Survivorship of *Daphnia pulicaria* and *Daphnia pulex* in the presence of *Pimephales promelas*

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Abstract

The freshwater zooplankter *Daphnia* plays an integral role in food web interactions; consequently, it has developed a multitude of adaptations that aid in survival. In this study, survivorship in the presence of a fish predator between two species of *Daphnia*, *Daphnia pulicaria* and *Daphnia pulex*, was compared. Both species differ morphologically in ways that either help or hinder their evasiveness to predators. Four lakes and four vernal ponds were sampled for *D. pulicaria* and *D. pulex* respectively, and *900 Daphnia* were taken from each sample. Six tanks were used for feeding trials, half which were experimental tanks containing one minnow of the species *Pimephales promelas* each (tanks 2, 4, 6) and half which were control tanks containing no minnows (tanks 1, 3, 5: Fig. 1). Additionally, the contents of the control and experimental tanks were identical and as follows: (1, 2) 300 *D. pulicaria*, (3, 4) 300 *D. pulex*, and (5, 6) 150 *D. pulicaria* and *D. pulex*. Minnows were allowed to feed for five hours. Survivorship of *D. pulicaria* was significantly higher than survivorship of *D. pulex* in containers where both species were present (chi-square analysis, $p<0.05$; two factor t-test, $p<0.05$). Survivorship of *D. pulicaria* was not significantly different from survivorship of *D. pulex* in containers where the two species were kept separate (two factor t-test, $p<0.05$). This indicated that the minnows used were exhibiting behavior that aligns with the optimal foraging theory. These results show that monitoring evolutionary adaptations of *Daphnia* is important for more specifically understanding the causes of species divergence amongst this zooplankter.

Introduction

Organisms that play a key role as prey for secondary consumers face the challenge of surviving long enough to reproduce. One organism that has been able to adapt well to this challenge is the freshwater zooplankter *Daphnia*. *Daphnia* species are present in lakes and ponds on every continent. This abundance makes them an organism of great interest as they play a pivotal role in food web interactions of several aquatic ecosystems. *Daphnia* species have the ability to feed on phytoplankton in a manner that is greater in scale and efficiency than other freshwater zooplankton (Miner et al. 2012). Moreover, members of the genus *Daphnia* are also a key source of prey for fish due to their slow movement in water and larger, more conspicuous size (Fisk et al. 2007). This has allowed *Daphnia* to acquire a unique and important position in the food webs of aquatic ecosystems (Miner et al. 2012).

Because they serve as a primary source of prey for many species, *Daphnia* have certain physiological and behavioral adaptations that aid in their survival. In lake environments where larger fish predators are present, *Daphnia* tend to be smaller than their pond counterparts where predators that are far smaller than fish, such as shrimp-like crustaceans called mysids or midge larvae such as that of the *Chaoborus* species, are present (Latta et al. 2011, Vanni 1986). Smaller prey are believed to be less conspicuous to their larger predators and thus more evasive, while larger prey are harder for their smaller predators to ingest (Pijanowska et al. 1993). Pigmentation differences between the two species may also make one species more apparent than the other if it is more heavily pigmented (Luecke and O'Brien 1983). Previous studies have focused on the micro-evolutionary changes in a particular species of *Daphnia* in response to fish predators that are introduced for the first time, finding that *Daphnia* evolve to maintain a smaller body size (Fisk et al 2007, Latta et al. 2007). Fitness studies have also been performed on *Daphnia* species native to different aquatic environments, however such studies have not been performed between *Daphnia* species that are morphologically distinct (Pijanowska et al. 1993). This study is unique in that is tests whether the morphological differences between the two species of *Daphnia* being studied can be attributed to predation differences between habitat types.

In this study, I tested the survivorship between two different species of *Daphnia*, *D. pulicaria* and *D. pulex*. *D. pulicaria* were sampled from lake environments where fish are present and *D. pulex* were sampled from vernal pond environments where fish are absent. The objective of this study was to determine if morphological differences between the two species produces a fitness advantage for one species over the other when fish are present. Because their small size and translucence would help them evade fish predators, I hypothesized that *D. pulicaria* will have significantly higher survivorship over *D. pulex* in the presence of fish predators both when the two species are in the same environment and when the two species are separated.

Methods

Study Sites

Four lakes and four vernal ponds were chosen for sample collection based on previously recorded presence of *D. pulicaria* and *D. pulex* respectively. All lakes and vernal ponds are located on the University of Notre Dame Environmental Research Center (UNDERC) property located in Land O' Lakes, WI, with exception to Palmer Lake, which is just off the UNDERC property located in Land O' Lakes, WI. The lakes sampled were Palmer Lake, Bay Lake, Crampton Lake, and Tenderfoot Lake. The vernal ponds sampled were Vernal Pond 12, Vernal Pond N, Vernal Pond 23, and Vernal Pond 9.

Sampling and Experimental Design

D. pulicaria samples were collected from lakes using a vertical zooplankton tow net at depths of 6-8 m. *D. pulex* samples were collected from vernal ponds using a horizontal zooplankton tow net. After each sample was taken, 900 *Daphnia* from that sample were identified and separated out into four glass collection jars (two containing 300 *Daphnia* and two containing 150 *Daphnia*). These amounts were determined based on a previous survivorship study of *Daphnia* in the presence of mysids (Pijanowska et al 1993). Twelve minnows of the species *Pimephales promelas* were collected from a fifth lake nearby using minnow traps.

Six cylindrical Pyrex tanks measuring 20.7 cm in height and 28.2 cm in diameter were filled with 5 L of water from Tenderfoot Lake at the beginning of each trial. Three of the six tanks were made experimental tanks containing one minnow each that had been starved for at least 24 hours. Three tanks were used as controls and contained no minnows. Aside from whether they contained a minnow or not, the contents of the control and experimental tanks were identical and as follows: (1, 2) 300 *D. pulicaria*, (3, 4) 300 *D. pulex*, and (5, 6) 150 *D. pulicaria* and *D. pulex* (Fig. 1). Samples of *D. pulicaria* and *D. pulex* were paired together based on the time at which the samples were taken from their respective lakes or vernal ponds (the first lake sampled was paired with the first vernal pond sampled). Minnows fed on the *Daphnia* for five hours and were then removed and placed in an area separate from the remaining minnows not used in the trial. This was done to ensure that minnows were only used in the trials once. After the minnows were removed the water in each container was filtered through a 35 µm mesh filter to separate the remaining *Daphnia* out. The *Daphnia* were recounted to determine survivorship. Trials were repeated four times, once for each lake-vernal pond pair.

Statistical Analysis

A chi-square analysis was performed using a 2x2 contingency table to determine if survivorship differed between *D. pulicaria* and *D. pulex* for each trial where the two species where placed together in the presence of a minnow.

A Shapiro-Wilk normality test was performed to determine whether the data was normally distributed. Given that it was ($p>0.05$), a two-sample t-test was performed to determine whether the survivorship between *D. pulicaria* and *D. pulex* differed significantly when each species was alone in the presence of a minnow and when the two species were placed together in the presence of a minnow. All statistics were completed using R, version 2.11.0.

Results

Survivorship of *Daphnia* in the control tanks was consistently near or exactly 100 percent. Thus, the survivorship data collected from the controls was removed from the data pool. A Shapiro-Wilk normality test showed the data to be normal for both the experiments that contained an equal ratio of *D. pulicaria* and *D. pulex* ($p=0.330$) and for the experiments that contained only one species of *Daphnia* ($p=0.488$).

There was a significantly higher number of *D. pulex* consumed than *D. pulicaria* when *Daphnia* from Palmer Lake (68 out of 150 *D. pulicaria* consumed) were paired with *Daphnia* from Vernal Pond 12 (100 out of 150 *D. pulex* consumed), $c^2(1, N=300) = 13.00$ $p<0.001$, when *Daphnia* from Bay Lake (94 out of 150 *D. pulicaria* consumed) were paired with *Daphnia* from Vernal Pond N (131 out of 150 *D. pulex* consumed) $c^2(1, N=300) = 23.04$ $p<<0.001$, when *Daphnia* from Crampton Lake (70 out of 150 *D. pulicaria* consumed) were paired with *Daphnia* from Vernal Pond 23 (122 out of 150 *D. pulex* consumed), $c^2(1, N=300) = 37.63$ $p<<0.001$, and when *Daphnia* from Tenderfoot Lake (72 out of 150 *D. pulicaria* consumed) were paired with *Daphnia* from Vernal Pond 9 (101 out of 150 *D. pulex* consumed) $c^2(1, N=300) = 10.71$ $p<0.001$ (Tables 1-4).

Significant differences were seen between the survivorship of *D. pulicaria* and *D. pulex* when both species were present in equal proportions. Average survivorship of *D. pulicaria* was 0.493 and was therefore significantly higher, $t(5.68) = 3.82$, $p < 0.01$ than the 0.243 average survivorship of *D. pulex* (Fig. 2).

Average survivorship of *D. pulicaria* was 0.565 and average survivorship of *D. pulex* was 0.489 and did not differ significantly $t(5.97) = 1.139$, $p = 0.30$ when the two species were kept separate from each other (Fig. 3).

Discussion

The results obtained to determine survivorship of *D. pulicaria* compared to *D. pulex* were somewhat expected. I hypothesized that that *D. pulicaria* would have significantly higher survivorship than *D. pulex* in the presence of fish predators both when the two species were separated as well as when the two species were together in equal amounts.

The significantly higher survivorship of *D. pulicaria* compared to *D. pulex* was expected because the *D. pulicaria* sampled live in lakes where fish predators are present and thus have many adaptations to evade these predators. Such adaptations include maintaining a generally smaller size than *D. pulex* that live in habitats where fish predators are absent. It has been shown that *Daphnia* that live in habitats where fish predators are originally absent gradually evolve to acquire a smaller size when fish predators are introduced (Dodson 1989). It is thought that the smaller size of the zooplankton decreases their visibility to fish predators and thus is highly advantageous (Hulsmann et al. 2011). In contrast, the *D. pulex* sampled would be at a greater advantage if they maintain a larger size. This is because the predation pressure experienced by *Daphnia* in these habitats comes from predators such as carnivorous zooplankton and midge larvae such as that of the *Chaoborus* species (Vanni 1986). These predators are far smaller than fish and therefore would have a more difficult time digesting larger zooplankton. Thus there would be a preference for smaller, more easily digestible organisms and the large size of *D. pulex* would be advantageous. However, when fish predators were introduced, the large *D. pulex* were more conspicuous than their *D. pulicaria* counterparts. Therefore, the minnows consumed far more of *D. pulex* than *D. pulicaria*.

Differences in pigmentation between different species of *Daphnia* are another evolutionary adaptation that this zooplankter has developed. In areas where fish are present, *Daphnia* often maintain a nearly transparent pigmentation, which in conjunction with their small size, aids in the reduction of visibility to fish predators, which was an adaptation seen in the *D. pulicaria* sampled used in this experiment (Tollrian and Heibl 2004). However, the *D. pulex* sampled instead exhibited a very apparent pink-red pigmentation. This differentiation points to the habitat characteristics of the vernal ponds from which the *Daphnia* were taken. Not only do these ponds exhibit a complete absence of fish predators, but the environment there is also quite anoxic. Therefore a greater production of hemoglobin is necessary to compensate for the little dissolved oxygen present in the water, which in turn gives the *Daphnia* a pink-red color (Fox 1948). However, in the lake water used in this experiment, this pigmentation left *D. pulex* far more noticeable than *D. pulicaria*. Therefore, this partially explains the significantly higher survivorship of *D. pulicaria* over *D. pulex*.

Results obtained from the experiments where *D. pulicaria* and *D. pulex* were separated produced unexpected results since there was no significant difference between the survivorship of each species. There was overall a higher survivorship of *D. pulicaria* compared to *D. pulex*, and this is most likely due to the differing adaptions between the two species discussed above. Nevertheless, the lack of significant results more distinctly points to the feeding preference of the minnows. Only when the minnows were presented with the two species of *Daphnia* at the same time was a clear preference of either species shown. Other planktivorous fish have been shown to exhibit similar predation behavior by choosing larger, more conspicuous prey over smaller, less conspicuous prey when available but still eating the latter when larger prey is not present (Eggers 1982). In this experiment, *D. pulex* was likely preferred because time spent foraging for this species by the minnows was less than it was for *D. pulicaria*. This reduction in foraging time could be attributed to the adaptations *D. pulicaria* has evolved to evade predators. Additionally, the larger size of *D. pulex* offers a greater reward in terms of energy consumed per *Daphnia*. However, when the minnows were not given an option of species type, they consumed both species fairly equally. This would indicate that minnows' behavior aligned with the optimal foraging theory.

The significance shown in the differential survivorship of *D. pulicaria* and *D. pulex* shows that both zooplankton evolutionary adaptations as well as the feeding behavior of minnows continue to be an important topic of study. Because there are an abundance of factors that differentiate the two species, I propose that further studies focus on which of the factors is more important for predation, specifically focusing on size versus pigmentation. Analyzing pigmentation differences alone and their affect on predation would be would be of particular interest as well, specifically in regard to the level of dissolved oxygen in bodies of water. This factor is inclined to change over time due to factors such as increased urbanization that could lead to decreased water quality (Wenner et al. 2004) and it would be interesting to observe the effect that this has on the *Daphnia* in lakes with low dissolved oxygen but where fish predators are also present.

Daphnia are frequently exposed to both environmental and anthropogenic stressors no matter their aquatic habitat; however, they can also evolutionarily adapt to change rather quickly (Altshuler et al. 2011). Understanding how these factors may change, and why, is something that should be greatly considered in order to more specifically understand the causes of species divergence amongst this zooplankter.

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Tables and Figures

	Alive	Eaten
D. pulicaria	82	68
D. pulex	50	100

Table 1 Survivorship of *D. pulicaria* from Palmer Lake mixed with *D. pulex* from Vernal Pond 12 Survivorship of *D. pulicaria* was significantly higher than survivorship of *D. pulex* when both species were present in equal proportions (chi-square analysis, chi-square value=13.001, p=3.114e-3).

	Alive	Eaten
D. pulicaria	56	94
D. pulex	19	131

Table 2 Survivorship of *D. pulicaria* from Bay Lake mixed with *D. pulex* from Vernal Pond N Survivorship of *D. pulicaria* was significantly higher than survivorship of *D. pulex* when both species were present in equal proportions (chi-square analysis, chi-square value=23.040, p=1.587e-6).

	Alive	Eaten
D. pulicaria	80	70
D. pulex	28	122

Table 3 Survivorship of *D. pulicaria* from Crampton Lake mixed with *D. pulex* from Vernal Pond 23 Survivorship of *D. pulicaria* was significantly higher than survivorship of *D. pulex* when both species were present in equal proportions (chi-square analysis, chi-square value=37.630, p=8.551e-10).

	Alive	Eaten
D. pulicaria	78	72
D. pulex	49	101

Table 4 Survivorship of *D. pulicaria* from Tenderfoot Lake mixed with *D. pulex* from Vernal Pond 9 Survivorship of *D. pulicaria* was significantly higher than survivorship of *D. pulex* when both species were present in equal proportions (chi-square analysis, chi-square value=10.705, p=0.00107).

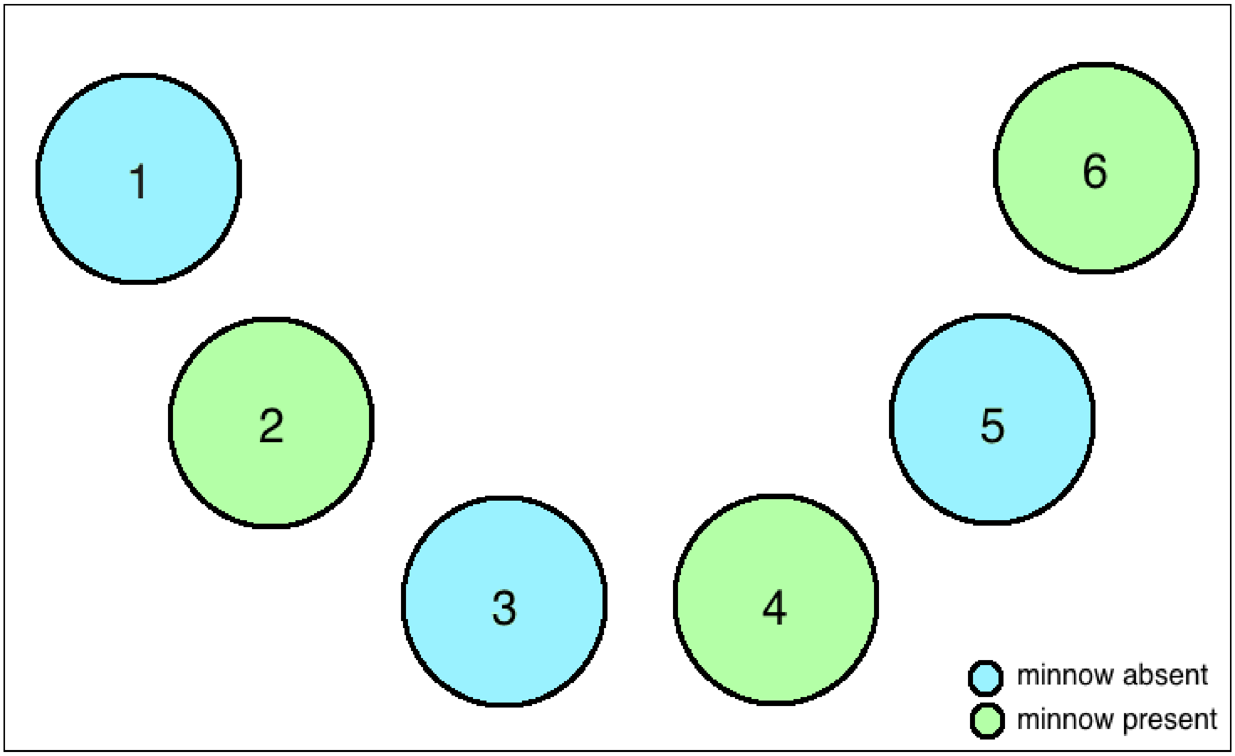

Figure 1 Experimental Setup. Each circle represents a cylindrical tank. Colors differentiate presence or absence of a minnow. Additional contents of each tank are as follows: (1, 2) 300 *D. pulicaria*; (3, 4) 300 *D. pulex*; (5, 6) 150 *D. pulicaria* and *D. pulex*.

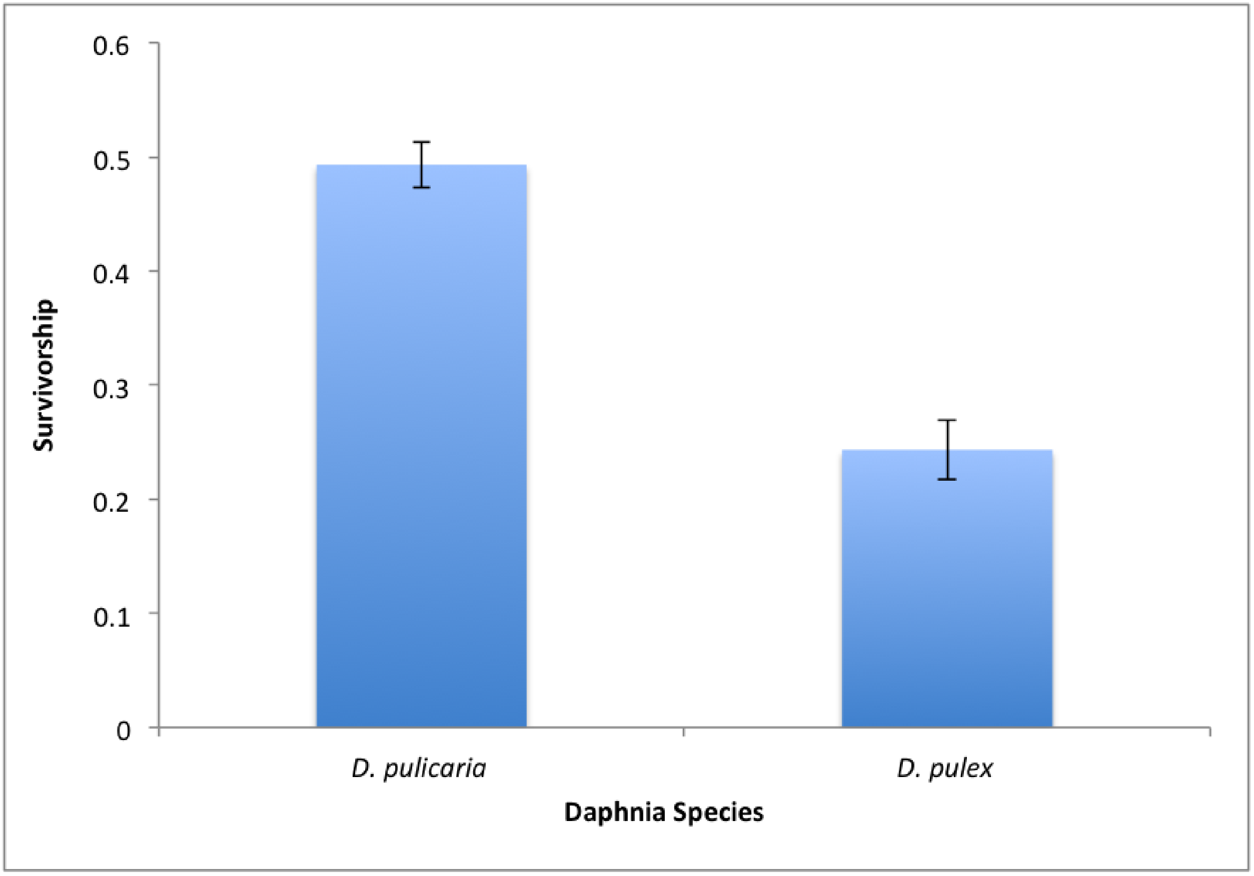

Figure 2 Survivorship of *D. pulicaria* and *D. pulex* when mixed. Survivorship of *D. pulicaria* was significantly higher than survivorship of *D. pulex* when both species were present in equal proportions (two sample t-test, p=0.00975). Error bars represent standard error.

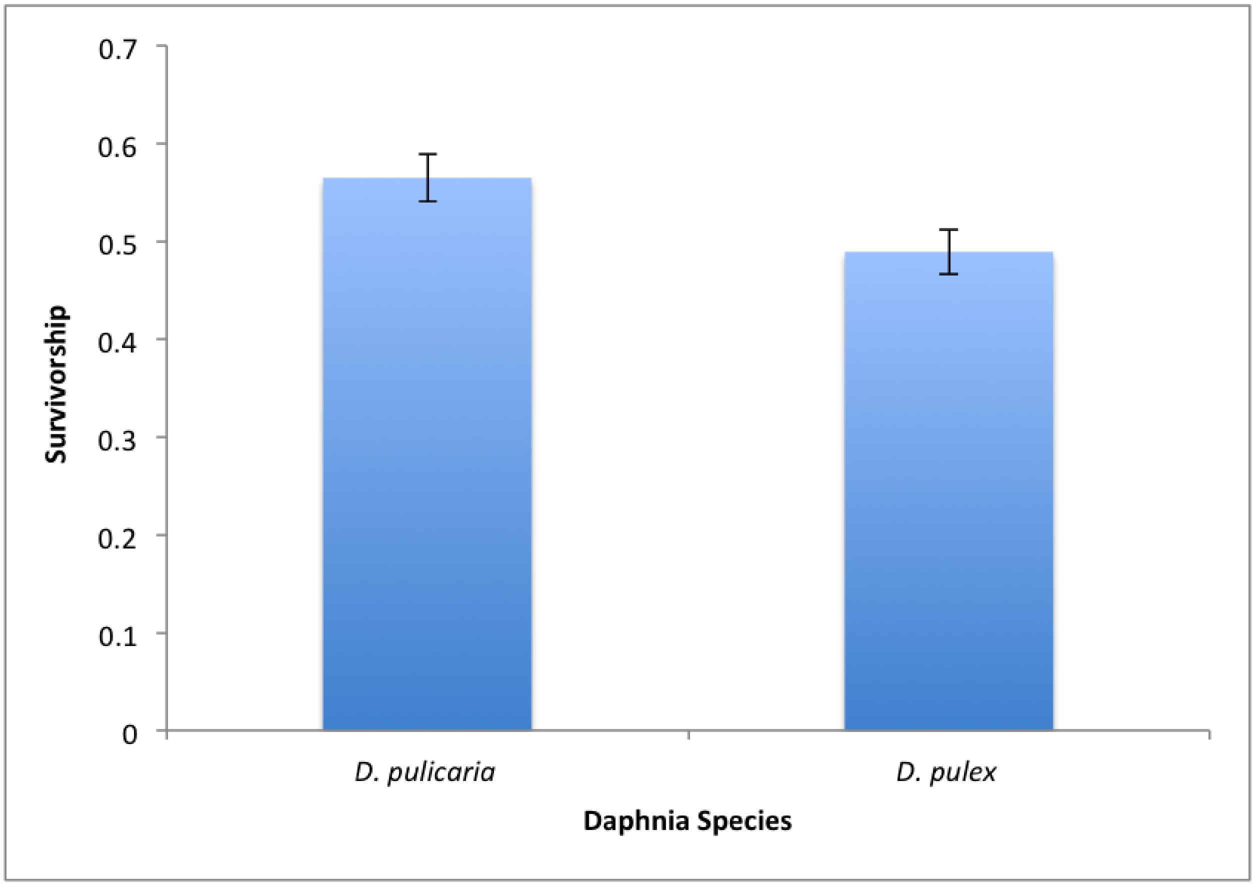

Figure 3 Survivorship of *D. pulicaria* and *D. pulex* when separated. Survivorship did not differ significantly between *D. pulicaria* and *D. pulex* when the two species were separate from each other (two sample t-test, p=0.2984). Error bars represent standard error.