

Exploring mechanisms explaining coexistence patterns of *Rhyacophila* species (Trichoptera) in streams near the Rocky Mountain Biological Laboratory

Abstract

Coexistence of closely related species can be explained by a number of biotic and abiotic factors. In Trichoptera (caddisflies), the patterns of coexistence of the species *Rhyacophila* in streams near the Rocky Mountain Biological Laboratory (RMBL) has been tracked for 43 years. This study is a continuation from previous student projects carried out in 2017 and 2018 to explore the mechanisms explaining patterns of coexistence of these caddisflies. In addition to coexistence patterns, data from previous studies include species preferences for streams of different altitude, size, water temperature, and the coverage of the nuisance diatom *Didymosphenia geminata*. The coverage of *D. geminata* varies among years (favored in low-flow years), and streams (favored in streams with lower levels of phosphorus). Previous studies considered those factors only at the scale of stream reaches, but microhabitat preferences within stream reaches by different species of *Rhyacophila* remain to be investigated. Another previously unexplored factor is resource partitioning of prey by these predatory caddisflies. Questions asked in this study include: 1) Do certain pairs of species coexist in stream reaches by partitioning microhabitats (specifically, current velocities, water depths, substrate particle sizes or coverage of *Didymo*?) 2) Do *Rhyacophila* species shift microhabitats in the presence of potential competitors? 3) Does partitioning of food (prey) resources explain coexistence of pairs of species of *Rhyacophila*? We addressed those questions by continuing surveys in seven out of eight long-term stream sites near RMBL and adding measurements of microhabitat and diet preferences of coexisting *Rhyacophila* species. We also surveyed eight small streams in the Upper East River drainage while waiting for water levels to be safe in the larger, long-term sites. Results showed that two species that typically coexist at warmer water sites (*R. brunnea* and *R. valuma*) did not partition microhabitats, while the two species that coexist in colder water sites (*R. alberta* and *R. hyalinata*) preferred different water depth and substrate microhabitats. We were unable to test effects of *Didymo* during this very high-water year. In addition, while we could not distinguish specific prey species in the gut contents of these caddisflies, one of each pair of coexisting species had significantly larger mouth gapes than the other, suggesting that they may partition prey species (larger: *Baetis* vs smaller: chironomids).

Introduction

The ranges of many species, both terrestrial and aquatic are being affected due to climate change. Therefore, it is important to understand the factors affecting the distribution and abundance of species in our changing world. The choice of species niche is affected by multiple aspects of their environments, including food resources, predators, and habitat features influencing their fitness. For example, Tingley et al. (2009) found this to be the case in a study conducted with many species of birds who adjusted their niche to maximize their fitness. According to the study, different species of birds tracked the precipitation and temperature to which they were best adapted (Tingley et al. 2009). Limited range of species is a classic concept that was first described by Grinnell (1917), as a “physiological... narrow range of environmental

conditions” where the California thrasher can thrive. The movement of species to occupy new ranges can give clues to the effects as climate change continues.

In addition, with niche changes, new combinations of species may come into contact, potentially leading to competition between those species for resources. Such competition can be avoided by partitioning space, food resources or development time (Grinnell, 1917). But in some cases, dominant species can outcompete other species, sometimes resulting in high stakes reductions in fitness, like Intraguild Predation (Sparrow-Scinocca, 2017). The central focus of this study is to further our understanding about how some closely related species that are potential competitors are able to coexist while others do not.

Veech et al. (2014 and 2016) developed analytical methods for describing species co-occurrence patterns of distribution to determine if two species were positively or negatively associated. Those methods have been used by previous studies of coexistence of closely related species, which may also provide possible hypotheses for why those pairs of species may not be found together very often. One hypothesis explaining coexistence of closely related species is that partitioning food sources is one important process that can reduce the negative effects of potentially competing species (Lavandier and Cereghino, 1995). A negative consequence of not having enough resources for early instar stonefly larvae could result in smaller females who need the fat in order to produce eggs (Peckarsky and Cowan, 1991). The inferior competitors will be smaller while the superior competitor becomes larger, and in some cases competing for the same prey may lead to intraguild predation for resources. In another example, two predatory invertebrates, *Sweltsa onkos* (a stonefly) and *Rhyacophila vilox* (a caddisfly) were studied in a controlled environment. The authors (Sircom and Walde, 2009) found that *R. vilox* consumed *S. onkos* when the abundance of *S. onkos* was high. Other factors to consider in explaining coexistence of closely related species are partitioning of time of development and how the habitat may influence reproductive strategies (Southwood, 1977). Species that partition time of greatest growth and development may actually be able to share microhabitats without coming in direct competition.

There are many examples of resource partitioning, or time to avoid competition in various species. To avoid competition, two species of caddisfly, *Rhyacophila valuma* and *R. brunnea*, were found in higher abundance in the same streams at different times of the season (Yang, 2018). In a classic review by Southwood, partitioning development between early season and later season explained how some closely related species may not negatively affect one another, because reproduction happens at different times. Competition is reduced and coexistence in the same habitats may be facilitated (Southwood, 1977). Other microhabitat features that can be partitioned by closely related species include position in the stream on different stone surfaces and the flow rate and substrate particle sizes. In a study by Georgian and Thorp (1992), the authors found that different species of net spinning caddisflies had different preferences for stream microhabitats. In general, however, net-spinning caddisfly larvae prefer higher flow sites, stable substrates, and rougher surface texture, which are the conditions that exist on the upstream and top positions of rocks (Georgian and Thorp 1992). Therefore, species that are inferior competitors may be displaced to less favorable microhabitats by superior competitors.

Species of the genus of caddisflies, *Rhyacophila*, prefer to inhabit cold, fast-moving and well oxygenated streams, although those specific niche requirements can vary among species. Studies conducted on patterns of coexistence in *Rhyacophila* (in 2017 by Bridgett Sparrow-Scinocca, and in 2018 by JaHyun Yang) revealed that many closely related species of *Rhyacophila* were found in the same streams near RMBL. Anywhere from, two, three, four, five, and sometimes six species of *Rhyacophila* larvae were found together. And some streams only typically have one species. Dodds and Hisaw (1925) observed that different species *Rhyacophila* in the Gunnison basin had specific elevation preferences, but also observed overlap in the species *R. coloradensis*, and *R. hyalinata*. These two species were observed in similar altitudes between 6,000 -11, 000 ft. The researchers also attributed preferences in altitude of streams to temperature needs, because higher altitude streams are generally colder than those at lower elevations (Dodds and Hisaw, 1925). Finally, Wiggins and McKay (1978) observed that the most abundant species of caddisflies were the ones whose niches were also abundant. They hypothesized that if the niches are vast, then many species will overlap as well.

This study will continue to attempt and understand why some closely related species may or may not coexist. Factors, both abiotic and biotic, that have been previously considered in the coexistence of closely-related *Rhyacophila* species are elevation, water temperature, water depth, size of streams, flow rates (discharge), and the proliferation of the nuisance diatom *Didymosphenia geminata* (*Didymo*), which covers the bottom of some streams in low flow years, potentially affecting the distributions of these caddisflies. It is possible that the recent environmental changes (e.g., protracted drought), which affects the extent of *Didymo* proliferation, could cause a shift in the ranges of these caddisflies. In a review article of the distributions of *Didymo*, Taylor and Bothwell (2014) provide clues about the conditions of the streams where *Didymo* proliferates, which might affect the suitability of habitat for *Rhyacophila* species. The authors further argue that species composition and the relative abundance of this and other diatom taxa are effectively used as indicators of changing environmental conditions. Therefore, investigating the relationship between *Didymo* proliferation and the distributions of *Rhyacophila* species could help us understand why some species do or do not coexist in streams around RMBL. In addition, the abundance of *Didymo* attracts chironomids, which could increase food resources for the caddisflies (Gillis and Chalifour, 2009), because chironomids are one of the favored prey items for *Rhyacophila* species (Thut, 1969). However, mayflies, which are another food source for *Rhyacophila*, may be negatively affected or unaffected by *Didymo* proliferations. Therefore, the effects of *Didymo* on these caddisflies (negatively or positively) is yet to be determined.

Previous studies conducted by Bridget and JaHyun in 2017 and 2018 have provided important background information about the biology and coexistence patterns of the four most abundant species of *Rhyacophila* in streams around RMBL. Their studies found *Rhyacophila alberta* and *R. hyalinata* were frequently found together at high elevation, smaller, colder streams with low conductivity. In 2017, Bridget observed that *R. hyalinata* not only negatively affected the growth rate of *R. alberta*, but the superior competitor also consumed by *R. alberta* (intraguild predation) (Sparrow- Scinocca, 2017). In the 2018 study, coexistence was observed in *R. brunnea* and *R. valuma*, which were found together in lower elevation, larger, warmer streams with larger substrate particle sizes (Yang, 2018). In addition, coexistence was possibly explained by partitioning of time, because larvae of *R. valuma* were more abundant in early

season samples, and *R. brunnea* were more abundant later in the season (Yang, 2018). JaHyun hypothesized that these two closely related species may be able to live in the same streams and avoid competition by staggering their development times. Different *Rhyacophila* species may emerge early or late depending on overwintering, the climate, and its ecological niche (Peckarsky et al., 1990).

The goals of this study are to extend the findings from studies of previous RMBL students carried out in 2017 and 2018 by partitioning microhabitats or food resources to potentially explain how species that have been found to compete (*R. alberta* and *R. hyalinata*), or have the potential to compete (*R. valuma* and *R. brunnea*) are able to coexist in the same streams. Because 2019 is a low *Didymo* year, we were not able to analyze the effects of proliferation of *Didymosphenia geminata* on the habitat partitioning by these species of caddisflies.

Study Sites

We sampled *Rhyacophila* from seven of eight long-term sites in the Upper East River Basin of Gunnison County, Colorado. There are three sites on Copper Creek (Second Crossing, above Judd Falls, above the bridge in Gothic), four sites on East River (Oregon Mine Road Crossing, near Avery, near Levi Cabin, and above the bridge), and one site on Rustlers Gulch. The Oregon Mine Road site was under ice and snow and could not be sampled. These sites are part of an unprecedented historical data set monitoring invertebrates since 1976 and provide an opportunity to look at long term trends. Streams have been sampled twice each summer, once during the early season and again in the later season to account for different phenologies of invertebrate species. We also sampled eight small streams in the same drainage basin while waiting for the water levels in the long-term sites (which are larger streams) to become safe for sampling. Those sites are Avery Creek, Marmot Creek, Lower Rock Creek, Upper East River, Lower Bradley Creek, Upper Bradley Creek, Gothic Creek, and B11 near the Avalanche Cabin (Figure 1 - map)

Methods

Collection Methods and Measurements of Environmental Parameters

For the long-term sites we used the same collection methods as previous years by using a Hoffman sampler consisting of a 0.104 m² area box fastened to a 364 µm mesh driftnet. The substrates inside the box were disturbed for one minute and benthic invertebrates were swept by the current into the net. All Diptera, mayfly, stonefly, and caddisfly larvae were sorted and identified in the field. We took four samples in different locations along each stream site. *Rhyacophila* specimens were preserved in vials of 70% ethyl alcohol for identification and further processing in the lab. All other organisms were released except for tiny specimens that could not be identified in the field.

For the smaller stream sites, a D-net with a 500 µm mesh was used. The substrate above the net opening was disturbed for one minute, as in the long-term sites. Only *Rhyacophila* were collected in the small streams and preserved in vials with 70% ethyl alcohol for lab analysis. Samples were taken at 3 different locations for each stream sampled.

Previous studies focused on the abiotic and biotic environments at the macrohabitat scale of stream reaches (water temperature, temperature-specific conductivity, stream size, discharge, *Didymo* cover, predator density and competitor density; Sparrow-Scinocca 2017, Yang 2018). Instead, for both the long-term sites and the smaller streams, we measured several abiotic factors at each sample taken in each stream to describe the microhabitats of the different species of *Rhyacophila*. Therefore, we described the attributes of *Rhyacophila* microhabitats by measuring selected parameters at each sample we took. The factors we measured for each sample were water depth (using a meter stick), current velocity (using a Marsh-McBirney flow meter), and substrate particle size (collecting the rocks that were inside the sampler when we collected the bugs and measuring their intermediate axis in mm). The abundance of *Didymo* was low to non-existent with high stream flow, so we were unable to compare *Didymo* growth in each sample.

We also attempted to use the scan method described by Martin and MacKay (1982) to dissect *Rhyacophila* to decode what prey were in their stomachs. The goal was to compare the abundance of the two major prey items preferred by *Rhyacophila* (chironomids and *Baetis* mayflies) in each sample as a second test of whether available food resources differ between microhabitats preferred by coexisting species of *Rhyacophila*. However, after dissecting over 20 individuals of different species, we learned that gut contents mostly consisted of unidentifiable food. Therefore, we modified another method from Martin and MacKay (1982) and estimated gut fullness by counting the number of abdominal segments that contained food. This method was only reliable with *R. valuma*, which we could inspect through the thin integument. We confirmed that this method was accurate by dissecting several specimens and obtaining the same results. Finally, using an ocular micrometer on a dissecting microscope, we measured the head capsule widths of all four species of *Rhyacophila* to test whether this surrogate of “gape width” differed between pairs of species that typically coexist at the same stream sites or in the same samples.

Data Analysis of Abundance and Environmental Associations

To test whether partitioning of microhabitat or food may explain the patterns of distribution of coexisting pairs of *Rhyacophila* species, we plotted the mean $\pm$ 2 SE for each environmental variable to compare microhabitats preferred by pairs of *Rhyacophila* species that typically coexist in the same streams (Yang and Peckarsky 2018). We tested whether pairs of coexisting species differed in environmental variables we measured at the microhabitat scale (water depth, current velocity, substrate particle size, and abundance of two types of potential prey species: *Baetis* mayflies and chironomids). We considered those means to be different if the $\pm$ 2 SE did not overlap for each variable at the microhabitat scale (per sample) instead of analyzing data at the macrohabitat scale, as was done in previous studies. Using these plots, we compared those preferences between pairs of species generally found together in the same streams to ask whether they coexist by occupying different microhabitats.

We also used bar graphs (mean $\pm$ 2 SE) to compare the microhabitats of each common species of *Rhyacophila* when it was found in a sample (microhabitat) by itself and in microhabitats with other potential competitors. That way we tested whether some species shift microhabitats or had

different aggregations with prey when in the presence of potential competitors. We considered microhabitat shifts to be significant if the 2 SE did not overlap.

Finally, we compared the gut fullness (number of abdominal segments containing food divided by 8, which was the maximum extent of segments that contained food) between *R. valuma* when it was collected with and without potential competitor species in samples to ask whether the presence of competitors affected gut fullness. We also calculated the ratio of head capsule widths for all *Rhyacophila* collected. We used this parameter to compare head morphologies of all species, with those with wider heads having gape potential for eating larger prey (*Baetis*) than those with smaller head widths (more suitable for eating chironomids). This comparison enabled us to generate hypotheses about possible partitioning of food resources between competing pairs of species. We considered gape widths to be significantly different between pairs of coexisting species if the ± 2 SE did not overlap.

Results

The species pairs that coexist were plotted together in a ± 2 SE test. The “Cold Water” species, *R. hyalinata* and *R. alberta*, significantly partitioned microhabitats with respect to water depth and substrate particle sizes. *R. hyalinata* preferred deeper microhabitats with larger substrate particle sizes, and *R. alberta* preferred shallower microhabitats with smaller substrate particle sizes (Figures 2a, 2c). While *R. hyalinata* microhabitats with slower current velocity than *R. alberta*, that difference was not significant (Figure 2b - ± 2 SE overlapped.) With the “warm water” species, *R. valuma* and *R. brunnea*, we found that there were no differences in preferences for water depth, current velocity, or substrate size (Figure 3a, b, c – bar graphs of mean ± 2 SE for the three microhabitat parameters: ± 2 SE overlapped for all parameters).

When we compared microhabitat preferences for each of the four species of *Rhyacophila* in samples in the absence of competitor species to samples in which competitor species were present, all species of *Rhyacophila* shifted to areas with slower current velocities in the presence of competitors (Figure 4a, b, c, d), but none of the species shifted depths, or substrate particle sizes.

The densities (per sample) of all four species were plotted for (Y) against prey densities (X – both *Baetis* and chironomid prey types) to test the relationships between prey and predator densities. We calculated R^2 to test the strength of each relationship. For the “cold water” species, the density for *R. hyalinata* increased as the density of *Baetis* increased, while the density of *R. alberta* did not change with *Baetis* density (Figure 5a). In contrast, when density of *R. hyalinata* was higher, the density of chironomids decreased, and again, the density of chironomids did not change in the presence of *R. alberta* (Figure 5b). The density of the “warm water” species did not change with the density of *Baetis*, but when plotted with chironomids, both the density of *R. valuma* and *R. brunnea* increased as the density of chironomids increased (Figure 5c).

Gut fullness was assessed in *R. valuma* (Figure 5d). The observation and dissection techniques from Martin and MacKay (1982) revealed no significant difference between the gut fullness of *R. valuma* in the absence or the presence of potential competitor species (± 2 SE overlapped). In addition, comparisons of prey density for *Baetis* and chironomids in

microhabitats where all *Rhyacophila* species were collected in sites where species had potential competitors, and where competitors were absent showed that *R. alberta*, *R. hyalinata*, and *R. valuma* aggregated in samples where the density of *Baetis* was high, and competitors were present (Figure 6a, b, c,). When chironomid densities were high, *R. alberta*, *R. brunnea* and *R. valuma* also aggregated in the presence of competitors (Figure 6d, e, f). When *R. hyalinata* was not in the presence of competitors, it aggregated in microhabitats with a high density of chironomids (Figure 6g).

Finally, comparisons of the head widths (as a surrogate of mouth gape) between pairs of coexisting species showed that between the “cold water” species, *R. hyalinata* had a significantly larger gape width than *R. alberta* (Figure 7a) and between the “warm water” species, *R. brunnea* had a larger gape width than *R. valuma* (Figure 7b).

Discussion

The results of this study provided several testable hypotheses regarding whether two pairs of species of *Rhyacophila* may be able to coexist in the same streams at the macrohabitat level, by partitioning resources at the microhabitats level or partitioning food resources. Two species that commonly coexist in colder streams (*R. alberta* and *R. hyalinata*) showed different preferences for water depth and substrate particle size, and a tendency to be found in different current velocities. However, the two species that commonly coexist in warmer streams (*R. brunnea* and *R. valuma*) showed no evidence of partitioning microhabitats for those same variables. We also observed that in the presence of competitors, all four species shifted their microhabitats to areas of slower flow and aggregated in microhabitats with higher prey densities (*Baetis* or chironomids). Attempts to distinguish different prey types in the stomach contents of these *Rhyacophila* species was not successful (most gut contents were not recognizable), so we were unable to test directly whether coexisting species partitioned food resources. However, a mouth gape analysis enabled us to hypothesize that both pairs of coexisting species may have mouthpart morphologies more suitable for eating different types of prey (large prey for *R. hyalinata* and *R. brunnea*, small prey for *R. alberta* and *R. valuma*). In the following discussion we speculate about possible mechanisms explaining our observations and develop hypotheses that can be tested in the future to understand how coexisting, closely related species avoid competition.

The two species that commonly coexist in colder streams (*R. hyalinata*, and *R. alberta*) showed preferences for two of the three microhabitat parameters that we measured: water depth and substrate particle size. These observations are consistent with the hypothesis that *R. alberta* may be avoiding coexisting in the same microhabitats as *R. hyalinata*, which has been shown in previous experiments to cause reduced growth rates of *R. alberta* (Sparrow-Scinnoca, 2017). Reduced growth rates can lead ultimately to smaller sizes when they pupate and eventually to reduced fecundity (Honěk, 1993) Therefore, coexisting with *R. hyalinata* could result in lower fitness for *R. alberta*, providing strong selection pressure for partitioning microhabitats. *R. hyalinata* also demonstrated intraguild predation of *R. alberta*, which would also place strong selection pressure on *R. alberta* to live in different microhabitats than *R. hyalinata*.

Observations of aggregation by these two species with different prey types and trends considering related to different mouth gape sizes also provided some indirect evidence consistent with the hypothesis that these two species have the potential to not have to compete for resources when they aggregate to areas with an increased amount of prey. The width of the larvae heads may give us clues to mouth gape sizes, and what prey may be easier to eat given the size of mouth parts. In an article by Ibanez et. al, the incisors of grasshoppers were measured, and they found that mouth parts are specialized towards the resources they are consuming (Kang, et al 1999 & Ibanez, et. al, 2013). *R. hyalinata* has the biggest mouth gape, making it most morphologically suited to eating larger prey, which could explain why this species was observed to aggregate in microhabitats with high *Baetis* densities (larger than chironomids). In later instars, this species had significantly wider mouth gapes than *R. alberta*, which may be more suited to eating smaller prey (chironomids). However, *R. alberta* did not aggregate in areas of high chironomid densities, except when in the presence of competitor species. More data are needed (e.g. experimental feeding trials) to test whether these two species partition prey resources.

In contrast to the colder water species, the pair of species that commonly coexist in warmer streams (*R. valuma* and *R. brunnea*) did not partition any of the microhabitat parameters that we measured. Both species aggregated in microhabitats with higher chironomid densities. These observations could be interpreted to mean that they do not compete and can coexist without affecting each other's fitness. It is unknown whether these two species compete, because experiments conducted by Sparrow-Scinnoca (2017) did not test for competition between these two species. Our observations suggest that aggregation of these two species of predators where prey resources are high occurs at the microhabitat scale, possibly decreasing the potential for interspecific competition.

Alternatively, this observation could possibly be explained by previous observations that these two species may partition development time. By comparing abundances and sizes of these two species in early and late summer collections, Yang (2018) hypothesized that *R. valuma* may develop as larvae, pupate and emerge earlier in the summer than *R. brunnea*. Our observation that *R. valuma* has smaller gape width than *R. brunnea* is consistent with the hypothesis that this species may have its greatest period of growth when *Baetis* is smaller and *R. brunnea* develops later in the summer when *Baetis* is large. *R. brunnea* may prefer to eat chironomids, which are the smaller prey type. However, aggregation of both species in microhabitats with high chironomid densities may be potentially explained by this being a very high-water summer when development of both species could be delayed (Finn & Poff, 2008).

Comparisons of microhabitats of pairs of coexisting species when they were collected in samples with and without potentially competing species provided some very interesting observations regarding the possible effects of interspecific competition on microhabitat or food preferences. All four species, *R. alberta*, *R. brunnea*, *R. hyalinata*, and *R. valuma*, switched to slower microhabitats in the presence of competitors. The cost of moving to slower flow areas could be higher for the species without filamentous gills (*R. hyalinata* and *R. valuma*), but these streams all are supersaturated with oxygen. Therefore, it is unlikely that moving to slower flow areas would cause respiratory stress (Peckarsky, unpublished data). *R. alberta* and *R. brunnea* have filamentous gills that help diffuse oxygen, which would make it possibly less costly to

move to slower flow areas of streams in response to avoiding interacting with potential competitors (Jacobsen, 2000). In addition, one possible disadvantage of fast flow is that it would allow for scouring and washing *Rhyacophila* downstream. None of these species are good swimmers, and *R. hyalinata* and *R. valuma* have two pairs of large anal claws that allow them to hang on to the substrate surfaces better than the other two species. It is interesting that all these species move to slower flow areas when coexisting in the same samples with interspecific competitors. Therefore, these microhabitat shifts to slower flow areas appear not to have any negative consequences.

In fact, such microhabitat shifts may benefit all these species because prey density of both *Baetis* and chironomids tends to be higher at slower flow areas, as illustrated in Figure 8. A switch to slower flow microhabitats in the presence of interspecific competitors could be advantageous to many *Rhyacophila* species. Consistent with this hypothesis, we also observed that all four species showed greater aggregations in microhabitats with high densities of prey when they coexisted in the same samples with potential interspecific competitors. This observation suggests that these species form multi-species aggregations in areas of high prey density, as has been observed in other studies (Sevenster, 1996). However, it remains unclear why these species would occupy faster flow microhabitats and show weaker aggregative responses in the absence of interspecific competitors. It is possible that multi-species aggregations in microhabitats with high prey density enables them to reduce interspecific competition if prey resources are less limited.

This study provides many opportunities for the future to test questions that are suggested by our observations. First, when we collected the *Rhyacophila* larvae, we collected small instars (I – III) during this high snow, late snow melt, high flow summer. It would be productive to test whether later instars of these species show greater tendency to partition microhabitats as they scramble to obtain resources to pupate, emerge as adults, and reproduce. Late snowmelt and high water have been shown to affect the development of the larvae of other. Species, and resources emerging at different times may cause a phenological shift that would affect the future of species success (Kudo and Ida 2013). Therefore, repeating these observations during lower water, drier summers may produce a different result. Second, while we tested for habitat partitioning at smaller scales than previous studies (Sparrow-Scinnoca 2017, Yang 2018), it is possible that we tested for microhabitat partitioning at scales that were not small enough to detect a difference between preferences of coexisting species. For example, substrate particle size preferences should probably be tested at the scale of individual rocks. When we turned over rocks to measure their sizes, we often noticed many species of *Rhyacophila*, as well as prey, that were attached to the rock.

Finally, the data that have been collected these past three years have answered some questions but left us with many more. Here are several examples: We need to implement a competition experiment with the two warm water species, with different prey types at different instars to answer the question of whether these pairs of coexisting species compete or can co-occur without negatively affecting each other's fitness. We also anecdotally observed that *Rhyacophila* species may be associated with accumulations of detritus, which are rare in these streams. They do not eat detritus but may use it as a good place to attach to the substrate.

Investigating this question may yield different results for preferred microhabitats. The mouth gape size versus prey size association could also be answered by an experiment.

We can conclude from our study that some pairs of closely related *Rhyacophila* species that coexist in streams near RMBL may partition microhabitat or prey resources to avoid competition, and others may not. There are several implications of this study that deserve further consideration. With climate change the streams and terrestrial habitats around RMBL are generally warming (Finn and Poff, 2008), and the frequency and intensity of extreme events are both increasing. This area is experiencing dramatic variation in the amount of snow accumulation and the timing of snowmelt and high flows. All those consequences of climate change can affect the developmental times and availability of microhabitats preferred by different *Rhyacophila* species that may prefer warmer or cooler water, faster or slower flows. Therefore, such climate change effects can influence the coexistence patterns of not only these caddisflies, but also of their prey species. Understanding the mechanisms of coexistence of these closely related species will be critical to predicting how they will respond to the challenges of living in a changing environment.

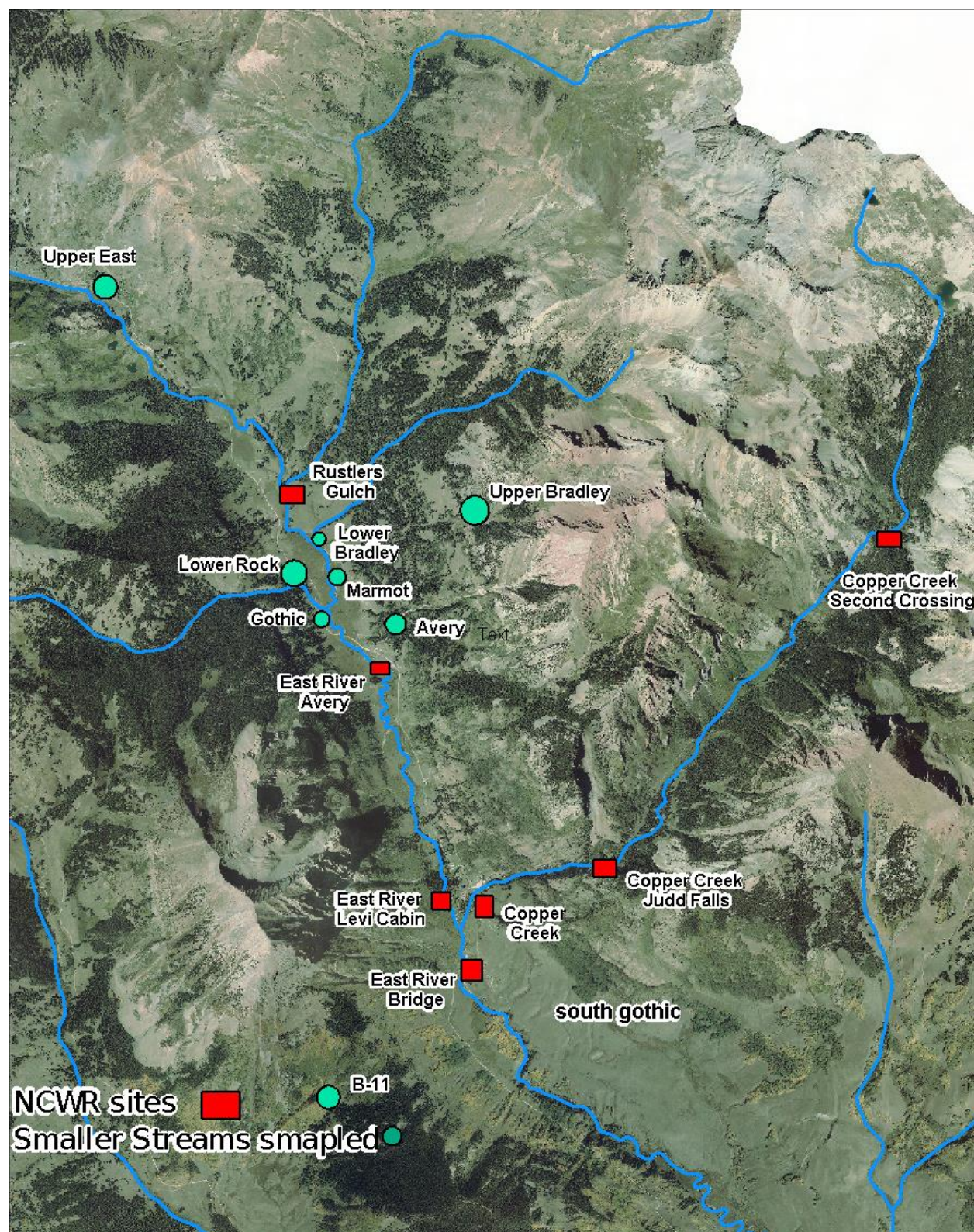


Figure 1 - Map of study sites used for this study. Red rectangles are for the NCWR sites that have been monitored since 1976. The teal circles show the small streams used while the flow in the larger streams returned to safe levels.

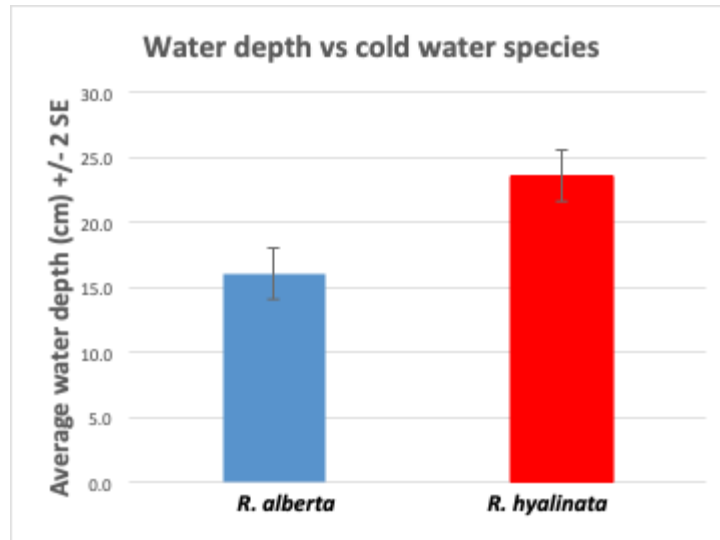


Figure 2A. Average $\pm$ 2 SE water depths (cm) for “cold water” species: *R. hyalinata* and *R. alberta* showed a significant difference in preferences for water depth. *R. hyalinata* preferred deeper microhabitats than *R. alberta*.

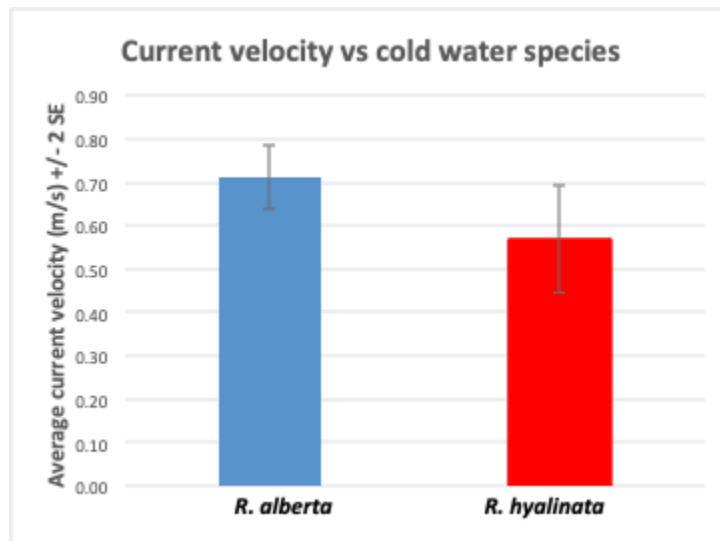


Figure 2B. Average $\pm$ 2 SE current velocities (m/s) for “cold water” species showed no significant differences in preferences for current velocities.

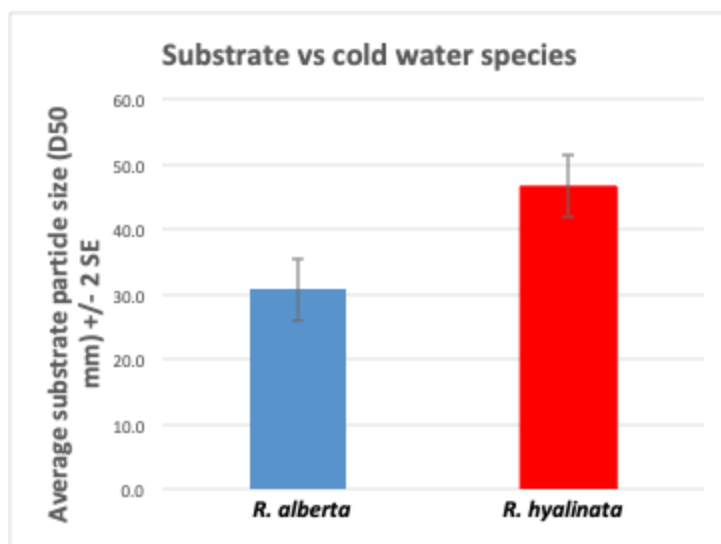


Figure 2C. Average $\pm$ 2 SE median substrate particle sizes (D50- mm) of microhabitats containing the two “cold water” species. *R. alberta* preferred significantly smaller substrate particle sizes than *R. hyalinata*.

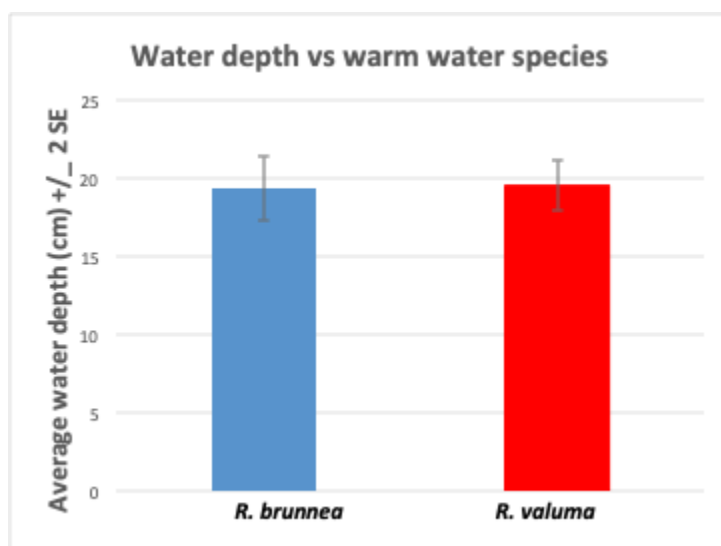


Figure 3A. Average $\pm$ 2 SE water depths (cm) of microhabitats containing the two “warm water species” (*R. brunnea* and *R. valuma*), showing no differences in microhabitat preferences between the two species.

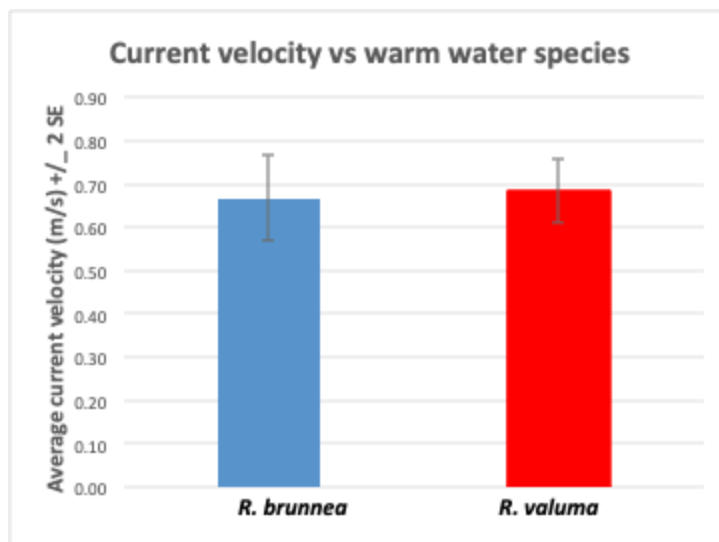


Figure 3B. Average $\pm$ 2 SE current velocities (m/s) of microhabitats containing the two warm water species (*R. brunnea* and *R. valuma*), showing no differences in microhabitat preferences between the two species.

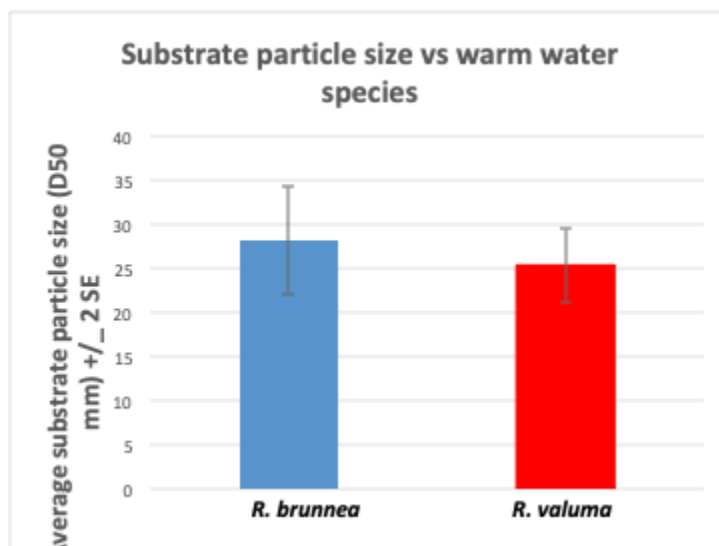


Figure 3C. Average $\pm$ 2 SE median substrate particle sizes (D50- mm) of microhabitats containing the two warm water species (*R. brunnea* and *R. valuma*), showing no differences in microhabitat preferences between the two species.

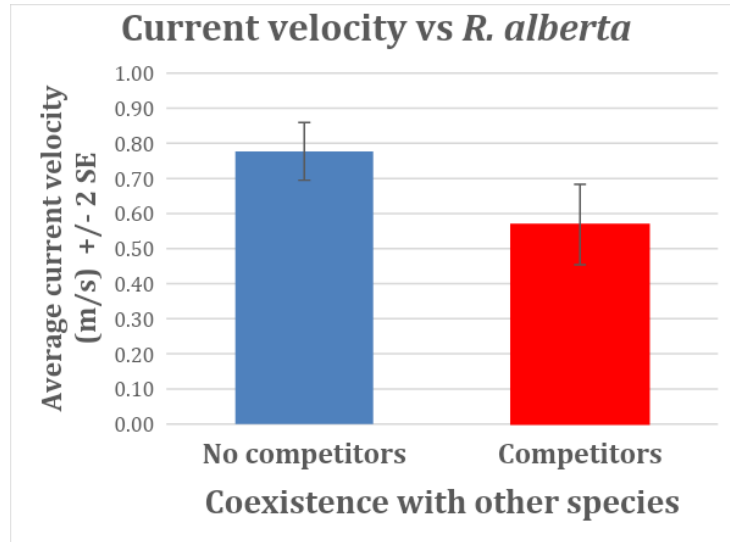


Figure 4a – *R. alberta* occupies microhabitats with slower average current velocities in the presence of competitor species of *Rhyacophila* when compared to microhabitats with no other species of *Rhyacophila* present.

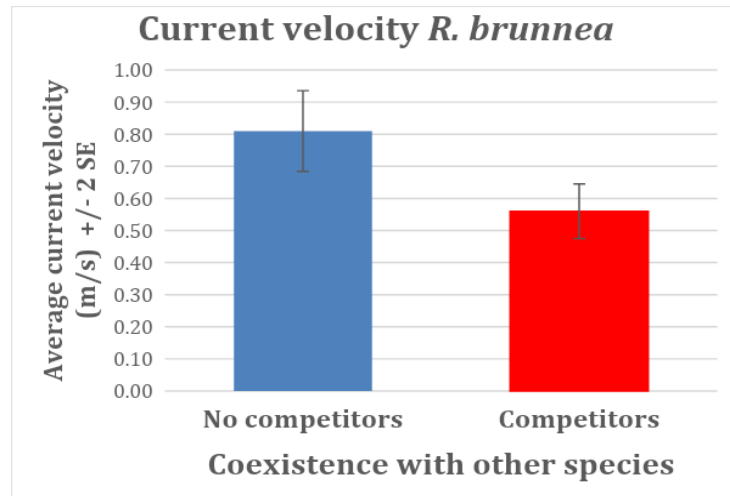


Figure 4b – *R. brunnea* occupies microhabitats with slower average current velocities in the presence of competitor species of *Rhyacophila* when compared to microhabitats with no other species of *Rhyacophila* present.

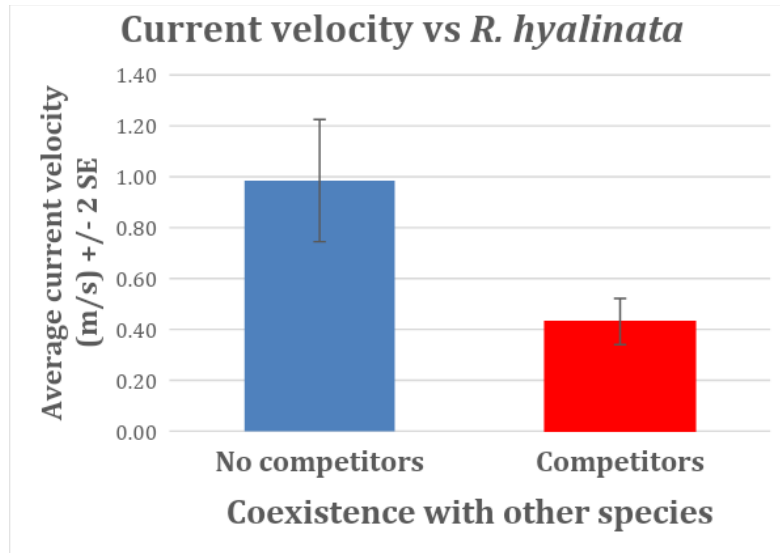


Figure 4c – *R. hyalinata* occupies microhabitats with slower average current velocities in the presence of competitor species of *Rhyacophila* when compared to microhabitats with no other species of *Rhyacophila* present.

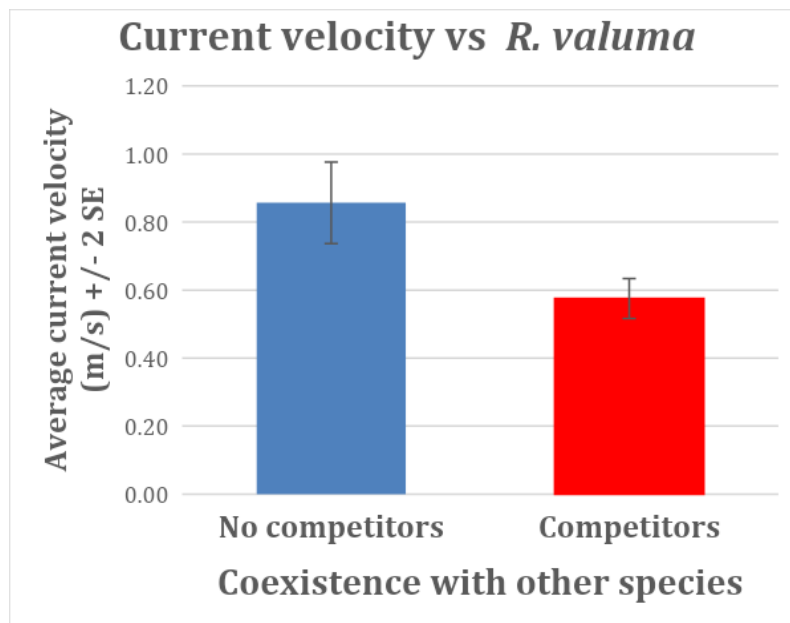


Figure 4d – *R. valuma* occupies microhabitats with slower average current velocities in the presence of competitor species of *Rhyacophila* when compared to microhabitats with no other species of *Rhyacophila* present.

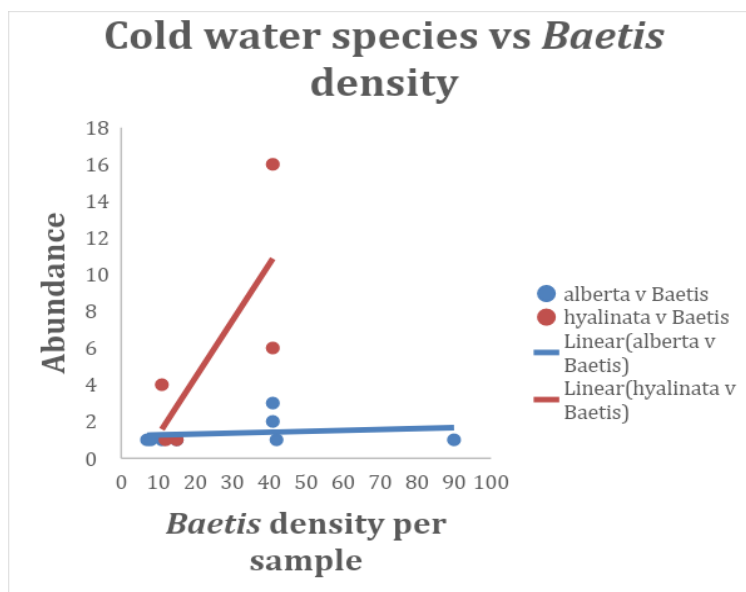


Figure 5a – Relationship between abundance (per sample) of the two “cold water” species of *Rhyacophila* over the range of densities of larger prey (*Baetis* mayflies). *R. hyalinata* density (per sample) increases with increasing *Baetis* density, but there was no relationship between *R. alberta* density and *Baetis* density.

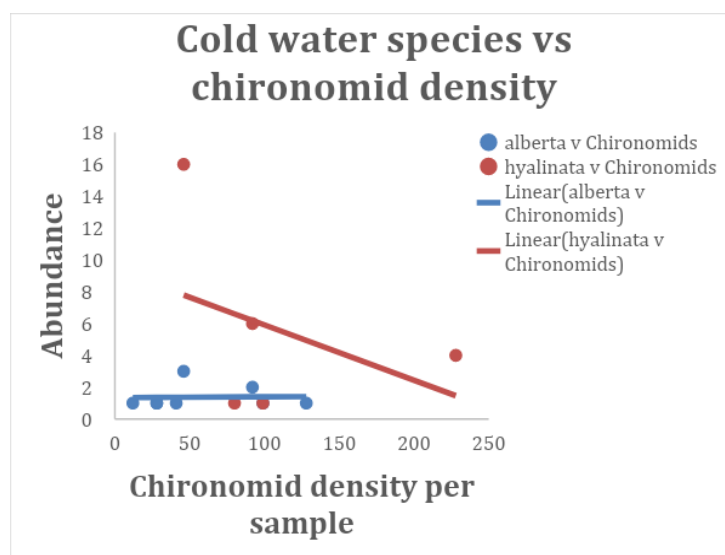


Figure 5b – Relationship between abundance (per sample) of the two “cold water” species of *Rhyacophila* over the range of densities of smaller prey (chironomid midges). *R. hyalinata* density decreases with increasing chironomid densities.

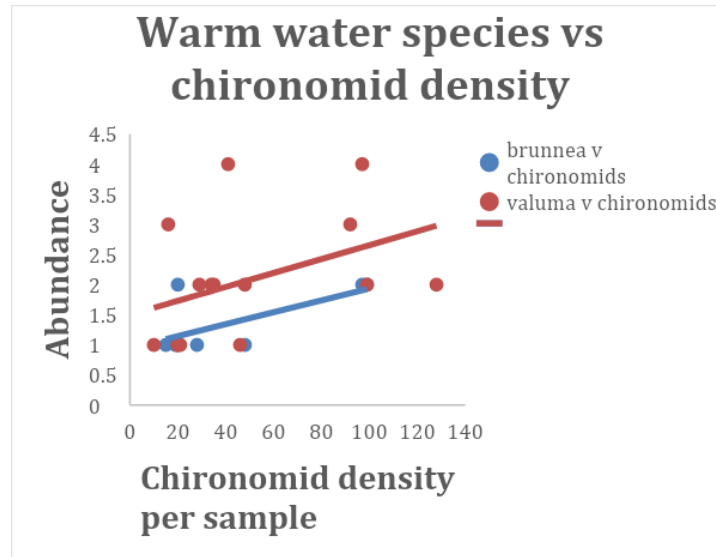


Figure 5c – Relationship between abundance (per sample) of the two “warm water” species of *Rhyacophila* over the range of densities of smaller prey (chironomid midges). Densities (per sample) of both *R. brunnea* and *R. valuma* increased with chironomid density.

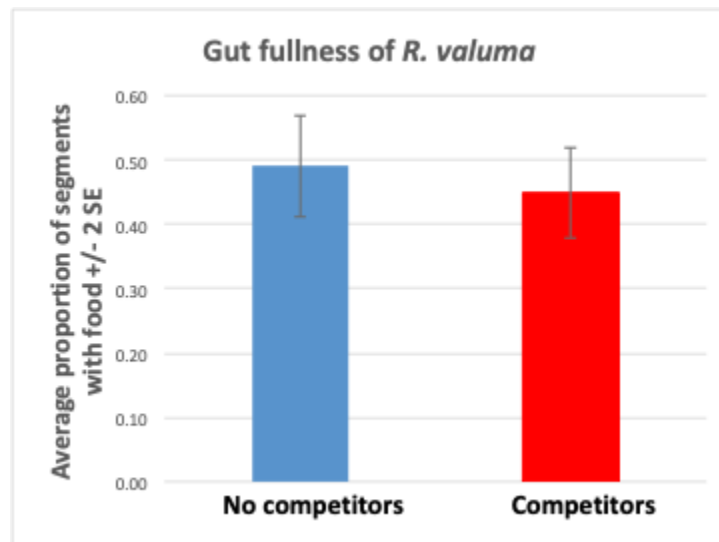


Figure 5d- The gut fullness of *R. valuma* in the absence, and presence of competitors showed no significant difference.

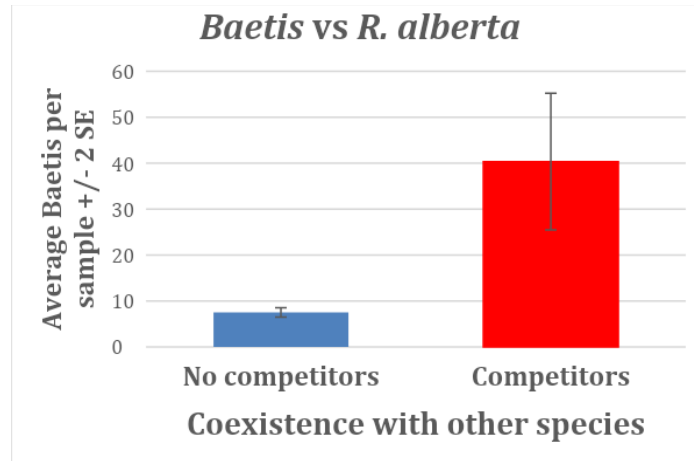


Figure 6a – Average density of *Baetis* prey (per sample) in microhabitats was higher where *R. alberta* was collected with coexisting species of *Rhyacophila*.

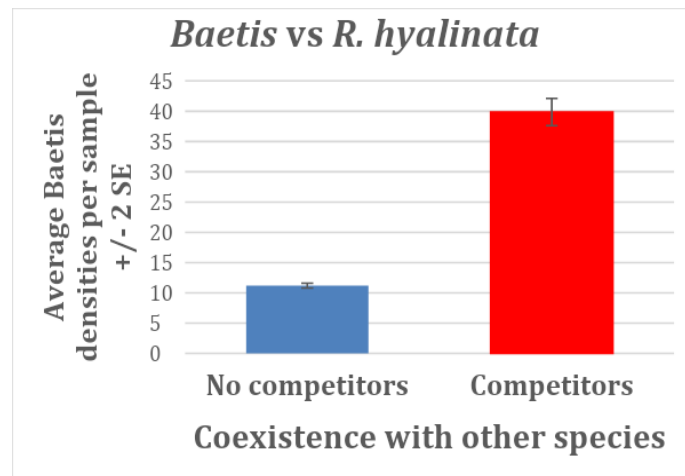


Figure 6b – Average density of *Baetis* prey (per sample) was higher in microhabitats where *R. hyalinata* was collected with coexisting species of *Rhyacophila*.

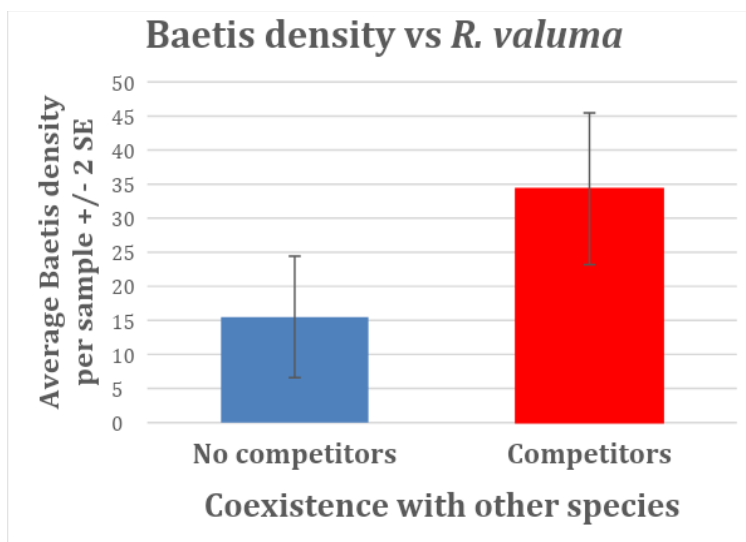


Figure 6c – Average density of *Baetis* prey (per sample) was higher in microhabitats where *R. valuma* was collected with coexisting species of *Rhyacophila*.

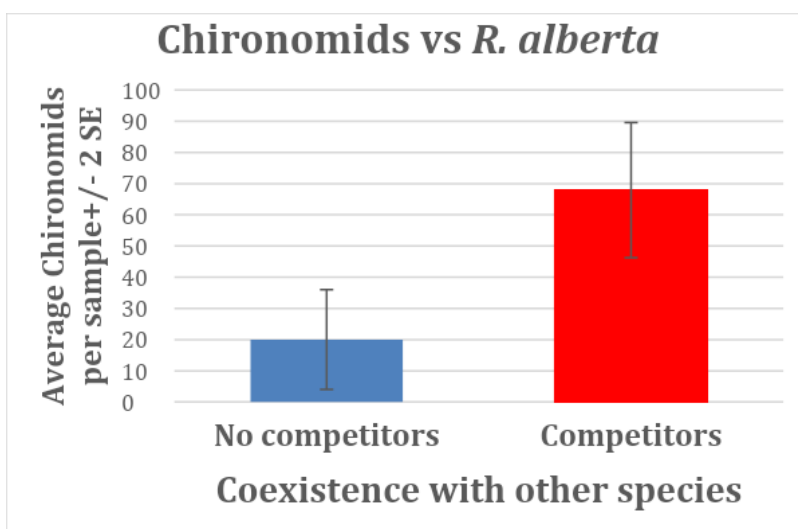


Figure 6d – Average density of chironomid prey (per sample) was higher in microhabitats where *R. alberta* was collected with coexisting species of *Rhyacophila*.

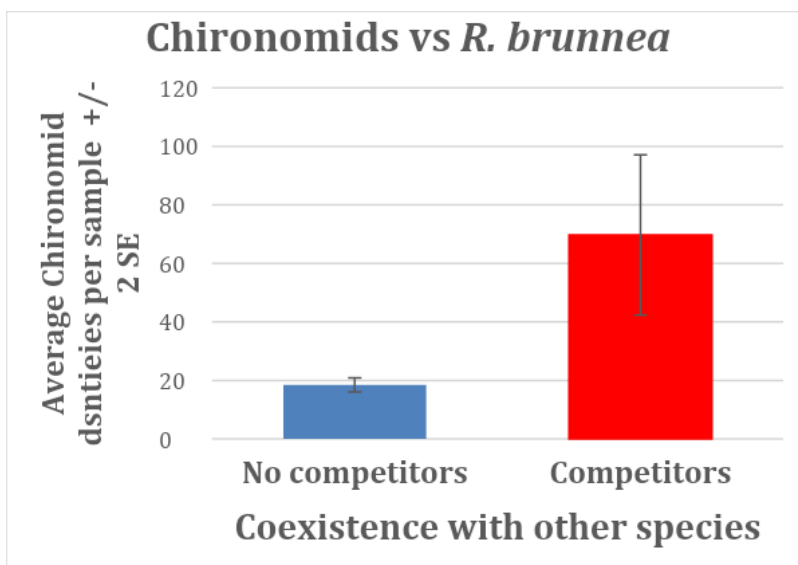


Figure 6e – Average density of chironomid prey (per sample) was higher in microhabitats where *R. brunnea* was collected with coexisting species of *Rhyacophila*.

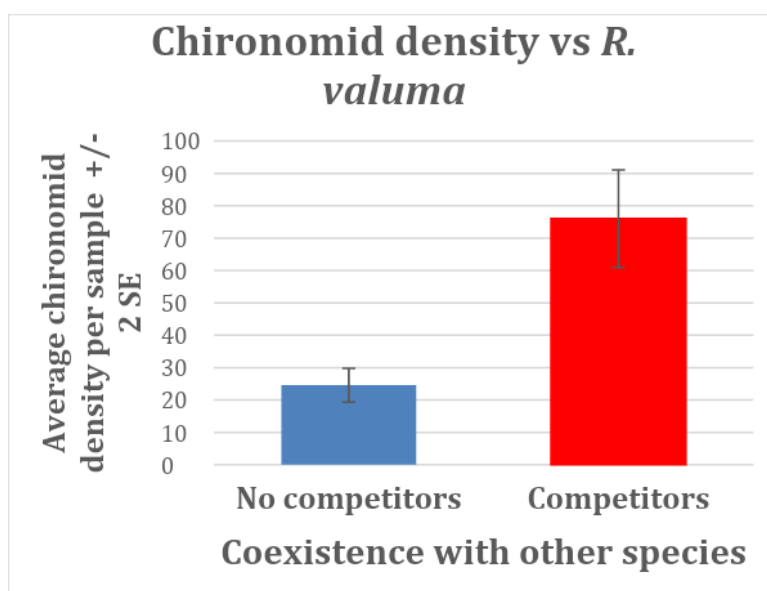


Figure 6f – Average density of chironomid prey (per sample) was higher in microhabitats where *R. valuma* was collected with coexisting species of *Rhyacophila*.

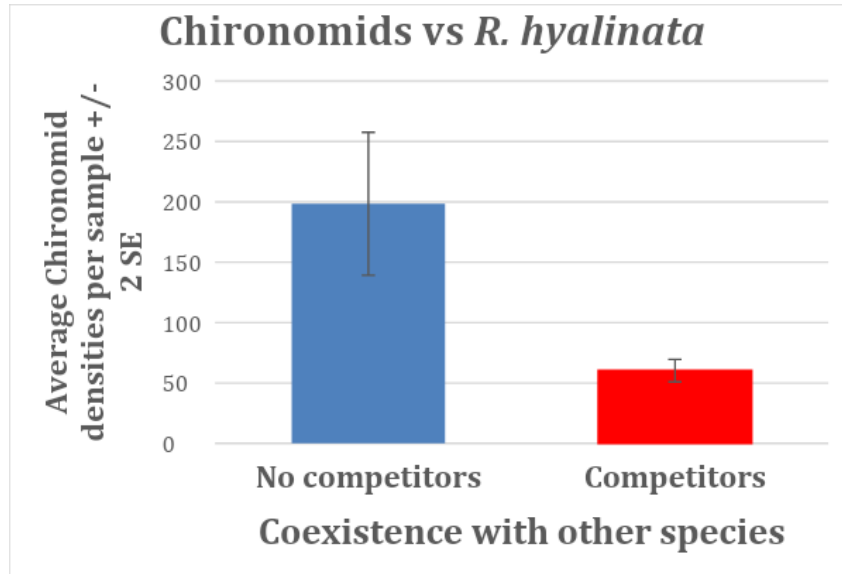


Figure 6g – Average density of chironomid prey (per sample) was higher in microhabitats where *R. hyalinata* was collected without coexisting species of *Rhyacophila*.

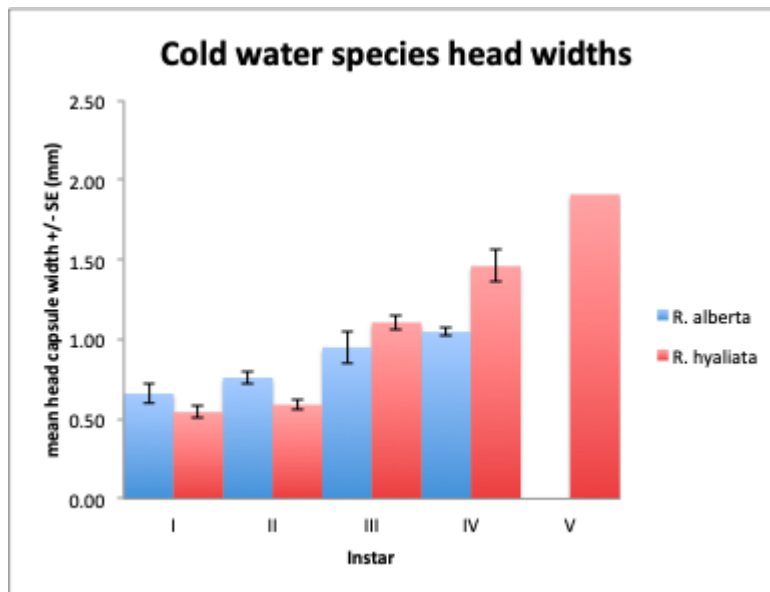


Figure 7a – Mean ($\pm$ 2 SE) head capsule widths of the two coexisting species of “cold water” *Rhyacophila*. Early (I, II) instar species do not differ in head capsule widths, but later instars (III, IV, V) *R. hyalinata* have significantly wider head capsules than *R. alberta*.

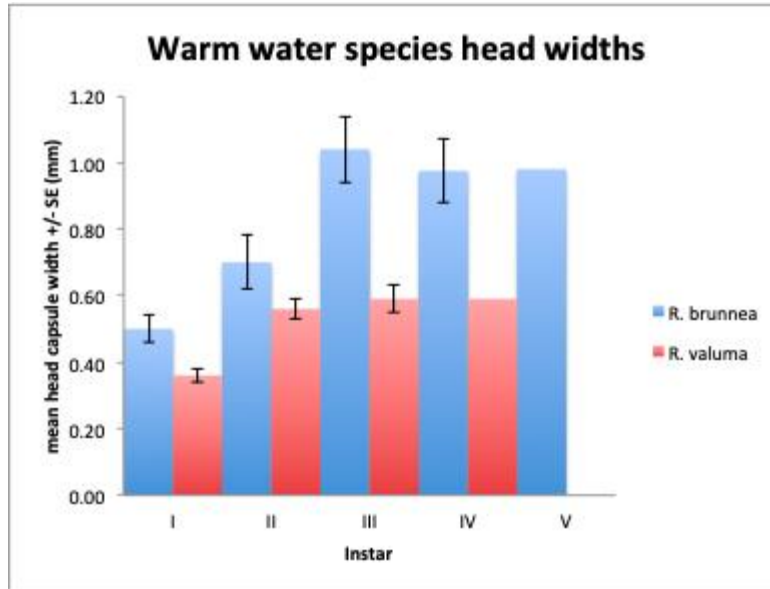


Figure 7b – Mean (+/-) head capsule widths of the two coexisting species of “warm water” *Rhyacophila*. All instars of *R. brunnea* have significantly wider heads than *R. valuma*.

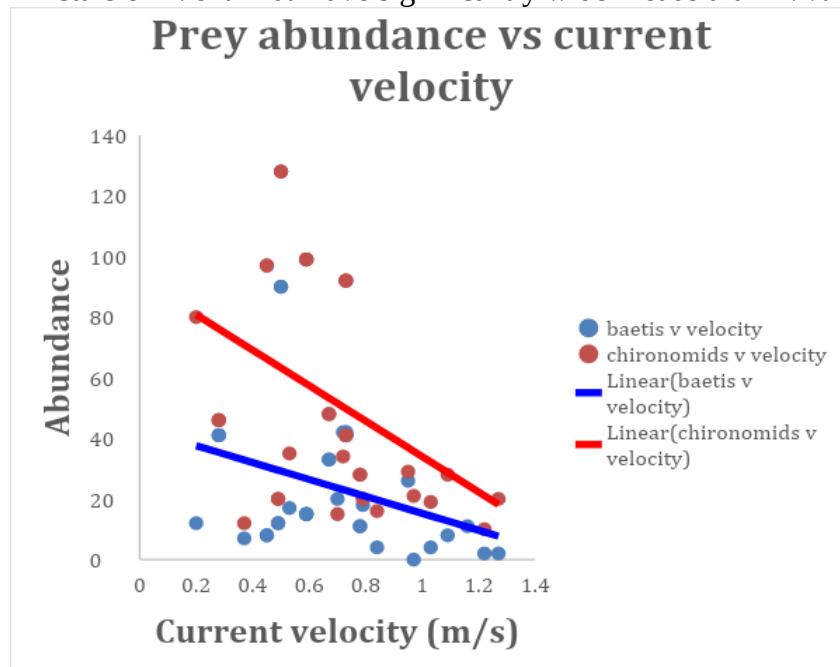


Figure 8 – Abundance per sample of the two main prey types consumed by *Rhyacophila*, *Baetis* (blue symbols) and chironomids (red symbols) as a function of current velocity. Both prey types are more abundant in areas of slower current velocity.

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