INTERACTIONS BETWEEN INTERMEDIATE PREDATORS AND PREY IN SUBALPINE PONDS

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ABSTRACT

The larval dragonfly species *Aeshna palmata* and the larval diving beetle species *Acilius sp.* are intermediate predators in subalpine pond system food webs that act as both predator and prey. Interactions between the two have not been explored. This study uses comparative feeding trials to discover their interactions with each other through competition or anti-predator behavior. It was found that the *Aeshna palmata* consumed a significant amount of Copepods, and Chironomids, while the *Acilius sp.* consumed significant amounts of all three prey (Copepods, Chironomids, and Chaobrids). There was no interaction found between the intermediate predators. This has important implications for the food web, such as guild significance and prey control.

INTRODUCTION

While there are top predators in some ecosystems, in others there are individuals that act as both predator and prey within their local food webs (Lima, 1998). These intermediate predators have been studied in various animals (invertebrates and vertebrates) and across different ecosystems, examining prey behavior in the presence of predators. In these studies, the prey engages in anti-predator decisions with trade-offs to its own fitness (Lima, 1998). This behavior results in prey becoming more challenging to locate and consume (Lima, 1998). According to optimal foraging theory, prey that provides the highest amount of energy per time spent should be preferred by predators (Dudova et al, 2019). Yet, the resulting selection of prey depends on the predator's nutritional demands and previous diet (Dudova et al, 2019). For intermediate predators, both of the previous statements apply; their consumption is dependent on both prey availability and present predators in the system.

This experiment will study intermediate predators at the Mexican Cut, a nature preserve

maintained by the Nature Conservancy and accessed through the Rocky Mountain Biological Laboratory (RMBL). In the Mexican Cut, there are multiple permanent, semi-permanent, and temporary ponds in the preserve (Wissinger et al 1999). Aquatic invertebrates have been studied for decades at RMBL; however, *Aeshna palmata* and *Acilius sp.* larvae are only known broadly based on their role in the food web of the pond systems at the Mexican Cut. Prey selectivity data are crucial for creating accurate maps of the topology of food webs, as well as predicting things such as species invasions, extinctions, and stability (Klecka & Boukal, 2012). This study aims to explore the feeding habits of *Aeshna palmata* and *Acilius sp.*, as much of the information known about wetland intermediate predators is from lower elevations. Also, it investigates what effects the presence of another intermediate prey has on the observed feeding habits. *Aeshna palmata* acts as a predator of certain aquatic invertebrates and zooplankton, while simultaneously being prey for the Arizona Tiger Salamander and aquatic invertebrates bigger than them (e.g., *Acilius* beetle larvae) (Wissinger et al 1999). *Aeshna* is one of the two dragonfly species found at the permanent to semi-permanent pond sites 1, 5, 9, and 12 (recently, a third species has been identified) (Wissinger et al 1999). The dragonfly larvae have been found to take multiple years to reach pupation evident by multiple year-class larvae (Wissinger et al 1999). Consequently, they can be vulnerable to predation based on their size class. *Acilius* is in the family Dytiscidae, which are predaceous diving beetles. Dytiscidae are often at the top of the aquatic invertebrate food webs and are known voracious predators of any organism they can fit their mandibles around (Ohba, 2009). *Acilius* is also an intermediate prey in the same pond systems as the *Aeshna palmata* (Wissinger et al 1999). However, there is not much known about the interactions between *Acilius* and Odonates (dragonflies and damselflies) at RMBL and subalpine ponds in general, despite their co-occurrence in pond food webs. We also know little about the feeding

habits and behaviors of dragonfly larvae and whether that varies among different dragonfly species.

This study investigates the feeding habits of *Aeshna* and *Acilius* via feeding trials and whether the presence of another intermediate predator changes these feeding habits. It is hypothesized that the beetle will feed more on the chironomids (midges) and less on the copepods and chaoborids (phantom midges). The reverse is hypothesized for dragonflies. This is expected because midges are benthic prey (spend most of their time in the benthos), while phantom midges and copepods are pelagic (swim around the water column). Observed behaviors in other pond systems for dragonfly larvae are that they like to perch at the top to hunt, thus consuming more of the pelagic prey, while beetles are observed to swim near the bottom of the ponds, thus consuming more benthic prey.

METHODS

In the Mexican Cut, there are permanent, semi-permanent, and temporary ponds where *Aeshna palmata, Acilius sp.,* chironomid and phantom midge larvae, and copepods were obtained for use in the experiments. Benthic larvae were captured using a d-net to sweep the benthos of the pond, whereas pelagic larvae and copepods were captured with a zooplankton net. A cumulative total of 18 *Aeshna palmata* (from pond 3) and *Acilius sp.* (from pond 8) were collected, respectively. Instar was kept consistent for each species. The *Aeshna palmata* were picked at a medium instar. The *Acilius sp.* were picked at their 3rd and final instar. The biomass will be calculated using the photos taken. A cumulative total of 300 chironomids, chaoborids, and copepods (100 each) was used as prey organisms.

There were four 12-quart sweater boxes set up for each replicate of the experiment, with a total of 6 replicates for the experiment. Each of the four sweater boxes contained different intermediate predators represented as follows: one with two *Aeshna palmata,* one with two *Acilius*, one with one each of the *Aeshna palmata* and *Acilius,* and a final one with no predators that acted as a control for background prey mortality. As *Aeshna palmata* prefers to hunt by perching, two strings were attached in each box to provide a climbing space. One end of the string was tied around a 2.5cm x 2.5 cm marble square; the other end was tied to a long bamboo stick. Two long bamboo sticks were stretched the length of the four sweater boxes to be the top anchor for both strings in all four treatments. As chironomids like to burrow into the benthos layer, a PVC pipe that stretched roughly the length of the sweater box was placed diagonally at the bottom to provide cover. The marble tiles previously mentioned also provided hiding space.

The number of both predators was counted beforehand, and each individual was kept in a sweater box and left alone for roughly 23 hours to reach similar hunger levels before treatments were set up. The order of which treatment went into which of the 4 sweater boxes was randomly chosen by blind picking one of four coordinate-labeled ping-pong balls (N.E.S.W.). After the predators were put in the treatments, 10 each of the chironomids and chaoborids were placed in each sweater box. Around 22 hours later, as a safeguard (since the number of chironomids and phantom midges were hard to count in the sweater box and the rough estimate of each was near the same), 10 copepods were added into each sweater box. Roughly 47 hours after the initial prey was put in, spot observations were noted. We went around to each tank, taking note of which of three behaviors they were exhibiting: swimming (out in the open), still (out in the open), or hiding. These observations were taken twice. Then the contents of the sweater box were transferred to a white tray, and the remaining prey were counted. Each of the remaining predators

was photographed in front of a grid so biomass could be estimated from established length-mass relationships.

The consumption of each species was analyzed with a separate two-way ANOVA, where the number of prey remaining was the response variable in the analysis, with the presence or absence of each predator and their interaction included as the predictor variables.

RESULTS

Prey were consumed by both predators, although the magnitude varied by species. Both dragonflies ($F = 58.13$, $P < 0.0001$) and beetles ($F = 126.37$, $P < 0.0001$) significantly reduced copepod abundance, but the magnitude of the reduction was lower in both the double beetle treatments (Fig 1). There was no significant interaction between the two intermediate predators ($F= 0.722$, $P= 0.405$).

Both dragonflies ($F = 21.13$, $P=0.0001$) and beetles ($F =12.03$, $P= 0.0024$) significantly reduced midge abundance, but the magnitude of the reduction was lower in both the double dragonfly treatments (Fig 2). There was no significant interaction between the two intermediate predators ($F= 3.09$, $P= 0.0938$).

There was a significant reduction in phantom midge abundance in the double beetle treatment ($F=4.59$, $P= 0.04463$). There was no significant reduction in the double dragonfly treatment ($F= 1.17$ $P= 0.2917$). There was also no significant interaction between the intermediate predators ($F= 0.11$, $P= 0.7386$). This is represented by Figure 3.

Predator species differed in their activity levels (Fig 4). The dragonflies were observed to be hiding 30 out of 36 times, out in the open but still 5 out of 36 times, and out in the open swimming 1 out of 36 times. The beetles were more active, observed to be hiding 2 out of 36

times, out in the open but still 13 out of 36 times, and out in the open swimming 21 out of 36 times.

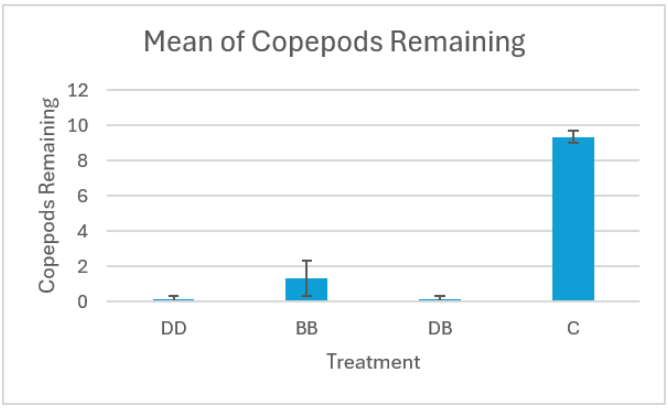

Figure 1: The mean number of copepods left for each treatment: DD (double dragonfly), BB (double beetle), DB (dragonfly and beetle), and C (control). Error bars represent +/- one standard error of the treatment mean.

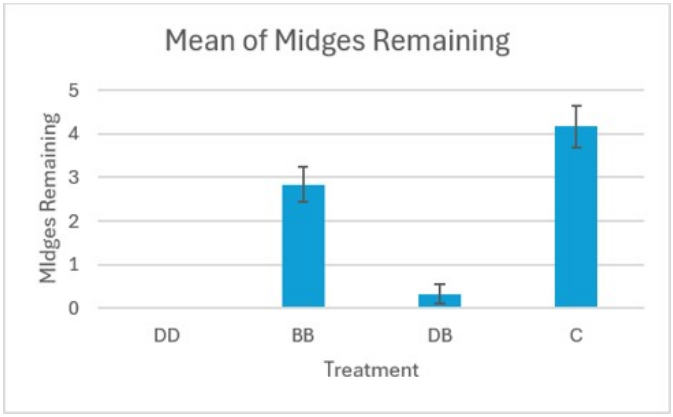

Figure 2: The mean number of midges left for each treatment: DD (double dragonfly), BB (double beetle), DB (dragonfly and beetle), and C (control). Error bars represent +/- one standard error of the treatment mean.

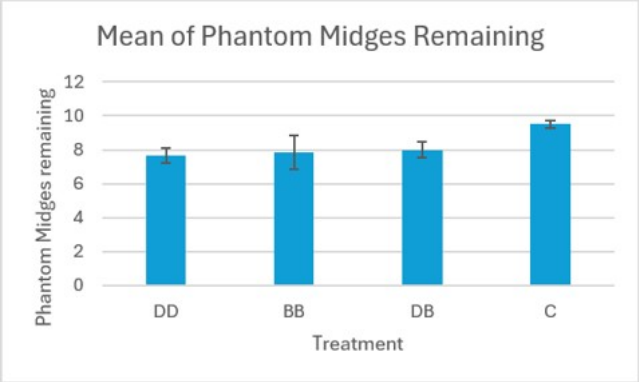

Figure 3: The mean number of phantom midges left for each treatment: DD (double dragonfly), BB (double beetle), DB (dragonfly and beetle), and C (control). Error bars represent +/- one standard error of the treatment mean.

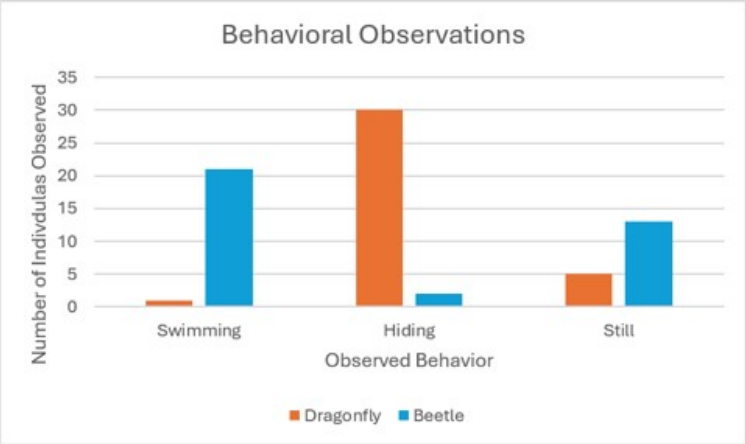

Figure 4: The total number of times each behavior was observed in each predator. Still and swimming were noted when the individual was out in the open.

DISCUSSION

This study aimed to gain insight into the feeding habits of *Aeshna* and *Acilius* larvae and, given their roles as intermediate predators, whether their presence influenced the others' feeding behaviors.

The dragonfly larvae were effective predators for both midges and copepods and were not effective predators for phantom midges. While the beetle larvae were effective predators for all three prey species. Thus, our hypothesis that the beetle larvae would feed on more chironomids (midges) and fewer copepods and chaoborids (phantom midges), while dragonfly larvae would feed more on copepods and phantom midges and less on midges, was not supported by the results. This could imply that the dragonfly larvae prey more heavily on copepods and midges in the pond systems, as their feeding behaviors appear more specialized in the experiment. This is supported by the fact that in both the copepod and midge treatments, the dragonfly larvae had higher prey consumption than beetle larvae. In contrast, beetles had a stronger impact on phantom midges, which may have been a consequence of their more active swimming behaviors. While the experiment had limited prey, it still showcased how similar niches were utilized differently between the two intermediate predators. As the dragonfly and beetle larvae are using the same resources, the predator guild would be diverse. In pond systems, the prey competition is different in each pond (especially in pond systems with the presence of the salamanders). These results give us insight into the predator’s prey selectivity, and when looking at a specific pond with only 2 out of the 3 prey, it gives us insight into what they might choose and at what rates.

In all the trials, there was no significant evidence that the two predators affected the others’ predation rates. That is, the presence of another intermediate prey does not change the other’s feeding habits; therefore, there’s less of a chance that adding another species would

change the predation rate and vice versa. While the predator guild would be diverse, there is no evidence that the predators affect each other, meaning that if one of the predators were lost, the predation rates of the other would be unaffected. This implies that the prey population, if other resources allow, would grow past normal population rates. While they did not affect the other's predation rate, it was observed after the experiment, in one of the dragonfly/ beetle treatments (number 14), after spot observations, that the previously alive beetle was no longer present. It is believed that the dragonfly larvae consumed the beetle larvae. This suggests interactions between these two intermediate predators may have occurred if the experiment duration was longer, or if they were depleted of food for longer. As it is common in odonates and diving beetles, food depletion may increase intraguild predation (Klecka & Boukal, 2012), which occurred after the experiment was run. In natural pond systems, encounter rates of predators and prey are going to be naturally smaller as there is more area for them to traverse. Therefore, predation rates would be less significant. There are ponds where the dragonfly and beetle larvae overlap with each other and the three given prey; however, there are also ponds where one or more species are not present. The results give us insight into what the predation rates would look like even without the presence of the other predator/ prey. The ponds also all have different habitats and population volumes. In natural conditions, there is less abundance of prey and lower encounter rates, meaning the likelihood of intraguild predation could be higher.

While the beetle larvae were shown to be statistically significant predators of the phantom midge, Figure 3 is seen to have a similar reduction in phantom midges' abundance in both the double beetle and dragonfly treatments. This is likely the case because there was a lot more variability in the beetle treatment, with some replicates having strong predation. This

suggests that an individual predator can be more or less efficient at preying, meaning individual variation is a factor to be considered.

The behavioral observations of these predators are likely mechanisms that explain variation in predation rates between species. Dragonfly larvae were found hiding or still for most observations, while the prey they were most effective at hunting were benthic prey. The beetles were observed to be out in the open most frequently and often found swimming. They were effective at preying on all the prey species. Previous observations of the behaviors of the dragonfly and beetle larvae were noted to have them both in significantly different zones of the ponds. Beetle larvae were noted to be benthic, while dragonfly larvae were noted to perch while hunting. The opposite was observed in the artificial tanks. The differences in their behaviors at the time of the experiment indicated the beetle larvae to be a great deal more active and out in the open compared to the dragonfly larvae, who were found most often at the bottom hiding. As the benthic prey stayed near the bottom, the less active dragonfly larvae were more likely to encounter the midges (in each of the double beetle tanks, no midges were remaining). While the more active beetle larvae consumed significant amounts of each prey. While in the natural pond systems, their position in the pond might prevent such predation rates, in the artificial ponds their activity level seemed to dictate the effectiveness of the predation overall and which prey they were more effective at consuming. It was noticed that the beetle larvae seemed to be reacting to their reflection, as they were seen to be bumping into the sides of the plastic bins; this could’ve affected their behavior and feeding habits. The fact that the beetles swam around more could be a factor in this observation, as both predators were subjected to the same conditions. In the pond systems, their location and activity levels would directly affect their prey selection. In smaller ponds, the encounter rates could be similar to those in the artificial tanks; therefore, predation

rates would likely stay the same, but in larger ponds, their prey encounter rates would be lower. Therefore, the effectiveness of their predation seen in the artificial tanks will have more impact on fitness.

An important limitation of this study is that observers were not able to see the tiny prey while doing quick observations. For this reason, at the 24-hour mark, when checking on the prey to see if there was a reduction in abundance to see if the experiment needed to be run for another 24 hours, the abundance couldn't be deciphered. This led to us adding copepods to ensure results. While the copepods were significantly reduced in abundance, this time difference in the availability of different prey species could have affected the results. Regardless, the experiment provided evidence that the intermediate predators were effective at consuming different prey and did not impact the predation of the other. This has implications for pond systems where, accounting for pond size and prey availability, there is a diverse predator guild with no intraguild effect, meaning that adding or removing a predator has no effects on overall predation, leading to cascading effects on prey abundance in the pond systems.

LITERATURE CITED

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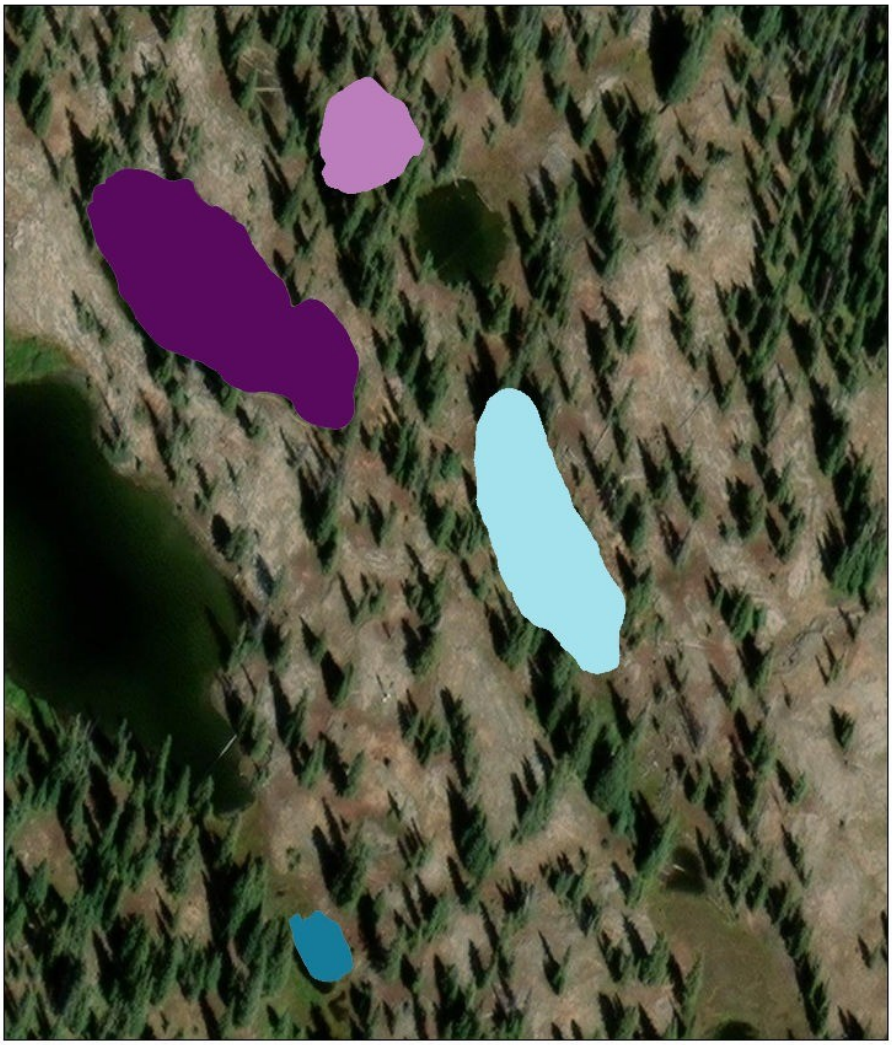
TABLES AND FIGURES

Collection Sites of Larvae



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1:1,469

0 0.01 0.01 0.03 mi

0 0.01 0.03 0.06 km

Collection Sites of Larvae

- L8- Acilius
- L3- Aeshna
- L10- Chironomids and Copepods
- L12- Phantom Midges

Microsoft, Vantor