

**Spatial and Temporal Patterns of Coexistence in Closely Related *Rhyacophila*
(Trichoptera) Species**

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Abstract

Environmental change affects the survival, interaction and co-existence of species within an ecosystem. We studied multiple species of *Rhyacophila*, a primitive genus of caddisfly that, in its larval state, lives at the bottom of cold, oxygen rich and fast flowing streams. Previous research has shown that six species, four of which are common, occur in the high montane, subalpine and alpine streams of the Upper East River Basin in the Gunnison National Forest. The goals of this study are: 1) to document the patterns of co-existence of different *Rhyacophila* species over space by sampling the same streams previously surveyed and to conduct species co-occurrence analyses to determine if there are any positive or negative associations between pairs of species; 2) to measure various environmental factors to obtain descriptive data about the typical niches of different species, which enabled us to generate hypotheses about potential causes of species co-occurrences; and 3) to conduct both seasonal and long-term temporal comparisons of *Rhyacophila* species co-occurrence patterns and environmental associations using the past 31 years of data. Results suggest that partitioning of space, time and competition are a few key mechanisms that can explain the observed patterns of *Rhyacophila* coexistence. Because *Rhyacophila* are particularly sensitive to environmental change, this study provides clues to climate change related impacts and serves as a comparative model for more threatened freshwater systems in Colorado.

Introduction

Environmental change affects the survival, interaction and co-existence of species within an ecosystem. Grinnell (1917) described a species' niche as its ultimate distributional unit. Environmental factors define a species' expected niche boundaries while species interactions and competition influence the realized range (Grinnell 1917). Climate change is affecting environmental conditions and species interactions and therefore causing range shifts in many species. For example, 48 out of 53 bird species in the Sierra Nevada mountains were found to track their climatic niche to cope with the changing environment (Tingley 2009). Range shifts and overlapping niches create new interactions among different species, including competition for resources and predation, which affect a species' ability to survive within a particular ecosystem (Tingley 2009). Therefore, with rapidly changing climate, it is even more vital to understand species coexistence and the factors affecting it. High alpine streams, in particular, which are fairly pristine, can serve as comparative models for more threatened systems.

Species co-occurrence analysis allows comparisons to be made between distribution patterns of pairs of species to determine if there are any specific positive or negative associations (Veech et al. 2016). Understanding these associations can enable us to explain patterns of distributions of species that have overlapping ranges. Such analyses can help to generate hypotheses to test how species interactions or niche preferences may affect the relative abundance of coexisting species and, consequently, their ability to thrive in various ecosystems. In addition, multivariate analyses are an excellent tool to better understand the potential mechanisms underlying these patterns of coexistence.

Caddisflies (Order: Trichoptera), like other aquatic macroinvertebrates, are an important bioindicator of water quality. They undergo complete metamorphosis, living their larval and pupal stages underwater and emerging as winged, terrestrial adults that resemble moths. While most caddisflies construct a mobile case out of benthic substrates for protective and respiratory functions or spin silk webs for suspension feeding, all species of *Rhyacophila*, our focal genus,

are free-living and case-less. They are the largest genus in Trichoptera and comprised of diverse, primitive and mainly predacious caddisflies that reside in cold, oxygen-rich, and fast-flowing waters (Thut 1969). *Rhyacophila* possess morphological adaptations including a pair of anal claws and use of silk to attach to surfaces (Resh and Solem 1996). Some species possess tracheal gills of various sizes. Larvae feed primarily on larvae of Chironomidae, Simuliidae, Trichoptera and Baetidae (mayflies) (Thut 1969). However, a few species including *R. verrula* found in our study streams are herbivorous, feeding on vascular plants and algae (Thut 1969). Adults oviposit under stones protruding from the stream surface, and larvae pupate under rocks within an enclosure constructed out of pebbles and silk. Because they lack cases, *Rhyacophila* are especially sensitive to environmental change and thus suitable models for our system (Voshell 2002).

Several spatial and temporal mechanisms have been observed to influence *Rhyacophila* species co-occurrence around the world (Thut 1969). For example, in Scandinavian streams 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). Flow rate combined with prey abundance was shown to affect rates of competitive interactions between two different species of *Rhyacophila* in France (Lavandier et al. 1995). Thut (1969) found certain species of *Rhyacophila* prefer slower flowing water and others faster current velocities. Another study from Canada illustrates that the presence or absence of predators and prey of *Rhyacophila* species may affect their seasonal activity and population density (Sircom et al. 2009). Specialization of feeding habits was found to facilitate coexistence of several *Rhyacophila* species in the same streams (Thut 1969), and predators were found to aggregate in areas of high prey density (Muotka 1993). Finally, the types and abundance of other mesopredators, including multiple species of *Rhyacophila* and stoneflies can increase the competition for resources and potentially increase intraguild predation (Huhta et al. 1999). Lavandier (1995) demonstrated how two species of *Rhyacophila* successfully coexist through slight differences in life histories and food habits, which reduces interspecific competition. Most of these studies have been conducted in Europe and North American *Rhyacophila* co-occurrence is not as well understood.

Previous surveys of eight streams in the Upper East River Basin of Gunnison County, Colorado from 1976 to the present time have shown that six species of *Rhyacophila* co-exist throughout the various streams in the basin, in different proportions with four being the most abundant (*R. alberta*, *R. brunnea*, *R. hyalinata*, *R. valuma*) and two being relatively rare (*R. coloradensis* and *R. verrula*) (Peckarsky, McIntosh, and Alvarez, unpublished data). The goals of this study are: 1) to document the patterns of co-existence of these closely related species of *Rhyacophila* over space by sampling the same streams previously surveyed and to conduct species co-occurrence analyses to determine if there are any positive or negative associations between pairs of different species; 2) to measure various environmental factors to obtain descriptive data about the typical niches of different species, to generate hypotheses about potential causes of species co-occurrences; and 3) to conduct seasonal and long-term temporal comparisons of *Rhyacophila* species co-occurrence patterns and environmental associations using the 32 years of data that are available for analyses (1976 – 2006 and 2018). Samples taken between 2007 and 2017 at these same eight sites can be added to this dataset as they are processed.

Methods

Study Sites

We sampled *Rhyacophila* from eight different sites in the Upper East River Basin of Gunnison County, Colorado (Fig. 1). They include 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 Rustler's Gulch (near the confluence with the East River). 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. Different *Rhyacophila* species may emerge early or late depending on overwintering dynamics, the climate, and its ecological niche (Peckarsky et al. 1990, Smith 1968). For example *R. coloradensis* emerges in early spring and fall (Smith 1968). Sampling two times per summer captures both early and late developing species.

Collection Methods and Measurements of Environmental Parameters

We used the same collection methods as previous years by using a Hoffman sampler built from a stainless steel box frame covering a 0.104 m² substrate surface area and fastened to a 365 μ mesh drift-net; the net was placed facing upstream to allow current to enter. We disturbed the substrates framed by the box sampler for one minute and the benthic invertebrates were flushed by the current through the net and into a collecting dolphin bucket on the downstream end. We then sorted and identified all collected macroinvertebrates, including mayfly, stonefly, caddisfly and true fly larvae, as well as some non-insect invertebrates (e.g. mites, flatworms, oligochaetes and ostracods). We took four samples at different locations at each site to account for spatial variability typical of streams. We preserved all *Rhyacophila* specimens in vials of 70% ethyl alcohol for identification in the lab. All other organisms were released except for individuals that we could not identify without microscopy.

We measured several abiotic factors in the streams to characterize the environment of the *Rhyacophila* species we collected. First, we installed Hobo data loggers at the bottom of the stream on the day of the first sample to continuously (hourly) measure water temperature; they were removed after our second samples during the last visit to each site when we took zigzag particle counts (see below) so that the period of time the loggers were submerged ranged from 27 to 40 days. On the date of each invertebrate sample, we used a conductivity meter to measure temperature-specific conductivity. We calculated stream size using drainage basin area estimates from a USGS web-based GIS application (StreamStats), which provides the area of land drained by each stream site. This parameter is an alternative to one-time measurement of discharge (the volume of water moving through a stream per unit of time) instead being independent of the date of the measurements. We selected these variables for our study because they were observed to be useful explanatory variables for distributions of these *Rhyacophila* species studied in 12 different streams by Bridget Sparrow-Scinocca in summer 2017 (Sparrow-Scinocca 2017). We also recorded the elevation using GPS data obtained from each site. After finishing the second set of invertebrate samples, we performed zigzag particle counts to measure substrate particle size distribution calculating the median size of 100 particles in each reach. We measured the percent

of 100 substrate particles that were embedded, having no space underneath for colonization of invertebrates. Finally, we used a glass viewing box to estimate the percent cover and thickness of *Didymosphenia geminata* at half meter intervals at three transects of each stream to calculate a mean standing crop index (product of percent cover and thickness).

Data Analysis of Species Co-existence

Species can form positive and negative associations based on their interactions. A positive association between species suggests that the presence of one species facilitates the presence of another, or that both species have similar environmental preferences (Veech et. al 2016). Positively associated species also have the potential to compete in the present time for the same resources. A negative association indicates that the presence of one species inhibits that of another species, thereby indicating past competition, or that two species prefer different suites of environmental conditions. A pair of species that are randomly associated indicates that neither affects the other's presence.

[Note: this analysis will be completed by Amanda Klemmer] To analyze coexistence patterns between pairs of *Rhyacophila* species we will run a probabilistic model of species co-occurrence (Veech 2014, Veech et. al 2016) in R with the assistance of Amanda Klemmer. In this model, species co-occurrence is represented as the number of sampling sites in which two species co-occur. Each species is assigned either a 1 to represent that 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 pairs of *Rhyacophila* species in the eight study streams. 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). The outcome is represented by the equation $E(N_{1,2}) = P(1) \times P(2) \times N$ (Veech et. al 2016). A frequency of co-occurrence significantly greater than expected indicates a positive association, and a frequency of co-occurrence significantly less than expected indicates a negative association. If the observed occurrence is not significantly different in comparison to the expected it was classified as a random association. These associations will enable us to predict which pairs of species are more likely to occur together and those that are not. We will apply this analysis not only to the data obtained in 2018, but also to the data from 1976 to 2006 to test whether observed patterns of co-occurrence persist over time.

Data Analysis of Pairwise Abundance Patterns and the Relationships between Abundance and Environmental Variables

While the co-occurrence analyses are based on presence and absence of species, associations between pairs of species can also be examined by comparing their abundance across sites. We examined potential correlations between the abundances of the pairs of the four most abundant species. Using the Hoffman Sampler enabled us to calculate densities of each species, and we used scatterplots to illustrate relationships between each pair of species, as well as

correlation analyses to examine if there were positive, negative or random correlations between the abundances of different species. [Formal correlation analyses forthcoming.]

To test whether environmental associations explain the patterns of distribution of different *Rhyacophila* species, we graphically examined the relationships between the environmental variables measured and the densities of the four most abundant *Rhyacophila* species and evaluated the strengths of those relationships by calculating R^2 in Microsoft Excel. We have reported only the relationships where a variable explained more than 20% of the variation in density of total *Rhyacophila*, species richness, and densities of the four most abundant species of *Rhyacophila*. We will implement two types of formal statistical analyses [forthcoming – by Amanda Klemmer]. First, we will conduct a multivariate analysis (non-metric multidimensional scaling: NMDS) to compare the environmental variables that favored each species of *Rhyacophila*. This analysis illustrates the distributions of different species in multivariate habitat space, according to the factors measured (drainage basin area, elevation, water temperature, conductivity, substrate particle size, *Didymosphenia* cover, competitors, and prey). This analysis will also enable us to observe whether species that do not co-occur have different environmental preferences and 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 formal analysis of niche preferences involves a model selection procedure. This protocol 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 will run a model selection procedure to determine which subset of environmental factors best explains the density of total *Rhyacophila*, species richness (number of species at a site), and the densities of each of the four most common *Rhyacophila* species.

Data analysis of seasonal differences in phenology among species

We tested whether the patterns of seasonal development differed between pairs of *Rhyacophila* species that were observed to share similar environmental preferences (*R. alberta* and *R. hyalinata*, *R. brunnea* and *R. valuma*: see Results) using two methods. First, we plotted the abundance of each pair of species collected in the early and late season samples to determine whether they were an early or late developing species. Second, we classified all *Rhyacophila* individuals into three size classes (small, medium and large) of the two pairs of species that shared environmental preferences to observe whether their size frequency distributions provided evidence of time partitioning (i.e. one species develops early and the other late). [Note: Amanda Klemmer will do formal statistical analyses of both of these sets of data.]

Analysis of long-term temporal patterns of co-occurrence

The long-term data set of presence/absence of *Rhyacophila* species at all eight sites enables us to formally analyze whether species co-occurrence patterns are consistent over time, or whether species “blink in and blink out” at various sites over time. Amanda Klemmer will implement formal analyses to test these questions using Co-occur in R. Those data are not included in this paper, but we describe the long-term patterns in general in the first part of the Results section.

Results

Six *Rhyacophila* species were found in the Upper East River Drainage Basin, four of which were common (*R. alberta*, *R. brunnea*, *R. hyalinata*, and *R. valuma*) and two rare (*R. coloradensis* and *R. verrula*) (Fig. 2). In the early sampling period, we collected a range of one to five different species numbering between 10 to 50 total *Rhyacophila* individuals at any one site. In the late sampling period there were between one and four different species and 10 to 125 total *Rhyacophila* individuals at any one site. In the following analyses, we will be focusing on the four most abundant species.

Cursory examination of the presence/absence patterns of each of the four most abundant species at the eight sites that have been sampled since 1976 indicates that some species appear in almost every year at some sites (i.e. *R. valuma* in five of the eight sites, *R. hyalinata* in two of the eight sites). Others (i.e., *R. alberta* and *R. brunnea*) “blink in and out” of the eight sites over the years of this study. The number of species collected at the eight sites also varies over the years, with some sites having as few as one species in some years and as many as five species in other years; although some sites consistently have only one species. The factors that vary annually within sites are stream hydrology (some are high water and others low water), associated temperature, and proliferation of *Didymosphenia geminata*. While annual variation in the date of peak flow (early or late) did not explain the variation in the numbers of *Rhyacophila* species collected, associations between different species and the environmental variables we measured in 2018 enable us to generate some hypotheses about what factors may be driving the annual variation in patterns of species occurrence of *Rhyacophila*.

In our graphical analyses of the strength of relationships between *Rhyacophila* abundance and environmental variables using the 2018 data, we found a few important parameters that best explain *Rhyacophila* total abundance and species richness. Three factors, with an R^2 cutoff above 0.2, have the potential to influence total *Rhyacophila* density (Fig. 3). *Rhyacophila* density strongly increased with increasing median substrate particle size (D50) of a stream and explained ~70% of the variation in total *Rhyacophila* (Fig. 3a). Total *Rhyacophila* density decreased with increasing density of competitors (i.e. stoneflies) and explained ~35% of the variation (Fig. 3b). Finally, total *Rhyacophila* density increased slightly with increasing density of chironomid prey. This weaker relationship explained ~20% of the variation (Fig. 3c). Among the other variables measured, we found no significant explanatory mechanisms for total *Rhyacophila* density. In addition, three factors, with an R^2 cutoff above 0.2, have the potential to influence *Rhyacophila* species richness (Fig. 4). *Rhyacophila* species richness increased slightly with increasing median substrate particle size, explaining ~28% of the variation (Fig. 4a), with increasing percentage of substrate embeddedness, explaining ~31% of the variation (Fig. 4b), and with increasing *Didymosphenia geminata* standing crop, explaining ~20% of the variation (Fig. 4c). Again, none of the other variables (i.e. stream size, temperature) measured were reasonable candidates to significantly explain variation in the total density or species richness of *Rhyacophila*.

Analyzing environmental preferences of the four common *Rhyacophila* species, we found that two pairs of species preferred similar conditions. *R. alberta* and *R. hyalinata* were often found together with a preference for streams of smaller size (drainage basin area below 25 km²) (Fig. 5a), higher elevations (Fig. 5b), lower conductivity (Fig. 5c), and colder temperatures (maximum numbers of both species below 8°C) (Fig. 5e). The second pair, *R. brunnea* and *R.*

valuma showed preferences for streams of medium size (maximum numbers of both species between a drainage basin area of 30 and 40 km²) (Fig. 6a), lower elevations with *R. brunnea* having a weaker preference (Fig. 6b), medium conductivity (Fig. 6c), medium temperatures (maximum numbers of both species between 10 and 11°C) (Fig. 6d), larger substrate particle sizes (Fig. 6e), and medium *Didymosphenia geminata* standing crop (Fig. 6f). It should be noted that many of these environmental variables are correlated and appear to track together; therefore, it is necessary to conduct a model selection procedure that will rank the most influential variables to *Rhyacophila* abundance patterns.

Because the two species pairs described in the above paragraph have similar environmental preferences, we would expect there to be a positive association between each pair; however, this is not the case. Conversely, for the species pairs that prefer different environmental conditions and thus have different spatial distributions, we would expect there to be negative associations; however, this is also not the case, suggesting that environmental preferences are not the only explanation for *Rhyacophila* coexistence. In the 2018 log transformed species association graphs (Fig. 7), we observed two positive relationships, three random, and only one negative relationship between pairs of *Rhyacophila* species. Within the two pairs that are expected to have positive associations (*R. alberta* vs. *R. hyalinata* and *R. brunnea* vs. *R. valuma*), only the former pair (Fig. 7a) followed this expectation, supporting the hypothesis that they have the potential to compete for shared resources. The latter pair had a random association (Fig. 7b), suggesting that variables other than environmental preferences explain their patterns of abundance. The remaining four species pairs prefer different environmental niches (Figs. 5 and 6), suggesting that they partition space, but only one pair, *R. hyalinata* and *R. valuma* had a negative association (Fig. 7f). *R. alberta* and *R. brunnea* had a positive association (Fig. 7c), *R. hyalinata* and *R. brunnea* had a random association (Fig. 7d), and *R. alberta* and *R. valuma* had a random association (Fig. 7e). Therefore, other factors than environmental variation must be involved in explaining the patterns of abundance.

Furthermore, we found that the patterns of association depend on the years and sites sampled. For example, observing the patterns of *Rhyacophila* coexistence in the historic Non-Consumptive Water Rights invertebrate data from 1976 on as well as data from other sites during 2006-7 and 2017, the lack of consistency in these patterns of association are more apparent. Some species occur in different streams over different years or blink in and out over time. Forthcoming analyses in R based on presence/absence of *Rhyacophila* species will enable us to tease out which associations are most consistent, and which ones are more ephemeral. The discrepancy in strength of species associations provides additional evidence that *Rhyacophila* species are not simply distributed based on environmental parameters (partitioning of space), but are also influenced by other variables.

Finally, time partitioning can offer an alternate explanation for these patterns of coexistence. In species that share the same space (environmental preferences) but are not positively associated, phenological differences in development of larvae within a season may allow species pairs to coexist without intense competition. We looked at two types of time partitioning: 1) abundance of the two pairs of *Rhyacophila* that do not partition space in the early vs. late season samples (Fig. 8); and 2) size frequency distributions of those same two pairs of species in early vs. late samples (Fig. 9). The early and late season comparison of abundance suggests that *R. alberta* and *R. hyalinata* do not partition time since densities between the early and late samples are very similar (Fig. 8a) while *R. brunnea* is more abundant in the late season

and *R. valuma* in the early samples and thus would support the hypotheses that those two species can coexist in the same streams by partitioning time of development (Fig. 8b). Alternatively, separating *Rhyacophila* species to the frequency of small, medium, large, and pupal size classes enabled us to compare which species matured earlier and later (Fig. 9). These data are consistent with the hypothesis that both species pairs with similar environmental preferences (*R. alberta* and *R. hyalinata*; *R. brunnea* and *R. valuma*) partition time. *R. alberta* develops to larger sizes earlier in the season (Fig. 9a) and *R. hyalinata* develops to larger sizes later in the season since there were no large individuals in the early sample (Fig. 9b). *R. brunnea* develops to larger sizes later in the season (Fig. 9c) and *R. valuma* develops to larger sizes earlier in the season (Fig. 9d). The abundance data for the latter species pair concur with the size distribution data that one explanatory mechanism for coexistence in space is through seasonal differences in development.

Discussion

Graphical analyses demonstrated that the variation in abundance and species richness of the four common *Rhyacophila* species is in part explained by a few correlated environmental factors; however, there is variability in environmental associations over time and in various sites, so there are other mechanisms to the coexistence patterns of *Rhyacophila* species. We also found inconsistencies over time and across various sites in *Rhyacophila* species associations. Here we will explore the mechanisms behind these patterns.

The top three factors that affected *Rhyacophila* density were substrate particle size, stonefly (competitor) density, and chironomid (prey) density. The benthic substrates are important habitats for larvae to pupate by building stone and silk enclosures under the rock (Wiggins 2015). Abundance and distribution of macroinvertebrates may also be affected by successful oviposition and survival of offspring which is influenced by protruding rock size and water velocity of the surrounding area (Lancaster et al. 2010). Thus all *Rhyacophila* species may have an affinity for streams with larger substrates if they provide better oviposition habitats. In addition, increased density of predatory stoneflies was associated with lower densities of total *Rhyacophila*. This observation suggests that the two types of predators may compete for common prey such as chironomid larvae (citation). The majority of the stonefly populations consisted of chloroperlids which are known to consume chironomids, which may also be the preferred prey of *Rhyacophila* larvae. Finally, increased chironomid densities were associated with increased *Rhyacophila* densities, a result that is also consistent with the hypothesis that *Rhyacophila* larvae achieve higher densities in streams where their favored prey are abundant.

The top three environmental factors that affected *Rhyacophila* species richness were substrate particle size, percent substrate embeddedness, and *Didymosphenia geminata* standing crop. Larger substrate particles were associated with increased *Rhyacophila* species richness potentially due to greater habitat heterogeneity. Larger stones have more surface area of various shapes and interstitial spaces, allowing more options for larvae to inhabit. Our data also showed that *Rhyacophila* species richness increases with greater substrate embeddedness, which is counterintuitive to our expectations since embedded rocks would have fewer interstitial spaces for macroinvertebrates to inhabit. One possible explanation for this unexpected result is that substrate embeddedness is highly correlated with substrate size since streams with larger median particle sizes have a larger percentage of embedded large rocks ($R^2 = 0.93$). Finally, *Rhyacophila* species richness increased slightly in streams of higher *Didymosphenia geminata* cover. It is

possible that streams with higher *Didymo* may increase the heterogeneity of habitats for macroinvertebrates. More likely, however, is that high *Didymo* streams have significantly greater densities of chironomids, which provide more food to support greater numbers of species of *Rhyacophila* (Anderson et al. 2015).

Because various environmental factors are correlated (e.g. stream size and elevation, temperature and conductivity, which have been previously related to *Rhyacophila* species abundances: Sparrow-Scinocca et al. 2017), we cannot definitively attribute patterns of abundance or coexistence to specific environmental variables (until we complete the formal statistical analyses). However, previous studies have shown that species of poikilotherms are often distributed by temperature gradients as it relates to metabolism. Water at colder temperatures dissolves more dissolved oxygen than warmer aquatic environments, and *Rhyacophila* are known to be a cold-water specialist (Thut 1969). Our data suggest that some species (*A. alberta* and *A. hyalinata*) need cooler temperatures than others (*R. brunnea* and *R. valuma*) to achieve optimal densities. Therefore, water temperature is a likely candidate explaining the partitioning of space between those two pairs of species. In addition, conductivity has the potential to be an important explanatory variable. It is an indicator of the productivity (amount of dissolved ions present in the water) of streams. It is possible that the higher elevation, cold water species can tolerate less productive streams better than the lower elevation, warm water species can, but these speculations need to be further tested with experiments.

Nonetheless, we can examine all of our data on the associations of *Rhyacophila* species pairs, and the associations between environmental factors vs each species to generate hypotheses potentially explaining which factors influence the coexistence patterns of species pairs. The two high elevation, cold water species (*R. alberta* and *R. hyalinata*) were positively associated, suggesting that they do not partition space, and thereby have the potential to compete in the present time. Consistent with that suggestion, Sparrow-Scinnoca et al. (2017) conducted experiments showing that *R. hyalinata* reduces the growth rate of *R. alberta* and consumes *R. alberta* (intraguild predation: IGP), which provides evidence that those two species do in fact compete, with *R. hyalinata* being the dominant competitor. However, the size frequency distributions of these two species differ, with *R. alberta* developing faster than *R. hyalinata*, which could reduce their exposure to competition, enabling them to coexist in streams with similar environmental conditions. Thus, we might expect a more strongly positive association between this pair of species if not for competition and the partitioning of development time.

The other two species that have similar environmental requirements, (*R. brunnea* and *R. valuma*) were randomly associated, even though we expected a positive association based on the lack of niche partitioning. We do not have information on competition between those two species, but do have strong evidence (based on both seasonal abundance patterns and size frequency distributions) that these two species have different phenologies. Therefore, partitioning of time of development prevents us from detecting a positive association between these species.

Finally, previous experiments (Sparrow-Scinnoca et al. 2017) showed that *R. brunnea* caused *R. alberta* to consume fewer *Baetis* prey, which provides evidence that these two species may compete for common food resources. From this interaction we would expect a negative association between the pair, but instead find a positive association, even though these species have different sets of environmental preferences (*R. brunnea* is a low elevation, warmer water species that develops later in the season, and *R. alberta* is a high elevation, colder water species

that develops earlier in the season). Their positive association in abundance, despite their contrasting environmental preferences and partitioning of development time, does suggest a potential for them to compete in streams where they coexist. However, the factors explaining their coexistence patterns are undoubtedly complicated, involving multiple mechanisms.

Previous studies of predatory stoneflies (Perlodidae) in these same high elevation streams have documented partitioning of development time and competition between closely related species (*Megarcys signata* and *Kogotus modestus*), which is relevant to the patterns of coexistence in *Rhyacophila* (Peckarsky and Cowan 1991). These studies found that competition between these two stonefly species led to asymmetry of body sizes over time with higher densities of stoneflies and that there was no effect on mean growth rate although stoneflies ate significantly more prey if the competitor species was absent. Competitive effects also decreased as larvae slowed feeding before emergence, allowing species to coexist once past the dominant growth stages (Peckarsky and Cowan 1991).

Other variables that we did not include in this study, such as food or recruitment limitation, may be involved in explaining coexistence of different species of *Rhyacophila*. Unexplored possible explanations for species associations include food partitioning between closely related *Rhyacophila* species or other competing organisms, recruitment and oviposition strategies of *Rhyacophila*. These unexplored variables provide future directions for this research.

Results suggest that partitioning of space, time and competition are a few key mechanisms that can explain the observed patterns of *Rhyacophila* coexistence. In the future, the long term Non-Consumptive Water Rights data set enables us to explore persistence or lack of persistence in patterns, which can be observed in the context of rapidly shifting environmental conditions. Particular sensitive macroinvertebrates like *Rhyacophila* can serve as bioindicators and deserve our continued attention and study.

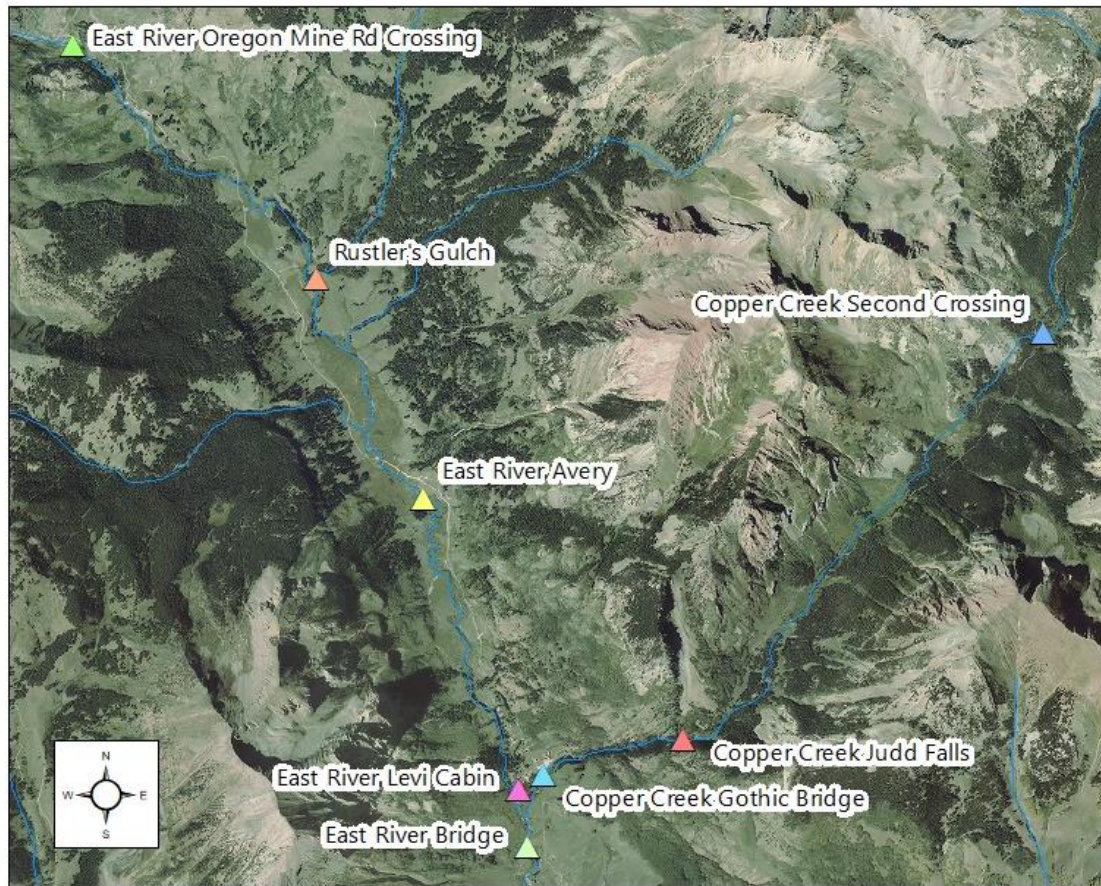


Figure 1 – Upper panel: Upper East River Drainage research sites for sampling *Rhyacophila* species, which have been used since 1976 for the Non-Consumptive Water Rights invertebrate survey. Lower panels: Left: Location of Gunnison County, Colorado, USA. Right: Location of the study area in Gunnison County.

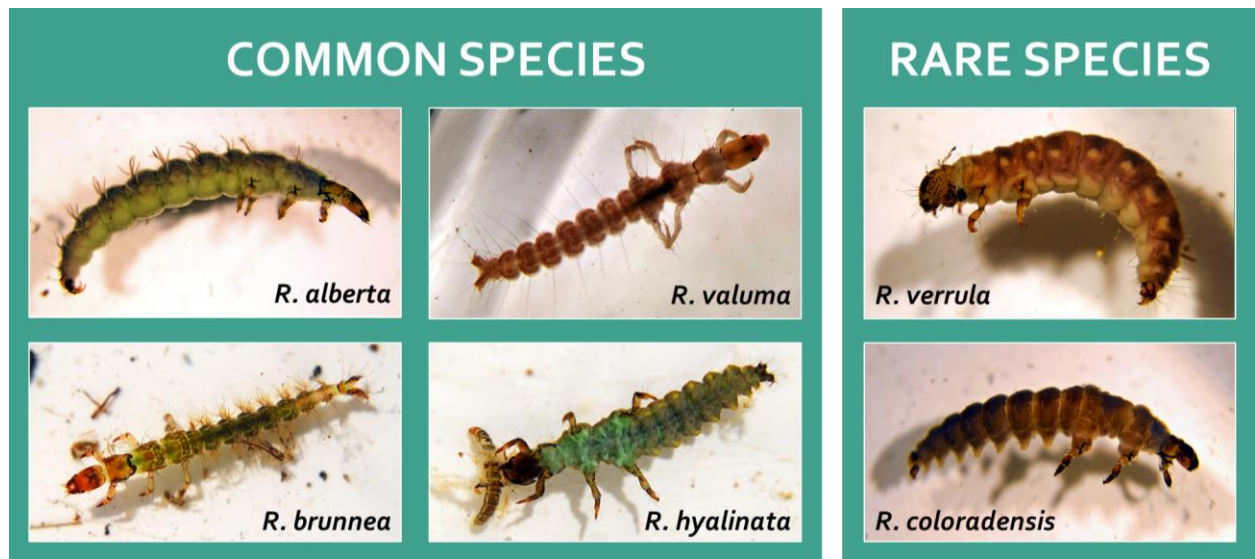


Figure 2 – Six *Rhyacophila* species in the Upper East River Drainage. Four species are common (*R. alberta*, *R. brunnea*, *R. hyalinata*, and *R. valuma*) and two are rare (*R. coloradensis* and *R. verrula*). Photo credits: JaHyun Yang.

Fig. 3a - Total *Rhyacophila* density increases with increasing substrate particle size

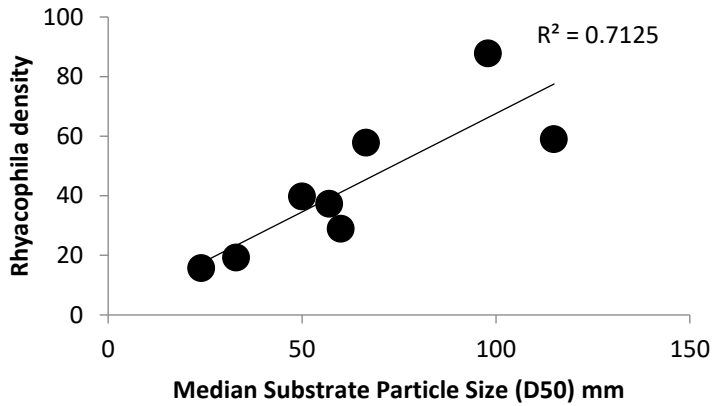
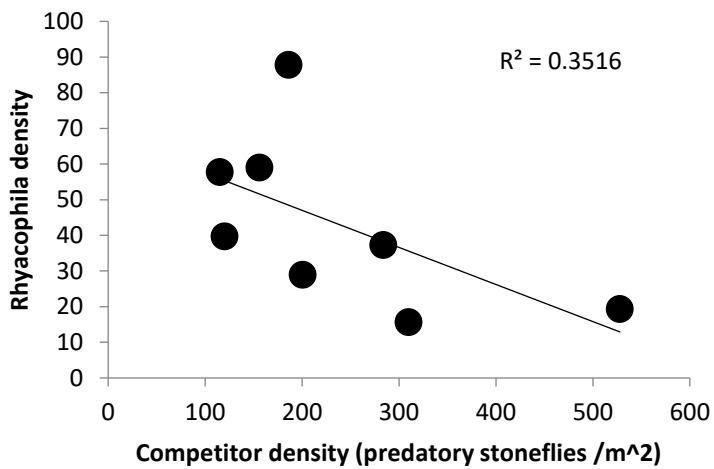


Fig. 3b - Total *Rhyacophila* density decreases with increasing competitor density



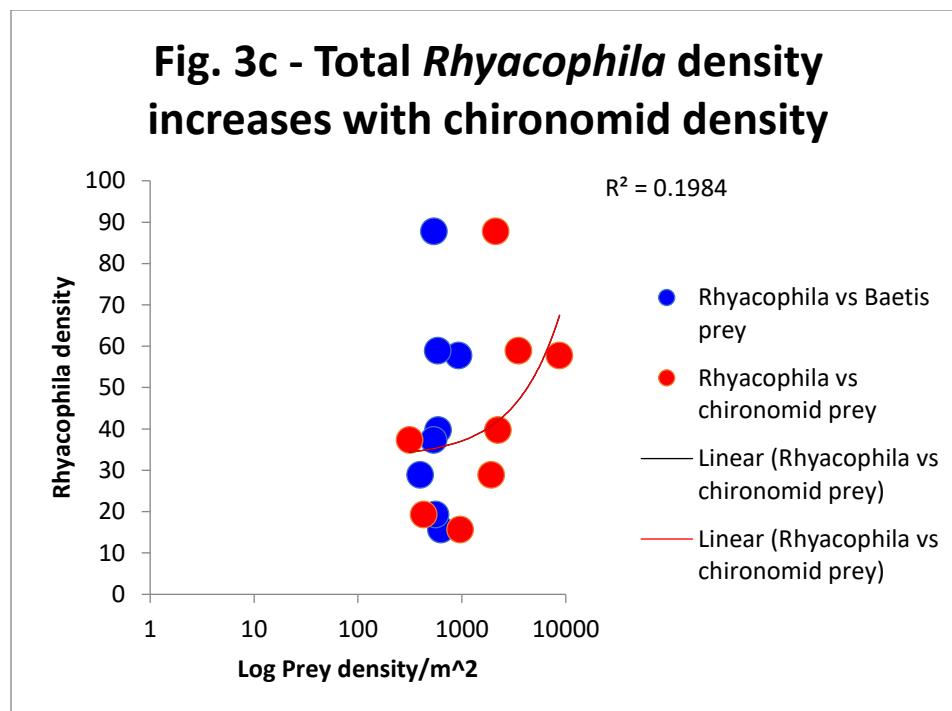


Figure 3 – Factors that best explain total *Rhyacophila* density (number of individuals of all species collected in four 0.104 m² samples). a. Median substrate particle size (mm), b. Competitor density (number of predatory stoneflies per m²), and c. Log transformed chironomid prey density (red symbols). *Baetis* prey density did not explain total *Rhyacophila* density. R^2 values are given for each relationship, and all relationships are linear.

Fig. 4a - *Rhyacophila* species richness increases with substrate particle size

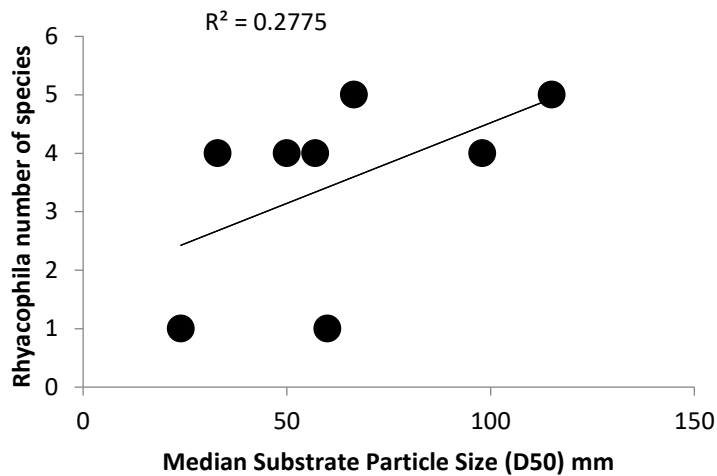
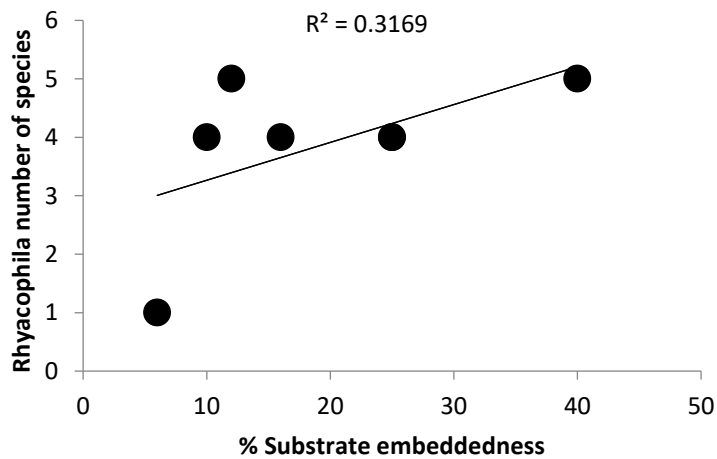


Fig. 4b - *Rhyacophila* species richness increases with substrate embeddedness



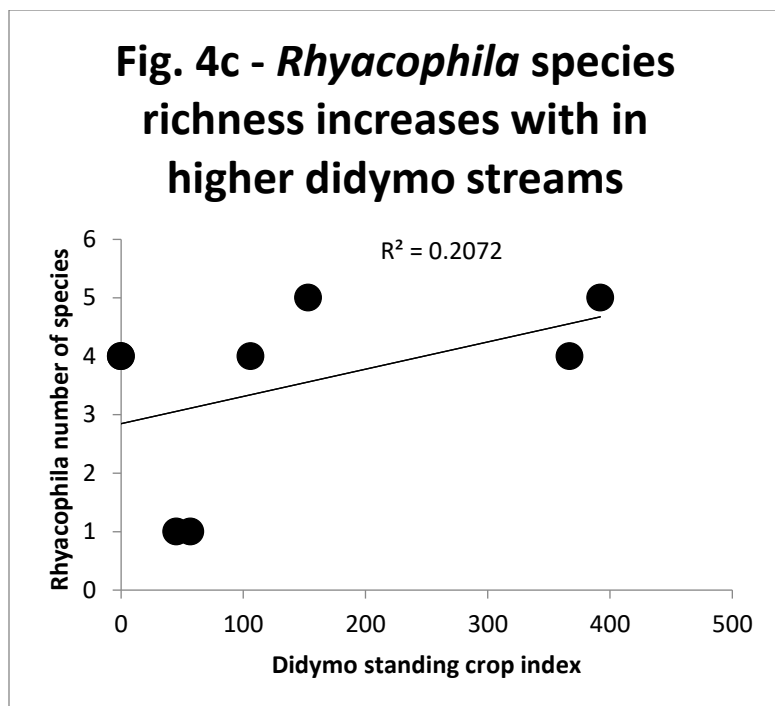


Figure 4 – Factors that best explain *Rhyacophila* species richness. a. Median substrate particle size (mm), b. Percent substrate embeddedness, and c. Standing crop index of *Didymosphenia geminata*. R^2 values are given for each relationship, and all relationships are linear.

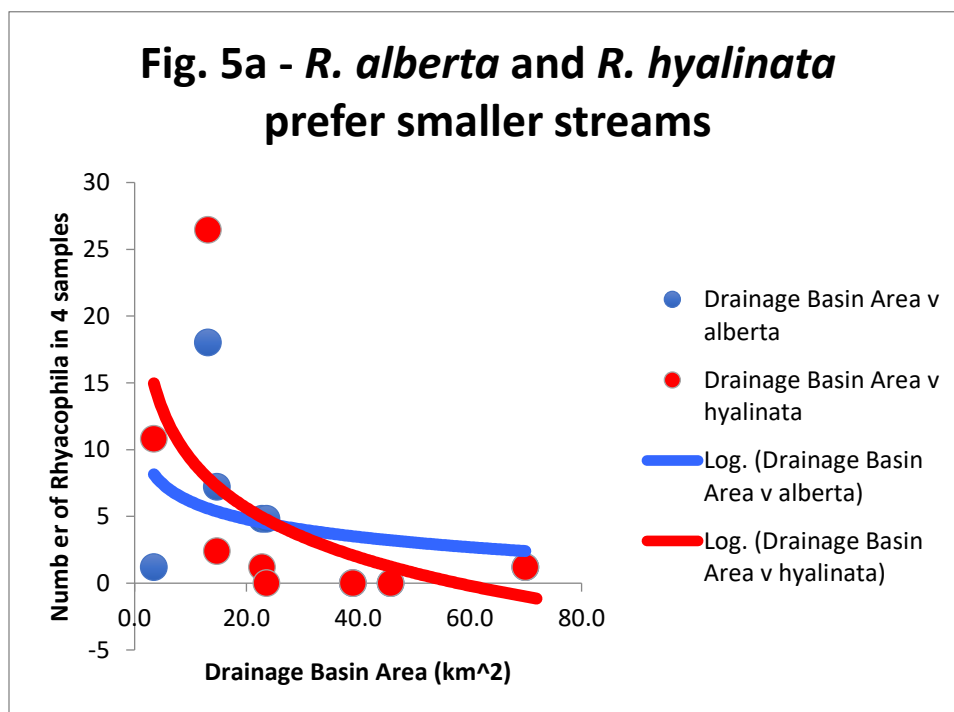


Fig. 5b - *R. alberta* and *R. hyalinata* prefer high elevation streams

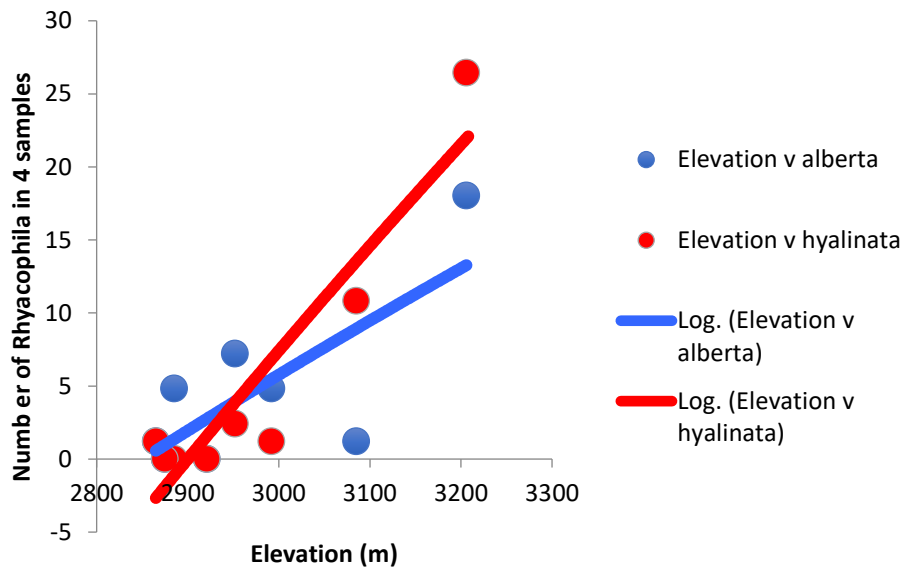
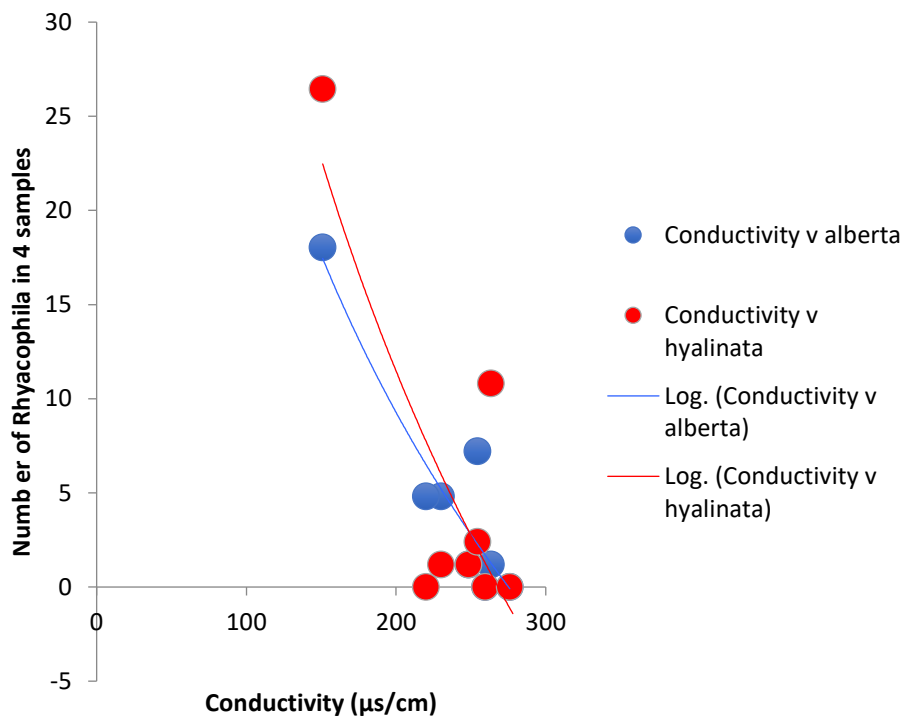


Fig. 5c - *R. alberta* and *R. hyalinata* prefer low conductivity streams



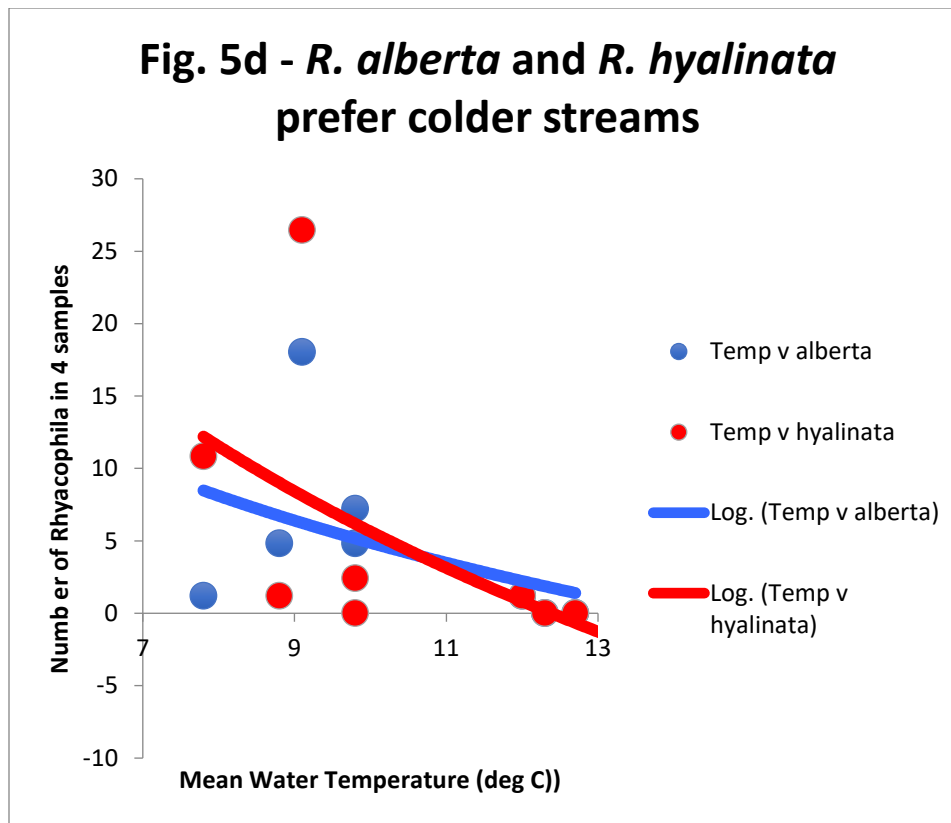


Figure 5 – Relationships between *Rhyacophila alberta* (blue symbols) and *R. hyalinata* (red symbols) (number of individuals in four 0.104 m² samples) and four different environmental variables. a. Stream size estimated as drainage basin area (km²); b. Elevation (m); c. Conductivity (μs/cm); and d. Mean water temperature (degrees C) over the first to the last sampling date in each stream. Lines represent best fit relationships (the form of which is given in the legend.)

**Fig. 6a - *R. brunnea* and *R. valuma*
prefer medium sized streams**

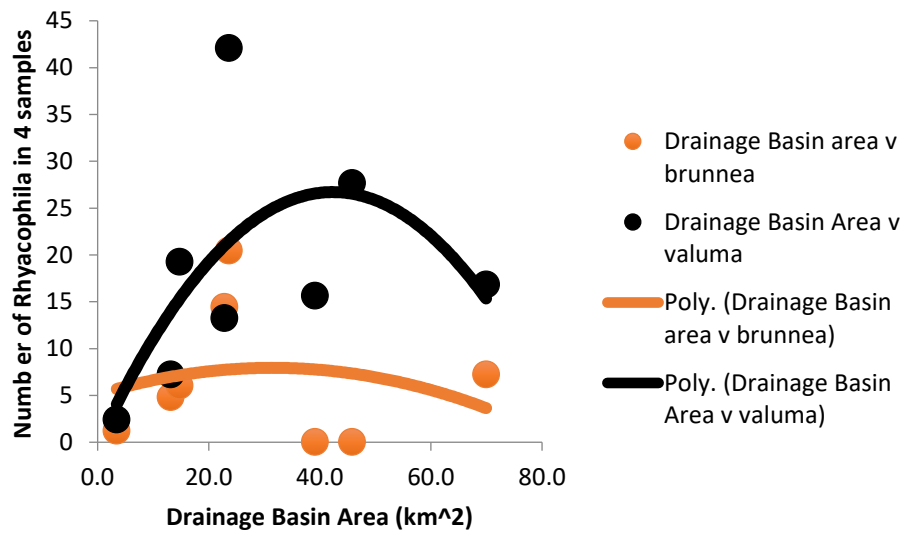


Fig. 6b - *R. valuma* prefers lower elevation streams

***R. brunnea* weak preference for lower elevation streams**

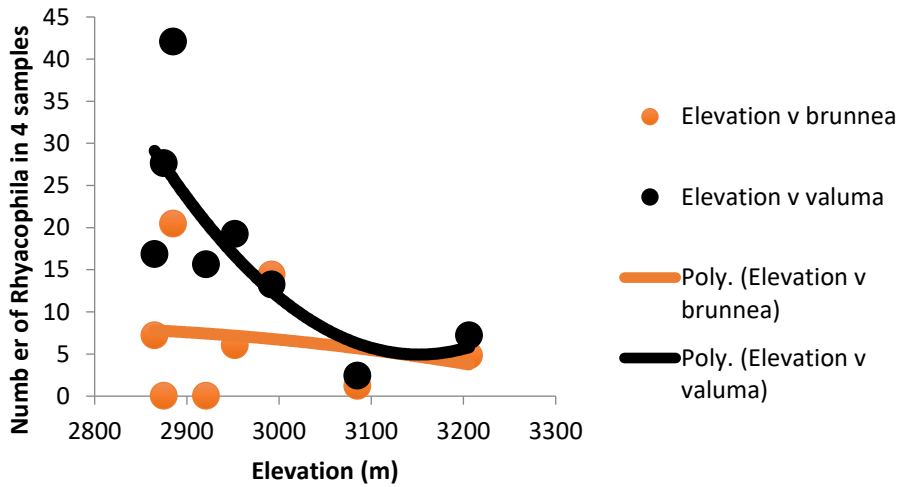


Fig. 6c - *R. brunnea* and *R. valuma* prefer medium conductivity streams

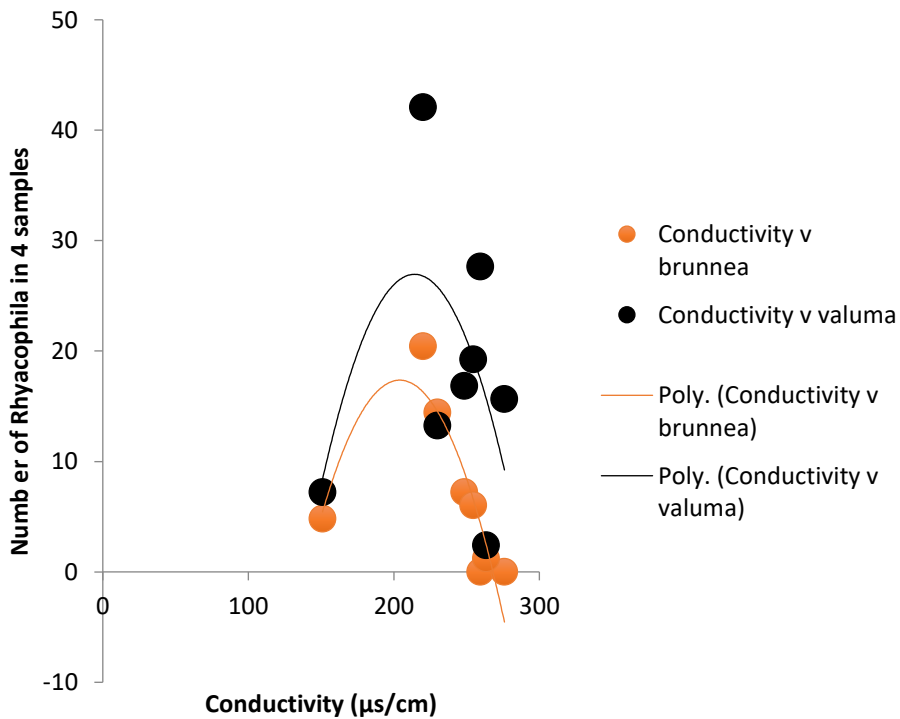


Fig. 6d - *R. brunnea* and *R. valuma* prefer medium temperature streams

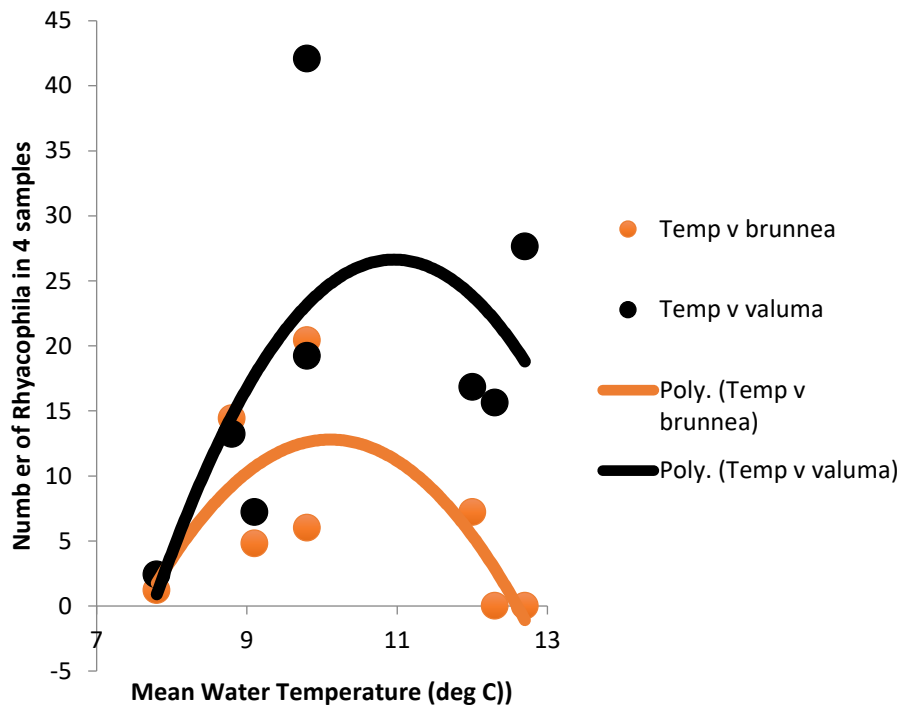
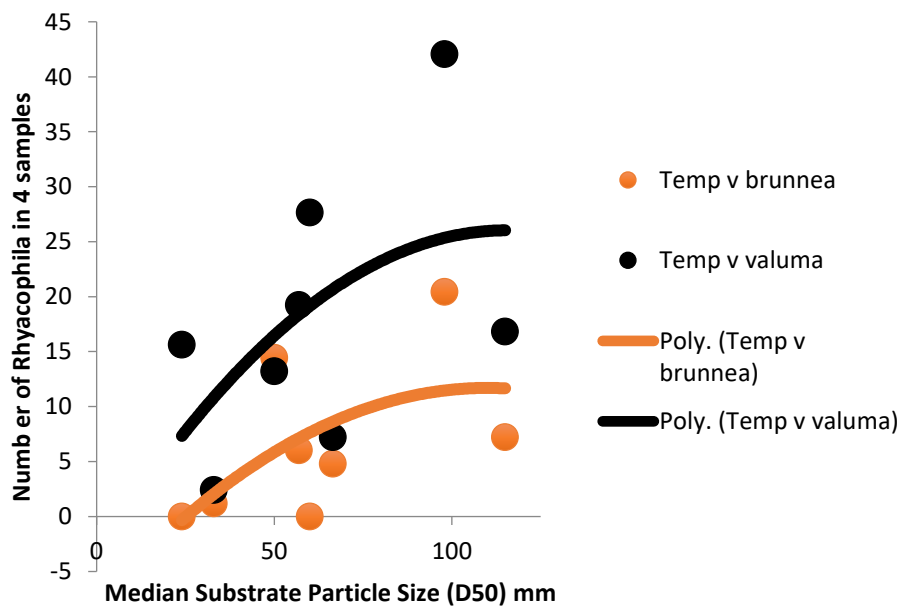


Fig. 6e - *R. brunnea* and *R. valuma* prefer larger substrate particle sizes



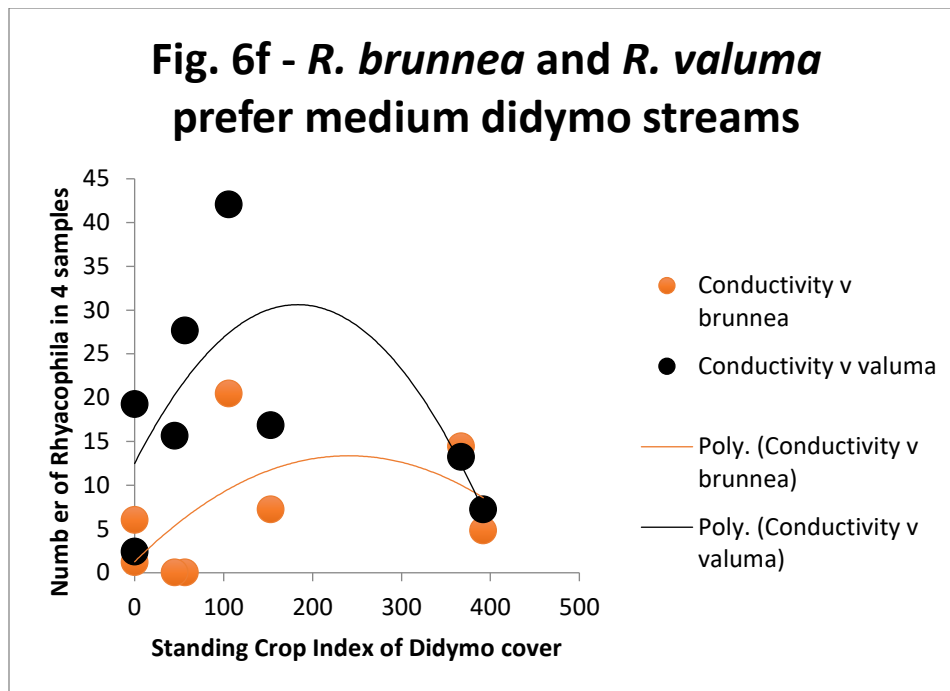
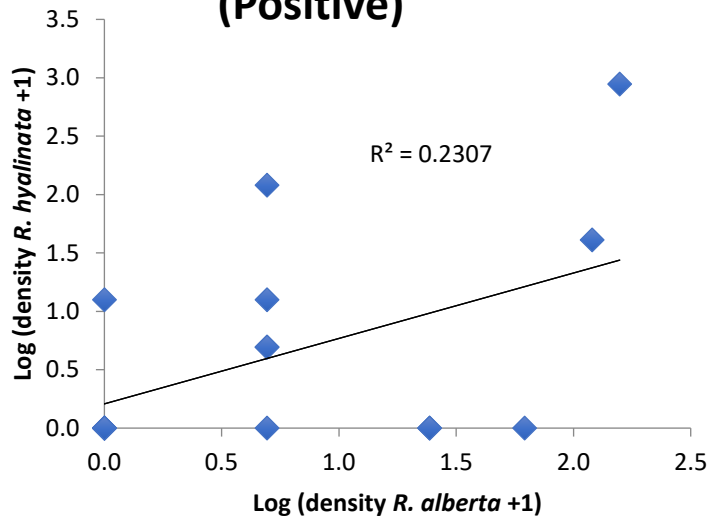
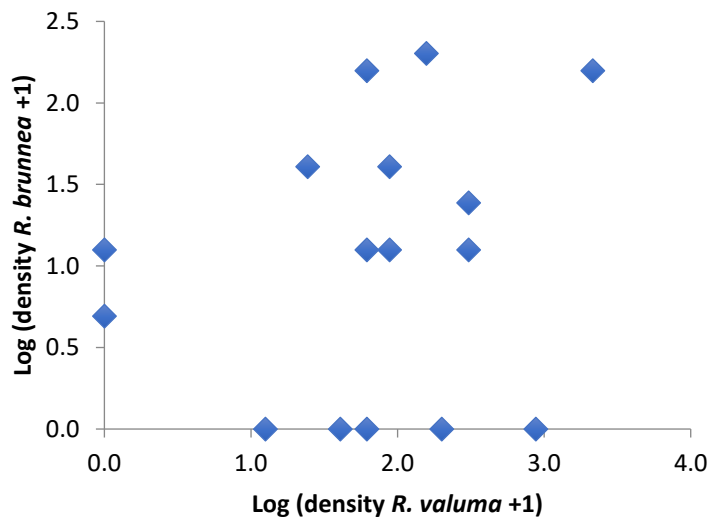


Figure 6 – Relationships between *Rhyacophila brunnea* (orange symbols) and *R. valuma* (black symbols) (number of individuals in four 0.104 m² samples) and six different environmental variables. a. Stream size estimated as drainage basin area (km²); b. Elevation (m); c. Conductivity (μs/cm); d. Mean water temperature (degrees C) over the first to the last sampling date in each stream; e. Median substrate particle size (mm); and f. Standing crop index of *Didymosphenia geminata*. Lines represent best fit relationships (the form of which is given in the legend.)

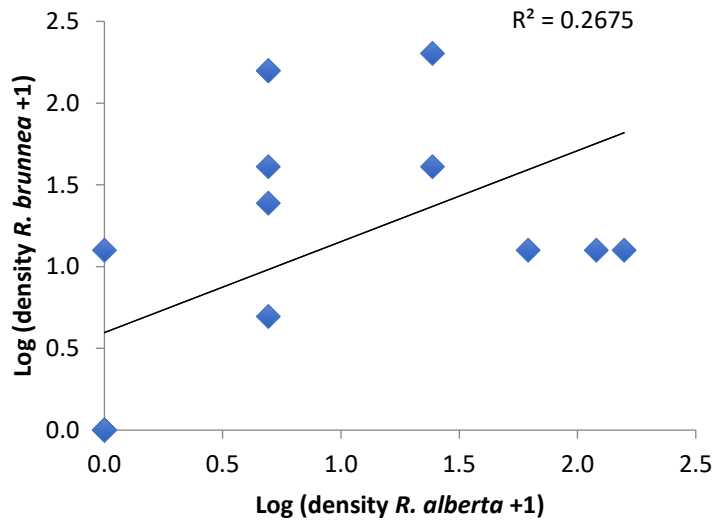
**Fig 7a - *R. alberta* vs. *R. hyalinata*
(Positive)**



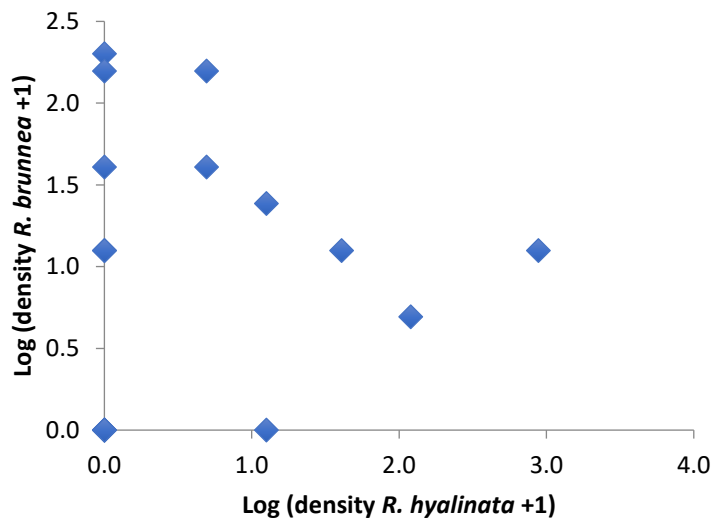
**Fig 7b - *R. valuma* vs. *R. brunnea*
(Random)**



**Fig. 7c - *R. alberta* vs. *R. brunnea*
(Positive)**



**Fig. 7d - *R. hyalinata* vs. *R. brunnea*
(Random)**



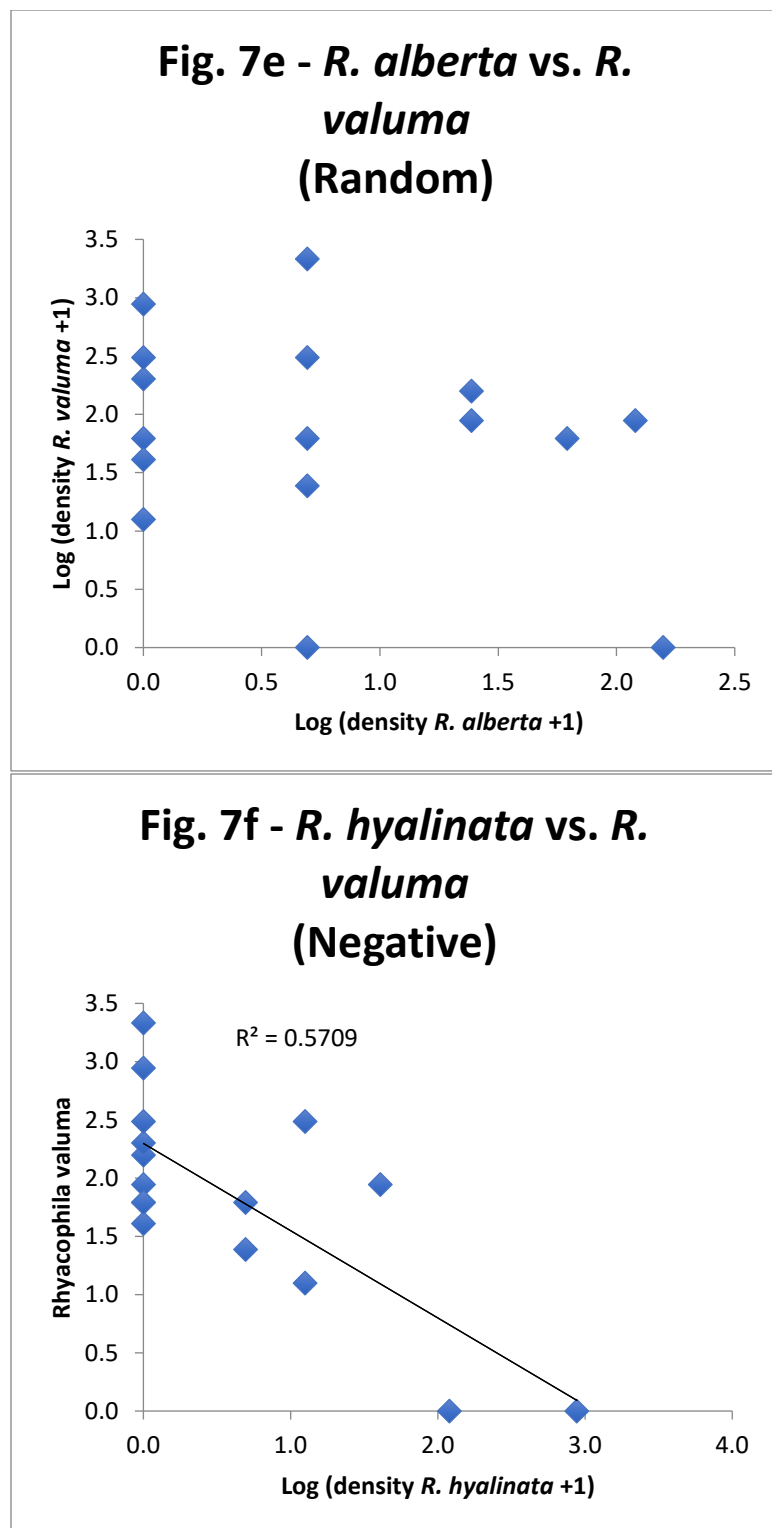


Figure 7 – Log transformed ($x+1$ and $y+1$) association graphs of six pairs of *Rhyacophila* species. a. Positive association between *R. alberta* and *R. hyalinata*; b. Random association between *R. valuma* and *R. brunnea*; c. Positive association between *R. alberta* and *R. brunnea*; d. Random association between *R. hyalinata* and *R. brunnea*; e. Random association between *R. alberta* and

R. valuma; f. Negative association between *R. hyalinata* and *R. valuma*. Lines represent best fit relationships.

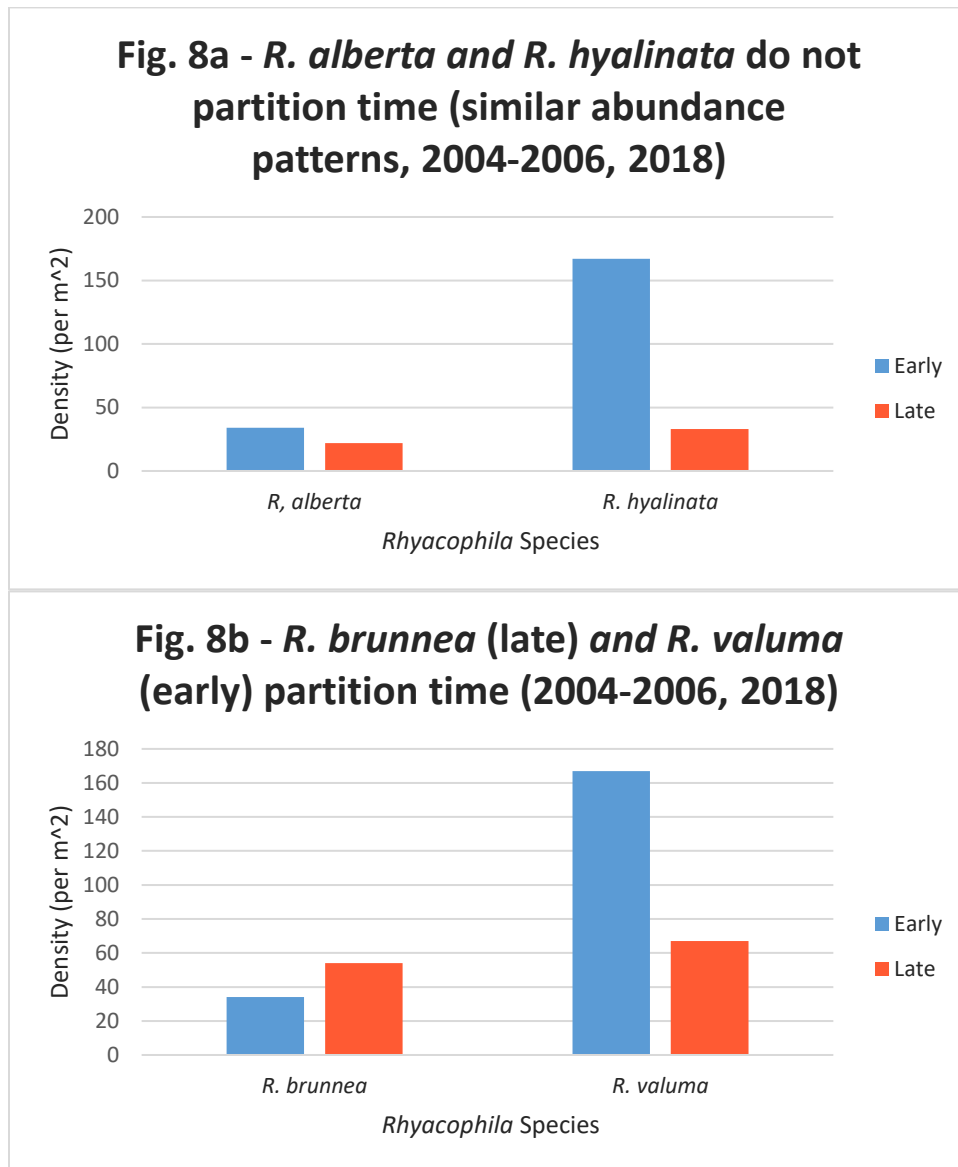


Figure 8 – Densities (number of individuals collected in four 0.104 m² samples) of two pairs of *Rhyacophila* species in early season samples (blue) and late season samples (red) at all eight sites over 4 years (2004-6 and 2018). a. *R. alberta* and *R. hyalinata* have similar abundance patterns in early and late season samples, indicating that they do not partition time; and b. *R. brunnea* is more abundant in late season samples and *R. valuma* is more abundant in early season samples, suggesting that these two species partition time.

**Fig. 9a - *R. alberta* size distributions
(Mature early)**

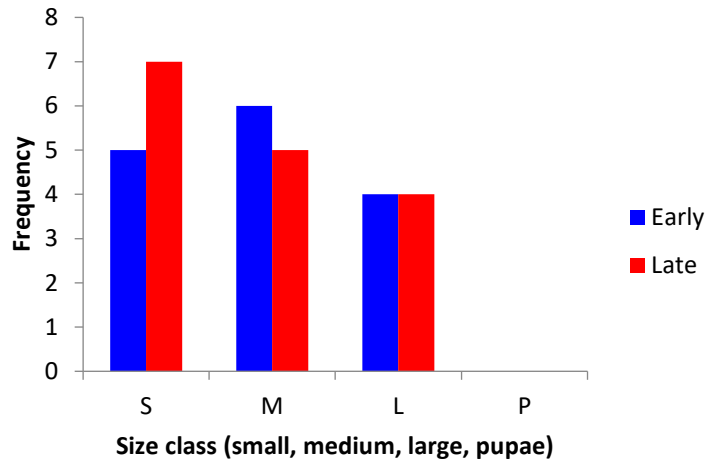


Fig. 9b - *R. hyalinata* size distributions (Mature late)

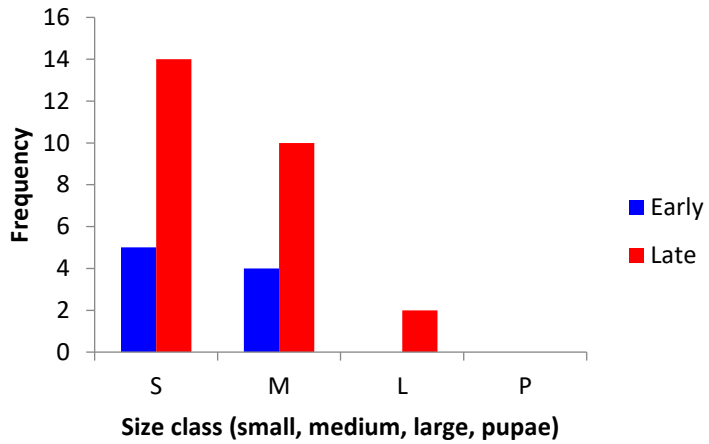
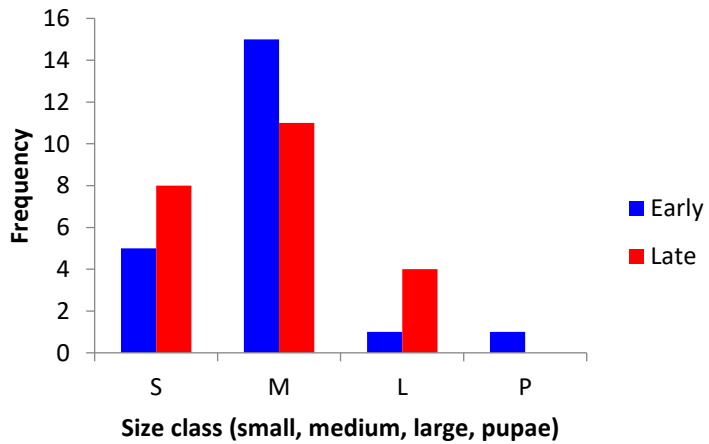


Fig. 9c - *R. brunnea* size distributions (Mature late)



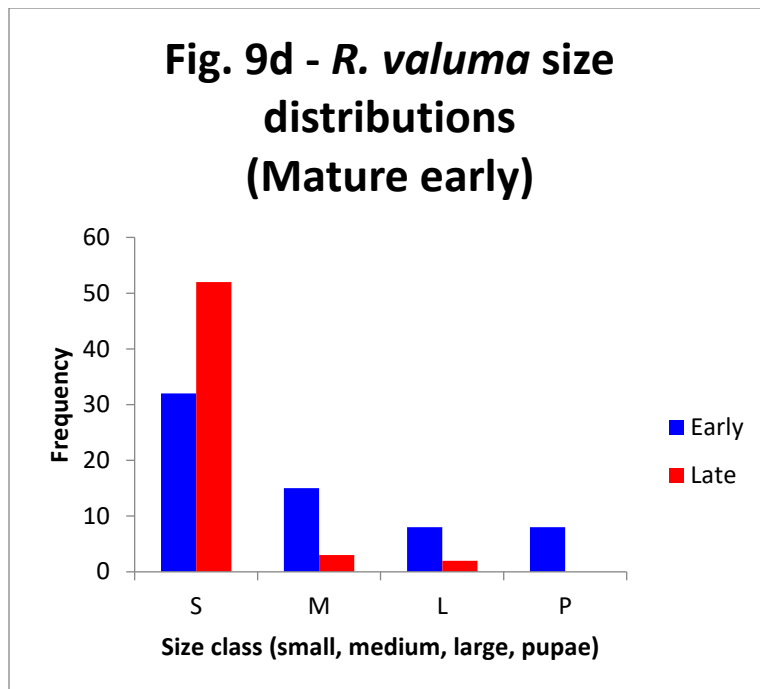


Figure 9 - Size frequency distributions of the four common *Rhyacophila* species classified as small (S), medium (M), large (L), and pupae (P) collected during early (blue) and late (red) season samples of 2018. Comparisons of size distributions in early and late season samples suggest that a. *R. alberta* develops to larger sizes early in the season, than b. *R. hyalinata* and c. *R. brunnea* develops to larger sizes later in the season than d., *R. valuma*, consistent with time partitioning between the two pairs of species that prefer similar environmental conditions.

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