

Species Co-occurrence Patterns and Mechanisms for *Rhyacophila* in High-Altitude Streams

Student: Bridget Sparrow-Scinocca

Mentor: Bobbi Peckarsky

Full Time Independent Research

Summer 2017

Abstract

Currently, due to global warming, we are seeing large range shifts in many different species across the world. As these species shift they are encountering new species and new environments. The way that a species interacts with these new species and environments will determine its ability to expand its range, preventing extinction by being successful in this new environment. *Rhyacophila* is a genus of caddisflies, which belong to a group of terrestrial insects that complete their aquatic larval stage in cold, fast-flowing and highly oxygenated freshwater streams. Previous research has shown that the distribution and abundance of species in this genus vary greatly across streams in the Upper East River Basin in Gunnison, Colorado. In this study we wanted to determine if there were any species co-occurrence patterns that we could identify that would explain the distribution of these species. We also wanted to test potential mechanisms such as species interactions and environmental variables to see if they would explain these distribution patterns. We did find both positive and negative association patterns between various species. *Rhyacophila alberta* seemed to be negatively associated with *Rhyacophila brunnea* and weakly positively associated with *Rhyacophila hyalinata*. Both *Rhyacophila alberta* and *Rhyacophila brunnea* seem to have environmental preferences that were opposite of each other and *Rhyacophila hyalinata* seemed to fall somewhere in the middle of the spectrum. Using an experiment we tested whether or not predation and growth rates of *Rhyacophila alberta* were affected by the presence of species it commonly co-existed with compared to species it rarely co-occurred with. We did find effects on both predation rate and growth rate depending on treatment. However, predation rate did not predict growth rate. This experiment shows that species distributions can be influenced by both interspecific and intraspecific species interactions and environmental factors.

Introduction

Species throughout nature are constantly interacting with each other and with their environment. These interactions can determine the ability of a species to be successful within a particular ecosystem. Species that co-exist have the potential to either directly or indirectly affect each other's overall fitness. Species competing for the same resources may have a negative effect on the fitness of another if they reduce their competitors' access to food, habitat or other resources. In contrast, some species may potentially rely on another for protection from predation, or for the creation of a suitable living habitat, and therefore could have a positive effect on each other's fitness. Currently, due to global warming, we are seeing range shifts occurring in many species and there is the potential for species to encounter new species and to have novel overlapping ranges as these shifts occur. With rapidly changing climate, it is even more important to understand how changing species interactions will affect the potential for species to survive.

Therefore, it is possible that species can form positive and negative associations based on these interactions. A positive association between species would suggest that the presence of one species facilitates the performance of another species within the same environment (Veech et. al 2016). A negative association between species would suggest that the presence of one species prevents or inhibits another species from living within the same environment or reduces its fitness (Veech et. al 2016). A pair of species can also be randomly associated, in which their

respective presence has no effect on the abundance or performance of the other (Veech et al. 2016).

Species co-occurrence analysis allows comparisons to be made between pairs of species to determine if there are any specific patterns in their associations. Understanding these associations can enable us to predict which species will occur together and which species will not, which can be crucial to explaining patterns of distributions of species that have overlapping ranges. Such analyses can further help us understand how species interactions can affect their relative abundance and ability to thrive in various ecosystems. In addition, manipulative experiments can be conducted following a species co-occurrence analysis that can help us understand the potential mechanisms underlying these patterns of coexistence.

Rhyacophila is a genus of caddisflies, which belong to a group of terrestrial insects that complete their aquatic larval stage in freshwater streams. They are typically found in fast-flowing, cold, highly oxygenated, stony rivers and streams. Although they only spend a portion of their life in the water, they are mesopredators that help shape the composition of the prey communities in stream ecosystems (Messner et al. 2009). Unlike other caddisflies, members of the genus *Rhyacophila* are free-living predators and they do not spin nets or build portable cases (Elliott 2005). In order to complete metamorphosis they create brown cocoons and they spin silk to create a protective gravel case that they attach to large stones on the stream bottom (Elliott 2005).

There are several potential environmental factors, including multiple species interactions that have been reported to influence *Rhyacophila* distributions within the stream. For example, some species of *Rhyacophila* have been shown to be the only large invertebrate predators that can withstand and attack prey at faster flow rates (Messner et al. 2009, Malmqvist et al. 1996). Also flow rate combined with prey abundance might influence rates of competitive interactions of two different species of *Rhyacophila* (Lavandier et al. 1995). In addition, the time of day and developmental stage (instar) have been shown to influence activity patterns of different species of *Rhyacophila* (Elliott 2005, Elliott 2006). In addition, the presence or absence of predators and prey may affect their levels of activity and population density (Sircom et al. 2009). Finally, the types and abundance of other mesopredators, including multiple species of *Rhyacophila*, can increase the competition for resources and potentially increase intraguild predation (Huhta et al. 1999).

Previous work done in the Upper East River Basin of Gunnison County, Colorado during 2006 and 2007 has shown that different proportions and abundances of species of *Rhyacophila* co-exist throughout the various streams in the basin (Peckarsky, McIntosh, and Alvarez, unpublished data). The goals of this project were: 1) to sample some of the same streams previously surveyed to compare the distributions of different species of *Rhyacophila* over space (different streams); we then conducted species co-occurrence analyses to determine if there are any positive or negative associations between different *Rhyacophila* species. 2) To measure various environmental factors to obtain descriptive data about the typical habitats of different species, to generate hypotheses about potential causes of species co-occurrences. 3) To implement a manipulative experiment to test whether competitive interactions between pairs of abundant species of *Rhyacophila* that either do or do not typically co-occur, affect predation rates on mayfly prey and growth rates of *Rhyacophila*. These protocols in combination will

enable us to examine species co-occurrence patterns and to test some potential mechanisms explaining distributions of *Rhyacophila* species in high-altitude streams.

Methods

Part 1: Patterns and Potential Mechanisms of Species Co-existence (Goals 1 and 2)

Study Sites

We sampled *Rhyacophila* from 12 different streams in the Upper East River Basin in Gunnison County, Colorado. Stream choices were based on previously known abundances from a survey conducted in 2006 and 2007. Six streams that had been previously recorded as having more than one species in relatively high abundance were sampled: Copper Creek, Lower Benthette Brook, Lower Bradley, Mosca, Rustlers Gulch, and Sylvanite (Peckarsky, McIntosh and Alvarez, unpublished data). Six streams that had been previously recorded as having only one species, or additional species that are extremely rare, were sampled: B3, Gothic, Marmot, Mossy, Queens, and Whiterock (Peckarsky, McIntosh and Alvarez, unpublished data). These surveys were completed within the within one week to avoid any variation in species abundances due to seasonal changes.

Collection of Organisms and Measurements of Environmental Parameters

We used a custom made box sampler (Hoffman Sampler) fastened to a 364 μm mesh driftnet and disturbed a known area of substrate (0.104m^2) inside the box for one minute, washing all of the invertebrates residing in that substrate into the net. We collected five Hoffman samples, which is the same method as the 2006 and 2007 surveys so that we can compare densities between years. Therefore, the total area of substrate sampled at each site was 0.52m^2 . We poured the contents of the drift net into sorting pans beside each stream and all *Rhyacophila* individuals were hand-picked, preserved in 70% ETOH in jars, brought back to the lab and identified to species under a dissecting microscope. So that abundances could be compared to those of other studies, we converted total numbers collected into densities by dividing raw numbers by 0.52m^2 .

To address the second goal, to understand more about the environment of *Rhyacophila* species, several biotic and abiotic factors were measured at each stream. The volume of flow (discharge) was calculated using standard methods to measure stream width, average depth and average current velocity across one transect at each site. Hobo light and temperature loggers were placed in all streams a week prior to sampling and for several weeks after sampling to obtain a continuous record of both light and water temperature. We used a conductivity meter to estimate temperature-specific conductance, which is a surrogate for stream productivity. Previous studies (Peckasky et al. 2001) enabled us to note the presence or absence of predatory salmonid fishes (mostly brook trout) at each site, which are the main predators of *Rhyacophila* larvae. The presence and relative abundance of other mesopredators such as predatory stoneflies (Perlodidae and Chloroperlidae) and flatworms were noted as either being rare, common or abundant. Those other species can potentially affect the level of competition for resources that the caddisflies would experience in the stream. The presence and relative abundance of potential prey species such as chironomids, simuliids, and *Baetis* mayflies were record as either being rare, common or abundant in the samples collected in each stream. Finally, we counted the

number of protruding rocks at each site to determine the amount of potential oviposition habitat for *Rhyacophila* females to lay their eggs.

Data Analysis of Species Co-existence

In order to analyze species coexistence we ran a probabilistic model of species co-occurrence (Veech 2013, Veech et. al 2016) in R. In this model, species co-occurrence is represented as the number of sampling sites in which two species will co-occur. Each species was assigned either a 1 if they are present in the stream or a 0 to signify their absence. This analysis compares the observed co-occurrence to the calculated expected co-occurrence of *Rhyacophila* in the streams if patterns of co-occurrence are random. The observed co-occurrence is represented by the actual number of sites where the two species of interest co-occur. The expected co-occurrence ($E(N_{1,2})$) is calculated by finding the product of the two species' probability of occurrence ($P(1), P(2)$) multiplied by the number of sampling sites (N). This is represented by the equation $E(N_{1,2}) = P(1) \times P(2) \times N$ (Veech et. al 2016). A positive association was obtained if the frequency of co-occurrence was significantly greater than expected. A negative association was obtained if the frequency of co-occurrence was significantly lower than expected. If the observed occurrence is nearly equal and thereby not significantly different in comparison to the expected it was classified as a random association. These associations will enable us to predict which species are more likely to occur together and those that are not.

Data Analysis of Relative Abundance

We examined potential correlations between the densities of the different species pairs collected in the 12 study streams. We constructed graphs (scatterplots) of each pair of species to examine visually if there are positive, negative or random associations between the abundances of different species. We used this graphical approach to decide which species to use in the experiment (see below). We conducted Pearson correlation analyses to test for positive, negative or random associations between the densities of the three most abundant species. This analysis provides the direction, strength and significance of those associations.

Data Analysis of Habitat Preferences

We implemented two types of analyses will to test whether preferences for different environmental factors explain the patterns of distribution of different *Rhyacophila* species. First, we conducted a multivariate analyses (non-metric multidimensional scaling; NMDS) to compare the environmental variables that favour each species of *Rhyacophila*, which illustrates compares the distributions of different species in multivariate habitat space, according to the factors we measured (flow, water temperature, light, conductivity, predators, competitors, prey and oviposition habitat). This analysis enables us to observe whether species that do not co-occur have different habitat preferences and will provide a visual illustration and a significance test of whether certain species cluster together or separate along axes describing the various environmental factors we measured.

The second analysis of habitat preferences will implement a model selection procedure. This analysis ranks environmental variables from highest to lowest with regard to the proportion of variation in the abundance of each species of *Rhyacophila* explained by each variable. We ran a model selection procedure to test which subset of environmental factors best explain the

density of total *Rhyacophila*, species richness, and the densities of each species of *Rhyacophila* individually.

Part 2: Mechanisms of Species Co-occurrence (Goal 3)

We implemented two experiments to test whether the presence of a second individual *Rhyacophila* affected the predation rate or the growth rate of a focal individual *Rhyacophila*. Species were selected based on observed co-occurrence and relative abundance of species in the 12 survey streams. We used the most abundant and ubiquitous species as the focal species (*Rhyacophila alberta* = Species A), a second abundant species with which *R. alberta* has a positive association (*R. hyalinata* = Species B), and a third abundant species with which *R. alberta* has a negative association (*R. brunnea* = Species C). We used as prey the mayfly *Baetis bicaudatus*, which as been shown to be consumed by *Rhyacophila* in previous experiments conducted at RMBL (Vance and Peckarsky 1997), and is easily contained within the experimental set up we used. Other potential common prey items (e.g. Chironomidae and Simuliidae) are small enough to escape through the mesh of the experimental channels or entangle all the inhabitants with silk, respectively!

Collection of Organisms

Experiments were completed during the last two weeks of July after we analyzed the species co-occurrence data. We collected individuals of *R. alberta*, *R. hyalinata* and *R. brunnea* from streams where they are abundant and as close to RMBL as possible (e.g. Copper Creek, B3 and Queens). We collected *Baetis* prey from Copper Creek and kept them cold in yogurt cups in coolers while we transported them back to the weatherport where the experiments were conducted. We placed individual *Rhyacophila* in vials with mesh covers so they would not eat each other, and also kept them cold for transport back to the Rocky Mountain Biological Laboratory where we set-up the experiment.

Experimental Set-Up

The design and set-up of weatherport system is described and illustrated in Peckarsky and Cowan (1991). Experiments were conducted in 60 15-cm diameter circular plexiglass chambers that are housed in a weatherport beside the East River at RMBL. This set-up allows for the experiment to take place under natural light and temperature conditions. Stream water originating from a small fishless tributary of the East River (B8) was filtered and delivered by gravity into the chambers. We controlled flow rate by adjusting gang valves to simulate natural flow rates in the habitat of *Rhyacophila* and their prey. We will line the bottom of the chambers with 6 small rocks (total of 360) collected from the East River as a refuge for predators and prey and a source of algal food resources for prey.

Experimental Design 1: Predation Rate Trials

This experiment tested whether the predation rates of individual *Rhyacophila* were affected by the presence of another individual. The same density of 6 individuals of *Baetis* mayfly larvae were added to each chamber for prey, and allowed to acclimate for 24 hours before adding the predators. 360 *Baetis* were collected for this experiment, and 60 extras were taken to the lab to estimate prey size (head capsule widths, which can be converted to dry

weights using known regression equations). We compared predation rates of *R. alberta* (the most common and focal species - A) feeding on *Baetis* prey in the absence of competitors, to its predation rates in the presence of a conspecific competitor (another *R. alberta*), a congeneric competitor with which it is positively associated (*R. hyalinata* - B) and a congeneric competitor with which it is negatively associated (*R. brunnea* - C). The response variable was *Baetis* mortality rate per predator per day, and trials were run for 24, 48 and 72 hours to test whether time feeding affected their predation rates. Using 60 channels we had 12 replicates of each of five treatments: A + 6 *Baetis*, 2A + 6 *Baetis*, AB + 6 *Baetis*, AC + 6 *Baetis*, 6 *Baetis*.

Comparisons of predation rates among the 4 treatments with predators show whether a second predator affects feeding of individuals of the focal species (A). We used control channels with prey and no predators to correct for losses of prey not due to predation. At the end of the allotted feeding periods, we counted and recorded all remaining caddisflies and prey from each channel after each trial. After each predation trial, we replenished *Baetis* to bring the initial prey densities back up to 6 per channel for the next predation trial and to ensure that prey were not limited for estimating the growth rates of remaining predators (Experiment 2).

Experimental Design 2: Growth Rate Trial

Over the entire period when we were estimating predation rates (7 days), and using the same individual *Rhyacophila* as in the predation trials, we tested the effect of the treatments on the growth rates of the remaining *Rhyacophila* (ones that did not get consumed during the feeding trials). The caddisflies measured for growth rates were those that continued to feed in the weatherport chambers over the period of a week. In this experiment the response variable is the mg of dry weight gained (or lost) by each *R. alberta* per day when feeding on *Baetis* by itself (A) or with a conspecific predator (AA), an congeneric competitor with which it is positively associated (AB) and a congeneric competitor with which it is negatively associated (AC). We omitted the channels where one of the two competitors disappeared some time during the 7-day experiment because in those cases, the original treatments were not maintained throughout the experiment. Growth rate is a better test for competition (or facilitation) between pairs of *Rhyacophila* than feeding rate, and is often used as a surrogate for fitness. Feeding rates may not predict growth rates because other factors (e.g., avoiding a competitor) may increase energy expenditure during foraging.

Data Analysis of Predation Rate

We tested for differences among treatments using a Mixed Model Two-Way ANOVA with treatment (A, AA, AB, AC) and time feeding (24h, 48h, 72h) as fixed factors, and individual *R. alberta* as a random factor. We omitted all channels in which one of the pair of *Rhyacophila* disappeared because we only wanted to use channels where the original treatments were maintained for the whole trial. This analysis tests whether having a competitor of the same or different species feeding in the channels affected the average predation rate of *R. alberta*. Pairwise comparisons between treatments also enabled us to test whether interspecific competition for prey is as strong as intraspecific competition for prey, and whether the strength of interspecific competition depends on whether species coexist in nature.

Data Analysis for Experiment on Growth Rate

We will create graphs of growth rate over time for species in different treatments. This will be done in order to determine which species succeeded better in our trials. We implemented two analyses to test the effects of treatments on growth rates of individual *R. alberta*: 1) tests for mean growth rate differences among treatments, and 2) the variation between the growth rates of the two *R. alberta* feeding together. For the first analysis we conducted a One-Way ANOVA testing whether the presence of a potential competitor affected the mean growth rate of the focal species (*R. alberta*). Pairwise comparisons between treatments tested whether interspecific competition was stronger than intraspecific competition, and whether the strength of those interactions depended on whether species normally co-occur. Second we conducted a T-test comparing the variation in growth rates between paired individuals of the same species. We randomly paired individuals from the A treatments with another A individual feeding by itself to use as a control to test whether differences between growth rates of the AA's feeding together differed randomly or were greater than the differences between the randomly paired individuals that grew by themselves for 7 days.

Results

(Statistical analysis still in progress. Results and Discussion Section are based on preliminary results.)

Part 1: Patterns and Potential Mechanisms of Species Co-existence (Goals 1 and 2)

Species Co-existence and Abundance Patterns

Throughout our collection in the 12 streams we found 6 different species of the genus *Rhyacophila*. We found individuals of *Rhyacophila alberta*, *Rhyacophila brunnea*, *Rhyacophila hyalinata*, *Rhyacophila valuma*, *Rhyacophila verrula* and *Rhyacophila coloradensis*. We found these species at different abundances and distributions throughout these 12 streams (Table 2). The most highly abundant species was *Rhyacophila alberta*, followed by *Rhyacophila brunnea* and *Rhyacophila hyalinata*. The preliminary graphs of this experiment suggest that different species have different relationships with each other. When we plotted abundance graphs it seemed that *Rhyacophila alberta* and *Rhyacophila brunnea* were negatively associated with each other (Fig 1). However, it seems that *Rhyacophila alberta* and *Rhyacophila hyalinata* are weakly positively or randomly associated with each other (Fig 2). There were not large enough sample sizes of other species to make these correlation graphs. A species co-occurrence analysis will be conducted to determine if any of these correlations result in the characterization of positive, negative or random associations between these species.

Influence of Environmental Variables

Preliminary graphs of various environmental variables were created to determine if preferences for different environmental factors explain the patterns and distributions of different species of the genus *Rhyacophila*. We will later conduct a model selection analysis which will rank these environmental variables from highest to lowest with regard to the proportion of variation seen in the abundance of each species of *Rhyacophila*, explained by each variable in the data matrix.

Continuous Environmental Variables:

Preliminary graphs show that the level of stream discharge may affect the type of species that are found there. *Rhyacophila alberta* seemed to be found in higher abundances at low flow rates (Fig 3). However, *Rhyacophila brunnea* and *Rhyacophila hyalinata*, seem to be found more commonly at faster flow rates, although this trend does not seem to be as strong (Fig 3).

Preliminary results suggest that mean temperature of the stream may have an effect on the distribution of *Rhyacophila alberta*, which seems to be at higher abundances in colder streams (Fig 4). *Rhyacophila hyalinata* had a weaker association with temperature, but still tended to be higher in colder streams in comparison to *Rhyacophila brunnea* which tended to be higher in warmer streams (Fig 4). Densities of *R. alberta* and *R. hyalinata* seemed to increase with an increase in the channel gradient percentage of the stream (Fig 5). However, there seemed to be only a slight influence of channel gradient on densities of *R. brunnea*, with them being only slightly more abundant in streams with a lower channel gradient percentage (Fig 5). *R. alberta* was found in higher abundances in streams with a higher conductivity (Fig 6). However, level of stream conductivity did not seem to have a significant effect on the distribution of *R. brunnea* and *R. hyalinata* (Fig 6). The density of oviposition substrates did not seem to have any significant effect on the distribution and abundance of the different species of *Rhyacophila*.

Categorical Environmental Variables:

Both abundance of prey species and abundance of competitor species were categorized at each stream as rare, common or abundant. These categories were then converted to numerical values with rare, common and abundant respectively equalling 1, 2 and 3. Total values seen in the graph represent the total abundance of the different prey and competitor species. The density of *Rhyacophila alberta* seemed to decrease with both an increase in prey and competitors (Fig 7, Fig 8), whereas the density of both *Rhyacophila hyalinata* and *Rhyacophila brunnea* did not seem to vary significantly with different abundances of prey. Vegetation cover was quite hard to quantify and therefore it is difficult to make conclusions about the effect it had on these 3 main species of *Rhyacophila*.

Part 2: Mechanisms of Species Co-occurrence (Goal 3)

Predation Rate

Predation rates on *Baetis* in each treatment did not seem to vary with time. The averages of predation rate for each treatment seem to be similar across the 24, 48 and 72 hour trials (Fig 9). A, AA and AB treatments seemed to have relatively similar predation rates across the entire experiment. However, the treatment AC consisting of 1 *R. alberta* and 1 *R. brunnea* seemed to have a reduction in feeding rates in comparison to the other treatments (Fig 9).

Growth Rate

All species of *Rhyacophila* on average grew throughout the experiment. Individual *R. alberta* in the A, AA and AC treatments seemed to have relatively similar predation rates across the entire experiment (Fig 10). However, the treatment AB consisting of 1 *R. alberta* and 1 *R. hyalinata*, seemed to have a reduction in growth rate in comparison to the other treatments (Fig 10). Individual *alberta* in the AA treatment had an interesting divergence in growth rates in which, in several replicates we saw, one individual had a much larger growth rate than the other individual which shared the same channel (Fig 10).

Intraguild Predation

Not many individual *Rhyacophila* in the various treatments ate each other, but there was some intraguild predation and it varied with the predator treatment. In treatment AB with 1 *R. alberta* and 1 *R. hyalinata*, 4 *R. alberta* went missing and no *R. hyalinata* went missing (Table 1). However, in treatment AC with 1 *R. alberta* and 1 *R. brunnea*, 3 *R. brunnea* went missing and 4 *R. alberta* went missing. It is thought that the missing ones were eaten by their partner in the channel (Table 1).

Discussion

Species distributions can be influenced by both interspecific and intraspecific species interactions and environmental factors. Environmental factors are quite often described as being the most important drivers in determining the range of a species. However, it is important to remember that the ability of a species to thrive in a particular ecosystem may be dependent on the interactions it has with other species living within the same habitat. Positive associations between species can allow species to take advantage of environments they could not inhabit on their own (Veech et. al 2016). Negative associations between species can exclude species from living in habitats that are environmentally suited to them (Veech et. al 2016). This means that species interactions can play a role in expanding or restricting the range of a species, and therefore should be looked at in combination with environmental factors in order to determine species co-occurrence patterns and mechanisms.

In this study we wanted to examine whether there were any patterns in the co-existence of different species of *Rhyacophila* in 12 streams in the Upper East River Basin. We compared densities of the various species to our most highly abundant species of *Rhyacophila alberta* in order to determine if there were any potential associations we could predict from a preliminary analysis. *Rhyacophila alberta* and *Rhyacophila brunnea* only co-occurred in 1 of the 12 streams. This led us to conclude that *R. alberta* and *R. brunnea* were likely to have a negative association with each other. *Rhyacophila hyalinata* was almost always found in streams that also contained *Rhyacophila alberta* which led us to predict that they would have a weakly positive or random association. These patterns led us to choose these 3 species as our focal species of *Rhyacophila* for this study. We then wanted to attempt to determine if these patterns were driven by environmental factors, species interactions or a combination of both.

For each of the 12 streams we took measurements of continuous variables such as stream discharge, mean temperature, sunlight, conductivity, channel gradient and density of oviposition rocks. We also took measurements of categorical variables such as abundance of prey, abundance of competitors, presence or absence of fish predators, and vegetation cover. This allowed us to try to determine if these species are partitioned in their habitat based on preferences for certain environmental variables. *Rhyacophila alberta* seemed to show preference for slower flowing, colder streams with higher levels of conductivity that occur on a steeper channel gradient. Whereas *Rhyacophila hyalinata* and *Rhyacophila brunnea* showed weaker trends and tended to be found over a broader range of these environmental variables. This is interesting because although *R. alberta* is by far the most abundant species, occurring in more streams than any other species, it seems to show a stronger preference for certain environmental conditions in comparison to *R. hyalinata* and *R. brunnea*. However, the more tolerant species *R. hyalinata* and *R. brunnea* seem to be constricted to a smaller number of streams. This may suggest that species interactions may play an important role in limiting their ranges to these

streams, when they could hypothetically be able to expand their range and tolerate the conditions in streams we did not find them in.

In order to determine more about the role of species interactions we decided to test if the feeding rate and growth rate of *Rhyacophila alberta* would change in the presence of different competitors. We found that *Baetis* predation rate was very similar in treatments where *R. alberta* was by itself, with another *R. alberta* and when it was with a *R. hyalinata*. These treatments represent interactions that *R. alberta* would typically encounter and therefore suggest that feeding rates are similar when it is paired with species it is used to encountering. However, predation rates were lower in all trials in the treatment where *R. alberta* was with *R. brunnea*, a species it does not typically co-occur with. This suggests that two species that do not commonly co-occur may have a stronger negative influence on each other's feeding rate in comparison to those that typically co-occur.

However, the effects of growth rate did not suggest the same effects of these treatments. When comparing growth rates of *R. alberta* across the various treatments we only see a small increase in growth rate in the treatment that contains *R. alberta* and *R. hyalinata*. This would suggest that there was more of an effect on growth when *R. alberta* is paired with an individual that it typically competes with. In this experiment predation rate did not predict the growth rate.

There are several potential explanations for why this may be true. The first is that this design does not test for which individual *Rhyacophila* ate the prey. Therefore we do not know which bug ate the *Baetis* that disappeared. It may not be an even split as we calculated, but could potentially be more one-sided in which one bug ate most or all of the prey and the other bug was not as successful at feeding. This would change the predation rate of individual bugs and, with these adjustments, may be a better predictor of growth rate. It is also possible that factors other than predation rate, such as energy expenditure, could have been affecting growth rate. Individuals of *Rhyacophila* in the trials may have been eating but may not have grown as much because they were using up energy to avoid the other competitor in the tank. This would lower their growth rate and makes it hard to determine that growth rate proportional to the amount of food intake. There are also size differences between the different species. This is how they naturally occur in the streams, but it may have an effect on how they grow and the rate at which they need to grow in order to reach adulthood. Therefore there may be several different reasons why predation rate might not equal growth rate. However, these trends can still show that there is some effect of species interactions on *Rhyacophila* and that further tests need to be done in order to determine exactly what these are.

In conclusion, it seems that *R. alberta* and *R. brunnea* have a reciprocal negative association. This can be concluded by looking at the reduced predation rates seen on *baetis* in AC treatments, the reciprocal intraguild predation, and also because they prefer different environmental variables. It also seems like *R. alberta* and *R. hyalinata* co-occur randomly. This can be concluded by looking at the reduced growth rate of *R. alberta* in the AB treatment and the one-sided intraguild predation observed of *R. hyalinata* on *R. alberta*. It is also very interesting that *R. hyalinata* seems to prefer certain environmental variables that match trends seen in *R. brunnea* and some that match trends seen in *R. alberta*. This suggests that species interactions in combination with preferred environmental factors might determine the ranges of different species in the genus *Rhyachophila*.

Future studies should include similar experiments with treatments of *R. brunnea* and *R. hyalinata* by themselves and with each other. This would allow us to compare the growth and predation rates of those species for all 3 species, which may give us a better idea about how these

species are interacting. It will be important in the future to conduct studies that focus on identifying both intraguild and *baetis* predation behaviour so that we can determine which individuals in each channel were feeding. It would be interesting to characterize behavioural interactions between the different species to determine how they are affected. Learning more about smaller scale habitat partitioning would be good in order to determine how often species that are found in the same stream actually interact. It would also be helpful to conduct experiments manipulating temperature, flow rate and other important environmental variables in order to see what each species prefers. Conducting experiments like this will help us be able to predict what environmental variables and species interactions will affect the distribution and size of species ranges in this changing global climate.

Appendix

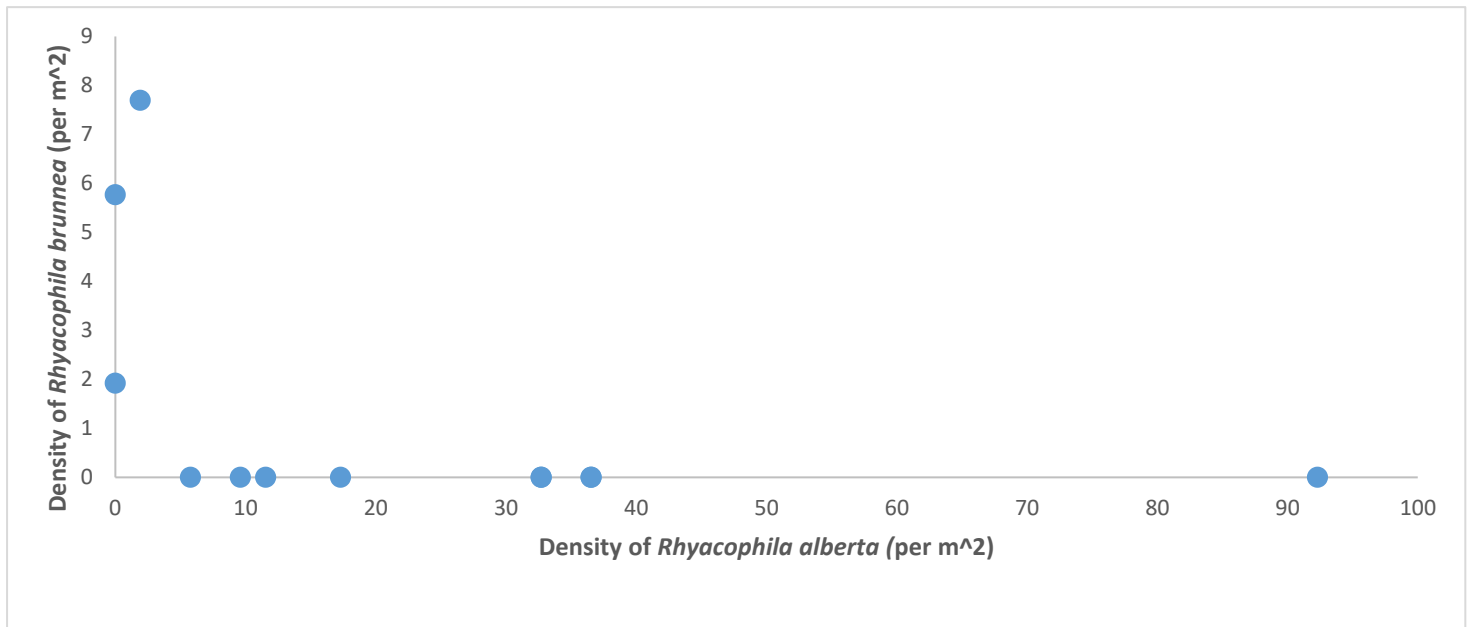


Figure 1 Density of *Rhyacophila alberta* and *Rhyacophila brunnea* per m².

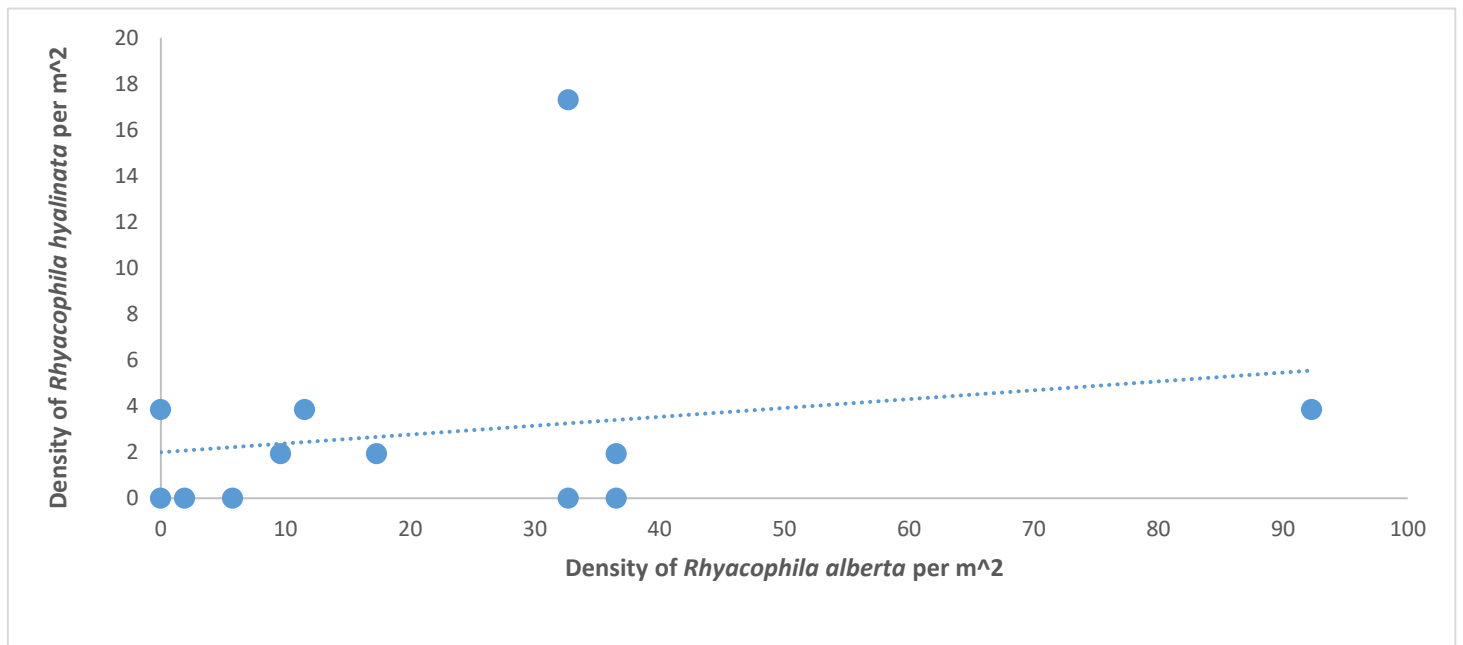


Figure 2 Density of *Rhyacophila alberta* and *Rhyacophila hyalinata* per m².

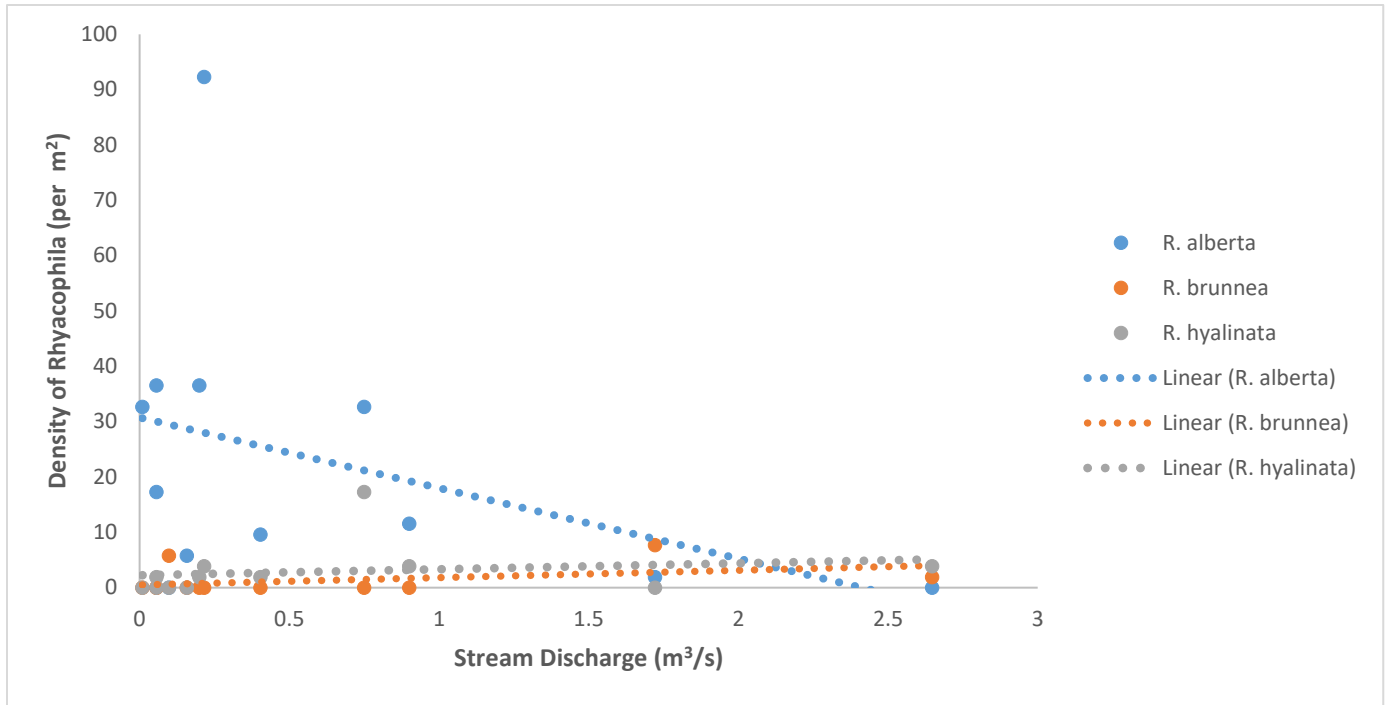


Figure 3 This is a plot of the abundance of the species *R. alberta*, *R. brunnea* and *R. hyalinata* for different levels of stream discharge.

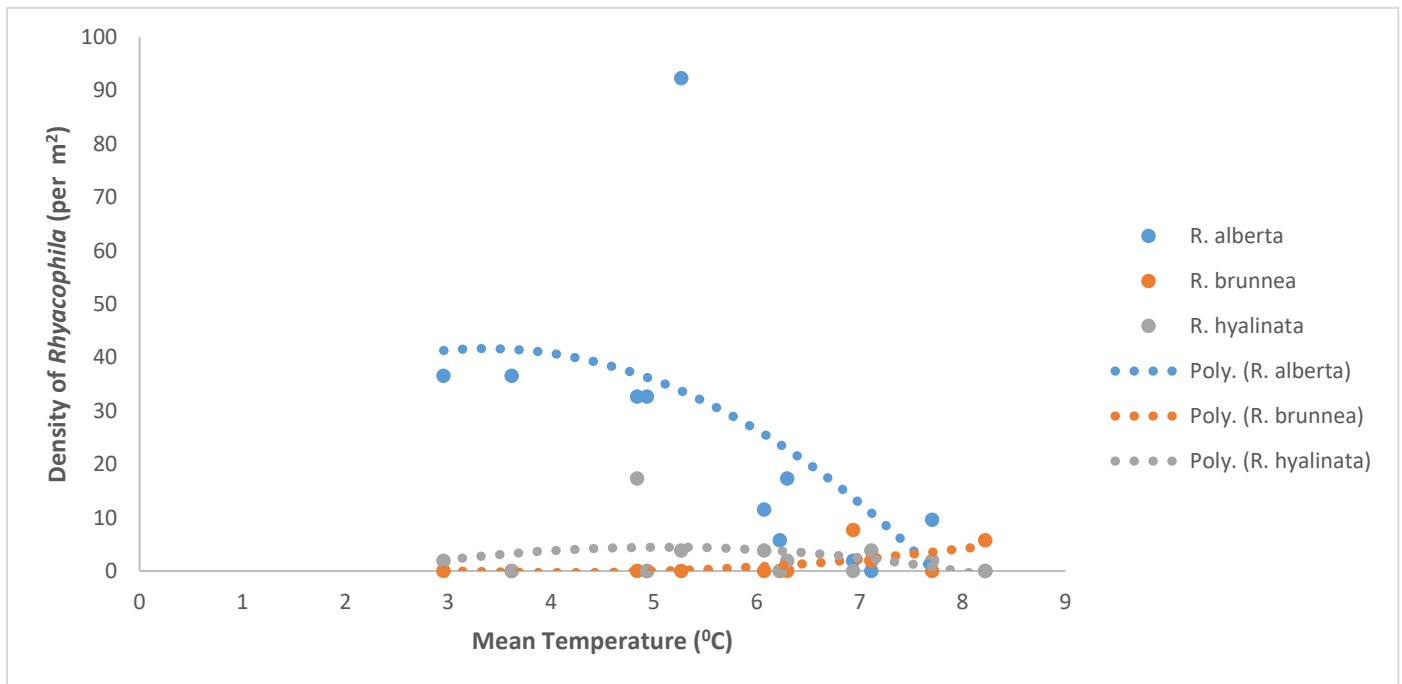


Figure 4 This is a plot of the abundance of the species *R. alberta*, *R. brunnea* and *R. hyalinata* against mean temperature.

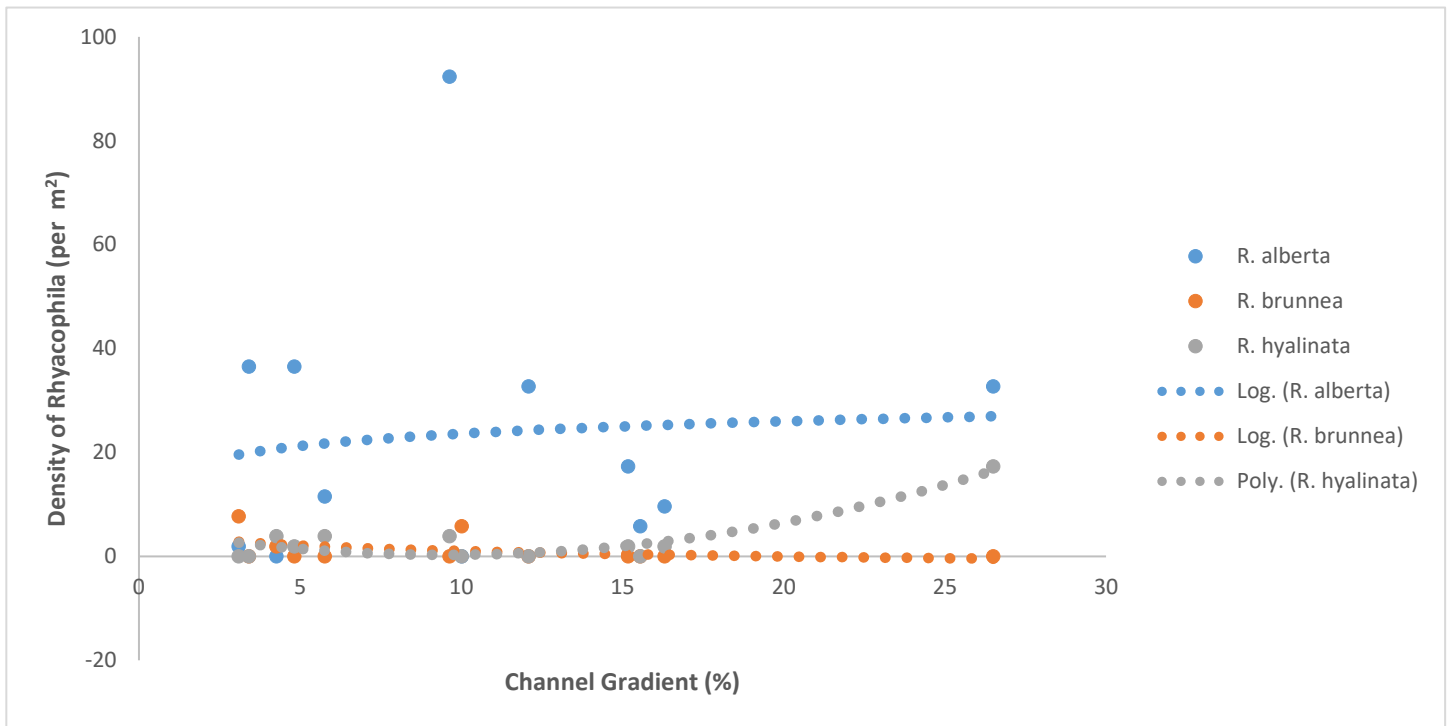


Figure 5 This is a plot of the abundance of the species *R. alberta*, *R. brunnea* and *R. hyalinata* for different levels of channel gradient

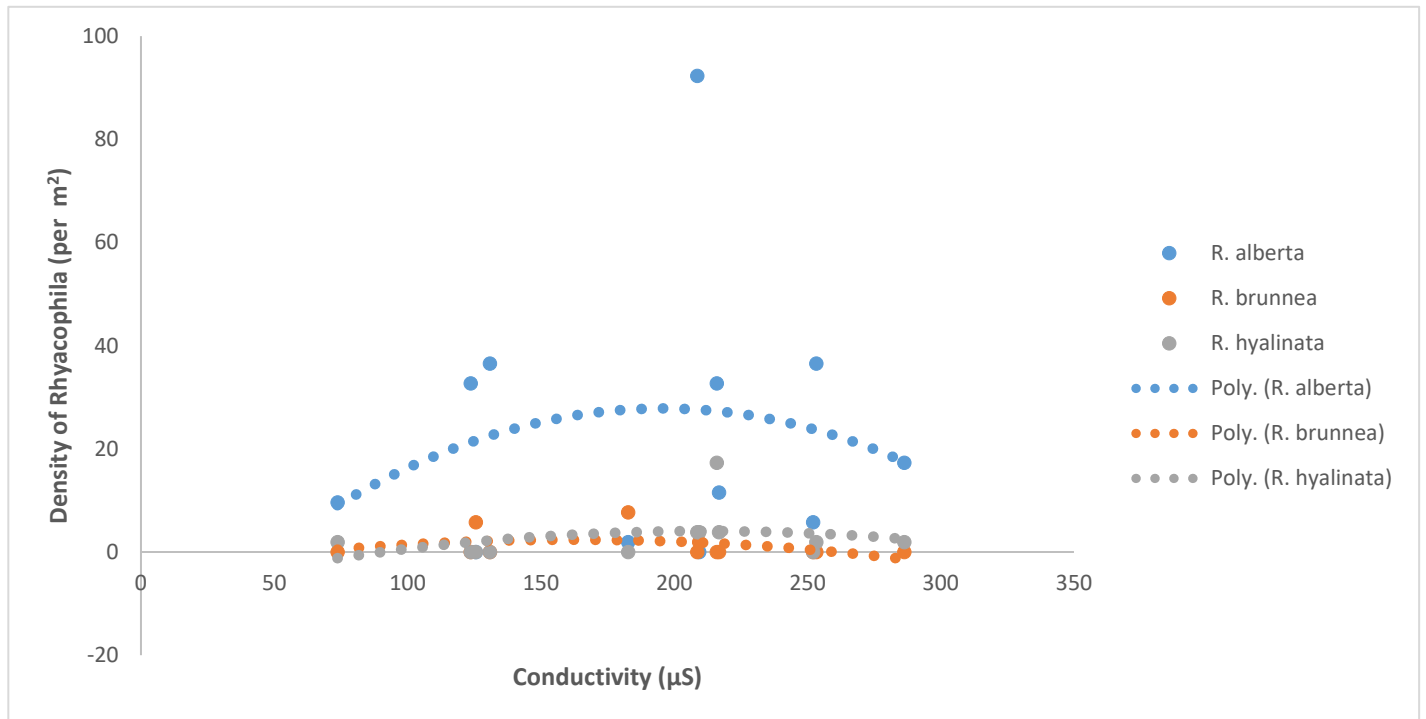


Figure 6 This is a plot of the abundance of the species *R. alberta*, *R. brunnea* and *R. hyalinata* for different levels of conductivity.

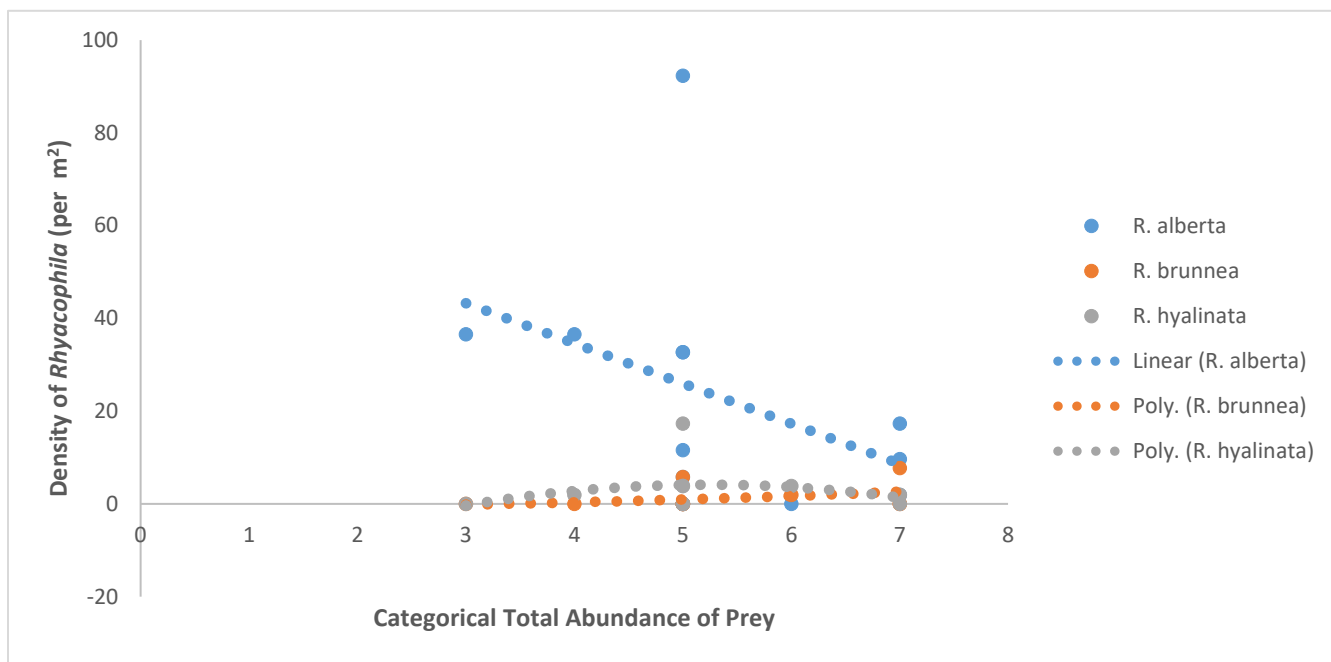


Figure 7 This is a plot of the abundance of the species *R. alberta*, *R. brunnea* and *R. hyalinata* for different abundances of prey species.

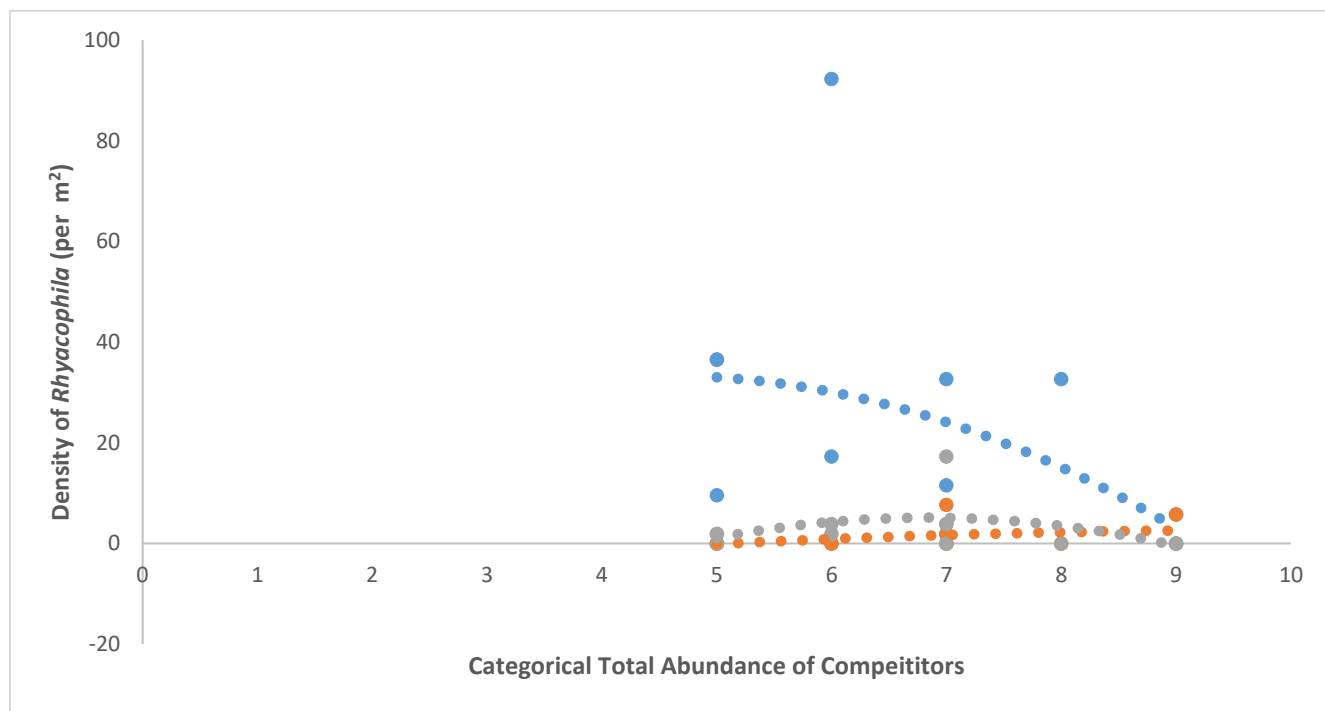


Figure 8 This is a plot of the abundance of the species *R. alberta*, *R. brunnea* and *R. hyalinata* for different abundances of competitor species.

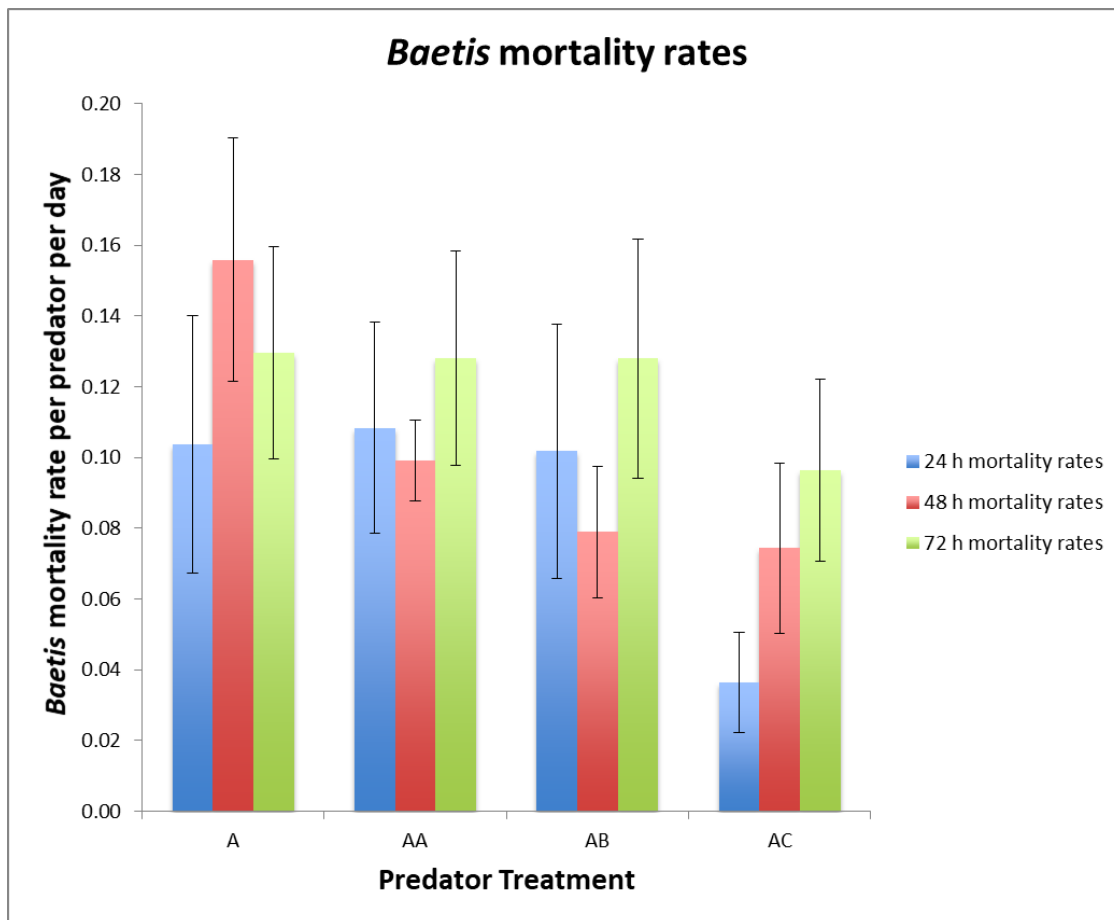


Figure 9 This graph displays the *Baetis* mortality rate for each treatment at the 24, 48 and 72 hour trials.

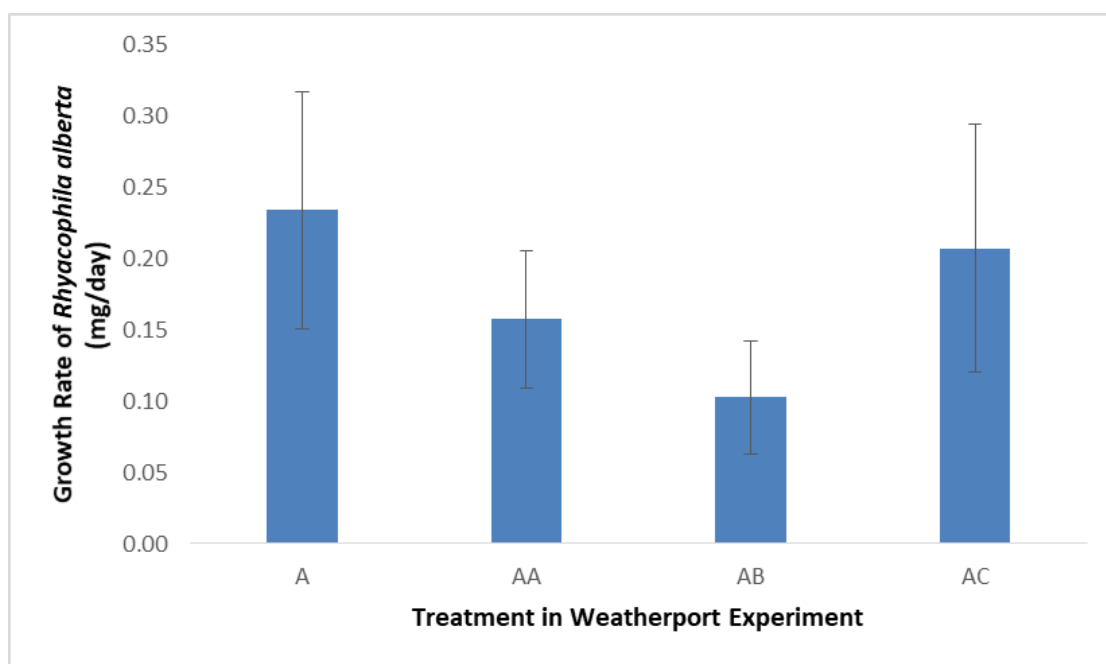


Figure 10 This graph displays the growth rates of *Rhyacophila alberta* in the various treatments.

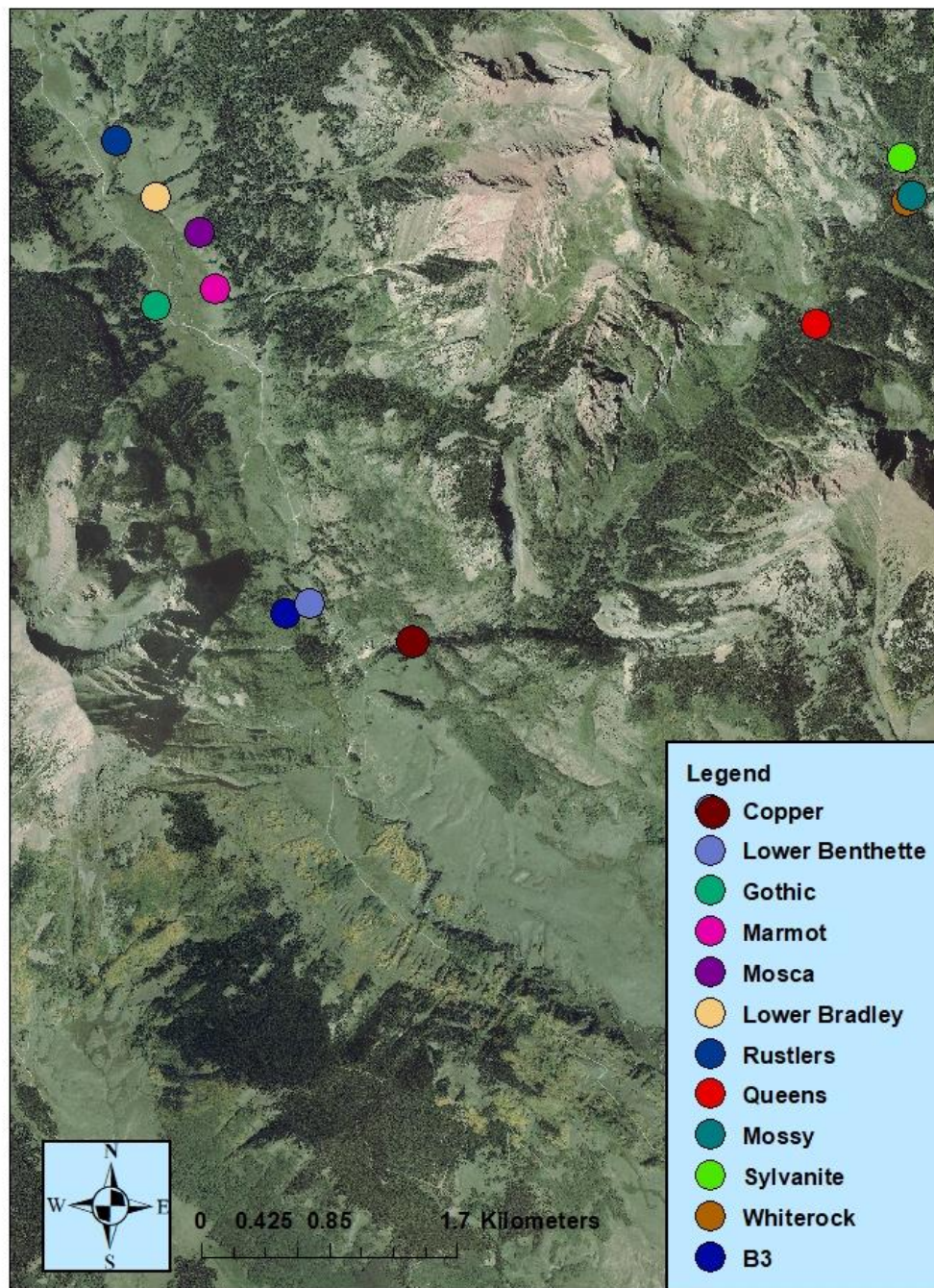
Treatment	A	A+A	A+B	A+C
<i>R. alberta</i> lost (A)	2	4	4	3
<i>R. hyalinata</i> lost (B)	--	--	0	--
<i>R. brunnea</i> lost (C)	--	--	--	4
Total in trials	12	24	12+12	12+12

Table 1 This table displays the amount of intraguild predation in the various treatments.

	<i>alberta</i>	<i>brunnea</i>	<i>hyalinata</i>	<i>valuma</i>	<i>verrula</i>	<i>coloradensis</i>
Stream						
B3	1	0	0	0	0	0
Copper	1	1	0	1	0	0
Gothic	1	0	1	1	0	0
Lbenth	0	1	0	1	0	1
Lbrad	1	0	1	1	0	0
Marmot	1	0	0	0	0	0
Mosca	1	0	1	0	0	0
Mossy	1	0	0	0	0	0
Queens	1	0	1	0	1	0
Rustlers	0	1	1	1	1	0
Sylvanite	1	0	1	0	0	0
Whiterock	1	0	1	0	0	0

Table 2 This table displays which species of *Rhyacophila* were present and absent at 12 different streams. 0 codes for *rhyacophila* being absent from the stream and 1 codes for being present.

Streams in Rhyacophila Species Co-occurrence Survey



Literature Cited

- Huhta A., Muotka T., Juntunen A. & Yrjonen M. 1999. Behavioural interactions in stream food webs: the case of drift-feeding fish, predatory invertebrates and grazing mayflies. *Journal of Animal Ecology*, 68: 917-927.
- Lavandier P. & Cereghino R. 1995. Use and partition of space and resources by two coexisting *Rhyacophila* species (Trichoptera) in a high mountain stream. *Hydrobiologica*, 301: 157-162.
- Elliott J. M. 2005. Contrasting diel activity and feeding patterns of four instars of *Rhyacophila dorsalis* (Trichoptera). *Freshwater Biology*, 50:1022-1033.
- Elliott J. M. 2006. Prey switching in *Rhyacophila dorsalis* (Trichoptera) alters with larval instar. *Freshwater Biology*, 51:913-924.
- Messner K.M., Juntunen A., Malmqvist B. & Muotka T. 2009. Predator-prey interactions in a variable environment: responses of a caddis larva and its blackfly prey to variations in stream flow. *Finnish Zoological and Botanical Publishing Board*, 46:193-204.
- Peckarsky B. L. & Cowan C. A. 1990. Consequences of larval intraspecific competition to stonefly growth and fecundity. *Oecologia*, 88: 277-288.
- Sircom J. & Walde J. S. 2009. Intraguild interactions and large-scale population patterns. *Journal North American Benthological Society*, 28: 649-658.
- Vance S.A. & Peckarsky B. I. 1997. The effect of mermithid parasitism on predation of nymphal *Baetis bicaudatus* (Ephemeroptera) by invertebrates. *Oecologia*, 110: 147-152.
- Veech J. A. 2014. The pairwise approach to analysing species co-occurrence. *Journal of Biogeography*, 41: 1029-1035.
- Veech J.A., Griffith D. M. & Marsh C. J. 2016. cooccur: probabilistic Species Co-Occurrence Analysis in R. *Journal of Statistical Software*, 69: 1-17.