antipredator defenses in nature by calculating the difference in population growth rate (r) between a responsive clone, i.e., a clone exhibiting antipredator defenses, and a nonresponsive clone exposed to predation. Overall, the costs of inducing a behavioral defense, in this case vertical migration, was associated with a trade-off in r , as the responsive clone suffered a 32.3 % reduction in

population growth (Boeing et al. 2005). Thus, the induction of a predator avoidance mechanism ensured survivorship but limited population growth. In accordance with Boeing et al. (2005), Loose and Dawidowicz (1994) found that predator induced migration incurred fitness costs, as fish exudates caused *Daphnia* to migrate to deeper, colder waters which negatively affected body size and reproduction. Riessen and Sprules (1990) documented costs of morphological antipredator defenses, showing that necktooth induction was demographically costly in terms of juvenile and adult developmental rates. They indicated that induced morphs would be at a disadvantage in the absence of *Chaoborus* predation, owing to the trade-off between the morphological defense and slower developmental time, which consequently increases prey vulnerability (Riessen and Sprules 1990). In accordance with this study, Black and Dodson (1990) found that there were demographic costs associated with neck spine induction, as spined morphs resulted in fewer offspring and reduced population growth as compared to typical morphs. These studies elucidate the importance of inducible phenotypic traits in the presence of predation while simultaneously illustrating the disadvantages of trade-offs in the absence of predation.

Although life-history theory predicts that organisms have limited energy budgets, which ensue in trade-offs in resource allocation, some studies indicate an absence of trade-offs in *Daphnia* phenotypic traits (e.g., Luning 1994, Weber et al. 2003). Multiple tenets have been proposed to account for the lack in trade-offs including pleiotropy or coupling in traits, which requires a certain combination of traits to co-occur (Baer and Lynch 2003) and favorable ambient conditions, which allow organisms to increase population growth without costs to survivorship (Glazier 2000). However, probably the most discussed tenet is the acquisition-allocation model of van Noordwijk and de Jong (1986). Generally, it is predicted that individuals that have the ability to either acquire more resources or more efficiently use acquired resources will be able to make investments in both growth and reproduction, as opposed to those individuals that acquire fewer resources, thereby negating potential trade-offs (Glazier 2000). Thus,

individuals with a greater energy budget are able to allocate more resources to more traits. However, this doesn't account for the discrepancy between traits that are predicted to involve trade-offs and actual observed trade-offs. As Roff and Fairbairn (2007) point out, expected trade-offs in phenotypic traits, which are typically quantified through negative statistical correlations, are often not observed in laboratory experiments. They ascribe this discrepancy in life-history theory to variability in resource acquisition, which is explained by the van Noordwijk and de Jong model, referred to as the "Y-model" (Roff and Fairbairn 2007). Essentially, the Y-model predicts that if resource acquisition is not fixed, i.e. there is a smaller variation in the amount of resources allotted to two particular traits, the covariance between them may be positive and consequently the statistical correlation between the traits will be positive, giving the impression that there is no trade-off (Roff and Fairbairn 2007). Mathematically, this is defined by Roff and Fairbairn (2007) using the Y-model. Consider 2 traits (X_1 and X_2) that are determined by the allocation of resources from a common, acquired resource pool (T). The amount of resources allotted to trait 2 (X_2) is limited, owing to the fact that a proportion (P) of the resource pool (T) is given to trait 1 (X_1), leaving $(1-P)T$ of the resource pool being allocated to trait 2. Using this model, the covariance in the two traits can be determined with the following equation:

$$\sigma^2_{x_1x_2} = \sigma^2_T [\mu_p (1-\mu_p) - \sigma^2_p] - \mu_T^2 \sigma^2_p, \quad (1)$$

where $\sigma^2_{x_1x_2}$ denotes the covariance between traits 1 and 2, μ designates the means, and σ^2 designates the variances (Roff and Fairbairn 2007, Glazier 2000). Thus, it can be seen that large variations in resource acquisition (σ^2_T) may lead to a positive covariance and consequently a positive correlation between traits, or if variation in resource acquisition is greater than variation in resource allocation, then one trait won't be favored over the other and no trade-off will be observed. The van Noordwijk and de Jong model is one way to account for the disparity in predicted vs. observed trade-offs. However, another alternative explanation is that induced responses may be independent of each other. Contrary to much of

the literature depicting cost-associations with inducible antipredator defenses, Luning (1992, 1994) showed that daphniids can induce independent life-history responses to *Chaoborus* kairomone, with no accompanying morphological responses. Life-history theory predicts that morphological defenses should incur costs in reproductive output, with the two being causally related in some way. However, Luning (1992, 1994) found that although *D. pulex* are known to exhibit neck tooth induction in the presence of predators, daphniids displayed a delayed maturity in response to predation threat which was independent of neck tooth induction. Similarly, Tollrian (1995) found that changes in life-history parameters were not a consequence of neck tooth induction, indicating a non-causal relationship between morphological defenses and life-history changes. Therefore, there may not be stringent cost associations with morphological defenses vs. life history traits. Rather, *Daphnia* may induce a variety of adaptive antipredator responses to predation that are not contingent upon trade-offs.

2.4 Chemosensation and metal stress

Inducible defenses are unequivocally important in reducing both prey mortality, vulnerability and assuring population growth. The inability to detect predator kairomone and induce defenses could have detrimental ecological implications. Recently, it has been discovered that heavy metals interfere with chemosensory abilities and the induction of antipredator defenses in a plethora of fauna including: rainbow trout (Scott et al. 2003), snails (Lefcort et al. 2000), tadpoles (Lefcort et al. 1998), and *Daphnia* (Hunter and Pyle 2004). More specifically, the impacts of copper contamination on chemosensation have been elucidated in several recent studies. As a trace metal, copper is essential in ecosystem functioning, however, at high concentrations copper becomes toxic and can bioaccumulate (Long et al. 2004). Pyle and Mirza (2007) assessed the impact of copper on chemosensory ability, i.e., the ability not only to detect predators but also to detect food resources and conspecifics, in multiple representative trophic levels, with leeches representing the benthos, *Daphnia* representing primary consumers, and two fish

species representing secondary consumers. Copper impaired chemosensory functioning at all trophic levels in the following ways: in leeches, high copper concentrations resulted in greater foraging times and less consumption of food resources; in *Daphnia*, individuals exposed to copper were unable to induce neck teeth in the presence of predator cue while control individuals were able to induce neck teeth; and in fish, copper impaired the ability to detect conspecifics, which are utilized as a predator avoidance cue (Pyle and Mirza 2007). Hence, in all representative trophic levels, copper impaired the ability to detect non-visual cues, indicating potential reductions in survivorship or fitness in the field.

Although copper has been known to inhibit the induction of morphological defenses such as neck teeth in *Daphnia* (Hunter and Pyle 2004), the effects of copper on *Daphnia* survivorship when exposed to a predator have never been directly tested, until recently. Mirza and Pyle (2009) analyzed the direct impact of copper on *Daphnia* survivorship by staging encounters with chaoborids. Daphniids exposed to both kairomone and copper produced neonates with fewer and shorter neck teeth, as compared to daphniids reared in kairomone only (Mirza and Pyle 2009). In addition, neonates exposed to copper had lower survival during staged encounters with *Chaoborus*, indicating that copper had a direct effect on prey survivorship (Mirza and Pyle 2009). Besides survivorship, copper has also impacted *Daphnia* behavior, as individuals exposed to increasing nominal copper concentrations showed decreases in positively phototactic behavior irrespective of the presence or absence of predator cue (Yuan et al. 2003). Recall that phototactic behavior is simply defined as migratory behavior which utilizes light as a reference point, with negatively phototactic individuals being located deeper in the water column and positively phototactic individuals being located near the surface (De Meester and Dumont 1989). The copper induced negative phototactic behavior demonstrated by Yuan et al. (2003) may have implications on *Daphnia* fitness, owing to the fact that better food quality and warmer temperature gradients are found near surface waters. Although negatively phototactic individuals are protected from visual predators due

to their deeper water refuge, they do suffer fitness costs imposed by colder temperatures and poor food quality (Loose and Dawidowicz 1994). As De Meester and Dumont (1989) argue, in the absence of visual predators, positively phototactic daphniids have the evolutionary advantage over negatively phototactic daphniids. Therefore, the ability of copper to induce behavioral responses, e.g. negative phototaxis has detrimental ecological implications.

2.5 Tolerance to metal stress

While several studies elucidate the inhibitory role of copper in *Daphnia* defense systems, survivorship and fitness, other studies indicate the potential for acclimation/adaptation to metal stressors resulting in increased tolerance, survivorship, and (or) potentially fitness. The three basic responses to a metal stressor are tolerance, avoidance, or mortality; the latter two responses result in a more metal tolerant population as sensitive lineages are extirpated or replaced by resistant lineages (Lopes et al. 2004). Resistance to aqueous metals can be achieved via two mechanisms: 1) Previous exposure to a sub-lethal concentration ensues in physiological acclimation and consequently metal tolerance or 2) Metal tolerance is genetically based and is a ramification of natural selection on genotypic variation in resistance to metals (Klerks and Weis 1987). The former mechanism is typically governed through phenotypic plasticity, i.e. individuals acclimate themselves to fluctuations in the environment. While both mechanisms of metal resistance allow individuals to tolerate metal stressors, metal adaptation may result in biodiversity loss due to strong selective pressures on tolerant-specific clones (Ponti et al. 2010). Hence, adaptation to metal stress may have an ecological price. Nevertheless, individuals with an acquired metal resistance have an advantage over non-tolerant individuals.

Several studies have documented either acclimation or adaptation to metals in algae (Myslik and Hutchinson 1971), isopods (Brown 1976), the benthic invertebrate, *Limnodrilus hoffmeisteri* (Klerks and Levinton 1989), and *Daphnia* (Le Blanc 1982). In *Daphnia* specifically, both adaptation and acclimation

to metals has been exhibited. Adaptation in *Daphnia* populations can be achieved through the hatching of sexually produced, genetically distinct resting eggs. Because most species of *Daphnia* are cyclic parthenogens, they produce both asexually and sexually throughout their lifetime, with the product of sexual reproduction being resting eggs (Cousyn et al. 2001). When ambient surroundings are favorable, resting eggs will hatch, thereby introducing new genotypes into the system (Cousyn et al. 2001). Several studies have shown adaptation to aqueous metals in distinct *Daphnia* clones and populations. Ponti et al. (2010) found that a monoclonal field population of *D. galeata* obtained from a historically copper contaminated lake (Lake Orta) was three times more tolerant to ionic copper concentrations than a field population of *D. galeata* taken from a reference lake. Due to the monoclonal structure of the population, the authors attributed the increased metal tolerance of Lake Orta daphniids to adaptation, with the toxic environment inducing selective pressures on the most tolerant genotype, i.e. the current population. Similarly, adaptation to acid mine drainage (AMD) was documented for two monoclonal populations of *D. longispina* (Lopes et al. 2006). Populations taken from AMD impacted sites, which contained highly acidic, metal contaminated effluents, displayed lower sensitivity and greater survivorship to AMD impacted waters as compared to populations taken from reference sites (Lopes et al. 2006). Because both acclimated and non-acclimated individuals from the AMD impacted sites exhibited higher tolerance and consequently greater survivorship, tolerance was attributed to genetically determined resistance, i.e. adaptation (Lopes et al. 2006). Agra et al. (2010) also found pollutant-induced selection and consequently adaptation in *D. longispina* clones acquired from a water reservoir contaminated by acid mine drainage. Comparing multiple lineages from both a clean water reference site and the polluted acid mine drainage site, Agra et al. (2010) found that only copper-sensitive clones were present in the reference site, while only copper-tolerant clones were present in the contaminated site. In addition, the authors found that clones obtained from the contaminated site exhibited no genetic variation in their tolerances to Cu and Zn

(Agra et al. 2010). Not only was metal adaptation by multiple lineages demonstrated, but there was little inter-clonal variation in metal tolerance suggesting that genetically variable populations can adopt similar metal resistance mechanisms.

Besides metal adaptation, acclimation to metals by *Daphnia* has also been observed. Using *D. magna* clones obtained from zinc contaminated sites, Muysen et al. (2002) compared the zinc tolerance of *D. magna* field clones to standard laboratory clones. Not only were clones obtained from the field more tolerant to zinc but they also displayed a greater reproductive output and larger body size than control, laboratory clones (Muysen et al. 2002). However, zinc tolerance and reproductive rate decreased in field clones when cultured under low zinc concentrations for multiple generations (Muysen et al. 2002). Because daphniids were unable to respond to the metal stressor after it had been removed for successive generations, the *D. magna* field clones exhibited acclimation rather than adaptation to metal stress. Nevertheless, the study demonstrated that field populations are not only able to acquire increased resistance to metal contaminants but may demonstrate greater fitness as a consequence of increased resistance.

Metal resistance in *Daphnia* has been corroborated by studies indicating both adaptation and acclimation, with tolerant individuals exhibiting greater survivorship and, in some instances, greater overall fitness. Thus, it may be assumed that irrespective of the mechanism of metal tolerance, individuals with an acquired resistance to metal stressors may be better apt to respond to additional, biotic stressors, e.g., predation, than non-tolerant individuals. The persistence of natural populations in contaminated habitats is determinant of the ability of those populations to tolerate a combination of abiotic and biotic stressors (Lopes et al. 2009). Therefore, in order to maintain ecosystem functioning, a population (or community) must exhibit co-tolerance in multiple stressor environments. But how can a community establish co-tolerance in a habitat simultaneously stressed by contaminant exposure and predation

pressures? According to the conceptual model developed by Vinebrooke et al. (2004) co-tolerance can be achieved when species tolerances are positively correlated. To elaborate, when 2 stressors, e.g., a metal contaminant and predation, enter a system either stressor has the potential to extirpate more sensitive individuals, leaving the less sensitive individuals in the population. If the remaining species' sensitivities to the stressors are positively correlated, then exposure to one stressor will result in an increased tolerance to the other stressor, i.e., co-tolerance (Vinebrooke et al. 2004). This scenario is deemed by Vinebrooke et al. (2004) as "stress-induced community tolerance." Evolutionary co-tolerance was demonstrated for a metal adapted *D. pulex* population from the severely metal polluted Sudbury region in Ontario (De Schampelaere 2010). When exposed to a novel stressor, *Microcystis*, metal adapted *Daphnia* exhibited higher fitness than non-adapted daphniids from a non-metal polluted lake in Dorset, Ontario (De Schampelaere 2010). Hence, it may be assumed that individuals that have adopted metal-resistance mechanisms, i.e. either adaptation or acclimation, in dually stressed environments may be able to exhibit co-tolerance.

However, some authors argue that just as inducible defenses incur a cost, so must metal tolerance mechanisms. Fitness costs incurred from an adaptive tolerance to cadmium was observed for the midge, *Chironomus riparius* (Postma et al. 1996) and the fruit fly, *Drosophila melanogaster* (Shirley and Sibley 1999). Loss of genetic diversity has also been indicated as a cost associated with adaptive metal tolerance. Reduced genetic variability is a consequence of pollutant-induced selection, which alters the allele frequencies of genes in a population so that only the most tolerant genotypes remain (Van Straalen and Timmermans 2002). While adaptive tolerance to the contaminant is achieved, the trade-off is that the population experiences a marginal decrease in genetic variability, which decreases population resilience. Arguably then, organisms who have adopted metal tolerance mechanisms may be less apt to tolerate novel stressors when metal stressors are removed.

Kelly Lake (lat 46°26'N, long 81°05' W), is a historically metal contaminated lake located in Sudbury, Ontario. Prior to the 1970s, the Sudbury region was a severely industrially damaged area, with tens of thousands of tons of Cu, Ni, and Fe being released into the atmosphere via smelters, leading to the consequential acidification of many lakes within and around the area (Gun et al. 1995). The implementation of emission reduction legislation in the 1970s facilitated the chemical recovery of these lakes, including Kelly Lake (Gun et al. 1995). While chemical recovery has been achieved in the area, biological recovery, e.g. zooplankton recovery, in the lake systems remains limited (Valois et al. 2010). As Valois et al. (2010) indicate, zooplankton recovery varies according to region and species and can be limited by abiotic stressors such as metals. *Daphnia* have a relatively low copper sensitivity (U.S. EPA 1985). Brix et al. (2001) emphasize that organisms exhibit varying degrees of copper sensitivity due to several mechanistic reasons including: membrane permeability, different metal regulation strategies, and allometric differences, i.e. differences in the amount of permeable membrane relative to overall body size. Data obtained in 2008 estimated nominal, ambient copper concentrations for Kelly Lake at 18.3 µg/L (Inglis 2009). This copper concentration exceeds Ontario's Provincial Water Quality Objective (PWQO) of 5 µg/L for copper (MOEE 1994) and is only slightly lower than the copper concentration shown to produce acute toxicity in *Daphnia magna* (U.S. EPA 1985). In addition, chemosensory impairment in *Daphnia* has been documented at a copper concentration of only 5 µg/L (Hunter and Pyle 2004). Therefore, it is important to understand not only acute toxicity effects of copper but also the effects of chronic copper stress on chemosensation, as impairment of inducible defenses may negatively influence biological recovery. Kelly Lake is an appropriate system to test the sensitivity of *Daphnia* populations to metal stress, particularly copper, thereby determining if daphniids have acquired metal resistance and the implications of that resistance in a multi-stressor environment.

2.6 Thesis objectives

The objectives of this study were: 1) To determine if *D. pulicaria* clones obtained from Kelly Lake respond to *Chaoborus* predation threat by inducing antipredator morphological and life history traits; 2) To determine if a nominal, environmentally relevant copper concentration impedes the ability of *Daphnia* to respond to predator cue, where “environmentally relevant” is defined as a bioavailable copper concentration that may be encountered in the field (Bossuyt and Janssen 2003); 3) To determine if there is any inter-clonal variation in *Daphnia* responses. We hypothesized that a) Kelly Lake clones have adopted a metal tolerance strategy and therefore are able to respond to predation threat in both the presence and absence of copper exposure and b) Due to their genetic variability, morphological and life-history responses to *Chaoborus* cue will vary among clones.

Chapter 3

Fleeing predation: the effect of copper on inducible antipredator defenses in *Daphnia pulicaria* clones from a historically metal contaminated lake

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ABSTRACT

Antipredator defenses are ubiquitous in aquatic ecosystems. In the widely studied *Chaoborus-Daphnia* predator-prey system, *Daphnia* elicit a variety of phenotypically plastic responses to *Chaoborus* including: morphological, life history, and behavioral responses. While these inducible defenses benefit the prey, metal contaminants have been shown to interfere with chemosensory functions, thereby inhibiting antipredator defenses and decreasing survivorship. However, in lakes with a history of metal contamination, such as Kelly Lake in Sudbury, Ontario, there is evidence to suggest that *Daphnia* may have adapted to high, ambient copper concentrations. Using seven distinct *Daphnia* clones that were hatched from resting eggs from Kelly Lake, we examined morphological and life history traits when clones were exposed to either a nominal concentration of copper, kairomone, or a combination of both. As expected, clones displayed a variety of inducible responses in both kairomone-control and kairomone-copper treatments, which was attributed to genetic variability. Expected trade-offs in life history traits were not always observed, suggesting that inducible traits may be coupled. Furthermore, in contradiction to life history theory, one clone exhibited both increased somatic growth and increased reproductive output, indicating that clones likely adopted adaptive strategies to stressors rather than eliciting trade-offs in traditional traits. Our results indicate that environmentally relevant copper concentrations do not inhibit the induction of antipredator defenses in *Daphnia* from Kelly Lake, and we conclude that Kelly Lake *Daphnia* have developed an adaptive tolerance to copper. Adaptation to copper contamination may have implications for resilience in natural Kelly Lake populations.

INTRODUCTION

With the increase in anthropogenic pollutants, ecotoxicological studies have become concerned with not only how individuals respond to toxicant stress, but how populations and communities respond to stress as well. Conventional ecotoxicology is limited in its analysis of the effects of pollutants on natural populations because it fails to account for genetic variability, often using only one or two isolates per study (De Schamphelaere et al. 2010), and because it fails to account for the combined effects of anthropogenic and natural stressors, e.g. competition or predation (Lopes et al. 2009). The resilience of a population depends not only on its ability to tolerate a single, anthropogenic stressor, but on the ability of a population to co-tolerate both abiotic and biotic stress (Lopes et al. 2009).

Adaptation to metal stress, resulting in increased tolerance to metal contaminants, has been documented for algae (Myslik and Hutchinson 1971), isopods (Brown 1976), the benthic invertebrate, *Limnodrilus hoffmeisteri* (Klerks and Levinton 1989), and *Daphnia* (Le Blanc 1982). While adaptive tolerance to metals benefits populations within contaminated habitats, it often comes at an ecological price, as pollutant-induced selection acts on tolerant genotypes, thereby reducing the overall genetic diversity within the population and potentially leaving the resultant population susceptible to novel stressors (Van Straalen and Timmermans 2002, Lopes et al. 2009). However, in some instances, tolerance to one stressor may result in decreased sensitivity to another stressor, i.e. co-tolerance (Vinebrooke et al. 2004) The mechanism of co-tolerance is explained by Vinebrooke et al. (2004) as the outcome encountered when species' tolerances to 2 stressors are positively correlated such that resistance to one stressor ensues in increased resistance to the other stressor. Thus, it is imperative to understand the effects of multiple stressors on ecosystems in order to determine the resilience of natural populations.

Several studies have documented adaptation to metal stress in *Daphnia* populations (e.g. Ponti et al. 2010; Agra et al. 2010; and Lopes et al. 2003). Yet, few studies have examined the combined effects of

anthropogenic and biotic stress on *Daphnia*. Studies that did examine the effects of combined stressors, i.e. copper exposure and predation pressure, on *Daphnia* found that copper impeded the ability of daphniids to induce antipredator defenses and decreased overall survivorship (Yuan and Michaels 2003; Hunter and Pyle 2004; Mirza and Pyle 2009). However, extrapolating these results to natural populations is difficult due to the low genetic variability and because they do not account for potential adaptation to copper in the *Daphnia* clones, which may affect clonal responses.

Kelly Lake in Sudbury, ON is a historically metal contaminated lake that has experienced chemical recovery (Gunn et al. 1995). Nevertheless, its copper concentration exceeds the Ontario Provincial Water Quality Objective (PWQO) of 5 µg/L (MOEE 1994). The most recent water chemistry data estimates nominal copper concentrations at 18.3 µg/L (Inglis 2009). Species mean copper acute values and species mean copper chronic values were estimated at 20.2 µg/L and 8.4 µg/L, respectively, for *Daphnia magna* clones (U.S. EPA 1985). Thus, Kelly Lake provides an opportunity to test the assumption that *Daphnia* originating in the lake have adapted to metal contamination, and therefore, are able to exhibit co-tolerance to copper stress and predation stress. The specific aims of this study were: 1) To determine if *D. pulicaria* clones obtained from Kelly Lake respond to *Chaoborus* predation threat by inducing antipredator morphological and life history traits; 2) To determine if a nominal, environmentally relevant copper concentration (10 µg/L) impedes the ability of *Daphnia* to respond to predator cue; and 3) To determine if there is any inter-clonal variation in *Daphnia* responses. We hypothesized that a) Kelly Lake clones have adopted a metal tolerance strategy and therefore are able to respond to predation threat in both the presence and absence of copper exposure and b) Due to their genetic variability, morphological and life-history responses to *Chaoborus* cue will vary among clones.

MATERIALS AND METHODS

Study site

Kelly Lake, located in Sudbury, ON (lat 46°26'N, long 81°05'W), is a 340.8 hectare eutrophic lake situated approximately 10 km south of the Copper Cliff smelting complex (Figure 1). It has a shoreline perimeter of 15.5 kilometers and a maximum depth of 18 meters (Figure 1a). Due to its proximity to the International Nickel Company (Inco Ltd.) mining and smelting complex, Copper Cliff, and the local creosote plant, Kelly Lake is characterized as a severely metal-contaminated lake, with sediments rich in ore deposits of copper, nickel, palladium, iridium, and platinum (Pearson et al. 2002). Concomitant with metal deposition, nutrient influxes from Junction Creek, which historically acted as a point-source of raw sewage effluent, contributed to the high phosphorus content in Kelly Lake sediment (Pearson et al. 2002) and consequently its eutrophic nature. The most recent water chemistry data taken for the lake was in 2008 and indicated the following measurements: pH=7.13, ionic copper=18.3 µg/L, Phosphorus= 25.2 µg/L, DOC=4.9 mg C/L, Alkalinity=39.0 mg/L CaCO₃ (Inglis 2009).

Sediment coring and processing

On May 17, 2010, five Glew gravity cores (25 cm in length, 10 cm in diameter) were taken from the center of Kelly Lake main basin at a depth of approximately 18 m. Sediment cores were extruded and sectioned into 1-cm intervals in situ. Sectioned cores were placed in Whirl-Pak® bags and placed in coolers. Upon arrival to the laboratory, collected sediment samples were transferred to the refrigerator.

Ephippia retrieval

Sediment samples were sieved using a 212 µm mesh. A dissecting scope was employed to search the retained organic matter for ephippia. *D. pulicaria* ephippia were identified according to size and shape. All initial identifications were verified using adult hatchlings. Viable eggs were counted, placed in weigh boats containing FLAMES solution (Celis-salgado et al. 2008), and incubated at 10° C with a 14L:10 D

photoperiod. Diapausing eggs were monitored every other day for hatchlings for the first week and then monitored daily for subsequent weeks.

Maintenance of animals

Individual, hatched neonates were placed in 500 mL jars in COMBO culture medium (Kilham et al. 1998) at 21°C under a 16:8 L:D photoperiod and were fed *Chlamydomonas* spp. (approximately 3 mL/week; cell density, 2.8×10^6 cells/mL). Water changes were conducted 3 times/week. The 7 *Daphnia* strains were cultured for approximately one month prior to experimentation in order to reach a sustainable population size of approximately 50 individuals/strain.

Kairomone preparation

A mixture of fourth instar *Chaoborus flavicans* and *Chaoborus trivittatus* were collected from Otter Lake in Dorset, ON (lat 45° 18' N, long 79° 56' W). Due to their similar sizes, these species likely exhibit similar size-selective predation. Therefore, it was deemed unnecessary to separate the two species. Prior to experimentation, animals were maintained in COMBO culture medium (Kilham et al. 1998), fed brine shrimp once a week, and were stored at 8° C to prevent pupation. Stored chaoborids were fed *Daphnia* neonates one week before experimentation to certify that predator cue was prey-specific.

Experimental chaoborids were kept in the incubator at a temperature of 21° C. Kairomone treatment was prepared by placing 10 *Chaoborus* into 1 L of COMBO, a concentration indicative of high predation risk (Mirza and Pyle 2009). Pupating individuals were replaced with fourth instar *Chaoborus* to maintain kairomone quality. Individual *Chaoborus* larvae were fed 3 neonates daily (half-way through the experiment, this was reduced to 2 neonates daily due to neonate density restrictions) to ensure that kairomone was exuded. Neonates were consumed almost immediately upon contact with chaoborids, thereby preventing the potential for prey-released alarm cues, as described by Laforsch et al. (2006) and ensuring that elicited cues were predator-mediated. To ensure that kairomone concentrations did not

fluctuate throughout the experiment, 50% water changes were conducted every other day on *Chaoborus* conditioned water.

Stock solution preparation

Copper solution was prepared from the salt, cupric sulfate pentahydrate ($\text{CuO}_4 \cdot 5\text{H}_2\text{O}$, Fisher Scientific, Ottawa, ON) dissolved in 1 L of double deionized water. Exposure solutions were prepared via serial dilutions using double deionized water to yield the desired concentration. All exposure solutions contained a nominal copper concentration of $10 \mu\text{g L}^{-1}$ (10 ppb), as this was observed to be the lowest sub-lethal copper concentration capable of inducing an anti-predatory response in *Daphnia pulicaria* (Inglis 2009). Metal analyses were deemed unnecessary, owing to the fact that no concentration specific effects were intended to be analyzed but rather, the relative sensitivity of clones to contaminant exposure wished to be understood, as per Lopes et al. (2004). All *Daphnia* clones were fully acclimated to copper prior to experimentation.

Experimental protocol

Exposures were conducted in 20 mL glass test tubes at 21° C under a 16:8 light to dark photoperiod. To test the effects of predator stress and copper stress on *Daphnia* morphology and life history, daphniids were exposed to predator cue, copper, or a combination of the two stressors using a two-factor experimental design. In total, there were 4 treatments: a control treatment (K- Cu-), a copper control treatment (K- Cu+), a kairomone control treatment (K+ Cu-), and a kairomone copper treatment (K+ Cu+). Single, gravid females were placed into test tubes containing 10 mL of one of the 4 treatment solutions. Test tubes were monitored daily for neonates and *Chlamydomonas* spp. was added daily at a cell density of 10^5 cells/ml; water changes were conducted every other day.

To eliminate maternal effects, responses were monitored starting with the F2 generation. The first brood of neonates released by an experimental, gravid female was discarded. When the female released her

second brood she was discarded and her neonates were selected to continue exposure to the treatment. To account for variation in responses between broods, both the first generation neonates and the second generation neonates were preserved in ethanol and later examined for inducible morphological defenses.

Morphological measurements and life-history parameters

Morphological measurements were taken on a dissecting scope. To assess size at first reproduction, body length, i.e. the length from the top of the head to the end of the caudal spine was measured (Figure 2). To determine whether or not tail spine length was an inducible trait, caudal spine length was measured on all mature daphniids and relative tail spine length (tail spine/body size, with body size being the length from the top of the head to the bottom of the carapace) was also measured (Figure 2). Relative lengths were determined to ensure that longer tail spines were not a function of larger body sizes. I defined “mature” as the time at which a daphniid first produced eggs. Morphological measurements were taken using the ImageJ program (Abramoff et al. 2004). Spatial calibration of all images was achieved before conducting measurements.

Because *Chaoborus* predation is known to induce changes in life-history strategies, the following life history parameters were determined for each clone: time to first reproduction, mean size of neonates per brood, and average number of neonates per clutch. All measurements were taken into the F2 generation; however, to account for variation between broods both the F1 and F2 generation were preserved and analyzed.

Statistical Analyses

To assess variation in the *Chaoborus* response in the absence of copper contamination, student t-tests were used to compare morphological and life-history responses between + kairomone and –kairomone treatments for all clones, n= 101. In total, there were 7 responses: size at first reproduction (SFR), time to first reproduction (TTFR), tail spine length, total number of first brood neonates, average size of first

brood neonate, total number of second brood neonates, and average size of second brood neonates. Normality was verified visually using boxplots and histograms and confirmed using the Shapiro-Wilks normality test, while homogeneity of variances was confirmed using Levene's test. P-values were adjusted using the Benjamini-Hochberg test (Benjamini and Hochberg 1995).

To determine if copper influenced the response of *Daphnia* to *Chaoborus*, I then conducted two-way ANOVAs on raw data for the 7 response variables for each of the clones. Assumptions of normality and homogeneity of variances were tested by observing plots of the fitted vs. residual values, which showed heteroscedasticity in the data and by using Levene's test. P-values were adjusted using the Benjamini-Hochberg test to control for the false-discovery rate. All statistical analyses were conducted in R (R Developmental Core Team 2008).

Results

Responses to kairomone in the absence of copper

Individual clonal responses to *Chaoborus* spp. kairomone were highly variable, with all but one clone (clone 2) inducing a significant morphological (and) or life history response (Table 1). Only two out of seven clones (clone 4, 7) responded to predator cue by inducing a morphological response, i.e. changing their size at first reproduction (SFR). Interestingly, these clones displayed opposite responses, with clone 7 being significantly larger at SFR while clone 4 was significantly smaller at SFR (Table 1, Figure 4). All other clones elicited life-history alterations in response to predator cue. Clones 1, 6, and 7 responded to kairomone by significantly increasing the average size of neonates in both the first and second broods, while clone 3 responded by significantly decreasing the average neonate size in the first and second broods (Table 1, Figures 6 and 8). Changes in average neonate body size were accompanied by a

significantly shorter time to first reproduction in clone 3 only (Table 1). Clone 5 induced a solitary life history response: significantly increasing the average size of second brood neonates (Table 1).

Joint stressor (Kairomone-Cu) effects

All clones that responded to kairomone also displayed a significant *Chaoborus*-copper interaction for at least one of the response variables (Table 2). Clones that responded either morphologically or via life-history alterations to kairomone in control treatments reacted in the following ways to joint stressors: 1) gained a response in kairomone-copper treatments not observed in kairomone-control treatment, 2) lost a response in kairomone-copper treatment observed in kairomone-control treatment, or 3) altered a response, i.e. displayed an opposite response in kairomone-copper treatment to the one observed in kairomone-control treatment (Table 4). Of the seven clones, 6 gained a morphological (and) or life history response in the presence of copper and kairomone. Clone 7 was the only clone to respond morphologically, significantly lengthening its relative tail spine length in the *Chaoborus*-copper treatment. (Table 2, Figure 5). All other clones induced life-history responses in copper-kairomone treatments not observed in control-kairomone treatments, with clones 1 and 6 significantly reducing their time to first reproduction, clone 6 significantly increasing the average number of first brood neonates, clones 2, 4, and 5 significantly decreasing the average size of first brood neonates, and clones 1 and 7 significantly increasing the average number of second brood neonates (Table 2, Figures 3, 7, 6, and 9).

While some clones induced responses in joint stressor treatments not observed in control-stimulus treatments, other clones lost their ability to respond to kairomone in copper. For example, although clone 3 displayed significant reductions in time to first reproduction, average neonate size and a significant increase in the average number of second brood neonates in *Chaoborus*-control treatments, no responses to predator cue were induced in *Chaoborus*-copper treatments (Table 2). In concordance with clone 3,

clones 1, 5, 6, and 7 were unable to significantly alter the average size of second brood neonates in *Chaoborus*-copper treatments, despite their ability to significantly increase average size of second brood neonates in *Chaoborus*-control treatments (Table 2, Figure 8). Furthermore, the morphological response of clone 7 to predator cue was hampered by copper, as the ability to significantly increase SFR in the presence of *Chaoborus* kairomone was lost with the addition of copper stress. Finally, clone 4 displayed a loss of response for SFR and average number of second brood neonates via its inability to significantly reduce SFR and average number of second brood neonates, as observed in *Chaoborus*-control treatments (Table 4).

The only clone to display an altered response, i.e. an opposing life-history strategy in control vs. copper-stimulus treatment, was clone 1. Contrary to the control, clone 1 exhibited a significant reduction in the average size of first brood neonates in the presence of joint stressors (Table 4, Figure 6).

Solitary stressor (Cu) effects

In some instances, clones did not respond to predator cue but instead responded to copper stress. For example, clones 1, 4, and 7 significantly decreased their average number of first brood neonates when exposed to copper but did not exhibit a response to kairomone (Table 2, Figure 7). Clone 4 displayed an additional copper-induced response by significantly decreasing the average size of first brood neonates compared to treatments without copper (Table 2, Figure 6). Clone 5 also displayed a copper-induced response, significantly reducing its time to first reproduction in copper-control treatment (Table 2, Figure 3).

While some clones responded solely to copper stress, others elicited a copper stress response that was commensurate with the predator stress response. For example, clone 1 significantly increased the average size of first brood neonates in response to predation threat. Commensurate with the predator stress response, clone 1 significantly increased the average size of first brood neonates in the presence of copper

stress (Table 2, Figure 6). Additionally, clone 1 maintained its kairomone response by significantly increasing the average size of second brood neonates in copper (Table 2, Figure 8). Similarly, clones 6 and 7 responded to predation threat by significantly increasing the average size of second brood neonates, a response that was mimicked in copper (Table 2, Figure 8). Clone 7 displayed an additional maintained response, as the induction of significantly larger bodied first brood neonates was observed in both predation threat and copper stress treatments (Table 2, Figure 6).

Trade-offs

All of the clones, with the exception of clone 5, exhibited significantly positive correlations in at least one of the traits expected to incur trade-offs, e.g. time to first reproduction and size at first reproduction (Table 3). However, only clone 7 displayed a significant negative correlation, indicating a trade-off between average number of first brood neonates and average size of first brood neonates, with smaller bodied individuals being a consequence of larger brood sizes ($t = -2.1024$, $p = 0.04$).

Discussion

Predator-induced phenotypic plasticity is common in *Daphnia* spp. In accordance with other studies, e.g. Black and Dodson 1990, Spitze 1991, our clones exhibited a large extent of variation in their responses to *Chaoborus* predation threat including: significant increases and decreases in size at first reproduction, average neonate size, average number of neonates per brood, and time to first reproduction. Most of these responses were commensurate with typical *Chaoborus*-induced phenotypic traits, e.g. shorter time to maturation, larger body size at first reproduction, and lower reproductive output (Stibor and Luning 1994, Weber et al. 2003); however, some clones displayed atypical responses to kairomone. Rather than increasing its size at first reproduction, a trait commonly induced in the presence of gape-limited predators, clone 4 significantly decreased its size at first reproduction, while all other remaining clones failed to induce a morphological response. Because induced defenses are often associated with costs to

the prey (Boersma et al. 1998), it may be assumed that clones who did not invest energy in somatic growth, i.e. clone 4, most likely allocated resources to reproduction, thus exhibiting a trade-off in traits. However, in the case of clone 4, there was a significant reduction in both body size and reproductive output. Contrary to clone 4, clone 7 exhibited significant increases in both SFR and size of neonates, again elucidating the apparent lack of trade-offs in responses. If a cost-benefit association is not a driving force behind the induction of morphological vs. life-history traits, then what is?

According to Baer and Lynch (2003), some traits have an inherent relationship, e.g. physiological, mechanical, energetic, that requires them to be expressed simultaneously and disallows the combination of other traits. Boersma et al. (1998) adds that clones may express combined or coupled responses as an adaptive tactic. Therefore, the apparent lack in trade-offs could be attributed to coupled traits, or what some authors deem pleiotropy, e.g. Spitze (1991). Evidence for coupled traits came from the significant positive correlations between SFR and average size of first brood. For multiple clones, a smaller size at maturation was indicative of a smaller brood size, irrespective of the treatment, indicating that clutch size is likely a function of body length and may be a coupled trait. However, Roff and Fairbairn (2007) attribute the lack of concordance in observed vs. expected trade-offs to variability in resource acquisition, which, when quantified using the resource acquisition-allocation model proposed by van Noordwijk and de Jong (1986), consequently generates a false statistical impression that no trade-off exists among the two traits. In other words, if a fixed amount of resources or energy is allocated to one trait, then a trade-off can be expected; however, if the amount of energy acquiesced by the two traits varies, i.e. it's not a fixed allocation, then positive correlations between those traits will ensue (Roff and Fairbairn 2007, Glazier 2000). Thus, it is possible that trade-offs weren't properly quantified in the statistical model.

An alternative explanation for the observed lack in trade-offs could be what Weber et al. (2003) coin, "the *Chaoborus* paradox." The *Chaoborus* paradox contradicts classical postulates dictating that

daphniids will lower their reproductive output as a consequence of increased growth by showing that, in some instances, resources are allocated to both somatic growth and reproduction (Weber et al. 2003). The *Chaoborus* paradox was documented by Weber and Declerck (1997) who indicated that trade-offs between *Daphnia* growth and reproduction were negligible, as daphniids grew faster in kairomone treatment but did not reduce their reproductive output. Similarly, Spitze (1991) concluded that increased reproductive output incurred no physiological costs. Life history responses induced by clone 7, i.e. larger SFR and increased reproductive output, confirm the *Chaoborus* paradox. Thus, the variation in responses to predation threat may be attributed to both genetic variability in inducible responses and variation in the coupling of traits, indicating that daphniids may have adopted differing adaptive strategies to predation, rather than eliciting trade-offs in traditional traits.

Effects of multiple stressors on inducible traits

Daphnia clones displayed a high degree of plasticity in their responses to predation threat, but what happened to inducible traits when an additional stressor, i.e. copper, was added to the system? Metal contaminant exposure has been known to interfere with chemosensation by disrupting chemical signaling between predator and prey, often with detrimental consequences to the prey (Pyle and Mirza 2007, Mirza and Pyle 2009, Hunter and Pyle 2004, Leoni and Garibaldi 2009). While former studies have focused on the inhibitory role of copper, it can be debated that multiple stressors, i.e., predation threat and contaminant exposure, may be additive or multiplicative, with potentially adaptive synergistic effects (Hanazato and Dodson 1995). Thus, it may not be appropriate to label contaminants as purely inhibitory. Rather, the mechanisms behind energy allocation in multiple stressor environments should be better understood.

Overall, our clones responded to multiple stressors in 3 general ways: 1) clones gained a response 2) clones lost a response or 3) clones altered a response. Variation in inter-clonal responses is most likely

due to changes in the pattern of energy allocation as a consequence of copper contamination and *Chaoborus* predation threat. For all of the morphological and life-history traits observed, (with the exception of time to first reproduction), a significant interaction between copper and kairomone was detected, indicating a synergistic effect (Hanazato and Dodson 1995). It is generally assumed that *Daphnia* have a limited energy budget and that additional stressors may sequester energy reserves originally allocated to growth or reproduction (Hanazato and Dodson 1995). Hence, the synergism seen between copper and kairomone may indicate trade-offs in tolerance vs. growth/reproduction. For example, there was a significant interaction for size at first reproduction for clone 4, with the two stressors inducing a significantly smaller body size than the control. Since a smaller body size increases vulnerability to *Chaoborus* predation, clone 4 may be allocating resources to copper tolerance instead of investing in somatic growth. In a similar vein, synergistic effects were observed for the average size of first brood neonates for clones 1 and 5. Both clones significantly decreased the size of their neonates, while maintaining their body size, again indicating that energy may be allocated to copper tolerance rather than reproduction. Consider the trade-off hypothesis: increased metal tolerance may be expensive in terms of energy and other resources (Harper et al. 1997). There is a caveat though; clones came from a historically metal contaminated lake and therefore were predicted to have an evolved tolerance to copper contamination. Most likely then, there is a cost associated not only with increased tolerance to copper exposure, but also with an adaptive tolerance to copper contamination.

Several authors dictate that adaptive tolerance, like inducible defenses, must incur a cost. Illustrating this concept, Shirley and Sibley (1999) showed that genetically determined Cd tolerance incurred fitness costs in *Drosophila melanogaster*. Similarly, Ward and Robinson (2005) showed that an evolved resistance to cadmium exposure in *D. magna* was both physiologically and genetically costly, as tolerant individuals exhibited reduced body size and reduced genetic variability compared to non-tolerant individuals. Fitness

costs of evolved metal tolerance were further quantified in a study by Agra et al. (2010), illustrating that populations of *D. longispina* obtained from a site historically exposed to copper mine drainage displayed lower feeding rates compared to non-tolerant individuals from reference sites. Because clones exhibited responses typically associated with reductions in fitness, e.g. reduced body size, and in some instances, lower reproductive output, it may be inferred that Kelly Lake daphniids have an evolved tolerance to copper exposure and trade-offs in fitness are indicative of costs associated with their metal adaptation. Alternatively, fitness costs can be explained by the metal requirement hypothesis. The metal requirement hypothesis postulates that individuals may utilize a tolerance mechanism via reduced or less efficient metal uptake/utilization, which consequently, may result in reduced fitness due to micronutrient deficiencies (Harper et al. 1997, Agra et al. 2010). If tolerant daphniids are decreasing their metal uptake as an adaptive means in highly contaminated sites, those same individuals, when transplanted to ‘cleaner’ sites may suffer reduced filtration rates and subsequently reduced fitness. The nominal copper concentration in Kelly Lake is 18.3 µg/L (Inglis 2009). Experimental exposures contained copper concentrations of approximately 10 µg/L. It may be possible that daphniids from Kelly Lake have adopted a tolerance mechanism and employed that mechanism in exposures. This would explain both the apparent lack of trade-offs in inducible responses and reduced fitness traits, i.e. smaller body size and reduced reproductive output, exhibited in some clones exposed to the dual stressors, copper and kairomone.

Yet, genetic variability in the degree of copper tolerance cannot be overlooked as a probable explanation for the high degree of inter-clonal variation in induced responses. Baird et al. (1990) notes that there is considerable genetic variability in responses to metal stress; more importantly, metal tolerance is not dominated by a general mechanism but rather is mediated through individual clones’ abilities to cope with a toxicant stressor (Baird et al. 1990). Therefore, individual clones may have

varying degrees of copper tolerance and may be utilizing alternative tolerance mechanisms, which would account for the variation in induced responses. However, to fully analyze whether or not our daphniids exhibited a definite adaptive tolerance to copper exposures, thereby indicating an evolved, genetically based tolerance to copper, we would need to take into account heritability. As Morgan et al. (2007) point out, heritability is the precursor in differentiating between adaptation and physiological adjustments, i.e. phenotypic plasticity or acclimation, in populations. In order to determine inter-clonal genetic differentiation for copper tolerance, we would need to monitor inducible responses in later-generation offspring. This comparison, combined with a reference population as per Lopes et al. (2004), would provide evidence for adaptive tolerance to copper contamination.

While we did not directly test the copper tolerance of a *Daphnia* population from a non-polluted reference cite, a former study conducted by Inglis et al. (2009) showed that a *D. pulicaria* population obtained from a non-metal contaminated lake located in Chaffey's Locks, ON was inhibited in its ability to respond to *Chaoborus* kairomone when exposed to a nominal Cu concentration of 10 µg/L. Thus, we have evidence to support our hypothesis that Kelly Lake *Daphnia* have adapted to copper contamination. To further support our hypothesis, adaptation to metal contamination in the Sudbury region has recently been documented for a population of *D. pulex* (Shaw et al. unpublished data cited in De Schamphelaere et al. 2010). Interestingly, when the Cd adapted population from Sudbury was exposed to a novel stressor, *Microcystis*, individuals exhibited greater fitness in the presence of either stressor in contrast to non-adapted individuals, indicating an evolved co-tolerance to metal contamination and cyanobacterial stress (Glaholt et al. unpublished data cited in De Schamphelaere et al. 2010). Co-tolerance to multiple metal contaminants has been documented for heterotrophic and autotrophic biofilms as well, with pre-existing exposure to copper resulting in an increased tolerance to zinc and vice versa (Tlili et al. 2011). The pollutant induced co-tolerance was attributed to "positive species co-tolerance" coined by Vinebrooke et

al. (2004). The positive species co-tolerance hypothesis postulates that if individuals' sensitivities to stressors are positively correlated, increased resistance to one stressor is a consequence of exposure to an alternate stressor (Vinebrooke et al. 2004). Therefore, pre-existing exposure to a metal pollutant, e.g. copper, may result in a decreased sensitivity to a novel stressor, e.g. predation. Assuming Kelly Lake *Daphnia* have evolved an adaptive tolerance to copper contamination, the inter-clonal variation in responses to dual stressors, i.e. copper exposure and predation threat, could be attributed to mechanisms of co-tolerance rather than costs associated with tolerance. This has ecotoxicological implications, as co-tolerance to predation and contaminant exposure may positively influence the resilience of natural populations from Kelly Lake and should be considered when making ecological risk assessments.

Effects of copper as a solitary stressor

Life history and morphological responses to copper control treatments were similar to responses in the co-stressor treatments. Interestingly though, some clones who expressed a negative kairomone response in control treatments induced life-history responses in copper control exposures, indicating that copper is likely a more important factor in influencing clonal responses than kairomone. This again provides evidence for adaptation to copper, as clones elicited similar life history responses (decreased reproductive output, delayed maturity) in copper control treatments, which likely were adaptive life history strategies in response to copper contamination. Koivisto and Ketola (1995) found that *D. pulex* significantly reduced their time to maturity when exposed to nominal copper concentrations. In addition, Lopes et al. (2009) showed that *Daphnia* populations that were adapted to acid mine drainage responded more strongly to applied chemical stress, as opposed to applied predation threat, indicating that inherent adaptive mechanisms to metals were induced more frequently than antipredator defense mechanisms. Thus, we have further support to indicate that clones have developed an adaptive tolerance to copper contamination, which consequently results in copper-induced, adaptive life history responses.

Conclusion

While former studies emphasize the inhibitory role of copper on inducible anti-predator defenses, our results indicate that copper does not impede the ability of clones to respond to predation threat. Rather, variation in inter-clonal responses to multiple stressors may be due to a variety of factors including: trade-offs in energy allocation between copper tolerance and morphological/life-history responses, inherent tolerance mechanisms that decrease nutrient uptake (and consequently fitness), or genetic variability in response traits due to variability in tolerance. We have evidence to suggest that Kelly Lake *Daphnia* exhibit a genetically based resistance to copper exposure and that this resistance may result in co-tolerance to predation stress and copper contaminants. Future studies should analyze effects of exposure on later-generation juveniles to verify the adaptive nature of these traits.

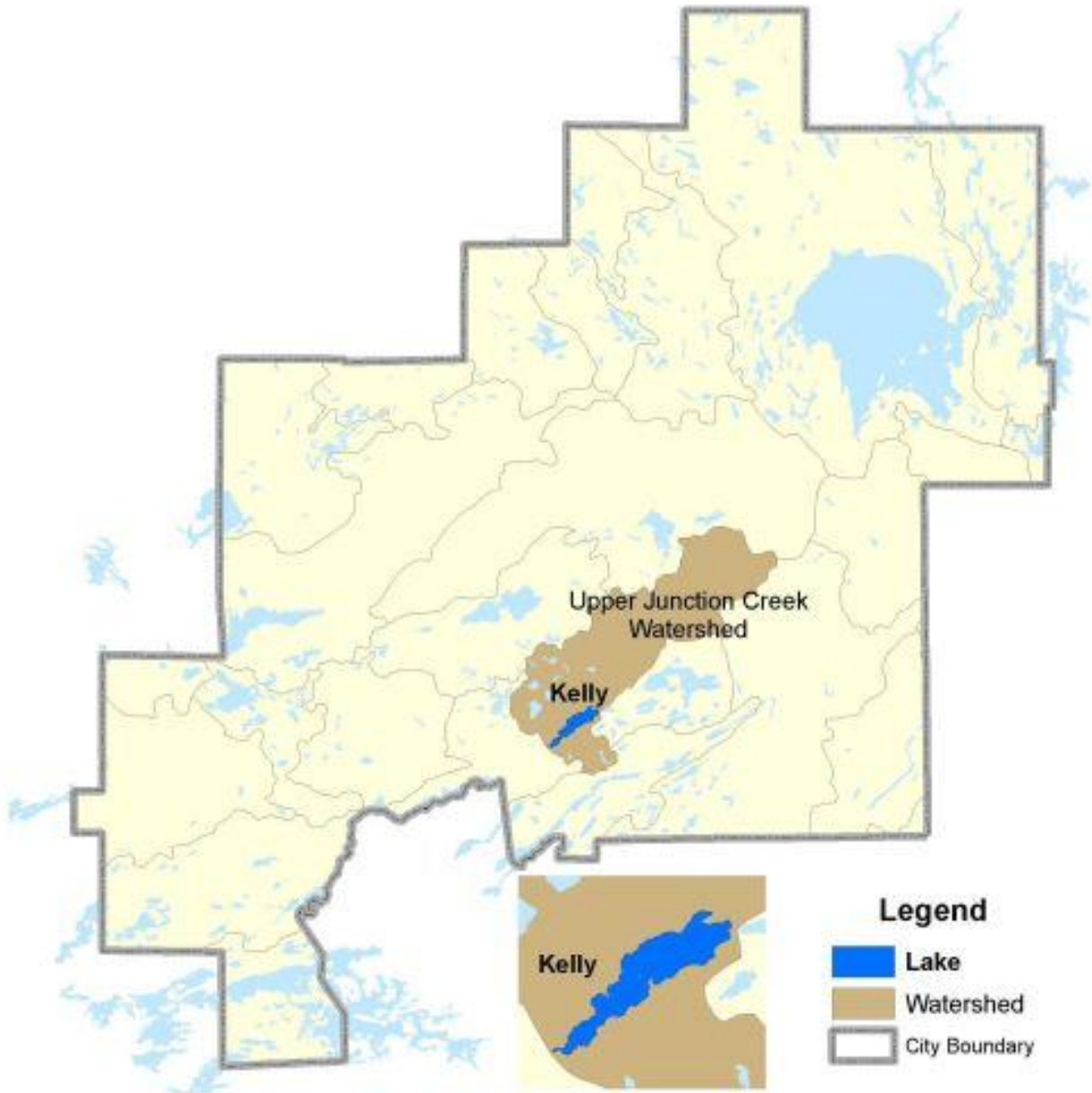


Figure 1. Watershed map of Kelly Lake indicating the location of the lake within the City of Greater Sudbury boundary.

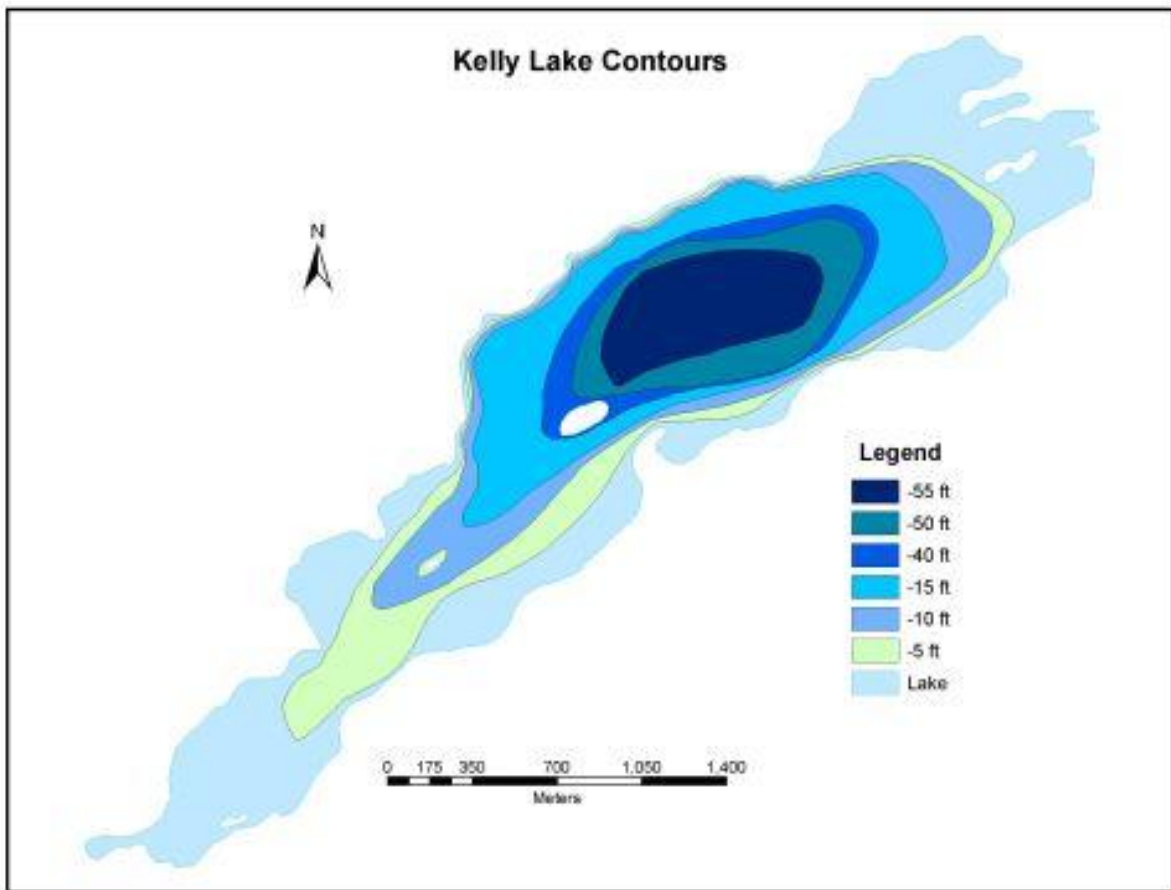


Figure 1a. Bathymetric map of Kelly Lake indicating lake morphometry.



Figure 2. Image of *D. pulicaria* illustrating how morphological measurements were taken using the ImageJ program. Long, red arrow indicates total body length measured from the top of the head to the end of the caudal spine. Short, red arrow indicates caudal spine length measured from the base of the carapace to the end of the tail spine. Relative tail spine length (Tail spine length/Body length, where body length= measurement from the top of the head to the bottom of the carapace) was also measured.

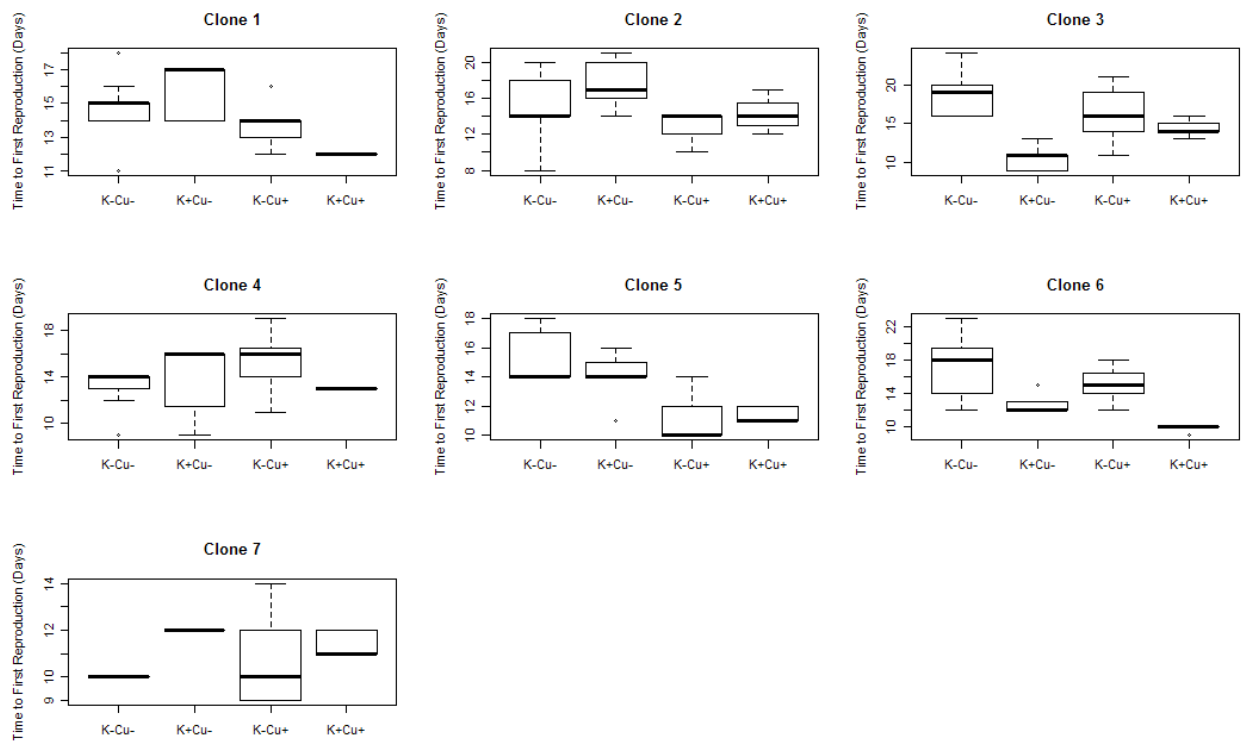


Figure 3. Boxplots indicating time to first reproduction (days) for individual clones in control (K-Cu-), kairomone-control (K+Cu-), copper-control (K-Cu+), and kairomone-copper (K+Cu+) treatments. Significant kairomone effects were observed for clones 3, 6, and 7 while significant copper effects were observed for clones 5 and 6. Solid lines= median response, vertical bars= maximum and minimum values, points=outliers.

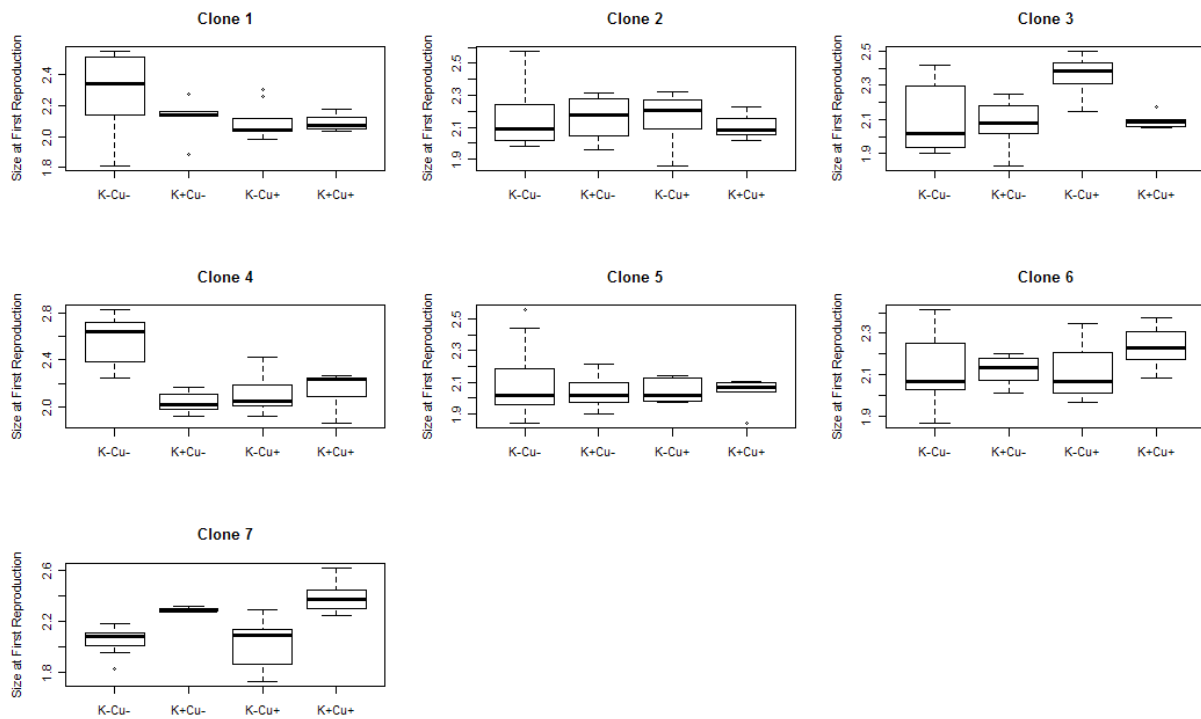


Figure 4. Boxplots indicating size at first reproduction (mm) for individual clones in control (K-Cu-), kairomone-control (K+Cu-), copper-control (K-Cu+), and kairomone-copper (K+Cu+) treatments. Significant kairomone effects were observed for clones 4 and 7, while significant copper effects were observed for clone 4. Solid lines= median response, vertical bars= maximum and minimum values, points=outliers.

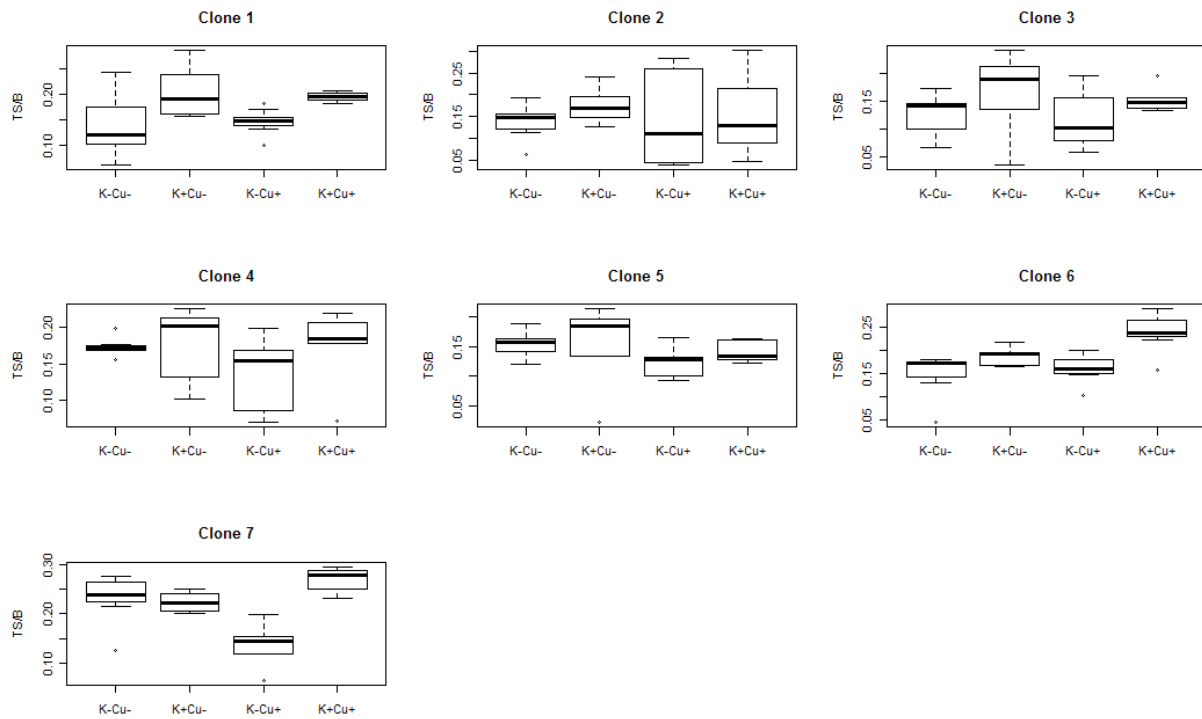


Figure 5. Boxplots indicating relative tail spine length (Tail Spine/Body Size) in mm for individual clones in control (K-Cu-), kairomone-control (K+Cu-), copper-control (K-Cu+), and kairomone-copper (K+Cu+) treatments. Significant kairomone effects were observed for clones 1, 6, and 7. No significant copper effects were observed. Solid lines= median response, vertical bars= maximum and minimum values, points=outliers.

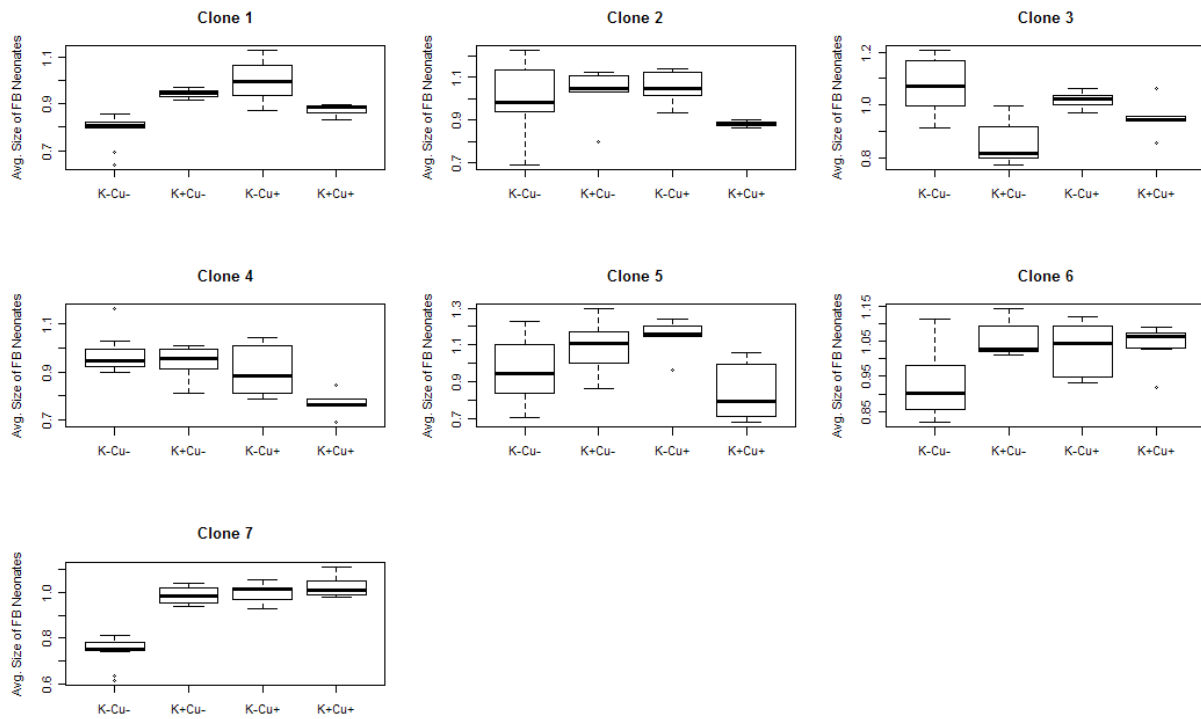


Figure 6. Boxplots indicating average size of first brood neonates (mm) for individual clones in control (K-Cu-), kairomone-control (K+Cu-), copper-control (K-Cu+), and kairomone-copper (K+Cu+) treatments. Significant kairomone effects were observed for clones 3 and 7 while significant copper effects were observed for clones 1, 4, and 7. Solid lines= median response, vertical bars= maximum and minimum values, points=outliers.

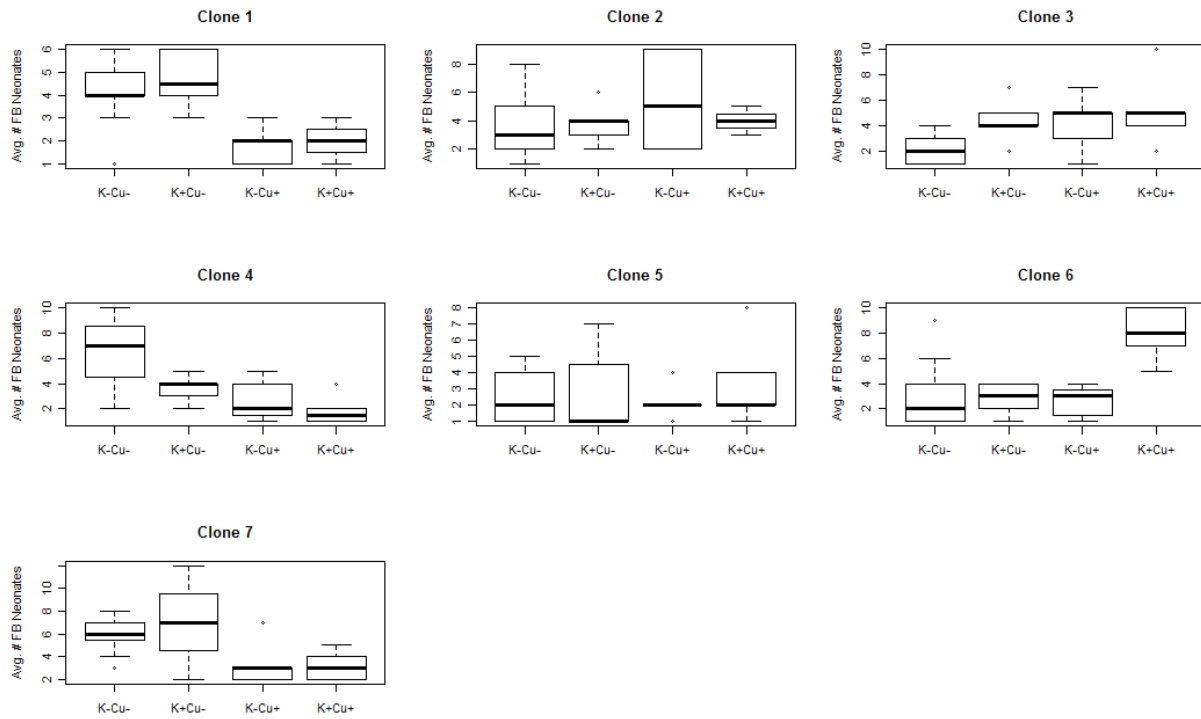


Figure 7. Boxplots indicating the average number of first brood neonates for individual clones in control (K-Cu-), kairomone-control (K+Cu-), copper-control (K-Cu+), and kairomone-copper (K+Cu+) treatments. Significant kairomone effects were observed for clone 6 while significant copper effects were observed for clones 1, 4, 6, and 7. Solid lines= median response, vertical bars= maximum and minimum values, points=outliers.

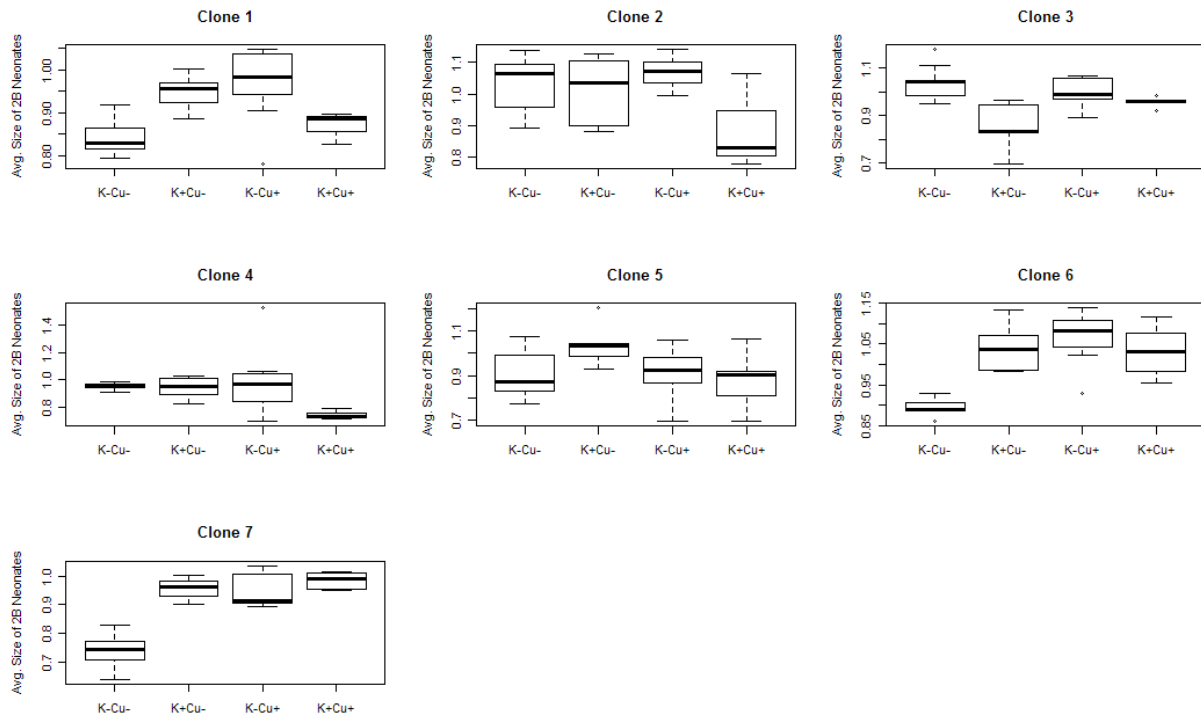


Figure 8. Boxplots indicating average size of second brood neonates for individual clones in control (K-Cu-), kairomone-control (K+Cu-), copper-control (K-Cu+), and kairomone-copper (K+Cu+) treatments. Significant kairomone effects were observed for clones 3, 6, and 7 while significant copper effects were observed for clones 5 and 6. Solid lines= median response, vertical bars= maximum and minimum values, points=outliers.

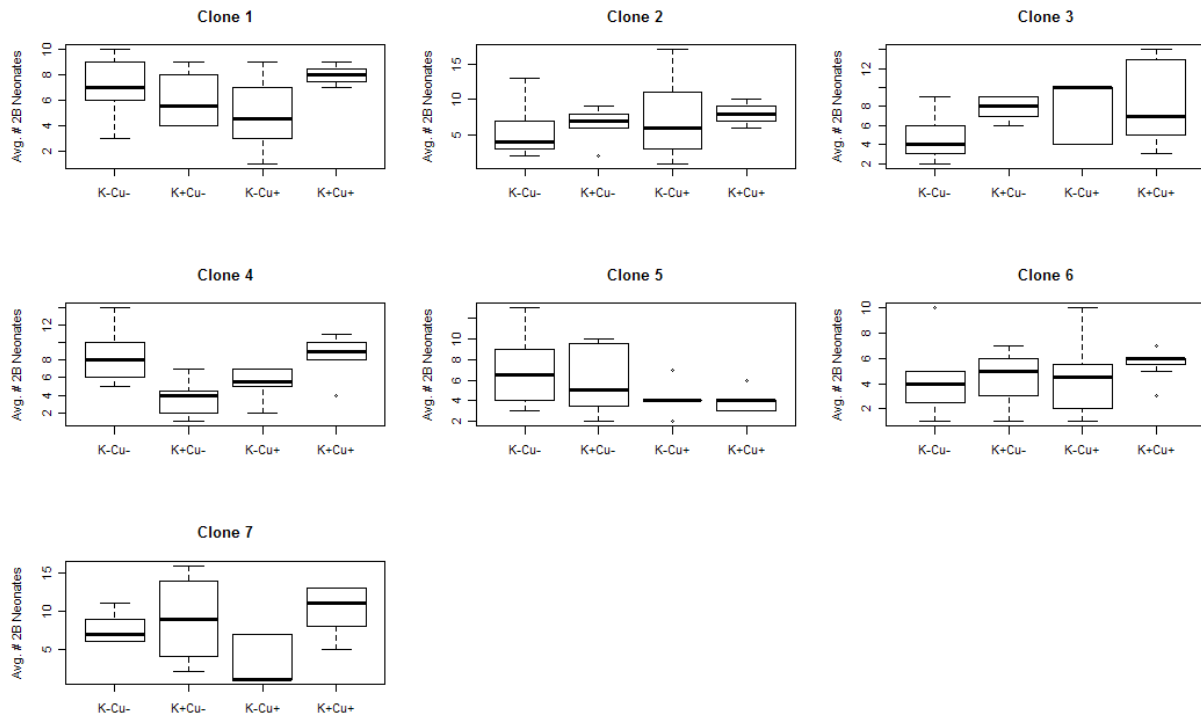


Figure 9. Boxplots indicating average number of second brood neonates for individual clones in control (K-Cu-), kairomone-control (K+Cu-), copper-control (K-Cu+), and kairomone-copper (K+Cu+) treatments. No significant kairomone or copper effects were observed. Solid lines= median response, vertical bars= maximum and minimum values, points=outliers.

Table 1. Student's t-Test of clonal responses to *Chaoborus* kairomone in control (K+ Cu-) treatment. Symbols: + and – denote a significant *Chaoborus* response (adjusted p-value < 0.05) and a non-significant *Chaoborus* response (adjusted p-value > 0.05), respectively. SFR= Size at first reproduction, FB=First Brood, 2B= Second Brood, TFR=Time to First Reproduction.

Clone	SFR	Tail Spine Length (mm)	Avg. #	Avg. Size	Avg. #	Avg. Size	TFR	(n)
			Neonates FB	Neonates FB	Neonates 2B	Neonates 2B		
1	–	–	–	+	–	+	–	15
				<0.001***		0.001**		
2	–	–	–	–	–	–	–	15
3	–	–	–	+	+	+	+	14
				0.008**	0.026*	0.019*	<0.001***	
4	+	–	–	–	+	–	–	14
	0.002**				0.026*			
5	–	–	–	–	–	+	–	17
						0.018*		
6	–	–	–	+	–	+	–	12
				0.034*		0.011*		
7	+	–	–	+	–	+	–	14
	<0.001***			<0.001***		<0.001***		

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 2. ANOVA results for individual clonal responses. P-values, adjusted using the Benjamini-Hochberg method, are shown in parentheses (), with significant p-values in bold. SFR=Size at first reproduction, RTS=Relative tail spine length (mm), Avg. # FB= Average number of neonates in the first brood, Avg. Size FB=Average size of first brood neonates, 2B=Second brood, TFR=Time to first reproduction.

Clone	Response	Ch		Cu		Ch-Cu	
		F	(P)	F	(P)	F	(P)
1	SFR	.895	(.483)	4.360	(.110)	1.191	(.400)
2	SFR	.064	(.801)	.078	(.782)	.101	(.752)
3	SFR	2.874	(.246)	4.502	(.110)	2.592	(.400)
4	SFR	12.155	(.006)	8.621	(.050)	19.969	(.001)
5	SFR	.692	(.483)	.303	(.782)	.158	(.752)
6	SFR	1.656	(.368)	.605	(.777)	1.587	(.400)
7	SFR	32.624	(.000)	.178	(.782)	1.395	(.400)
1	RTS	9.081	(.014)	.002	(.960)	.134	(.886)
2	RTS	.999	(.384)	.007	(.960)	.096	(.886)
3	RTS	2.948	(.177)	.088	(.960)	.006	(.938)
4	RTS	1.520	(.321)	1.4349	(.424)	1.286	(.625)
5	RTS	.117	(.735)	2.008	(.396)	.310	(.886)
6	RTS	18.644	(.001)	4.125	(.188)	1.614	(.625)
7	RTS	9.402	(.014)	6.300	(.139)	20.350	(.001)
1	Avg. # FB	3.051	(.163)	26.874	(.000)	.211	(.649)
2	Avg. # FB	.163	(.690)	1.645	(.629)	.678	(.629)
3	Avg. # FB	5.391	(.088)	3.253	(.120)	.572	(.649)
4	Avg. # FB	4.824	(.088)	14.686	(.002)	1.924	(.623)
5	Avg. # FB	.924	(.484)	.033	(.857)	.211	(.649)
6	Avg. # FB	14.208	(.006)	6.419	(.032)	12.885	(.010)
7	Avg. # FB	.413	(.614)	12.966	(.003)	.410	(.649)
1	Avg. Size FB	.631	(.507)	25.257	(<.001)	26.483	(.000)
2	Avg. Size FB	.985	(.466)	.273	(.803)	2.759	(.131)
3	Avg. Size FB	16.989	(.001)	.063	(.803)	3.919	(.087)
4	Avg. Size FB	5.989	(.054)	13.916	(.002)	2.415	(.133)
5	Avg. Size FB	.371	(.548)	.084	(.803)	11.081	(.006)
6	Avg. Size FB	4.770	(.069)	2.437	(.231)	3.838	(.087)
7	Avg. Size FB	68.653	(<.001)	51.036	(<.001)	20.532	(.000)

1	Avg. # 2B	.507 (.665)	1.358 (.446)	4.908 (.127)
2	Avg. # 2B	.158 (.694)	.918 (.488)	.002 (.962)
3	Avg. # 2B	3.509 (.264)	2.209 (.395)	.809 (.663)
4	Avg. # 2B	1.252 (.639)	.617 (.512)	18.470 (.001)
5	Avg. # 2B	.331 (.665)	4.453 (.321)	.026 (.962)
6	Avg. # 2B	.598 (.665)	.337 (.567)	.305 (.819)
7	Avg. # 2B	6.583 (.123)	2.018 (.395)	3.584 (.166)
1	Avg. Size 2B	.356 (.556)	8.405 (.018)	17.536 (.001)
2	Avg. Size 2B	3.715 (.095)	.357 (.556)	3.272 (.085)
3	Avg. Size 2B	14.262(.004)	.497 (.556)	5.204 (.050)
4	Avg. Size 2B	5.253 (.054)	1.915 (.250)	4.925 (.050)
5	Avg. Size 2B	2.415 (.156)	2.333 (.245)	3.278 (.085)
6	Avg. Size 2B	5.254 (.054)	17.696 (.001)	16.783 (.001)
7	Avg. Size 2B	67.082(<0.001)	42.229 (< 0.001)	18.822 (.001)
1	TTFR	.460 (.503)	5.493 (.064)	6.443 (.063)
2	TTFR	3.596 (.216)	3.687 (.121)	.251 (.621)
3	TTFR	24.897(<0.001)	.018 (.892)	8.575 (.058)
4	TTFR	1.021 (.375)	1.164 (.407)	3.116 (.157)
5	TTFR	1.503 (.325)	28.652 (< 0.001)	.728 (.562)
6	TTFR	30.368(< 0.001)	7.164 (.047)	.257 (.621)
7	TTFR	12.255(.004)	.247 (.727)	3.322 (.157)

Table 2 continued. ANOVA results for individual clonal responses. Adjusted p-values are shown in parentheses (), with significant p-values in bold. SFR=Size at first reproduction, TS=Tail spine, Avg. # FB= Average number of neonates in the first brood, Avg. Size FB=Average size of first brood neonates, 2B=Second brood, TTFR=Time to first reproduction. P-values were corrected using the Benjamini-Hochberg test.

Table 3. Pearson's correlation response variable matrix indicating correlations between measured responses for individual clones (C1-C7) in all treatments. All correlations were positive, with the exception of clone 7, which is indicated with an *. SFR= Size at first reproduction, TFR= Time to first reproduction, FB and 2B= First and second brood, respectively. Significant p-values are presented in parentheses (). Dashed lines indicate no correlation was present.

	TFR	Avg. Size FB	Avg. # FB	Avg. Size 2B	Avg. # 2B
SFR	C7 (<0.001)	C7 (0.012)	C1 (0.004) C2 (<0.05) C4 (<0.001) C6 (.004)	-----	-----
TFR	-----	-----	-----	-----	-----
Avg. Size FB	-----	-----	C7* (0.04)	C1 (<0.05) C2 (<0.01) C3 (<0.05) C6 (<.001) C7 (<0.001)	-----
Avg. #FB	-----	-----	-----	-----	C2 (<0.01)
Avg. Size 2B	-----	-----	-----	-----	-----
Avg. # 2B	-----	-----	-----	-----	-----

Table 4. Summary of responses analyzed in t-tests for individual clones in control treatments (K- Cu-, K+Cu-) and copper treatments (K-Cu+, K+Cu+). Arrows indicate direction of the response in kairomone-control treatment and kairomone-copper treatments and significance, $p < 0.05$. TFR= time to first reproduction, SFR=size at first reproduction, Tail=tail spine length (mm), #FB=average number of first brood neonates, Size FB=average size of first brood neonates, 2B=second brood. = Gained a response in copper treatments not observed in controls, = Lost a response in copper treatments observed in controls, and = Altered response or opposite response in copper treatments compared to controls.

Clone	TFR	SFR	Tail	#FB	#2B	Size FB	Size 2B
1 _{control}	 ↓				 ↑	 ↑	 ↑
1 _{copper}						 ↓	
2 _{control}							
2 _{copper}						 ↓	
3 _{control}	 ↓				 ↑	 ↓	 ↓
3 _{copper}							
4 _{control}		 ↓			 ↓		
4 _{copper}						 ↓	
5 _{control}							 ↑
5 _{copper}						 ↓	 ↑
6 _{control}	 ↓					 ↑	 ↑
6 _{copper}			 ↑	 ↑			 ↑
7 _{control}		 ↑			 ↑	 ↑	 ↑
7 _{copper}			 ↑				 ↑

Chapter 4

General Discussion and Implications of Research

Increases in anthropogenic pollutants has ensued in ecotoxicological studies aimed at assessing the ability of individuals, populations, and subsequently ecosystems to respond to chemical stressors; however, conventional ecotoxicology is limited in its ability to analyze long-term effects of chemical exposure on natural populations because it fails to account for genetic diversity within populations, often limiting analyses to a single genotype (De Schampelaere et al. 2011). Conventional ecotoxicological studies also fail to account for the presence of multiple (2 or more) stressors in aquatic systems. Natural populations are rarely only exposed to abiotic stressors, but rather, are typically exposed to a combination of abiotic and biotic stress, e.g. chemical contamination and predation (Lopes et al. 2009). Therefore, the ability of a natural population to maintain ecosystem functioning is largely dependent on the interactive effects between abiotic and biotic stressors (Lopes et al. 2009). Using seven distinct *D. pulicaria* clones from a historically metal contaminated lake, I analyzed the responses of individual daphniids to a single abiotic stressor, i.e. a nominal concentration of copper, a single biotic stressor, i.e. *Chaoborus* predation threat, and a combination of the two stressors. As expected, *Daphnia* displayed a large degree of phenotypic plasticity in their responses to *Chaoborus* kairomone; however, contrary to my expectations, several clones failed to elicit trade-offs in traits, with one clone allocating energy to both physiological growth and reproductive output. The observed lack in expected trade-offs was hypothesized to be a consequence of the following: 1) Inherent biological relationships, e.g. physiological or energetic, required traits to be coupled or pleiotropic (Baer and Lynch 2003), and the simultaneous expression of coupled traits was an adaptive strategy to predation pressure (Boersma et al. 1998) or 2) Investments in both somatic growth

and reproductive output, as exhibited by clone 7, were responses congruent with “the *Chaoborus* paradox” in that there were no apparent costs associated with increased fitness (Weber et al. 2003). Although the lack of trade-offs in inducible traits contradicted classical life history theory postulates, it was concluded that individual clones developed their own adaptive strategies to predation pressure and that variation in clonal responses was likely an artifact of genetic variability.

Knowing that Kelly Lake daphniids responded to biotic stress, I sought to understand the impact of combined stressors on individuals, and subsequently populations. Similar to kairomone control treatments, copper exposure and predation pressure elicited a variety of inducible responses in clones. Clones responded to co-stressor treatments by either gaining a response not observed in kairomone control treatments, losing a response observed in kairomone control treatments, or exhibiting an opposite response to the one observed in kairomone control treatments. While several studies indicate that copper exposure impedes the ability of *Daphnia* to respond to predation threat, thereby increasing prey vulnerability and decreasing survivorship (Hunter and Pyle 2004, Pyle and Mirza 2007, Mirza and Pyle 2009), this study illustrates that clones are able to elicit genetically variable responses to predation in the presence of copper, thereby demonstrating that copper does not inhibit the ability of clones to induce antipredator responses.

Interactive effects of copper exposure and predation pressure were observed for all of the measured responses (with the exception of time to first reproduction), thereby indicating synergistic effects. Folt et al. (1999) defines synergy as a combined effect elicited by multiple stressors whose impact is greater than the effect elicited by each individual stressor. Hanazato and Dodson (1995) explain that the outcome of synergistic effects is often associated with trade-offs in energy allocation. Clones that displayed synergistic effects also exhibited decreases in somatic growth and reductions in neonate body size. Because *Chaoborus* are gape-limited predators, reducing body size in the presence of the predator or

reducing neonate size would leave both parent and offspring more vulnerable to predation; therefore, the observed reductions in size can likely be attributed to trade-offs in energy allocation between copper tolerance and life history traits. Of course, this conclusion is based on the assumption that Kelly Lake *Daphnia* have evolved a genetically based tolerance to copper contamination. Assuming that adaptation did take place, the observed reductions in mature body size and neonate size, which may be associated with fitness costs, could be attributed to a specific metal tolerance mechanism. The metal requirement hypothesis postulates that individuals may utilize a tolerance mechanism through reduced or less efficient metal uptake/utilization, which consequently, may result in reduced fitness due to micronutrient deficiencies (Harper et al. 1997, Agra et al. 2010). Therefore, Kelly Lake daphniids may utilize this tolerance mechanism as means of coping with severely contaminated waters. However, when transplanted to more favorable ambient conditions, i.e. experimental exposures, the metal tolerance mechanism, if elicited, could result in reduced filtration rates and consequently reduced fitness, which would explain the observed reductions in body size.

Yet, it is also possible that Kelly Lake *Daphnia* respond to copper stress by inducing specific life history responses. Koivisto and Ketola (1995) found that *D. pulex* clones respond to copper stress by significantly reducing their time to first reproduction. Congruent with their study, I found that clones significantly delayed their time to first reproduction and decreased their reproductive output in copper control treatments. Therefore, Kelly Lake *Daphnia* may have acquired adaptive life history strategies as a copper tolerance mechanism which may be induced in the presence or absence of predation threat.

Irrespective of the mechanism of metal tolerance, this study supported the hypothesis that Kelly Lake *Daphnia* display an adaptive tolerance to copper exposure. Although I did not directly compare Kelly Lake *Daphnia* inducible defenses with inducible defenses from a reference population, a past study conducted by Inglis et al. (2009) indicated that a population obtained from a lake removed from industrial

pollution was inhibited in its life history responses by a nominal copper concentration of 10 µg/L, thereby suggesting that Kelly Lake *Daphnia* have adapted to copper exposure.

Adaptation in chemically stressed environments has ecotoxicological implications, as pollutant-induced selection can contribute to losses in genetic variation and consequently biodiversity (Van Straalen and Timmermans 2002). Strong selection pressures acting on the most chemically tolerant genotypes can alter the allele frequencies of genes, leading to reductions in overall genetic variation within a population, i.e. genetic erosion (Van Straalen and Timmermans 2002). Thus, while tolerant genotypes will be fitter in a contaminated environment, they may incur fitness costs in uncontaminated environments. If abiotic stressors cease or chemical recovery of contaminated lakes is facilitated, it may result in the extirpation of chemically tolerant populations. While pollutant-induced selection may have implications for biodiversity at a local scale, it may also have regional implications. It is well known that regional interactions of zooplankton communities are mediated by dispersal of individuals between habitats (Cottenie et al. 2003). Although dispersal assists in structuring local communities, it also mediates regional species richness, which was documented by Bengtsson (1989) who showed that interspecific competition in a metapopulation of *Daphnia* resulted in higher extinction rates and limited regional species richness. If pollutant-induced selection acts on metal tolerant genotypes at a local scale, dispersal of those genotypes to new environments may lead to competitive exclusion by superior genotypes, resulting in limitation of species richness on a regional scale.

Alternatively, adaptation in local populations may contribute to population resilience via species co-tolerance. Co-tolerance is the ability of individuals to form resistance to multiple stressors. According to Vinebrooke et al. (2004), species co-tolerance occurs when species sensitivities are positively correlated such that exposure to one stressor results in a decreased sensitivity to the other stressor. Co-tolerance has been documented for a *D. pulex* population originating in a metal contaminated lake in

Sudbury, ON (De Schamphelaere et al. 2011). Due to their adaptive tolerance to cadmium pollution, *D. pulex* individuals from a historically metal contaminated lake exhibited greater survival and fitness when introduced to the novel stressor, *Microcystis*, than *D. pulex* individuals originating in a non-metal contaminated lake (Glaholt et al. cited in De Schamphelaere et al. 2011). In addition, Tlili et al. (2011) documented co-tolerance to the chemical stressors, copper and zinc, in autotrophic and heterotrophic biofilm populations that underwent long-term exposure to a combination of Cu and Zn pollutants. Therefore, long-term exposure or adaptation to metal contaminants may facilitate the ability of populations to exhibit co-tolerance, which consequently, contributes to population resilience. While adaptation to metal contaminants may result in genetic erosion, the resilience of a population in a contaminated habitat is largely contingent on its ability to tolerate multiple stressors.

This study provides evidence to suggest that Kelly Lake *Daphnia* have developed an adaptive tolerance to copper contamination. When applying a combination of stressors, i.e. predation pressure and copper exposure, to *Daphnia* clones cultured in the laboratory, I found that individuals induced both morphological and life history responses, suggesting that daphniids are not inhibited by copper exposure and that natural populations in Kelly Lake are likely eliciting co-tolerance mechanisms to copper and *Chaoborus* predation threat. Co-tolerance in Kelly Lake *Daphnia* may account for the persistence of this population in a contaminated habitat.

Summary

1. Variation in kairomone-induced responses is largely influenced by inter-clonal genetic variability
2. Clones have developed their own adaptive strategies to copper stress, and therefore are able to elicit antipredator defenses in the presence of copper exposure

3. Decreases in body size and reproductive output may be associated with costs of adaptation to copper
4. Ability of individuals to respond to joint stressors (kairomone and copper) indicates the potential for co-tolerance to copper and *Chaoborus* predation in natural Kelly Lake populations

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Appendix A

Replicate	Clone	Cu	Chaob	SFR	Tail.Spine	Total.Neo.FB	FB.Avg.ne0	Total.Neo.2B	2B.Avg.ne0	Time.to.FR	Body	TS.Body
1	1	copper	pred	2.1775	0.375	3	0.894167	7	0.896429	12	1.8025	0.208044
2	1	copper	pred	2.035	0.3325	1	0.83	9	0.885556	12	1.7025	0.195301
3	1	copper	pred	2.0675	0.3175	2	0.8875	8	0.826563	12	1.75	0.181429
1	1	control	pred	1.885	0.42	6	0.913833	8	0.9695	14	1.465	0.286689
2	1	control	pred	2.1575	0.2975	4	0.93275	6	0.956833	17	1.86	0.159946
3	1	control	pred	2.13	0.2875	4	0.955	4	0.92375	17	1.8425	0.156038
4	1	control	pred	2.14	0.34	5	0.9306	4	0.88725	17	1.8	0.188889
5	1	control	pred	2.1375	0.3475	3	0.955333	5	1.0038	17	1.79	0.194134
6	1	control	pred	2.275	0.44	6	0.971833	9	0.955778	14	1.835	0.239782
1	1	copper	no_pred	2.04	0.2625	2	0.991	1	0.906	14	1.7775	0.147679
2	1	copper	no_pred	1.9825	0.2525	2	1.0025	3	0.781667	14	1.73	0.145954
3	1	copper	no_pred	2.1125	0.245	3	0.936	9	1.022111	16	1.8675	0.131191
4	1	copper	no_pred	2.045	0.2725	2	0.869	8	0.94175	16	1.7725	0.153738
5	1	copper	no_pred	2.0525	0.3	1	0.918	4	1.037	14	1.7525	0.171184
6	1	copper	no_pred	2.258	0.204	2	1.0615	6	0.943	13	2.054	0.099318
7	1	copper	no_pred	2.0325	0.2525	2	0.963	4	0.988	14	1.78	0.141854
8	1	copper	no_pred	2.0325	0.2475	1	1.126	7	1.047571	13	1.785	0.138655
9	1	copper	no_pred	2.035	0.3125	1	1.043	5	1.0432	14	1.7225	0.181422
10	1	copper	no_pred	2.303	0.304	3	1.066	2	0.977	12	1.999	0.152076
1	1	control	no_pred	2.515	0.144	4	0.695	9	0.846111	15	2.371	0.060734
2	1	control	no_pred	2.546	0.379	5	0.798	8	0.867188	15	2.167	0.174896
3	1	control	no_pred	2.416	0.22	4	0.8525	10	0.86575	18	2.196	0.100182

4	1	control	no_pred	2.528	0.271	6	0.858333	10	0.8285	15	2.257	0.120071
5	1	control	no_pred	1.8125	0.2275	1	0.640417	3	0.919167	16	1.585	0.143533
6	1	control	no_pred	2.2275	0.154	6	0.814375	5	0.816	14	2.0735	0.074271
7	1	control	no_pred	2.338	0.235	4	0.800833	6	0.794583	15	2.103	0.111745
8	1	control	no_pred	2.135	0.4125	3	0.8225	7	0.810357	11	1.7225	0.239478
9	1	control	no_pred	1.9825	0.39	4	0.805	7	0.83	11	1.5925	0.244898
1	2	copper	pred	2.085	0.095	5	0.865	10	0.83	17	1.99	0.047739
2	2	copper	pred	2.02	0.4675	4	0.885625	6	0.77875	12	1.5525	0.301127
3	2	copper	pred	2.2275	0.255	3	0.9	8	1.063875	14	1.9725	0.129278
1	2	control	pred	2.1975	0.2925	3	1.121333	7	1.127143	18	1.905	0.153543
2	2	control	pred	1.96	0.22	2	1.1065	2	1.1055	21	1.74	0.126437
3	2	control	pred	2.045	0.2625	4	1.02975	9	1.089111	20	1.7825	0.147265
4	2	control	pred	2.3175	0.365	4	1.0595	8	0.9825	16	1.9525	0.18694
5	2	control	pred	2.28	0.3725	4	1.03775	6	0.87875	16	1.9075	0.195282
6	2	control	pred	2.1625	0.42	6	0.8	7	0.899286	14	1.7425	0.241033
1	2	copper	no_pred	2.325	0.1825	9	1.015778	11	1.140727	14	2.1425	0.085181
2	2	copper	no_pred	2.09	0.08	5	0.9362	7	1.096571	14	2.01	0.039801
3	2	copper	no_pred	1.86	0.2225	2	1.074	1	1.046	10	1.6375	0.135878
4	2	copper	no_pred	2.167	0.447	2	1.1215	3	0.993667	12	1.72	0.259884
5	2	copper	no_pred	2.2475	0.0975	9	1.139222	17	1.103353	14	2.15	0.045349
6	2	copper	no_pred	2.27	0.5	5	1.0238	5	1.0346	14	1.77	0.282486
1	2	control	no_pred	2.2425	0.2575	8	0.929688	13	0.892308	18	1.985	0.129723
2	2	control	no_pred	1.9975	0.3225	3	0.69375	2	0.93125	8	1.675	0.192537
3	2	control	no_pred	2.015	0.2725	2	0.94125	3	0.956667	14	1.7425	0.156385
4	2	control	no_pred	1.98	0.255	2	1.2235	7	1.093571	12	1.725	0.147826

5	2	control	no_pred	2.085	0.28	1	0.978	3	1.139	14	1.805	0.155125
6	2	control	no_pred	2.315	0.2525	4	1.13175	9	1.065444	20	2.0625	0.122424
7	2	control	no_pred	2.0925	0.275	2	1.163	4	1.13675	14	1.8175	0.151307
8	2	control	no_pred	2.573	0.264	5	0.9854	7	1.060143	16	2.309	0.114335
9	2	control	no_pred	2.165	0.1275	5	1.0868	3	1.073	18	2.0375	0.062577
1	3	copper	pred	2.1725	0.355	4	1.06125	14	0.955786	14	1.8175	0.195323
2	3	copper	pred	2.0825	0.27	5	0.9574	5	0.9182	15	1.8125	0.148966
3	3	copper	pred	2.0975	0.285	10	0.9433	3	0.964333	16	1.8125	0.157241
4	3	copper	pred	2.0575	0.25	5	0.8552	13	0.958846	13	1.8075	0.138313
5	3	copper	pred	2.05	0.24	2	0.9395	7	0.982857	14	1.81	0.132597
1	3	control	pred	2.18	0.4225	4	0.799375	9	0.698611	9	1.7575	0.240398
2	3	control	pred	1.83	0.065	4	0.818125	8	0.827813	9	1.765	0.036827
3	3	control	pred	2.02	0.3225	7	0.773929	6	0.831667	11	1.6975	0.189985
4	3	control	pred	2.2475	0.3925	2	0.9185	7	0.963286	11	1.855	0.21159
5	3	control	pred	2.08	0.2475	5	0.9972	9	0.946667	13	1.8325	0.135061
1	3	copper	no_pred	2.498	0.185	5	1.0242	10	0.9671	21	2.313	0.079983
2	3	copper	no_pred	2.312	0.215	1	1.037	4	1.069	16	2.097	0.102527
3	3	copper	no_pred	2.387	0.324	3	0.969667	4	0.8915	11	2.063	0.157053
4	3	copper	no_pred	2.43	0.136	5	1.0612	10	1.0579	19	2.294	0.059285
5	3	copper	no_pred	2.15	0.3525	7	1.001429	10	0.9892	14	1.7975	0.196106
1	3	control	no_pred	2.02	0.15	4	1.07275	6	1.109667	21	1.87	0.080214
2	3	control	no_pred	1.9225	0.2825	1	1.2	2	1.0475	16	1.64	0.172256
3	3	control	no_pred	1.9	0.235	1	1.167	3	0.948	16	1.665	0.141141
4	3	control	no_pred	2.422	0.219	3	1.066333	5	1.0454	20	2.203	0.09941
5	3	control	no_pred	1.935	0.2775	1	1.206	2	0.974	16	1.6575	0.167421

6	3	control	no_pred	2.298	0.143	2	0.9945	4	1.041	19	2.155	0.066357
7	3	control	no_pred	2.413	0.307	1	0.915	7	0.985571	20	2.106	0.145774
8	3	control	no_pred	1.9675	0.245	2	0.9595	4	1.17675	18	1.7225	0.142235
9	3	control	no_pred	2.115	0.1925	4	1.0715	9	0.992333	24	1.9225	0.10013
1	4	copper	pred	2.23	0.4	1	0.845	10	0.715	13	1.83	0.218579
2	4	copper	pred	2.25	0.3525	2	0.78625	8	0.716563	13	1.8975	0.185771
3	4	copper	pred	2.265	0.3525	1	0.7625	11	0.723409	13	1.9125	0.184314
4	4	copper	pred	2.0825	0.315	4	0.75875	9	0.725833	13	1.7675	0.178218
5	4	copper	pred	2.25	0.385	2	0.755	9	0.788056	13	1.865	0.206434
6	4	copper	pred	1.855	0.125	1	0.6875	4	0.7575	13	1.73	0.072254
1	4	control	pred	2.1625	0.3625	4	0.99775	5	1.0178	16	1.8	0.201389
2	4	control	pred	1.945	0.34	4	0.81075	2	0.836	9	1.605	0.211838
3	4	control	pred	1.915	0.3525	2	0.8895	1	0.953	9	1.5625	0.2256
4	4	control	pred	2.0125	0.2375	4	0.95575	4	0.9525	16	1.775	0.133803
5	4	control	pred	2.1525	0.2475	5	0.9312	7	0.825143	14	1.905	0.129921
6	4	control	pred	2.0025	0.185	3	1.01	4	1.027	16	1.8175	0.101788
7	4	control	pred	2.05	0.36	3	0.991	2	0.9945	16	1.69	0.213018
1	4	copper	no_pred	1.9125	0.2675	5	1.013	5	0.9362	16	1.645	0.162614
2	4	copper	no_pred	2.1525	0.3575	1	1.004	5	1.064	14	1.795	0.199164
3	4	copper	no_pred	2.0575	0.17	2	0.942	7	0.943143	16	1.8875	0.090066
4	4	copper	no_pred	2.423	0.311	5	0.8245	5	0.75	17	2.112	0.147254
5	4	copper	no_pred	2.0125	0.295	3	0.786667	2	1.0275	16	1.7175	0.171761
6	4	copper	no_pred	1.9925	0.13	2	0.815	7	0.692143	11	1.8625	0.069799
7	4	copper	no_pred	2.22	0.17	2	0.805	7	1.530357	19	2.05	0.082927
8	4	copper	no_pred	2.0425	0.29	1	1.043	6	0.9865	14	1.7525	0.165478

1	4	control	no_pred	2.831	0.47	7	1.027571	14	0.981929	14	2.361	0.199068
2	4	control	no_pred	2.665	0.36	10	0.8991	11	0.940545	14	2.305	0.156182
3	4	control	no_pred	2.773	0.406	8	0.938375	8	0.97475	14	2.367	0.171525
4	4	control	no_pred	2.644	0.382	7	0.903714	5	0.9516	14	2.262	0.168877
5	4	control	no_pred	2.53	0.365	9	0.947444	5	0.9514	14	2.165	0.168591
6	4	control	no_pred	2.245	0.3375	2	1.1645	9	0.909222	12	1.9075	0.176933
7	4	control	no_pred	2.244	0.332	2	0.9665	7	0.968857	9	1.912	0.17364
1	5	copper	pred	1.84	0.255	2	0.79125	6	0.694167	12	1.585	0.160883
2	5	copper	pred	2.0375	0.285	8	0.713438	4	0.80875	12	1.7525	0.162625
3	5	copper	pred	2.101	0.249	4	0.679375	3	0.904333	11	1.852	0.134449
4	5	copper	pred	2.103	0.228	1	1.06	4	0.9165	11	1.875	0.1216
5	5	copper	pred	2.072	0.235	2	0.9925	3	1.062333	11	1.837	0.127926
1	5	control	pred	1.9375	0.04	7	0.860429	10	1.0411	16	1.8975	0.02108
2	5	control	pred	2.02	0.315	1	1.022	4	0.97275	14	1.705	0.184751
3	5	control	pred	2.0025	0.345	1	1.114	3	0.996	14	1.6575	0.208145
4	5	control	pred	2.0875	0.325	1	1.234	5	0.9306	14	1.7625	0.184397
5	5	control	pred	1.8975	0.335	4	0.97875	2	1.203	11	1.5625	0.2144
6	5	control	pred	2.11	0.2475	1	1.297	9	1.030556	14	1.8625	0.132886
7	5	control	pred	2.2175	0.265	5	1.109	10	1.0397	16	1.9525	0.135723
1	5	copper	no_pred	2.1425	0.3025	2	1.1435	2	0.696	10	1.84	0.164402
2	5	copper	no_pred	2.0175	0.1825	1	1.204	4	0.925	10	1.835	0.099455
3	5	copper	no_pred	2.1275	0.2425	2	1.243	4	0.86575	10	1.885	0.128647
4	5	copper	no_pred	1.97	0.23	2	1.1575	4	0.98225	12	1.74	0.132184
5	5	copper	no_pred	1.9825	0.1675	4	0.963	7	1.055714	14	1.815	0.092287
1	5	control	no_pred	1.97	0.245	2	1.226	3	0.887333	14	1.725	0.142029

2	5	control	no_pred	2.439	0.302	4	1.00625	9	0.989667	17	2.137	0.14132
3	5	control	no_pred	2.0575	0.2875	3	1.101333	6	1.0015	18	1.77	0.162429
4	5	control	no_pred	1.8425	0.2475	1	0.857	7	1.072143	14	1.595	0.155172
5	5	control	no_pred	2.1475	0.34	5	1.1944	6	0.938	14	1.8075	0.188105
6	5	control	no_pred	1.975	0.3025	1	0.885	4	0.773125	14	1.6725	0.180867
7	5	control	no_pred	2.558	0.287	4	0.708125	13	0.830962	17	2.271	0.126376
8	5	control	no_pred	1.9525	0.27	2	1.02125	3	0.828333	14	1.6825	0.160475
9	5	control	no_pred	1.96	0.275	1	0.8375	9	0.794722	14	1.685	0.163205
10	5	control	no_pred	2.185	0.235	1	0.82	7	0.86	14	1.95	0.120513
1	6	copper	pred	2.0825	0.4675	6	0.916833	6	0.955833	9	1.615	0.289474
2	6	copper	pred	2.1925	0.4	5	1.0642	7	1.031429	10	1.7925	0.223152
3	6	copper	pred	2.3775	0.4925	10	1.0917	6	1.1165	10	1.885	0.261273
4	6	copper	pred	2.3025	0.44	10	1.0823	3	1.095667	10	1.8625	0.236242
5	6	copper	pred	2.2325	0.47	8	1.028	6	0.954333	10	1.7625	0.266667
6	6	copper	pred	2.155	0.2925	8	1.064	5	1.06	10	1.8625	0.157047
7	6	copper	pred	2.31	0.44	10	1.0322	6	1.011833	10	1.87	0.235294
1	6	control	pred	2.0125	0.285	2	1.093	5	1.1326	12	1.7275	0.164978
2	6	control	pred	2.135	0.3475	4	1.027	1	0.985	13	1.7875	0.194406
3	6	control	pred	2.18	0.3525	3	1.144	7	0.982857	15	1.8275	0.192886
4	6	control	pred	2.2	0.3925	1	1.022	6	1.072	12	1.8075	0.217151
5	6	control	pred	2.0725	0.2975	4	1.0125	3	1.035667	12	1.775	0.167606
1	6	copper	no_pred	2.0375	0.1875	3	1.085	10	1.0218	17	1.85	0.101351
2	6	copper	no_pred	2.349	0.332	3	1.062667	3	1.127333	15	2.017	0.164601
3	6	copper	no_pred	1.99	0.2875	1	0.933	1	0.929	12	1.7025	0.168869
4	6	copper	no_pred	2.04	0.2625	2	1.1225	5	1.1394	18	1.7775	0.147679

5	6	copper	no_pred	2.1	0.35	3	1.027333	4	1.061	14	1.75	0.2
6	6	copper	no_pred	2.1325	0.34	4	0.9525	5	1.0788	16	1.7925	0.189679
7	6	copper	no_pred	1.965	0.2625	4	0.94125	6	1.084167	14	1.7025	0.154185
8	6	copper	no_pred	2.28	0.303	1	1.105	1	1.087	15	1.977	0.153263
1	6	control	no_pred	2.275	0.26	9	0.844722	10	0.8595	19	2.015	0.129032
2	6	control	no_pred	2.227	0.093	6	0.817917	4	0.89	23	2.134	0.04358
3	6	control	no_pred	1.865	0.2725	1	1.115	5	0.886	14	1.5925	0.171115
4	6	control	no_pred	2.0175	0.3	1	0.975	5	0.8875	12	1.7175	0.174672
5	6	control	no_pred	2.07	0.315	2	0.985	3	0.900833	14	1.755	0.179487
6	6	control	no_pred	2.414	0.322	2	0.86375	2	0.90875	18	2.092	0.15392
7	6	control	no_pred	2.0375	0.3025	1	0.9025	1	0.9275	20	1.735	0.174352
1	7	copper	pred	2.305	0.525	3	0.987	10	1.0099	12	1.78	0.294944
2	7	copper	pred	2.4525	0.49	2	1.0075	12	1.014667	11	1.9625	0.249682
3	7	copper	pred	2.38	0.53	2	1.05	13	0.953154	11	1.85	0.286486
4	7	copper	pred	2.2425	0.49	3	1.111667	8	0.977375	12	1.7525	0.279601
5	7	copper	pred	2.375	0.445	5	0.9798	13	1.001615	11	1.93	0.23057
6	7	copper	pred	2.625	0.565	4	1.0135	5	0.9498	11	2.06	0.274272
1	7	control	pred	2.2875	0.4575	7	0.940286	16	0.966063	12	1.83	0.25
2	7	control	pred	2.32	0.39	12	1.00025	2	0.901	12	1.93	0.202073
3	7	control	pred	2.2825	0.4325	2	1.0375	12	0.957417	12	1.85	0.233784
4	7	control	pred	2.285	0.3975	7	0.963	6	1.0025	12	1.8875	0.210596
1	7	copper	no_pred	2.0925	0.13	2	0.9275	7	0.894	10	1.9625	0.066242
2	7	copper	no_pred	1.725	0.22	2	0.968	1	0.913	9	1.505	0.146179
3	7	copper	no_pred	2.29	0.38	7	1.019429	7	1.007	14	1.91	0.198953
4	7	copper	no_pred	2.14	0.285	3	1.052667	1	0.908	12	1.855	0.153639

5	7	copper	no_pred	1.8625	0.2	3	1.013667	1	1.037	9	1.6625	0.120301
1	7	control	no_pred	2.1775	0.3875	6	0.8125	6	0.757917	10	1.79	0.21648
2	7	control	no_pred	2.0525	0.395	8	0.615	9	0.638611	10	1.6575	0.238311
3	7	control	no_pred	2.08	0.395	6	0.751667	9	0.784722	10	1.685	0.234421
4	7	control	no_pred	1.97	0.3725	4	0.633125	6	0.67375	10	1.5975	0.233177
5	7	control	no_pred	2.1275	0.46	7	0.7485	6	0.795	10	1.6675	0.275862
6	7	control	no_pred	2.06	0.42	7	0.739643	10	0.759	10	1.64	0.256098
7	7	control	no_pred	2.0775	0.3675	8	0.793125	6	0.717917	10	1.71	0.214912
8	7	control	no_pred	1.8225	0.205	3	0.804167	11	0.692955	10	1.6175	0.126739
9	7	control	no_pred	2.085	0.445	6	0.753333	6	0.828333	10	1.64	0.271341
10	7	control	no_pred	1.955	0.3875	5	0.7685	7	0.720357	10	1.5675	0.247209
11	7	control	no_pred	2.1825	0.4675	7	0.769286	7	0.742143	10	1.715	0.272595

