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In the Toolik Lake region of arctic Alaska, ponds contain one of two distinct zooplankton communities. Small ponds are typically dominated by *Daphnia pulex* and medium and large ponds are typically dominated by the larger daphnid, *Daphnia middendorffiana*. An invertebrate predator, *Heterocope septentrionalis*, exists in the medium and large ponds with *D. middendorffiana*. *D. pulex* were exposed to *Heterocope* kairomone in laboratory and *in situ* experiments in an effort to explain why these two species do not coexist in nature. An increase in average egg number per brood was observed when *D. pulex* were exposed to predator kairomone both in laboratory and *in situ* studies. In laboratory studies, *D. pulex* increased size at 24 hours old in response to *Heterocope* kairomone. Behavioral changes, such as an alternation in vertical position, were not observed when *D. pulex* and *D. middendorffiana* were exposed to predator kairomones.

THE LIFE HISTORY, MORPHOLOGICAL, AND BEHAVIORAL CHANGES OF  
TWO ARCTIC DAPHNIDS TO KAIROMONE FROM THE INVERTEBRATE  
PREDATOR *HETEROCOPE SEPTENTRIONALIS*

by

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APPROVAL PAGE

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## CHAPTER I

### INTRODUCTION

Predators can influence community structure. Predators can eliminate prey, but the ability of prey to sense and respond to the predator's presence may determine prey's survival. Chemical signals, called kairomones, have been shown to induce changes in prey. Kairomones from both invertebrate and vertebrate aquatic predators may induce life history, morphological, and behavioral changes in various species of *Daphnia* (see Tollrian and Dodson 1999, Laas and Spaak 2003 for review). Kairomone studies allow researchers to observe changes in prey and attribute these changes to phenotypic plasticity, not selective predation. The life history, morphological, and behavioral changes take place to increase prey survival and may allow predator and prey to coexist.

Changes in daphnid life histories have been noted in various species exposed to a variety of potential predator kairomones. To combat predation threats induced by invertebrate predator kairomones, daphnids may alter time to reproductive maturity (Dodson and Havel 1988, Black 1993), have higher numbers of offspring (Black 1993), and have faster reproductive rates (Black 1993, Tollrian 1995). Daphnids may also adopt differing life history strategies when responding to vertebrate predator kairomones. They may have more offspring per clutch (Sakwinska 2002), an earlier reproductive maturation



(Weber and Declerck 1997, Sakwinska 2002), a smaller size at first reproduction (Weider and Pijanowska 1993), or produce ephippial eggs (Slusarczyk 1995).

Morphological changes in daphnid size and structures may also occur when daphnids are exposed to predator kairomones. When exposed to invertebrate predator kairomone, daphnids may increase body size and width (Tollrian 1995), form neckteeth (Krueger and Dodson 1981, Black 1993, Tollrian 1995), increase helmet size (Bungartz and Branstrator 2003), or form elongated tail spines (Dodson 1989, Tollrian 1990, Brancelj *et al.* 1996, Caramujo and Boavida 2000). These morphological changes have been shown to effectively reduce invertebrate predation (Krueger and Dodson 1981, Havel and Dodson 1984, Vega 1995, Caramujo and Boavida 2000). Daphnids respond differently to kairomones from visual predators, such as fish (Confer *et al.* 1978, Kettle and O'Brien 1978). Generally, *Daphnia* exposed to fish kairomones are smaller than those not exposed to fish kairomones (Sakwinska 2002), effectively reducing the predator's ability to locate the prey.

Behavioral changes may also occur when daphnids are exposed to predator kairomones. Daphnids may occupy higher positions in the water column to avoid invertebrate predators that occupy lower positions in the water column (Dodson 1988, Beeton and Bowers 1982). Smaller daphnids have been shown to avoid surface waters when exposed to kairomone from a surface dwelling invertebrate predator (Dodson 1988). Daphnids may move horizontally to avoid high concentrations of predator kairomone (Watt and Young 1994, Kleiven *et al.* 1996). Daphnids may also aggregate (Pijanowska and Kowalczewki 1997) and swim at similar speeds (Jenson *et al.* 1998)

when in the presence of a fish kairomone. Prey aggregation has been shown to effectively reduce predation (Hamilton 1971). Daphnids have also been shown to alter vertical position and vertical migration patterns in response to predator kairomones. Alterations in vertical position and vertical migration by daphnids are attempts to reduce spatial overlap with predators (De Meester 1993, Von Elert and Pohnert 2000, Hendry and Burns 2001). Daphnids that alter vertical position in response to predators are more likely to survive and coexist with the predator.

While various studies have investigated many invertebrate and vertebrate kairomone effects on daphnids, prey responses to kairomone from the arctic predatory calanoid copepod, *Heterocope septentrionalis*, have only recently been explored. This predator is of special interest because it may be instrumental in determining the distinct zooplankton community types that occur in ponds of the Toolik Lake region of arctic Alaska (O'Brien 2001). Ponds are typically inhabited by one of two daphnids; *D. middendorffiana* or *D. pulex*. *D. middendorffiana* and *Heterocope* commonly coexist in medium and large ponds. A kairomone experiment showed that *D. middendorffiana* exposed to *Heterocope* kairomone are significantly larger at birth than *D. middendorffiana* exposed to fish kairomone or no predator kairomone (Green 2005).

A second arctic daphnid, *D. pulex*, inhabit smaller ponds, do not coexist with *Heterocope*, and rarely occur with *D. middendorffiana*. *D. pulex* are highly vulnerable to *Heterocope* predation (Hebert and Loaring 1980, Luecke and O'Brien 1983, Dodson 1984), and are more susceptible than *D. middendorffiana* to *Heterocope* predation because of their smaller size, weaker carapace, and ineffective escape abilities (Luecke

and O'Brien 1983, Dodson 1984). Even though *D. pulex* are better resource competitors than *D. middendorffiana* (Dzialowski and O'Brien 2004), most arctic ponds in the Toolik Lake region are inhabited by *D. middendorffiana* and *Heterocope*. Predator addition experiments have shown that *Heterocope* can effectively eliminate *D. pulex* within several years of predator colonization (O'Brien 2001) in medium and large ponds. Even though it has been demonstrated that *Heterocope* can eliminate *D. pulex* from an arctic pond, the life history, morphological, or behavioral changes that *D. pulex* may undergo in the presence of this voracious predator have not been examined.

Studying the effects that *Heterocope* kairomone has on *D. pulex* may give insight as to why these zooplankters do not coexist in the arctic. *D. pulex* life history responses to *Heterocope* kairomone were investigated by observing changes in egg number and body size. We hypothesized that *D. pulex* would increase egg numbers in response to *Heterocope* kairomone. This strategy might allow *D. pulex* to outnumber *Heterocope* predation. Since *Heterocope* predation decreases as *D. pulex* size increases (Luecke and O'Brien 1983, Dodson 1984), we hypothesized that *D. pulex* would increase in size in response to *Heterocope* kairomone. *D. pulex* and *D. middendorffiana* were photographed and observed in field and laboratory experiments to determine morphological changes in response to *Heterocope* kairomone and *D. pulex* and *D. middendorffiana* behavioral changes were noted by alterations in daphnid vertical position when exposed to *Heterocope* kairomone. We did not expect to observe morphological changes in response to *Heterocope* kairomone because previous studies have indicated arctic *D. pulex* do not have a response to other predator kairomones (Luening 1995). Because *Heterocope* are

susceptible to UV light (Luecke and O'Brien 1981), we expected *Heterocope* to avoid the upper portions of the water column. We then expected both daphnids to avoid the portion of the water column that *Heterocope* inhabit. The life history, morphological, and behavioral alterations that arose in *D. pulex* were compared to changes in *D. middendorffiana* to see if the alterations could further explain why *D. middendorffiana* can coexist with *Heterocope* while *D. pulex* cannot coexist with *Heterocope*. A predation experiment was also performed to determine if *D. pulex* exposed to *Heterocope* kairomone were less vulnerable to *Heterocope* predation. Because we did not expect morphological changes to occur when *D. pulex* was exposed to *Heterocope* kairomone, we did not expect any difference in the predation rates of *D. pulex* exposed to *Heterocope* kairomone and *D. pulex* not exposed to *Heterocope* kairomone.

## CHAPTER II

### METHODS

#### *Study Location*

Research was conducted in the summers of 2005 and 2006 at the Toolik Lake Research Station in northern Alaska ( $68^{\circ}38'00''\text{N}$ ,  $148^{\circ}36'15''\text{W}$ ) located about 20 kilometers north of the Brooks Mountain range along the Dalton Highway. *D. pulex* life history, morphological, and behavioral characteristics were analyzed in four ponds. Berm Pond IIb and Berm Pond III are located less than five meters apart on a deserted gravel road formed during the construction of the trans-Alaskan pipeline. Berm Pond IIb and III are permanent ponds similar in age, size, depth, invertebrate communities, substrate, and abiotic parameters (Table 1). *D. pulex* life history, morphological, and behavioral characteristics were also analyzed in Pond A and Pond B. These ponds, collectively referred to as the Road Ponds, are located along the access road of the Toolik Lake Field Station alongside the Dalton Highway. These ponds are shallower and smaller than the Berm Ponds. Ponds A and B are ephemeral and lost substantial volumes of water during the summer. The Road Ponds are similar to one another in substrate, depth, invertebrate communities, and abiotic parameters (Table 1).

### *Life History and Morphological Experiment*

The life history and morphological experiment was completed in 2005 (Experiment 1 and 2, Table 2). The objective of this experiment was to determine if *D. pulex* would respond to *Heterocope* kairomone by altering size or egg number. A plankton net was used to collect *D. pulex* from a *Heterocope* free pond, Berm Pond IIb. *D. pulex* were immediately brought back to the lab and 50 egg bearing females were placed into an Erlenmeyer flask filled with filtered water from Berm Pond IIb. These organisms were closely monitored and when they gave birth, their daughters (generation 2) were housed in cylindrical Plexiglass chambers (CPCs) (O'Brien and Kettle 1981) and placed into one of three 20 liter aquaria filled with filtered water from Berm Pond IIb. Plankton netting was affixed to two ends of the CPCs and allowed for the retention of *D. pulex* and the exposure of the organisms to *Heterocope* kairomone. In later experiments, the CPCs retained *Heterocope* and protected the surrounding *D. pulex* from *Heterocope* predation. The aquaria housing the CPCs were placed into a water bath and maintained at 16 degrees Celsius. *D. pulex* were monitored for egg appearance and the release of young (generation 3). When third generation offspring were 24 hours old, their length, from the eyespot to the base of the tail spine, was measured using a dissecting microscope. Twenty, third generation *D. pulex* were then arbitrarily placed into one of two treatments, *Heterocope* kairomone water or kairomone free water. Kairomone free water was collected from Berm Pond IIb and *Heterocope* kairomone water was collected from Foggy Pond 14. Two replicates of each type of kairomone water were housed in three 20 liter aquaria. Every fourth day, organisms were measured with a dissecting

microscope to the nearest tenth of a millimeter and egg number was noted. When the daphnids gave birth (generation 4), those organisms were again placed into the same treatment water as their mothers. Generation four organisms were observed every four days and size and egg number was noted. Seventy five percent of the treatment waters was changed every four days to ensure organisms were continually exposed to predator kairomone. At the time of water changes, nitrogen was added as  $(\text{NH}_4)_2\text{SO}_4$  and phosphorus was added as  $\text{KH}_2\text{PO}_4$  to ensure ample algal growth.

#### *Statistical Analysis- life history and morphological experiment*

Daphnid size and egg number were compared between treatments for fourth generation *D. pulex* using a t-test in SPSS (Version 14.0). Egg number was also compared between treatments using a t-test.

#### *Behavioral Experiment*

The behavioral experiment took place during the summer of 2006 (Experiment 3, Table 2). This experiment was designed to show if the vertical position of *D. pulex* and *D. middendorffiana* in the presence and absence of *Heterocope* kairomone differed. The experimental organisms were collected from Berm II or Dam Pond with a plankton net on the morning of the vertical position experiment and approximately 200 organisms of a species were placed in a beaker filled with their home water until the beginning of the experiment. *Heterocope* treatment water was conditioned by allowing seven *Heterocope* of similar size to inhabit an aquarium filled with seven liters of predator free, glacial melt water from a stream near Galbraith Lake, for about 20 hours prior to the start of the vertical position experiment. Control water was conditioned by aging seven liters of

predator free glacial melt water in an aquarium for about 20 hours prior to the beginning of the experiment.

The experimental chamber consisted of a Plexiglas® water bath housing six polycarbonate vertical position tubes (VPTs) 110 centimeters tall and 4.8 centimeters in diameter. The tubes were marked in two centimeter increments down from the top of the VPT to 30 centimeters and in 10 centimeter increments from 30 centimeters down to 110 centimeters. The bath allowed temperature regulated water to flow around the tubes without contaminating the treatments. Temperature was regulated to 15 degrees Celsius. A 250 watt flood lamp was mounted above the chamber to provide a light cue for the daphnids, and the sides of the chamber were wrapped in black plastic to simulate natural conditions of light entering from above. Twenty five juvenile *D. pulex* or *D. middendorffiana* were placed into a VPT that contained either the control water or the *Heterocope* kairomone water. Each treatment had three replicates. Experimental organisms were allowed to acclimate to the treatment waters for one hour prior to the beginning of the experiment. Observations on the vertical position of the organisms were made every thirty minutes for two hours after the acclimation period. The following day, the experiment was repeated with the other daphnid species.

*In situ* vertical position of *D. pulex* in Berm Pond II was also determined. This is a permanent, *Heterocope* free pond. Using a net affixed to a pole, horizontal plankton tows were taken at 10 centimeter increments throughout the water column. *D. pulex* densities were then calculated and compared between depths.



In an effort to explain possible daphnid vertical responses, the vertical position of *Heterocope* was determined both in the laboratory and *in situ*. Vertical position of *Heterocope* was determined in the VPTs by placing 15 *Heterocope* into each of three VPTs filled with predator free water. *Heterocope* were allowed to acclimate for 30 minutes. Vertical position of the *Heterocope* was then noted at 30 minutes increments for 1.5 hours.

*Heterocope* vertical position was also determined in two fishless ponds, Camp Pond and E6. The maximum depths of these ponds are approximately 1 meter and 2 meters respectively. Horizontal tows of a known distance were taken at differing depths using a plankton net. Because both Camp Pond and E6 also contain *D. middendorffiana*, vertical position of *D. middendorffiana* was examined in conjunction with *Heterocope* vertical position.

#### *Statistical Analysis-Behavioral Experiment*

In SAS (Version 9.1.3, SAS Institute, Cary, NC), a Kruskal-Wallis test was used to determine if daphnid vertical distributions differed between species and treatment. In SPSS, analysis of variance (ANOVA) was used to determine any statistical differences in the vertical positions of *D. pulex*, *D. middendorffiana*, and *Heterocope* both in the field and in the chamber.

#### *In situ Experiment*

During the summer of 2006, *Heterocope* kairomone was added to two small *D. pulex* ponds to determine if *D. pulex* would alter life history and morphology in response to *Heterocope* kairomone *in situ*, as they did in the laboratory (Experiment 4, Table 2).

*Heterocope* kairomone was added to Berm Pond IIB and Berm III served as its reference and *Heterocope* kairomone was also added to Pond B and Pond A served as its reference. Prior to the *Heterocope* kairomone addition, three horizontal plankton tows were taken on two separate sampling days, three days apart, in both *Heterocope* kairomone addition ponds (Berm IIB and Pond B) and reference ponds (Berm III and Pond A). The samples were preserved in 75% ethanol, and returned to the laboratory for analysis. *Heterocope* kairomone was added to the treatment ponds by placing 10 *Heterocope* in each of five CPCs. Shade cloth was wrapped around the CPCs to reduce possible *Heterocope* mortality due to UV light. Shade cloth covered CPCs were scattered arbitrarily around the experimental ponds. Five empty CPCs were also scattered arbitrarily in the reference pond. *Heterocope* were replaced every three days to ensure *D. pulex* were continually exposed to *Heterocope* kairomone. Three horizontal tows of differing depths were taken every three days from each pond for the duration of the summer and preserved. The samples were later examined with a dissecting microscope for daphnid density, size from the eyespot to the base of the tail spine, tail spine length, resting egg presence and egg number.

#### *Statistical Analysis-In situ Experiment*

*D. pulex* were classified as coming from ephemeral or permanent ponds, and egg number was compared between *D. pulex* from *Heterocope* kairomone addition ponds and reference ponds using ANOVA. Tail spine length was compared between treatments using linear regression.

### *Predation Experiment*

The predation experiment was conducted in the summer of 2006 to determine if *D. pulex* responded to *Heterocope* kairomone by altering morphology or behavior that was not measurable using a microscope (Experiment 5, Table 2). *D. pulex* were collected from Berm Pond II for the predation experiment. Offspring of these organisms were placed in CPCs housed in aquaria filled with either *Heterocope* conditioned treatment water or control water. *Heterocope* treatment water was conditioned by allowing three *Heterocope* from Dam Pond to inhabit 12 liters of kairomone free water. *D. pulex* were monitored and their offspring were collected. Ten 1.2 millimeter *D. pulex* that had either been conditioned to *Heterocope* kairomone or control water were placed into each of three CPCs housed in aquaria. Two *Heterocope* from Dam Pond were starved for six hours and placed into the containers filled with *D. pulex*. After 12 hours, any remaining *D. pulex* were counted and *Heterocope* feeding rates were determined using the equation

$$FR = \frac{-\ln(P_T/P_I)}{XT}$$

where  $P_T$  is the final concentration of *D. pulex*,  $P_I$  is the initial concentration of *D. pulex*,  $X$  is the concentration of *Heterocope*, and  $T$  is the time elapsed during the experiment in days (Dodson 1975). Ten *Heterocope* conditioned *D. pulex* or ten control *D. pulex* were placed into CPCs with no *Heterocope* to serve as controls. The measurements were replicated three times.

### *Predation Experiment- Statistical Methods*

Feeding rates of *Heterocope* on *Heterocope* conditioned *D. pulex* and *D. pulex* that had not been conditioned to *Heterocope* kairomone were compared using ANOVA.

## CHAPTER III

### RESULTS

#### *Life History*

At 24 hours old, *D. pulex* exposed to *Heterocope* kairomone were significantly larger ( $p=0.001$ ) than *D. pulex* not exposed to *Heterocope* kairomone (Figure 1).

Average *D. pulex* body size in the presence of *Heterocope* kairomone was about 0.85 millimeters in length while control organisms measured only 0.78 millimeters. *D. pulex* exposed to *Heterocope* kairomone also produced approximately two times more eggs than *D. pulex* not exposed to *Heterocope* kairomone ( $p=0.001$ ) (Figure 2).

#### *Morphological*

Adding *Heterocope* kairomone did not change daphnid tail spine length. Throughout the summer, no morphological defense, such as the appearance of neck teeth, spines, or helmets, appeared in *D. pulex* exposed to *Heterocope* kairomone.

#### *Behavioral*

The vertical positions of both *D. pulex* and *D. middendorffiana* in the presence and absence of *Heterocope* kairomone were concentrated in the upper 10 centimeters of the VPTs. The vertical position of *D. pulex* was not significantly different in the presence or absence of *Heterocope* kairomone (Figures 3A and B) and the vertical position of *D. middendorffiana* was not significantly different in the presence or absence of *Heterocope* kairomone (Figures 4A and B). However, *D. middendorffiana* did occupy

a significantly higher position in the water column than *D. pulex* ( $p=0.003$ ) (Table 3).

Vertical position of *D. pulex* and *D. middendorffiana* *in situ* was also determined. In Berm Pond II, *D. pulex* were not found in the upper 10 centimeters of the water column, and their numbers steadily increased in deeper samples and were most dense at the bottom of the pond (Figure 5). In contrast, *D. middendorffiana* avoided the water's surface and scattered arbitrarily throughout the rest of the water column.

In the VPTs in the laboratory, there were significantly more *Heterocope* in the upper 10 centimeters than the other strata ( $p<0.010$ ) (Figure 6). However, in pond measurements, *Heterocope* behaved differently. In Camp Pond, *Heterocope* were scattered throughout the water column. At 20 centimeter increments, there were no significant differences in *Heterocope* vertical position (Figure 7). In E6, there were also no significant trends in *Heterocope* distributions (Figure 8).

#### *In situ*

No life history, morphological, or behavioral changes were seen in the *D. pulex* from Berm Pond IIb exposed to *Heterocope* kairomone. At the beginning of the summer, *D. pulex* from Berm Pond IIb produced on average, about 12 eggs per brood. Egg numbers steadily decreased throughout the duration of the experiment. The average egg number per brood for the summer in Berm Pond IIb was 8.0 eggs, while average egg number per brood in Berm Pond III was 7.1 (Figure 9).

In Pond B, where *Heterocope* kairomone was added, an increase in egg number was noted towards the end of the sampling period. On July 25 and 28, there was a

significantly higher average egg number in Pond B compared to Pond A ( $p=0.000$ ) (Figure 10).

#### *Predation Experiment*

There was no significant difference in the predation rates of *Heterocope* on *D. pulex* that had been conditioned to *Heterocope* kairomone versus *D. pulex* that had not been conditioned to *Heterocope* kairomone.

## CHAPTER IV

### DISCUSSION

#### *Life History*

In the laboratory study, *D. pulex* exposed to *Heterocope* kairomone increased average size at birth and produced more offspring. Because *Heterocope* and *D. pulex* do not coexist, these responses may seem ill adapted, but increasing size and egg number may ensure *D. pulex*'s survival if *Heterocope* invade a *D. pulex* pond. As *D. pulex* increase in size, they become less susceptible to *Heterocope* predation (Luecke and O'Brien 1983, Dodson 1984) while increasing egg numbers may allow *D. pulex* to compensate for the mortality caused by *Heterocope* predation. These phenotypic changes may allow *D. pulex* to survive until the invading *Heterocope* die out. The mechanism that prevents *Heterocope* from surviving in these small *D. pulex* ponds is unknown, but presumably, *D. pulex* will only have to survive in the presence of *Heterocope* for at most, the summer.

#### *Morphological*

Since smaller *D. pulex* and *D. middendorffiana* are most susceptible to *Heterocope* predation (Luecke and O'Brien 1983, Dodson 1984), any morphological changes would have appeared during juvenile stages. Increasing tail spine length is a successful morphological defense commonly adopted by other daphnid species (Dodson

1989, Tollrian 1990, Brancelj *et al.* 1996, and Carmujo and Boavida 2000). However, no significant differences were found between the tail spine length of *D. pulex* exposed to *Heterocope* kairomone and those not exposed to kairomone. The lack of *D. pulex* morphological response could be from the lack of exposure of *D. pulex* to *Heterocope* kairomone in the wild or because *D. pulex* simply do not have a morphological response to *Heterocope* kairomone. Another explanation for this lack of response could be *D. pulex* do not budget energy for morphological responses to kairomone from any predator. Various clones of *D. pulex* grow neck teeth in response to *Chaoborus* kairomone but arctic *D. pulex* do not show this response (Luening 1995). Induction of morphological defenses could simply be too costly in an environment where zooplankton in small ponds only have a short growing season.

#### *Behavioral*

Neither *D. pulex* nor *D. middendorffiana* responded to *Heterocope* kairomone by altering their vertical position. In laboratory experiments, both species were concentrated in the upper 10 centimeters of the water column, but a test of vertical position *in situ* indicated daphnids do not tend to occupy surface waters. *D. pulex* and *D. middendorffiana*'s utilization of the upper portion of the water column in laboratory experiments may indicate the light cue was inadequate for pond dwelling daphnids. Interestingly, lake dwelling *D. middendorffiana* exposed to the same light cue as pond dwelling *D. middendorffiana* utilized deeper depths in the VPTs suggesting that the light cue was adequate for lake dwelling *D. middendorffiana*.



Because *Heterocope* are susceptible to UV light (Luecke and O'Brien 1981), one would assume *Heterocope* would occupy deeper positions in the water column, but tests of *Heterocope*'s vertical position in fishless ponds did not indicate *Heterocope* avoided the upper portions of the water column. In fact, *Heterocope* vertical distribution was similar throughout the water column. It appears that without vertebrate predators to regulate *Heterocope*'s utilization of the photoic zone, daphnids cannot avoid *Heterocope* in the water column. Knowing *Heterocope*'s vertical position in ponds, we would not expect daphnids to respond to *Heterocope* kairomone by preferring to occupy certain depths.

#### *In situ*

While *D. pulex* from Berm Pond IIb did not respond to *Heterocope* kairomone, *D. pulex* from Pond B did have a phenotypic response. Because Pond B is smaller than Berm Pond IIb and the same number of *Heterocope* was added to both ponds, *D. pulex* from Pond B were exposed to a higher concentration of *Heterocope* kairomone. Previous studies have shown that daphnids exposed to higher predator kairomone concentrations have a greater response than those exposed to lower concentrations (Van Gool and Ringelberg 1998, Tollrain 1993). The larger size of *D. pulex* from Berm Pond IIb compared to those from Pond B could also explain the lack of response to *Heterocope* kairomone in Berm Pond IIb organisms. Because *D. pulex* from Berm Pond IIb were larger and had more eggs, they presumably were already allocating maximum energy to growth and reproduction. They might not have had the extra energy to increase already high egg numbers. Because the increased egg number was only seen in *D. pulex* from

Pond B and not in *D. pulex* from Pond A, the change may not in actuality have been a response to the *Heterocope* kairomone. Perhaps this pond was inhabited by different clones of *D. pulex* that simply had more eggs per brood late in the summer than *D. pulex* clones in Pond A.

This experiment did give insight into the differences in the life history strategies adopted by *D. pulex* inhabiting permanent versus ephemeral ponds. *D. pulex* from the Berm Ponds had higher egg numbers per brood than *D. pulex* from the Road Ponds. This difference may be explained by food availability or water temperature. Daphnids produce the most eggs at lower temperature and higher food resources (Guisande and Gliwicz 1992, Giebelhausen and Lampert 2001). The Berm Ponds both had a maximum depth of 1.1 meters while Pond A maximum depth was only 28 centimeters and Pond B maximum depth was only 36 centimeters. At the deeper portion of the Berm Ponds, the temperatures were cooler than the surface waters (Table 2). The lower temperatures in the Berm Ponds could explain the increased egg numbers in those daphnids compared to daphnids from the Road Ponds. Another possible explanation for higher egg numbers in the Berm Ponds is the larger *D. pulex* size. Larger daphnids commonly have higher egg numbers (Brambilla 1982).

Because energy devoted to reproduction is determined when *D. pulex* are one day old (Mikulsk *et al.*, 2004), the *D. pulex* from Berm Pond IIb may not have had enough time to make a life history response. Because arctic *D. pulex* reach reproductive maturity at about two weeks after birth (personal observation), effects from the *Heterocope* kairomone would not be detected until at earliest 14 days after the *Heterocope* kairomone

addition and at most four weeks after *Heterocope* kairomone addition. *D. pulex* from Berm Pond IIb may simply not have had enough time to respond to *Heterocope* kairomone.

Other interesting information gathered from this study included the patchy distribution of *D. pulex* within ponds. Every sampling date, three separate horizontal tows were taken at arbitrary locations in the ponds. These locations varied in both depth and distance from the shoreline. Previous studies have shown that daphnids prefer pelagic habitats (Gliwicz 1992, Lauridsen *et al.* 2001). Because the replicate samples were arbitrarily taken at differing depths and locations in the ponds and *D. pulex* densities varied between samples from the same dates, this lends support to the phenomenon that daphnids tend to neglect certain sub-habitats within ponds while exploiting others.

#### *Predation Experiment*

The predation experiment was designed to show any behavioral or morphological changes in *D. pulex* that had been exposed to *Heterocope* kairomone. While no significant differences in predation rates were found between treatment and control daphnids, some behavioral differences may not have been detected due to the experimental design. For example, if *D. pulex* increased their swimming speed in response to *Heterocope* kairomone, this response could not have been used to its full potential in the small CPCs. Another possible *D. pulex* response not measurable in this experiment was aggregation. Aggregation has been shown to effectively reduce predation (Hamilton 1971). Because only ten *D. pulex* were placed into each CPC, an aggregation of adequate size to reduce *Heterocope* predation may not have been able to

form. Alternatively, *D. pulex* exposed to *Heterocope* kairomone may not have had any morphological or behavioral response to *Heterocope* kairomone.

#### *Future Studies*

Further investigation is needed to fully understand the complex relationship between *D. pulex*, *D. middendorffiana*, and *Heterocope*. While this study shows *D. pulex* from one pond respond to *Heterocope* kairomone, understanding why these animals responded but others did not is important. This study also gives insight into the differences in life history and morphology of *D. pulex* inhabiting ephemeral and permanent ponds. Further studies should look deeper into these differences to look at why these different strategies are adopted.

#### *Conclusions*

This study showed that *D. pulex* responded in the laboratory to *Heterocope* kairomone water from Foggy Pond 14 by increasing size at 24 hours old and producing more eggs per brood. *In situ*, *D. pulex* also responded to *Heterocope* kairomone by increasing average egg number per brood. Even though *D. pulex* and *Heterocope* do not coexist in arctic ponds, *D. pulex* do show phenotypic plasticity in response to *Heterocope* kairomone. The ability of *D. pulex* to respond to the kairomone may allow *D. pulex* to survive a *Heterocope* invasion.

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## Appendix: Tables and Figures

Table 1: Experimental pond abiotic characteristics. Temp is temperature measured in degrees Celsius, SpCond is conductivity measured in MilliSiemens per centimeter, zone is where the parameter was taken in the pond, pH is measured in units, LDO% is percentage of dissolved oxygen saturation, max depth in the maximum depth of the pond, and surface area is the approximate surface area of the pond measured in meters squared.

Pond	Date	Temp °C	SpCond mS/cm	Zone	pH Units	LDO% Sat	Max Depth m	Surface Area m <sup>2</sup>	Pond Type
A	7/25/2006	13.88	0.3324	surface	7.67	126.5	0.28	60	Ephemeral
B	7/25/2006	14.16	0.1448	surface	7.57	115.3	0.36	128	Ephemeral
Berm IIB	7/26/2006	11.43	0.0674	surface	5.92	79	1.1	120	Permanent
Berm IIB	7/26/2006	6.04	0.3353	pelagic	5.87	70.5			
Berm III	7/26/2006	12.05	0.0647	surface	6.49	73.9	1.1	182	Permanent
Berm III	7/26/2006	9.53	0.076	pelagic	6.37	64			

Table 2: Summary of experiments, organisms studied, responses, locations, and variables measured.

Experiment	Organism	Response	Location	Variables Measured
1	<i>D. pulex</i>	Life History	Laboratory	Size Egg #
2	<i>D. pulex</i>	Morphological	Laboratory	Body position
	<i>D. middendorffiana</i>		<i>In situ</i>	Body features
3	<i>Heterocope</i>	Behavioral	Laboratory	Vertical Position
	<i>D. pulex</i>		<i>In situ</i>	
	<i>D. middendorffiana</i>			
4	<i>D. pulex</i>	Life History	<i>In situ</i>	Egg #
		Morphological		Size
		Behavioral		
5	<i>D.pulex</i>	Morphological	Laboratory	Survival
		Behavioral		

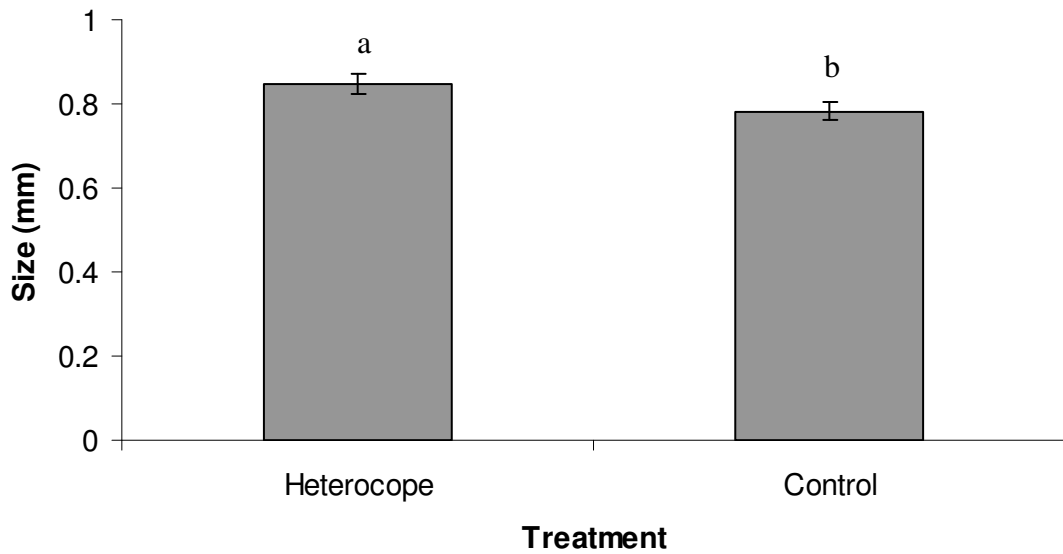


Figure 1: Size of *D. pulex* at 24 hours old exposed to *Heteroscope* kairomone or no *Heteroscope* kairomone. Error bars represent standard error.

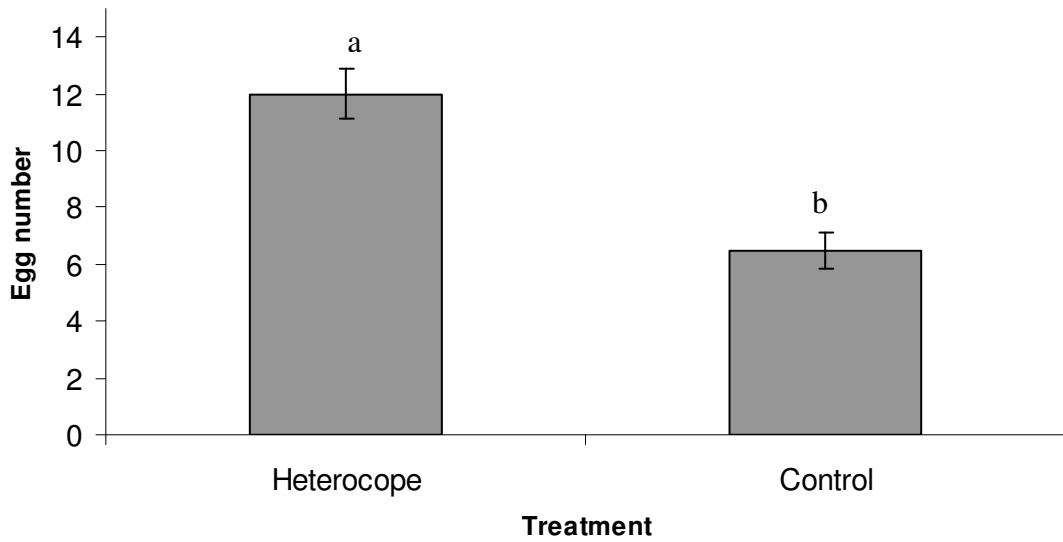


Figure 2: Average egg number per brood in *D. pulex* exposed to *Heteroscope* kairomone and *D. pulex* not exposed to *Heteroscope* kairomone. Error bars represent standard error.

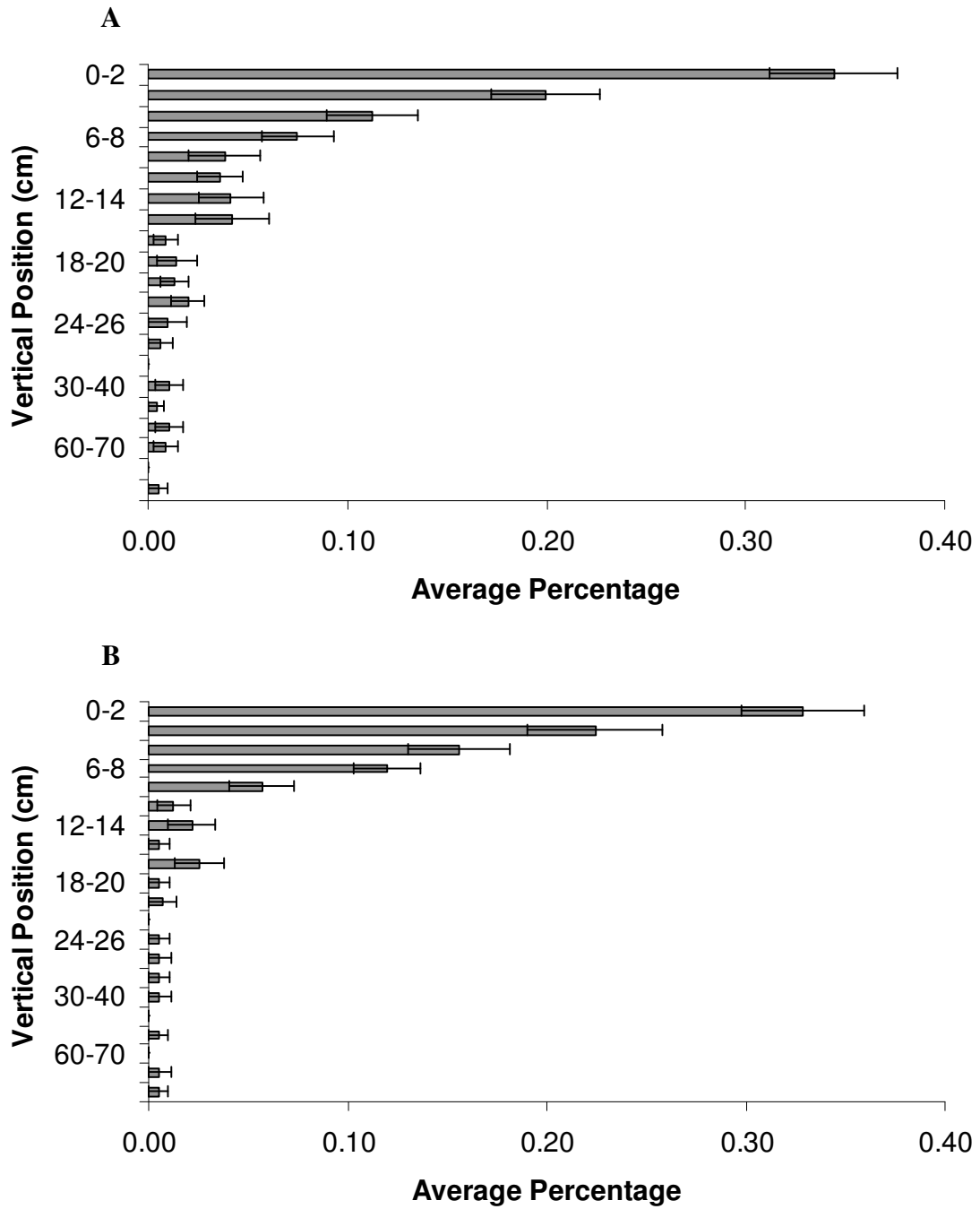


Figure 3: (A) Average percentage of *D. pulex* in strata in control treatment and (B) average percentage of *D. pulex* in strata in *Heterocope* treatment. Error bars represent standard error.

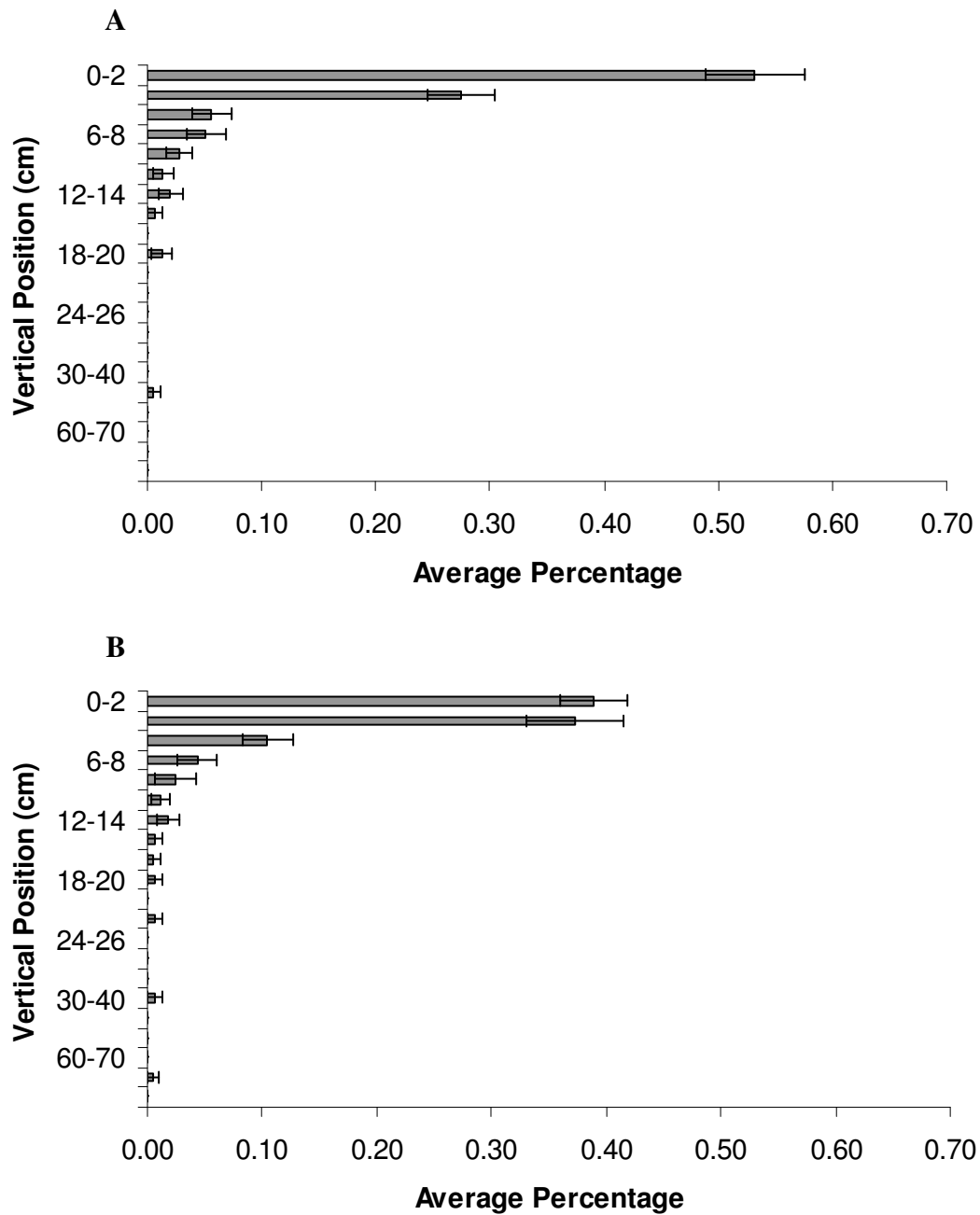


Figure 4: (A) Average percentage of *D. middendorffiana* in strata in control treatment and (B) average percentage of *D. middendorffiana* in strata in *Heterocope* treatment. Error bars represent standard error.

Table 3: P values of vertical position differences between control (c) and treated (t) daphnids.

	<i>D. pulex</i> (c)	<i>D. middendorffiana</i> (t)
<i>D. pulex</i> (t)	0.316	0.003
<i>D. middendorffiana</i> (c)	0.000	0.08

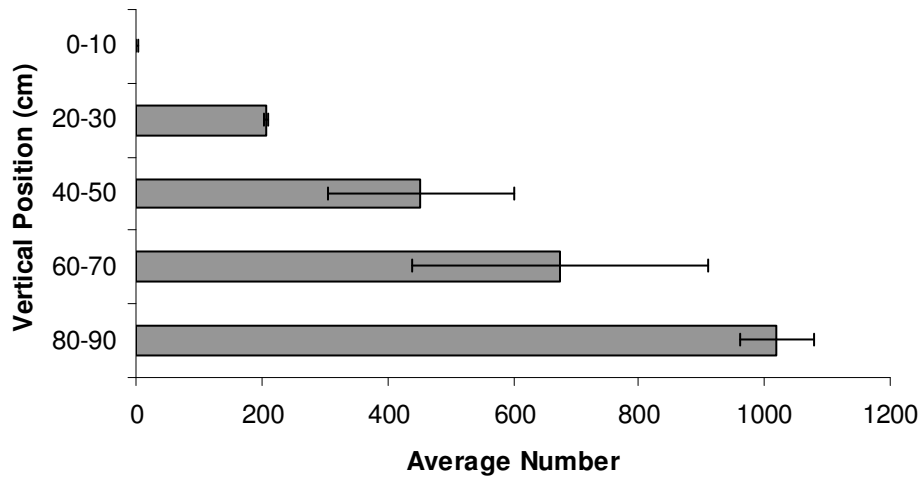


Figure 5: Average vertical position of *D. pulex* in Berm Pond II. Error bars represent standard error.

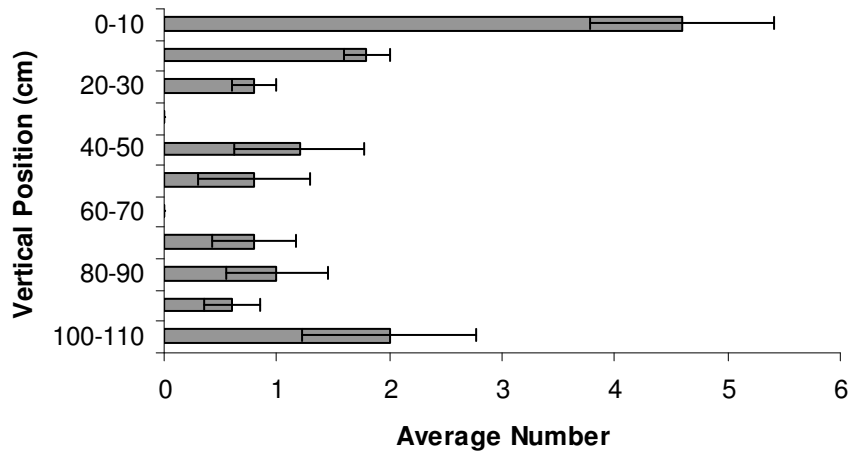


Figure 6: Average vertical position of *Heterocope* in laboratory. Error bars represent standard error.

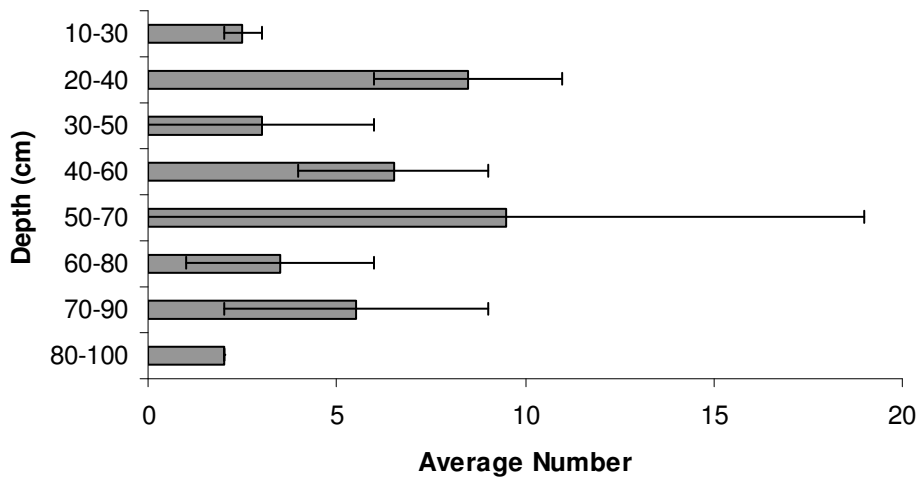


Figure 7: Vertical position of *Heterocope* in Camp Pond. Error bars represent standard error.

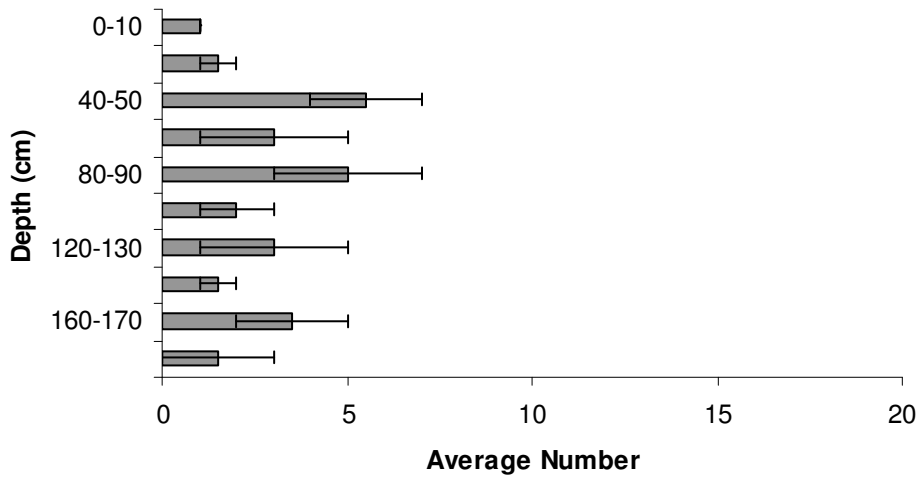


Figure 8: Vertical position of *Heterocope* in E6. Error bars represent standard error.

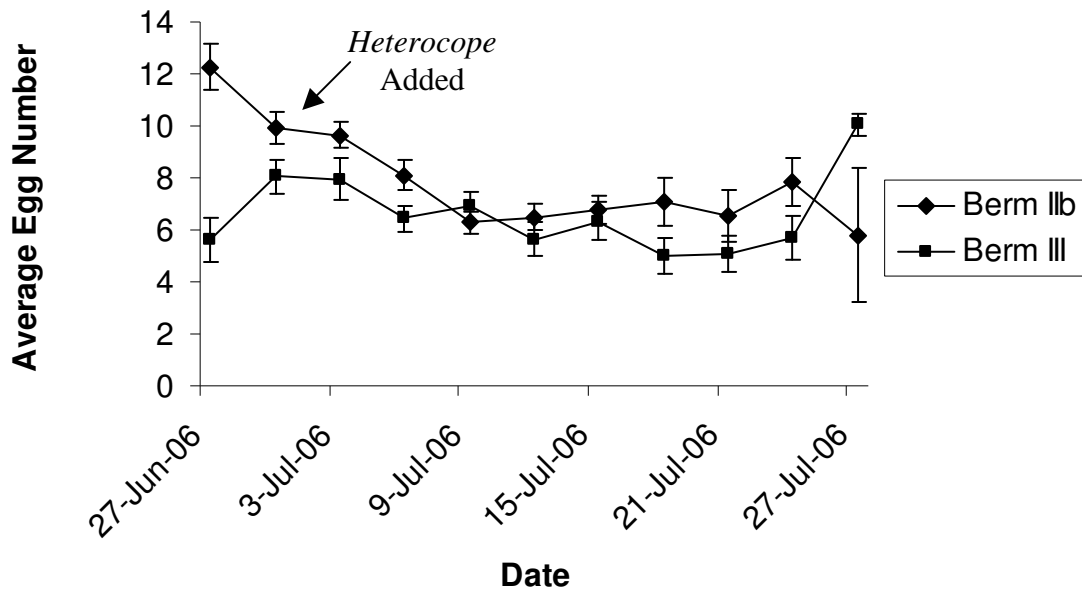


Figure 9: *D. pulex* average egg number in the Berm Ponds. Error bars represent standard error.

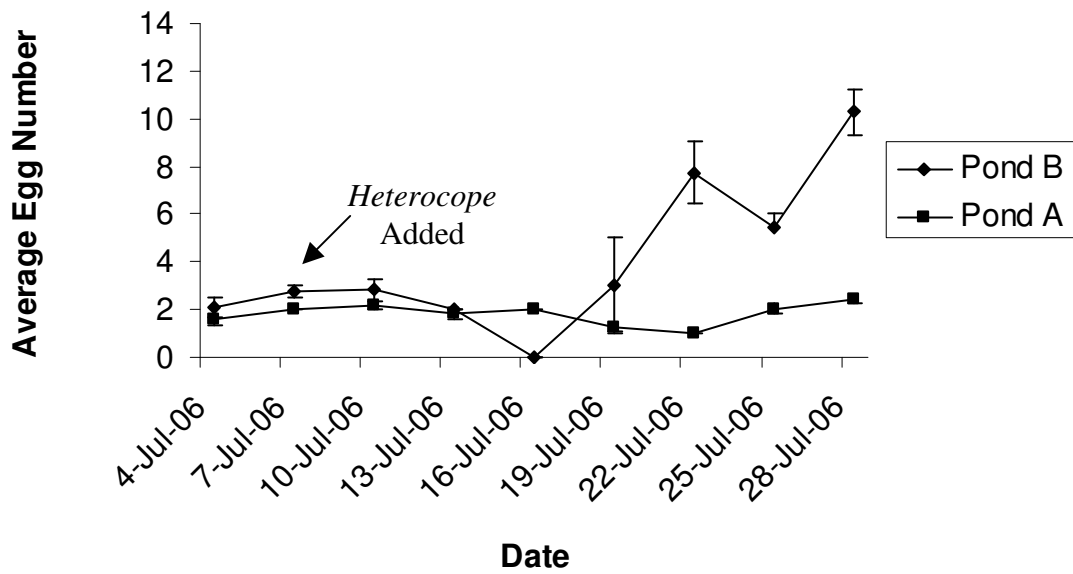


Figure 10: *D. pulex* average egg number in the Road Ponds. Error bars represent standard error.