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Variation in copper effects on kairomone-mediated responses in *Daphnia pulicaria*

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ABSTRACT

Chemical signals play an integral role in many predator–prey relationships but their effectiveness can be altered by environmental conditions. Prey species can detect predator kairomones, which induce anti-predator defenses. An example of this predator–prey relationship exists between *Daphnia* spp. and *Chaoborus* spp.; however, when living in water contaminated with low concentrations of copper (Cu) *Daphnia* can fail to respond to *Chaoborus* kairomone and, in turn, become more susceptible to predation. This has implications for *Daphnia* living in regions with Cu contamination, such as areas where mining activity has resulted in increased levels of metals in the surrounding lakes. We examined kairomone-mediated responses of multiple *Daphnia pulicaria* clones obtained from 8 lakes in Ontario, Canada, in the absence and presence of environmentally-relevant Cu concentrations. Life history traits and morphological anti-predator defenses were assessed using neonates collected from mothers that were exposed to kairomone and Cu treatments.

We found that kairomone-mediated responses and Cu-tolerance varied among *D. pulicaria* clones. Clones exposed to kairomone, in the absence of Cu additions, had diverse responses, including larger neonates, delayed reproduction, or altered brood size relative to no-kairomone controls. These kairomone-induced responses act as antipredator defense strategies against *Chaoborus* by preventing predation or stabilizing population growth. When exposed to Cu, two clones were able to respond to kairomone, while four clones no longer induced a response to kairomone. This variation in non-lethal effects of Cu on aquatic organisms suggests that toxicity tests should incorporate multiple genotypes and include predator–prey interactions.

1. Introduction

Chemical cues mediate many inter- and intra-specific interactions among aquatic animals. The ability to detect chemical cues is important because they can provide information that is essential for survival (Brönmark and Hansson, 2000). A kairomone is a type of chemical cue that is specifically involved in communication that occurs between different species (Dicke and Grostal, 2001; Whittaker and Feeny, 1971). Many predator–prey relationships are mediated by the release and detection of kairomones and the different responses that are induced by this type of chemical cue (Kats and Dill, 1998; Lima and Dill, 1990). A frequently studied example of a kairomone-mediated predator–prey relationship exists between the common waterflea, *Daphnia* spp. (prey) and phantom midge larvae, *Chaoborus* spp. (predator). Kairomone is released into the surrounding water from the digestive tract of *Chaoborus* when actively feeding on *Daphnia* (Krueger and Dodson, 1981). *Daphnia* can detect this kairomone and respond with antipredator defenses that increase their probability of survival (Kats and Dill, 1998). The chemical structure of the kairomone has yet to be determined, but it is thought to be a low molecular weight, non-olefinic hydroxy carboxylic acid (Tollrian and von Elert, 1994). How it interacts with chemosensory tissues is still unknown. There is evidence that acetylcholine, dopamine, and juvenile hormone are involved in life-history/morphological responses to invertebrate predator kairomone (Weiss et al., 2012, 2015) but little is known about the receptors involved in the initial perception of chemical cues. Chemosensors are located on the first antennae (Weiss et al., 2015) and the ability of *Daphnia* to sense its chemical environment is likely controlled by Gustatory receptor family (Gr) proteins (Peñalva-Arana et al., 2009).

Kairomone-induced antipredator defenses may protect *Daphnia* directly by preventing predation or indirectly by increasing population growth. The induction of morphological defenses in response to kairomone can increase *Daphnia* neonate survival by

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48–68% relative to those that do not induce any defenses (Havel and Dodson, 1987; Mirza and Pyle, 2009). The protection conferred by kairomone-mediated antipredator defenses is vital for the maintenance of *Daphnia* populations in aquatic ecosystems. *Daphnia* hold an essential position in aquatic food webs as primary consumers by providing a food source for many secondary consumers, while controlling populations of primary producers. Therefore, a decrease in *Daphnia* populations as a result of increased predation could cause cascading effects through the entire aquatic ecosystem (Carpenter et al., 2001).

Kairomone-induced defenses vary among *Daphnia* species and genotypes within a single species (Boeing et al., 2006; De Meester, 1996; Oram and Spitze, 2013; Spitze, 1992). These defenses can exist as changes in behavior, morphology, and/or life history traits (Kats and Dill, 1998). Variation in response to predators has been identified across a number of taxa (Lass and Spaak, 2003) and is likely important for organisms living in heterogeneous environments where they face complex and variable predator environments (Declerck and Weber, 2003; Stibor and Lampert, 2000).

There is increasing evidence that the ability of organisms to obtain information from their environment and respond to the presence of predators can be disrupted by environmental contaminants at concentrations below acute toxicity (Klaschka, 2008; Lürling and Scheffer, 2007). For example, kairomone-induced antipredator defenses were inhibited when *Daphnia pulex* was exposed to environmentally-relevant concentrations of Cu (2–4 μg/L is the Canadian Water Quality Guideline for the Protection of Aquatic Life (Canadian Council of Ministers of the Environment, 2007)) (Hunter and Pyle, 2004; Mirza and Pyle, 2009). Similar results have been reported for other aquatic animals where chemically-mediated responses were inhibited after exposure to low concentrations of Cu (Beyers and Farmer, 2001; Carreau and Pyle, 2005; McPherson et al., 2004). Although we do not have a complete understanding of the receptors involved in kairomone-perception (but see Miyakawa et al. (2015) and Weiss et al. (2015) for recent progress on neurological pathways involved in anti-predator defense), electroantennogram (EAG) measurements revealed that exposure to low concentrations of copper (7.5 μg/L) is capable of disrupting the olfactory response of daphniids (Simbeya et al., 2012).

Low concentrations of Cu can interfere with the ability of *Daphnia* to respond to kairomone under controlled laboratory conditions (Hunter and Pyle, 2004; Mirza and Pyle, 2009); however it is difficult to extrapolate this effect to *Daphnia* populations living in the wild because these laboratory-based results reflect only a single, laboratory-raised clone. It is likely the effects of metals on kairomone-mediated antipredator defenses vary among clones depending on their tolerance for the metal and the selective forces that exist in their natural habitat. Lopes et al. (2006) found populations of *Daphnia longispina* that originated from a Cu-contaminated environment were more tolerant to Cu stress, in comparison to a reference population from a non-contaminated site. Therefore, it is possible that the effect of Cu on anti-predator defenses in *Daphnia* varies across populations but this has not yet been examined.

We examined variation in response of 8 clones of *Daphnia pulicaria* to *Chaoborus* kairomone under controlled conditions and evaluated how Cu affects the ability of these clones to induce kairomone-mediated antipredator defenses. *D. pulicaria* originated from lakes in three regions in Ontario, Canada. We expected that Cu would impair *D. pulicaria* response to predator kairomone (Lürling and Scheffer, 2007) but this effect would vary across clones owing to past and current environmental conditions.

Fig. 1. Map of Ontario, Canada indicating general location of study lakes.

2. Materials and methods

2.1. Collection and maintenance of animals

All *D. pulicaria* clones were collected from lakes on the Canadian Shield in Ontario (Fig. 1; Table 1). Blue Chalk, Glen and Crown lakes are in the Muskoka region in south-central Ontario, Round Lake is on Queen's University Biological Station (QUBS) property, and Ramsey, Kelly, Simon, and Joe lakes are located in the City of Greater Sudbury, a region that is recovering from historical acid deposition and metal contamination (Gunn et al., 1995; Keller , 2009; Pyle et al., 2005). We collected *D. pulicaria* clones from the deepest location in Joe Lake during the summer of 2007 by towing an 80-μm mesh plankton net vertically through the water column. Dr. William Nelson (Queen's University, Kingston, Canada) provided the clone from Round Lake, which had been cultured in the laboratory since the summer of 2007. The remaining clones from Blue Chalk, Crown, Glen, Kelly, Ramsey and Simon lakes were obtained from the Dorset Environmental Science Center (DESC), Dorset, Canada, where they had been cultured since 2006. All the lakes used in our study contain a diverse assemblage of fish and invertebrate predators, including *Chaoborus* spp., (Arnott et al., 2006; Kirk, 1993; Wissel et al., 2003; B. Keller, Personal Communication).

Daphnia were cultured from 2007–2008 in the laboratory and maintained in FLAMES culture medium (Celis-Salgado et al., 2008) at 21 °C under a 16:8 light:dark photoperiod and fed *Chlamydomonas reinhardtii* (8.4×10^6 cells/week; 336 μg C/week) which were cultured in COMBO medium (Kilham et al., 1998). FLAMES culture medium was used to emulate low ion concentrations that *Daphnia* typically experience on Canadian Shield lakes (Celis-Salgado et al., 2008). We cultured the clones for multiple generations under these conditions to ensure that kairomone-mediated responses were the result of genotypic differences and not due to acclimation or maternal effects. Fourth instar *Chaoborus americanus* larvae (hereafter, *Chaoborus*) were collected from a fishless pond in North Bay, Ontario, Canada (46°25′ N 79°22′ W). *Chaoborus* were maintained as larvae in FLAMES culture medium at 8 °C to delay pupation and fed weekly with live brine shrimp nauplii, *Artemia salina*, prior to experimental use.

2.2. Stimulus preparation

We used two stimuli, control (FLAMES culture medium) and *Chaoborus* kairomone to evaluate the presence and type of antipredator response induced in *D. pulicaria* clones. *Chaoborus* were