Thermal variation and factors influencing vertical migration behavior in *Daphnia* populations

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SUMMARY

The antipredator behavior diel vertical migration (DVM), common in aquatic keystone species *Daphnia,* involves daily migration from warmer surface waters before dawn to cooler deeper waters after dusk. Plasticity in *Daphnia* DVM behavior optimizes fitness via trade-offs between growth, reproduction, and predator avoidance. Migration behavior is affected by co-varying biotic and abiotic factors, including light, predator cues, and anthropogenic stressors making it difficult to determine each factor's individual contribution to the variation in this behavior. This study aims to better understand this ecologically significant behavior in *Daphnia* by: (1) determining how *Daphnia pulicaria* thermal preferences vary within and among natural populations; (2) distinguishing the role of temperature verses depth in *Daphnia* vertical migration; and (3) defining how two anthropogenic stressors (copper and nickel) impact *Daphnia* migratory behavior.

Simulated natural lake stratification were constructed in 8 L (0.5 m tall, 14.5 cm wide) water columns to monitor under controlled laboratory conditions the individual effects of temperature gradients, depth, and metal stressors on *Daphnia* vertical migration. Three major findings are reported. First, while no difference in thermal preference was found among the four populations studied, within lake populations variability among isolates was high. Second, decoupling temperature and depth revealed that depth was a better predictor of *Daphnia* migratory patterns over temperature. Third, exposure to environmentally relevant concentrations of copper or nickel inhibited classic DVM behavior. These findings revealed the high variability in thermal preference found within *Daphnia* populations, elucidated the individual roles that depth and temperature have on migratory behavior, and showed how copper and nickel can interfere with the natural response of *Daphnia* to fish predator cues. Thus contributing to the body of knowledge necessary to predict how natural populations of *Daphnia* will be affected by climate related changes in lake temperatures and increased presence of anthropogenic stressors.

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1. INTRODUCTION

The micro-crustacean zooplankton genus *Daphnia* are ubiquitous in the world's lentic (e.g., lake and pond) ecosystems (Shaw et al., 2007), and are an integral component of aquatic food webs (i.e., keystone species) due to their central role in transferring the biomass of primary producer's (e.g. algae) up the food chain by being the primary food for smaller fish (Colbourne et al., 2004; Sarnelle, 2005). *Daphnia's* pervasiveness is due in part to their ability to tolerate a dynamic range of environmental conditions, in particular temperature. *Daphnia* migrate within the water column on a daily basis to avoid predation, a behavior called diel vertical migration (DVM), which exposes them to a wide range of temperatures (Gliwicz, 1986; Lampert, 1989, 1993; Loose, 1993; Sitch, 1989). Additionally, avoidance of inter- and intraspecific competition has also been linked to distinct patterns of *Daphnia* distribution within the water column (De Meester and Dumont, 1988; 1989; Dumont et al., 1985; Weider, 1984). As surface water temperatures continue to increase as a result of climate change, even large lentic systems such as the Great Lakes are predicted to become more variable, leading to changes in vertical mixing and the degree to which lakes will thermally stratify (Kling et al., 2003; McCormick, 1990; Snucins and Gunn, 2000; Thuiller, 2007; Waal et al., 2010). Thermal stratification in lakes results from seasonal climatic patterns which produce distinct thermal layers that decrease in temperature with depth (Wetzel, 2001), creating thermal niches. Thermal stratification not only influences the distribution of aquatic organisms, based on thermal optimums and ranges, but predator-prey dynamics as well (Wetzel, 2001). Therefore, changes in lake stratification are likely to impact the distribution and fitness of aquatic organisms such as *Daphnia*, which are heavily influenced by temperature (Heino et al., 2009; Mooij et al., 2005), thereby effecting ecosystem function due to their central role in aquatic ecosystems (Mooij et al., 2005; Thuiller, 2007; van de Waal et al., 2009).

Classic DVM behavior in *Daphnia* is a migration to deeper, darker, and cooler waters during the day to avoid visual predators and a migration to warmer surface waters during the night to feed when the risk of predation is reduced (Gliwicz, 1986; Lampert, 1989, 1993; Loose, 1993; Sitch, 1989). This negative photo-taxis behavior is triggered by a combination of light and predatory (i.e., kairomone) cues (Dawidowicz and Loose, 1992; Lampert, 1989, 1993; Loose, 1993). In the absence of light or predator cues, *Daphnia* remain in the warmer and food-rich surface waters which has been shown to lead to increased growth and reproduction rates in *Daphnia* (Dawidowicz and Loose, 1992; Lampert, 1989, 1993; Loose, 1993). Thus, indicating a fitness costs associated with DVM.

Lampert (1989) described three potential sources of fitness cost associated with diel vertical migration: 1) differences in food quality and quantity, 2) distance of migration (i.e., changes in depth), and 3) changes in temperature. The cost associated with food quality and quantity depends mainly on the assumption that algae is primarily found in the surface waters of the

euphotic zone (Gliwicz, 1986; Lampert, 1989, 1993; Loose, 1993; Sitch, 1989); however, recent studies have shown that high levels of algal biomass can exist outside the euphotic zone (Winder et al., 2003). Thus, putting into question the validity of the assumption that food availability is a true cost associated with DVM. While the effects of migration depth and temperature on *Daphnia* fitness have been well documented (e.g., Dawidowicz and Loose, 1992; Lampert, 1989, 1993; Loose, 1993), how these two factors independently influence the distance *Daphnia* migrate, in the presence of light and predator cues, is far less understood. This is because light, temperature, and depth are all naturally coupled (Loose and Dawidowicz, 1994; Lampert, 1989, 1993; Loose, 1993).

A cost associated with DVM not addressed in Lampert's (1989) paper is the disruptive effect chemical stressors, such as metals, can have on *Daphnia* migration patterns. Globally, metals are deposited in lakes through natural processes. However, over the past century elevated levels of metals are being loaded into lakes by anthropogenic activities resulting in potentially irreversible impacts on aquatic organisms and the ecosystems as a whole (Gunn et al., 1995; Keller, 2009; Keller et al., 1992; Keller and Piblado, 1986; Pyle et al., 2005). Studies have shown that metals in aquatic environments can cause physiological stress and impair behavioral and ecological activities (Atchison et al., 1987; Scott and Sloman, 2004). In *Daphnia*, environmentally relevant levels of Cu and Ni have been shown to disrupt their ability to detect the kairomone cue of predators, such as the *Chaoborus*, an invertebrate predator, and preventing the induction of defensive neckteeth on *Daphnia* (Hunter and Pyle, 2004; Mirza and Pyle, 2009). However, little is known about how kairomones of vertebrate predator, such as fish, and metals interact to influence thermal preferences associated with diel vertical migration of *Daphnia*. Furthermore, few if any studies have characterized the individual variation in thermal preferences within and among natural populations of *Daphnia*, to elucidate the environmental plasticity of the DVM behavior. Given the fitness consequences of DVM, it is critical to understand how genetic variation, biotic factors and abiotic factors contribute to phenotypic variation. Therefore, the goals of this study were to: (1) determine the thermal distribution of *Daphnia* isolates within and among natural lake populations; (2) elucidate the influence of depth and temperature variation on vertical migration behavior of *Daphnia* isolates; and (3) determine the impact of Cu and Ni on the vertical migration of *Daphnia* isolates with different thermal ranges, in the presence and absence of classic DVM conditions.

2. MATERIALS AND METHODS

2.1 Experimental Populations

All *Daphnia pulicaria* isolates used in this study were collected from lakes in the Canadian Shield region of Ontario, Canada. Three lakes located in Sudbury, Ontario, Canada (Joe: 46° 44" −81° 31", MacFarlane: 46° 25" −80° 59", and Simon: 46° 23" −81° 11") and one lake in Dorset, Ontario, Canada (Brandy Lake: 45° 06" −79° 31"). The lakes differ in their physiological and chemical attributes (Table 1). *Daphnia* from these lakes were genotyped to determine genetically distinct isolates, while being maintained for five years prior to conducting these studies under standard laboratory conditions (i.e., 20 °C, 16:8 hr light:dark cycle) as described in Shaw et al., (2006). Only adult *Daphnia*, defined as those with

embryos in their brood chamber, were used since adult *Daphnia* are susceptible to predation by fish and are more likely to exhibit migratory behavior responds to avoid predation (Lampert, 1993, 2006).

2.2 Experimental Water Columns

Experiments were conducted in one of eight glass columns (0.5 m tall, 14.5 cm diameter) filled with 8 L of the artificial lake media COMBO (Kilham et al., 1998; Fig. 1). To isolate the effects of depth and temperature on *Daphnia* vertical migration behavior we conducted laboratory experiments that decoupled these two naturally co-varying factors while controlling for other factors known to vary with depth in natural lakes to avoid confounding results. These controlled factors included light and predator cue levels, as well as food availability. The influence of food availability on diel vertical migration is unclear, due to the recent finding that algal biomass can be present outside the euphotic zone (Winder et al., 2003); therefore, we did not include food availability in our experimental design. The light sources were set-up to prevent any variation in light levels throughout the experimental water columns, with measurements taken throughout the water column on all eight columns before each experiment and showing no variation in light levels within and among water columns for any of the experiments (data not shown). The constant light levels throughout the experimental water column prevented any refuge, allowing the *Daphnia* to experience constant diel vertical migration behavior inducing conditions throughout the water column. The thermally stratified water column, constant level of light throughout the water column, control over the presence of kairomone, and the decoupling of temperature and depth (see Fig. 1) in our experimental design enabled us to examine the individual role temperature and depth play in determining the vertical migration response in *Daphnia* in a stratified system.

The thermal gradient was established by placing the water columns each filled with 20 °C COMBO into a 13 °C walk-in incubator with a 50-W aquarium heater (Penn-Plax© Cat. CH850) placed just under the surface of the water (Fig. 1). The aquarium heater needed to be set to 29 °C in order to acquire a temperature gradient that extended from 10 cm below the surface down to a depth of 35 cm, where temperature became a constant 13 °C for the remaining 15 cm to the bottom of the experimental column (Fig. 1). This thermal stratified configuration was found to be stable and reproducible as indicated by the small error bars associated with Figure 1. Experiments were initiated by placing *Daphnia* in the middle of the water column at an approximate depth of 25 cm in the 20 °C COMBO prior to establishing the thermal gradient. After 1 hr, the experimental thermal profile was stable. *Daphnia* were then provided ample time (i.e., 1 hr) to establish their thermal preference within the stratified water column prior to recording their position. Other experiments were conducted in the same way with the addition of one or more stressors (e.g., kairomone alone or in combination with Cu or Ni) being added and under light or dark conditions. The experiment was design to determine isolate the individual effects of the naturally co-varying factors (i.e., depth, temperature, presence/absence of light and fish kairomone) and anthropogenic metal stressors (i.e., Cu and Ni) on vertical migration behavior of the thermally distinct *Daphnia* isolates.

2.3 Defining Thermal Preferences

The use of behavior in artificial temperature stratified water columns was deemed the most appropriate way to study how thermal preference varies among genetically distinct *Daphnia* isolates within and among lakes due to the fact that our experimental design was consistent, reproducible, highly sensitive (i.e., able to show degree differences among isolates) and enabled us to move past our basic question of how thermal preferences vary within and among *Daphnia* populations to determine the independent effects depth and temperature have on *Daphnia* vertical migration behavior. However, in order to conduct our experiments using more than one individual *Daphnia* per experimental water column we needed to first determine whether or not *Daphnia* group dynamics (e.g., schooling effects) would influence individual isolates behaviors.

To determine if *Daphnia* isolates behavior would be influenced by the presence of other *Daphnia* we temporarily stained the gut tracks of *Daphnia* isolates that differed in their thermal preferences using an assortment of food coloring dyes. *Daphnia* were stained by put 5 drops of concentrated food coloring in a 150 mL beaker filled with artificial lake water and leaving the *Daphnia* to swim in the colored water for 1 hr prior to experimentation. *Daphnia* isolates of different thermal preferences and stained different colors were put together in various combinations (e.g., 1 warm group isolate with 4 cold group isolates or 1 cold group isolate with 4 warm group isolates) in a single experimental water column and each individual's thermal preference was determined. The results of each individual isolate of these group experiments were then compared to the results of testing individual isolates alone. No changes in thermal preference were found suggesting that group dynamics are not a variable of concern. However, the fact that the staining of *Daphnia* only lasted approximately 1 hour, made it difficult and error prone to differentiate the colored individuals by the 1 hour post treatment response measurement time, we conducted all experiments using 4–5 individual *Daphnia* isolates from a single thermal group in a water column and used all 8 water columns to increase experimental replication.

The full collection of 50 genetically distinct isolates representing the genetic diversity found within and among *D. pulicaria* populations of the 4 study lakes, were tested to determine each isolate's thermal preference. Each isolate was maintained under laboratory conditions for over 100 generations to eliminate maternal effects. Each test was conducted using five adult individuals per isolate and the position (i.e., depth) and its corresponding temperature in the water column was recorded for each individual *Daphnia* every hour for 6 hours. However, due to the lack of change in position by *Daphnia* after 1 hr we only report the 1 hr post-treatment results. Differences in thermal distribution among isolates within and between each lake were determined using a one-way analysis of variance followed by a Tukey post-hoc analysis (SigmaPlot 12, 2012).

A group of 12 isolates were chosen from the 50 isolates tested to represent the extremes in thermal preference and sorted into two thermally distinct groups, referred hereafter as the warm group and the cold group (Table 2). In general, the warm group represents *Daphnia* isolates with thermal preferences >17 °C, while the cold group isolates preferences <15 °C (Table 2). Four isolates were selected to represent the warm group and eight isolates were

chosen from the more common cold thermal preferences phenotype to represent the cold group.

2.4 Isolates Vertical Migration Response

To determine if our selected isolates making up the two thermal groups perform classic diel vertical migration, a set of experiments were conducted in the presence and absence of both light and fish kairomone. First, fish kairomone water was created by maintaining 10 goldfish (*Carassius auratus* of length 3 – 4 cm) in 1.5 liters of modified COMBO at 20 °C and fed ~100 mixed age *Daphnia* twice during over a 24 hr period (Weetman and Atkinson, 2002, 2004). Fresh kairomone stock was created daily by filtering the fish water through 0.45 μm membrane (Brewer et al., 1999; Loose and Dawidowicz, 1994; Sakwi ska, 1998). A final concentration of 0.2 fish L^{-1} was created by adding 0.25 L of the kairomone containing water to 7.75 L of kairomone free COMBO in the experimental columns (Brewer et al., 1999; Weetman and Atkinson, 2002, 2004). Next, the 2 hr initial set-up of the thermal stratified water column and *Daphnia* addition as previously described (see section 2.2) was conducted prior to any fish kairomone water slowly being added to the top of the water column using a pipette to prevent mixing of the water column, when applicable. The fish kairomone having made its way down through the stratified water column was evident by the response of *Daphnia* performing DVM through-out the water column, including the very bottom section. The position of the *Daphnia* was recorded just before any kairomone water would have been added (0 hr) and 1 hr after. A similar procedure was conducted in the absence of light and fish kairomone treatments as a control to determine if the *Daphnia* isolates migrated in the absence of classic diel vertical migration conditions. However, to avoid disrupting the circadian rhythm of the *Daphnia* these experiments were performed at night using a LED head lamp covered with a red filter (Kodak Number 25 Wratten). The red filter inhibited the transmission of light with wavelengths <160 nm, which is virtually undetectable by *Daphnia* (Loose and Dawidowicz, 1994; Scheffer et al., 1958). To determine whether or not each *Daphnia* isolate performed classic vertical migration behavior, Student t-tests were performed comparing 0 hr and 1 hr time points for each group in the presence and absence of light and kairomone (Systat Software SigmaPlot 12, 2012).

2.5 The Effects of Metal on Vertical Migration Experiment

The effects of environmentally relevant levels of Cu and Ni (5 μg Cu L^{-1} and 40 μg Ni L^{-1}; LWQP, 2004) were tested in the presence and absence of fish kairomone and light conditions on the two thermally distinct *Daphnia* isolate groups. The experimental procedure was the same as described above (see section 2.5), except that each metal was added during the set-up of the water column to allow for proper mixing throughout the water column. Test metal solutions were made from CuCl (Matheson Coleman and Bell) or $NiCl_2$ (J. T. Baker) dissolved in ultrapure water. To determine whether or not the addition of Cu or Ni to our experimental water columns in the presence and absence of both fish kairomone and light affected vertical migration behaviors we used two-way analyses of variance, followed by a Tukey post-hoc analysis (Systat Software SigmaPlot 12, 2012). Each analysis was done on the difference between initial starting position (0 hr) and final position (i.e., 1 hr post treatment application).

3. RESULTS

3.1 Variation in thermal preference

To determine how thermal preferences differ within and among natural *Daphnia* populations, the thermal distribution of 50 *Daphnia* isolates from four lake populations (Brandy, Joe, MacFarlane, and Simon; see method section) were assessed using the thermally stratified experimental water columns (Table 2). High variability in thermal preferences within lake populations was found, while no significant difference in thermal distribution was found among the four populations ($p = 0.312$; Fig. 2). Significant within lake differences in thermal preferences among *Daphnia* isolates were found in Brandy (n = 14; $p < 0.001$), Joe (n = 16; $p = 0.018$), and Simon (n = 14; $p = 0.078$) Lakes. However, no significant differences were detected among isolates from MacFarlane Lake (n = 6; $p = 0.805$; Fig. 2), possibly due to the low number of *Daphnia pulicaria* isolates collected (n = 6) being found in MacFarlane Lake, less than half the number of isolates found in the other three lakes (see Table 2). The observed variation in thermal preferences among isolates was further utilized to isolate the role temperature and depth play in vertical migration by using isolates with different thermal preferences and therefore initial starting position in the experimental water columns, to help unlock these two naturally coupled factors.

As a quality assurance measure, we tested whether *Daphnia* isolates selection behavior of initial starting position in the experimental water columns was based on temperature rather than depth. By reducing the heat applied to the water column we were able to move the stratification regime upwards and measured whether the *Daphnia* isolates would move to more shallow waters to stay at their selected temperatures or simply remain at their preferred depth. As expected, we found that all *Daphnia* isolates moved with their thermal preference (data not shown), validating our use of thermal preference behavioral response.

3.2 Effects of Light and Fish Kairomone on Vertical Migration

The influence of light and fish kairomone to stimulate diel vertical migration in *Daphnia* is well known (Wetzel, 2001); however, not all *Daphnia* exhibit this response. To determine if the *Daphnia* selected for the two distinct thermal groups exhibit the classic diel vertical migration behavior, we tested their response to kairomone and light in a full factorial designed experiment. Based on classical diel vertical migration behavior *Daphnia* should migrate downward in the presence of light and fish kairomone, but would exhibit no downward migration behavior in the absence of either light or fish kairomone. As predicted, no *Daphnia* exhibited any downward migration behavior in the absence of either light (-L) or kairomone (-K) (see Appendix S1 and S2) and all *Daphnia* isolates in warm and cold thermal groups exhibited the classic diel vertical migration behavior in the presence of kairomone and light using depth as the response variable (warm group: $p = 0.008$, Fig. 3b and cold group: $p = 0.01$, Fig. 3d). However, when using temperature as the response variable only the warm group migrated to a significantly lower temperature in the +L and +K treatment (warm group: $p = 0.003$, Fig. 3a and cold group: $p = 0.372$; Fig. 3c). This is due to the fact that the cold group started at the lowest thermal gradient (13 °C) in the experiment and therefore could not migrate to a lower temperature. This experimental design allowed for the decoupling of temperature and depth effects in determining the distance

Daphnia vertical migration, and showed that response to predator cues occurred whether or not a thermal gradient was present.

Warm group results using depth as the response variable were consistent with those obtained using temperature (see Fig. 3a and 3b), because temperature and depth were strongly correlated at temperatures above 13 °C (see Fig. 1); however, once the thermal gradient reached 13 °C depth became decoupled from temperature. Decoupling the effects of temperature and depth on *Daphnia* vertical migration behavior revealed three findings that would not have been found if temperature was the only response variable measured. Using depth as the response variable instead of temperature revealed that *Daphnia* in the cold group did in-fact perform diel vertical migration behavior under classic diel vertical migration conditions (+L, +K). The cold group *Daphnia* started at an average depth of 35 cm and temperature 13 °C and migrated to an average depth of 45 cm while temperature remained constant (Fig. 3d), resulting in a significant difference in final migration depth between the control (+L, -K) and +L, +K treatment ($p < 0.001$; Fig. 3d). In addition, depth as the response variable revealed that *Daphnia* from the warm group exposed to classic diel vertical migration conditions (i.e., +L, +K treatment) responded by ending their vertical migration at the onset of the lowest temperature thermal cline in the experimental water columns (i.e. 13 °C), but not the lowest depth in the experimental water column (Fig. 3a). Therefore, warm group isolates migrated only as far as temperature changes exist, stopped short of the maximum depth. Finally, average migratory distance traveled by *Daphnia* isolates in both warm and cold group under +L, +K treatment (12.25 cm ± 2.20 cm and 10.5 cm ± 1.22 cm, respectively) did not differ statistically ($p = 0.228$; Fig. 3b and 3d).

3.3 Effects of Metal on Vertical Migration

The direct effects of two metals on vertical migration of *Daphnia* were tested to determine if *Daphnia* migratory response would be impacted by the presence of either Cu or Ni in the water column. In this experiment, all warm and cold group isolates were exposed to each metal individually in the presence of light, with and without kairomone and the migration response was recorded. Due to the consistency found in the results of the two metals only the slightly clearer Cu results are presented in the main text of the paper, with Ni results provided as supplemental material (Appendix S2).

To determine if the metals Cu or Ni themselves caused *Daphnia* to migrate, the migration patterns with metals present but without fish kairomone were tested for all warm and cold group isolates. These experiments showed that while there was a trend of *Daphnia* having a lower initial starting position in the presence of metals, the presence of Cu or Ni did not result in *Daphnia* migration response (Fig. 3 and Appendix S2). Therefore, we then tested the effects of Cu and Ni on these *Daphnia* isolates natural response to light and the presence of fish predatory cues. We found that *Daphnia* isolates of both thermal groups predatory avoidance behavioral response (i.e., vertical migration) was inhibited in the presence of light and fish kairomone by both Cu and Ni (Fig. 3).

4. DISCUSSION

Three major findings emerged from these studies examining the influence of temperature, co-varying abiotic and biotic factors, and two anthropogenic stressors (i.e. Cu and Ni) on vertical migration within and among *Daphnia* populations: (1) comparison of *Daphnia* thermal preferences across the four lake populations showed no differences among populations (Fig. 2); however, for *Daphnia* isolates thermal preference differences within lake populations were common (Fig. 2); (2) depth rather than temperature was the more deterministic variable of *Daphnia* migration, since vertical migration occurred even in the absence of a temperature gradient (Fig. 1 and Fig. 3). (3) Under classic diel vertical migration conditions (i.e., +L, +K), but in the presence of environmentally relevant concentrations of either Cu or Ni, *Daphnia* predatory avoidance behavior (i.e. diel vertical migration) was inhibited (Fig. 3 and Appendix S1).

The four lakes in this study were selected because they share similar geological characteristics, all occur within the boreal environment of the Canadian Shield, and all are lakes that contain fish and therefore their *Daphnia* populations likely exhibit diel vertical migration behavior. Variation among all four lakes does exist in terms of size (area, volume, and depth), pH, and trophic state (Table 1). Even though these lakes differed in their physical and chemical attributes (Table 1), the *Daphnia* populations found in these lakes did not differ in their overall thermal preference ($p = 0.312$). This was surprising, because it suggests that the thermal preferences of *Daphnia* populations are independent of pH, depth, volume, area, and trophic status of their respective systems. Further examination showed that the lack a variation among populations was due to the similarities in the range of different thermal preferences among isolates of the various populations.

The studied *Daphnia* populations all had isolates that significantly differed in their thermal preference, except in MacFarlane Lake that yielded half as many genetically distinct *Daphnia* isolates (n = 6) as the other study lakes and only contributed cold thermal group isolates (Table 2). This high variability in the range of temperature preferences found among isolates within each lake's *Daphnia* population, suggests the existence of niche partitioning that could reduce intra-specific competition among *Daphnia* isolates by minimizing the resource allocation overlap in the water column as demonstrated in earlier seminal works (De Meester and Dumont, 1988; 1989; Dumont et al., 1985; Weider, 1984). Thermal partitioning through intra-specific competition among genetically distinct *Daphnia* isolates helps explain not only the high variability in thermal preferences found within lake *Daphnia* populations, but also how lakes that differ in their physical and chemical attributes can still have the same overall thermal range in their *Daphnia* populations.

This study also revealed that cold thermal group *Daphnia* in the presence of light and fish kairomone migrated to deeper waters, even in the absence of changes in temperature (Fig. 1 and Fig. 3d), suggesting that a change in temperature was not a necessary factor in determining the depth at which *Daphnia* migrate in response to predator cues. Furthermore, *Daphnia* isolates in the warm thermal group under classic diel vertical migration conditions started their migration higher in the water column (where temperatures were warmer), but migrated the same distance (~10 cm) as the cold thermal group. The average migration depth

took the warm group *Daphnia* just inside the 13 °C, stopping short of the experimental water column's bottom. This raises the questions: Why stop there? Why not migrate as deep as the cold group under the same +L, +K conditions? After all, migrating to these depths would not come at a cost due to changes in temperature. It is worth noting that while we report only results of *Daphnia's* position in the water column after 1 hr post treatment addition, we collected hourly data for 6 hrs post treatment addition. We only report the 1 hr post treatment depths because we found that the *Daphnia* remained at the same position over the 6 hr period (data not shown). The fact that *Daphnia* persisted at this final position for 6 hours also suggests this is a regulated behavior as *Daphnia* had ample time to migrate elsewhere. Our findings show that both cold and warm thermal group isolates traveled the same distance on average during their vertical migration despite ample time and space available for them to move further, suggesting that distance, not temperature, determines the final depth to which *Daphnia* isolates migrated.

Lampert (1989) observed that there are several possible sources of costs limiting diel vertical migration. Our study suggests that distance migrated, rather than temperature, dictates the depth to which *Daphnia* isolates vertically migrate when light conditions are held constant. Given the scale of this study, the migration distance of 10cm, it is difficult to directly translate these findings into something meaningful in the context of a natural lake environment. For example, the known effects of hydrostatic pressure on *Daphnia* migration (Lincoln, 1971) were not evaluated due to the size of our experimental chambers. However, our controlled laboratory study was designed to elucidate the naturally coupled mechanisms of light, depth, migration distance, and temperature to show how their roles differ in controlling the migration depth associated with *Daphnia* vertical migration behavior.

The present study also demonstrates that *Daphnia* isolates within a population occupy tight thermal ranges, which may represent niche-partitioning in response to intra-specific competition. However, the ability of individuals and populations to occupy alternative or wider thermal ranges through acclimation or adaptation will be important in determining how *Daphnia* populations will respond to thermal perturbations such as climate change. A study by Palaima and Spitze (2004) examined the height and breadth of temperature tolerance curves for 29 *D. pulex* complex genotypes and demonstrated that some *Daphnia* with high fitness at their optimal temperatures also had high fitness across a wide range of temperatures, supporting the hypothesis that some individuals within a population are able to alter their thermal location and maintain a high level of fitness. Therefore, any changes in thermal conditions of a lake whether from increased intra-specific competition due to a reduction in the number of thermally distinct strata, costs associated with the need to acclimate, or the bottlenecking associated with thermal selection, will likely result in a reduction in diversity and size (at least initially) of *Daphnia* lake populations.

The mechanism dictating the depth at which *Daphnia* vertically migrate to avoid predation is an important factor determining *Daphnia* fitness (e.g., how much energy is being devoted to predator avoidance versus reproduction). However, this defense mechanism depends on *Daphnia's* ability to perceive the predation threat by detecting kairomone cues. Any factor that interferes with this ability would prevent *Daphnia* from vertically migrating which would result in a dramatic increase in the rate of mortality. This study has demonstrated that

environmentally relevant levels of the metals Cu and Ni inhibit the classic vertical migration behavioral response exhibited by both warm and cold thermally constrained *Daphnia* groups in the presence of light and vertebrate predator cue. The lack of vertical migration by all isolates suggests that even at low levels metals are interfering with *Daphnia's* response to fish kairomone cues. Similar findings have been shown in other studies examining invertebrate predator kairomone and their influence on *Daphnia* neonate neckteeth development in the presents of these same metals (Hunter and Pyle, 2004; Mirza and Pyle, 2009), and fish kairomone influence on photo-tactic behavior of *Daphnia* in the present of Cu (Dang Kieu et al., 2001; Yuan et al., 2003). While our study confirms earlier findings about copper it adds nickel to the list of metals that at low concentrations inhibits *Daphnia's* responds to predatory fish cues. Furthermore, our results showed a surprising direct effect of metal on *Daphnia* positioning in the water column that we were unable to explain and warrants more research. The constant additions of copper and nickel into aquatic ecosystems around the world through atmospheric deposition suggests that disruptions to *Daphnia's* vertical migration response to predator cues will become more common and could result in an increased rate of fish predation and a dramatic decline in *Daphnia* populations globally.

5. CONCLUSION

These studies demonstrate how individual *Daphnia* genotypes can distribute themselves among the various thermal niches that result from stratification within a lake, and how this thermal niche partitioning is similar in lakes that differ in physical and chemical properties. Experiments designed to decouple the naturally co-varying factors of temperature and depth revealed that tracking changes in depth was a better predictor of *Daphnia* migratory behavior than changes in temperature. Furthermore, the results of these experiments also showed that regardless of initial thermal positions in the water column cold- and warm group *Daphnia* migrated a similar distance, supporting the hypothesis that the cost associated with distance migrated limits vertical migration behavior. Finally, these studies revealed the disruption of natural diel vertical migration behavioral response in *Daphnia* exposed to environmentally relevant levels of Cu or Ni. The addition of either of these common atmospherically derived anthropogenic stressors inhibits the response of *Daphnia* to fish kairomone in the presence of light, putting these non-migrating *Daphnia* at a greater risk of predation.

Collectively, these findings suggest diversity and overall size of *Daphnia* populations are dependent on highly stratified lentic systems and the absence of Cu and Ni pollution. However, a reduction in the variation in thermal stratification of lakes is predicted to occur in response to climate change (Heino et al., 2009; Mooij et al., 2005) and Cu and Ni loading into aquatic ecosystems is likely to continue (Keller, 2009; Keller and Piblado, 1986; Pyle et al., 2005); therefore, our findings predict a global decline in *Daphnia* diversity due to increased intra-specific competition within lakes and *Daphnia* populations will decrease in size as a result of increased predation pressure by fish. Because of *Daphnia's* global presence and its role as a keystone species in aquatic food webs, a significant decline in *Daphnia* diversity and population size could dramatically affect aquatic ecosystem function and stability (Lampert, 2006; Mooij et al., 2005; Thuiller, 2007; van de Waal et al., 2009).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- No variation in thermal preference among the four *Daphnia* populations.

- High within lake variability in thermal preference among *Daphnia* isolates.

- Depth was the better predictor of *Daphnia* migratory patterns than temperature.

- Exposure to low levels of Cu or Ni inhibited diel vertical migration behavior.
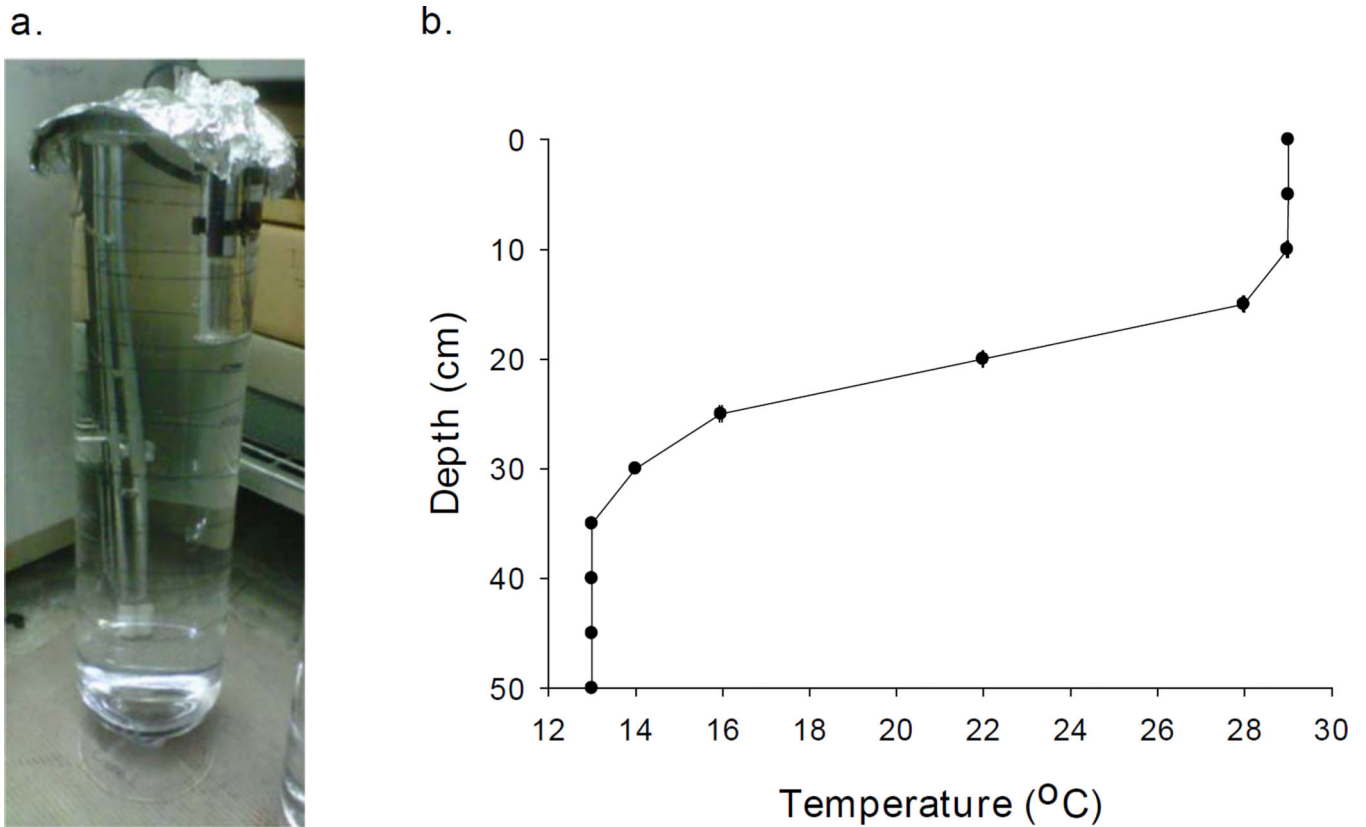
a.

b.

Figure 1.
a) Picture shows one of eight experimental water columns. b) The thermal stratification pattern of the experimental water column showing temperature (°C) declining with depth (cm). The thermal stratification pattern was consistent during and throughout each experiment, as indicated by the small standard error bars.
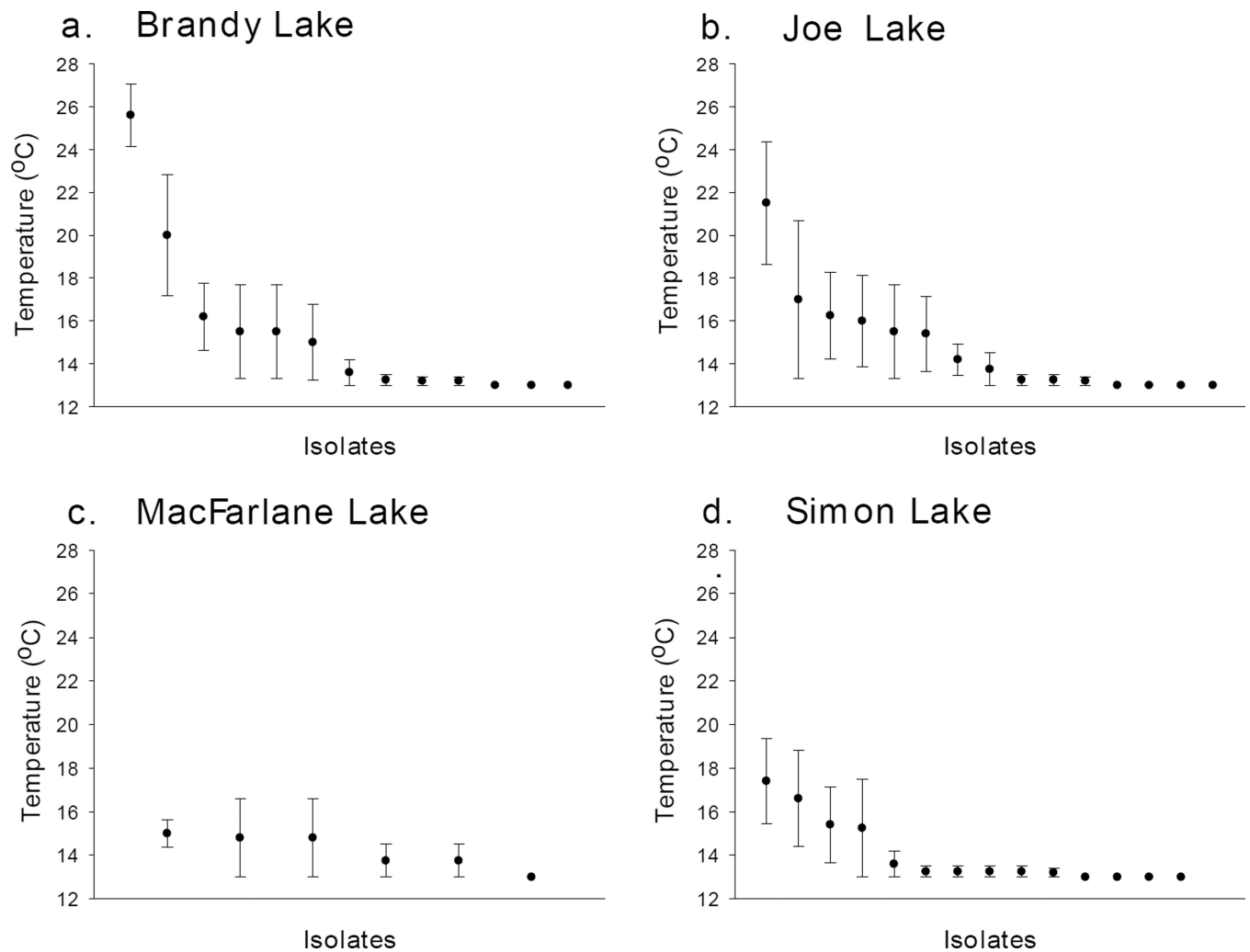
Figure 2.
Thermal preferences of the fifty isolate representing four lakes within the Sudbury and Dorset region of Ontario, Canada, tested in the presence of light and the absence of fish kairomone. For each isolate five individuals were tested and the data shown here represent the means ± standard error. The isolates that make up each lake's *Daphnia pulicaria* population showed significant variation in thermal preferences. However, no difference in thermal variation was found among the four lake populations.
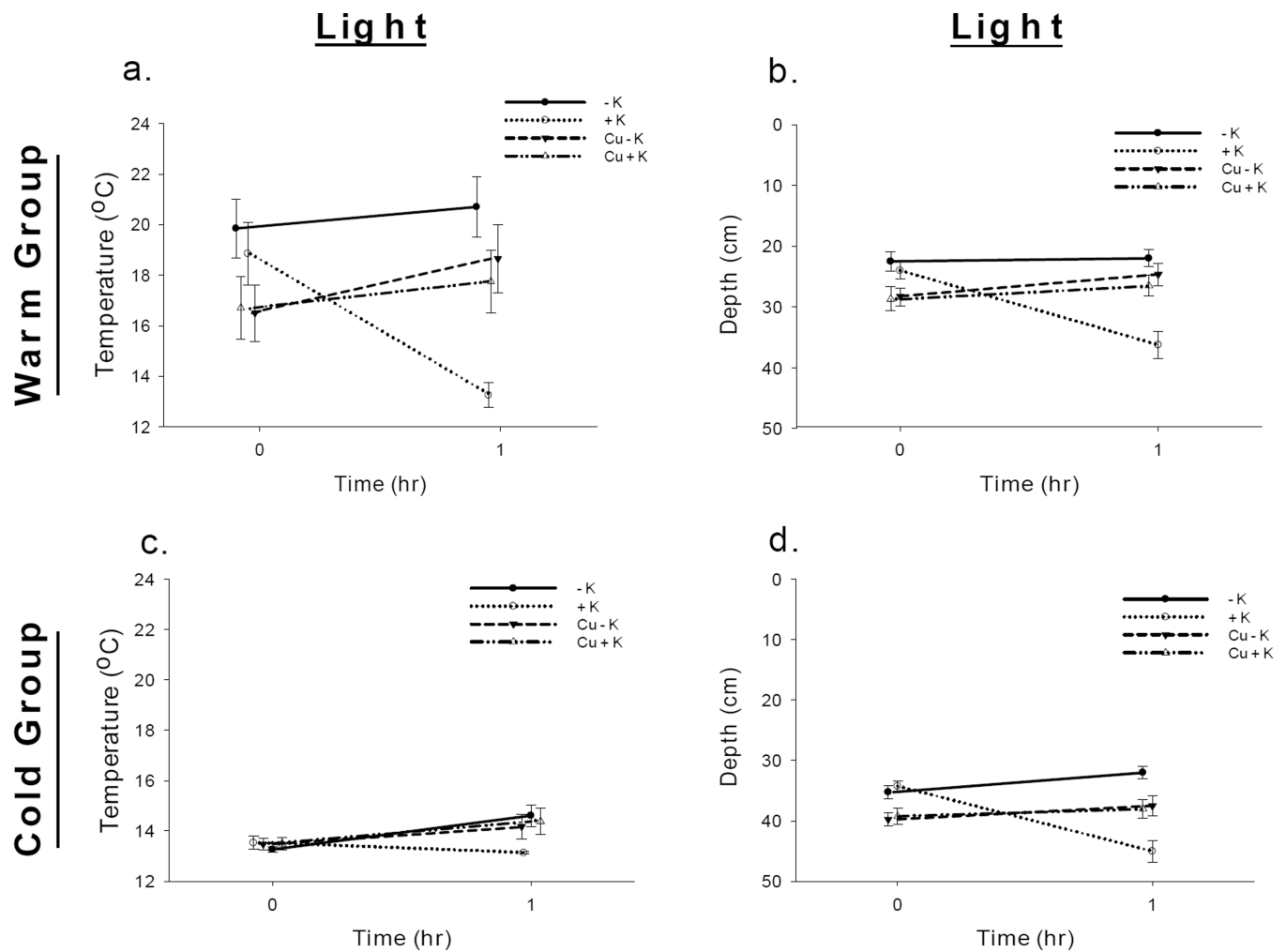
Figure 3.
Migration behavior in response to kairomone and copper. Mean, trajectory and standard error of depth or preferred temperature for both *Daphnia* thermal groups, selected based on their thermal preference profiles to making up the warm (n = 8) or cold thermal groups (n = 12), response (in the presence of light) to the presence or absence of either fish kairomone or metal (e.g., Cu). The response was measured as either a change in temperature (a and c) or depth (b and d). -K is the absence of fish kairomone and +K is the presence of fish kairomone.

Table 1

Physical and chemical properties associated with the four study lakes (DESC, 2004 and LWQP, 2002 & 2004).

Lake	Size (ha)	Volume (10⁴	Max Depth	pH	Trophic Status
Brandy	108	378	7.5	6.75	Oligotrophic
Joe	180	2012	34	6.60	Oligotrophic
MacFarlane	166	2990	18	7.53	Mesotrophic
Simon	102	1224	12	7.69	Eutrophic

Table 2

Summary of the isolates tested to determine thermal preference within and among natural *Daphnia* populations.

Lake	Isolate	Average Temperature	Standard Error	Thermal Group	Isolates Selected
Brandy	BR1	13.20	0.20	Cold	
Brandy	BR2	20.00	2.83	Warm	*
Brandy	BR3	13.00	0.00	Cold	
Brandy	BR4	13.87	0.62	Cold	*
Brandy	BR5	15.50	2.18	Cold	
Brandy	BR6	15.50	2.18	Cold	
Brandy	BR8	13.20	0.20	Cold	
Brandy	BR9	25.60	1.47	Warm	*
Brandy	BR10	13.60	0.60	Cold	
Brandy	BR11	13.00	0.00	Cold	*
Brandy	BR13	13.25	0.25	Cold	
Brandy	BR15	13.00	0.00	Cold	
Brandy	BR16	15.00	1.76	Cold	
Brandy	BR17	13.00	0.00	Cold	
Brandy	Mean	15.05	0.96	--	
Joe	J1	13.25	0.25	Cold	
Joe	J3	13.25	0.25	Cold	
Joe	J4	16.00	2.12	Cold	
Joe	J6	13.20	0.20	Cold	*
Joe	J7	13.75	0.75	Cold	
Joe	J8	13.00	0.00	Cold	
Joe	J11	14.20	0.73	Cold	
Joe	J12	15.40	1.75	Cold	
Joe	J13	15.50	2.18	Cold	
Joe	J14	13.07	0.07	Cold	*
Joe	J15	13.00	0.00	Cold	

Lake	Isolate	Average Temperature	Standard Error	Thermal Group	Isolates Selected
Joe	J16	13.00	0.00	Cold	
Joe	J17	17.00	3.67	Warm	
Joe	J18	13.00	0.00	Cold	
Joe	J19	21.50	2.87	Warm	*
Joe	J20	13.00	0.00	Cold	
Joe	**Mean**	**14.45**	**0.57**	--	
MacFarlane	MF1	13.00	0.00	Cold	*
MacFarlane	MF2	13.75	0.75	Cold	
MacFarlane	MF3	14.80	1.80	Cold	
MacFarlane	MF4	14.67	0.81	Cold	*
MacFarlane	MF5	14.80	1.80	Cold	
MacFarlane	MF6	13.75	0.75	Cold	
MacFarlane	**Mean**	**14.13**	**0.30**	--	
Simon	S1	15.40	1.75	Cold	
Simon	S2	13.00	0.00	Cold	
Simon	S3	13.00	0.00	Cold	
Simon	S4	13.00	0.00	Cold	
Simon	S5	13.20	0.20	Cold	
Simon	S6	13.25	0.25	Cold	
Simon	S7	13.00	0.00	Cold	*
Simon	S9	17.40	0.94	Warm	*
Simon	S12	15.25	2.25	Cold	
Simon	S13	13.25	0.25	Cold	
Simon	S14	13.60	0.60	Cold	
Simon	S16	13.25	0.25	Cold	
Simon	S17	13.25	0.25	Cold	
Simon	S20	14.73	0.81	Cold	*
Simon	**Mean**	**13.90**	**0.35**	--	