Species Interactions within and between the Inquiline Larval Fauna of *Sarracenia purpurea* L. : Competiton or Commensalism?

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Abstract:

Species interactions such as commensalism and competition greatly affect community structure and composition. The small and diverse communities present in the northern pitcher-plant *Sarracenia purpurea* L. (Sarraceniaceae) are ideal for the study of species interactions. This study investigated the relationships within and between the inquiline populations of the mosquito *Wyeomyia smithii* and the midge *Metriocnemus knabi* contained within the leaves of *S. purpurea.*

Samples of pitcher-plant leaf contents were taken from Waldron Fen, Emmet County, Michigan. The larvae present in each sample were identified, counted and measured. Average densities and larval lengths per unit volume were calculated by species type and dipteran association (i.e. cohabitation with other fly species). The data indicated that both interspecific competition and intraspecific competition may be present in the larval populations of *Metriocnemus knabi* and *Wyeomyia smithii*. The co-habitation of *M. knabi* and *W. smithii* resulted in reduced *M. knabi* mean larval length per unit volume when compared with the mean larval length per unit volume of *M. knabi* found alone, indicating the presence of interspecific competition (1.35 ±0.15 vs. 2.64 ±0.40, U= 747.5, $p<0.05$, $n=59$). The singly-occurring average length of *W. smithii* decreased with increasing density, indicating the presence of intraspecific competition within the population of *W. smithii* (Spearman's $\sigma = -0.302$, $p<0.05$).

Introduction:

Species interactions, such as competition and commensalism greatly affect community structure and composition. In a commensalism, the commensal species benefits from another species, but the other species, often called the host, is not affected by the interaction (Krebs 1985). Competion adversely affects both species populations, is limited to interactions between and among species of the same trophic level and can be either intraspecific or interspecific (Allaby 1998, Stiling 1999). Interspecific competition occurrs between individuals of different species while intraspecific competition occurs between individuals of the same species (Stiling 1999).

The small yet diverse communities present within the leaves of the northern pitcher-plant *Sarracenia purpurea* L. (Sarraceniaceae) are ideal for the study of species interactions (Miller et al 1994). *S. purpurea* is a insectivorous plant that commonly inhabits glacial peat bogs from Virginia North to Labrodor and North West to the District of Mackenzie (Fish and Hall 1978). Nectar is produced by the columnar leaves of the plant, luring insects to the hood of the leaf (Newell and Nastase 1998). Slick hairs on the inside of the hood cause insects to lose their footing and fall into the water-filled cavity of the leaf. Proteolytic enzymes secreted by the leaf act with bacterial exoenzymes to digest these captured insects, providing the plant with essential nutrients not available in the nutrient poor peatland soil (Bradshaw 1983).

Although insects that fall into the water column of *Sarracenia purpurea* are readily decomposed, the leaves of *S. purpurea* offer an unusual habitat for a variety of invertebrates, rotifers, cladocerans and bacteria (Nastase et al. 1991, Miller et al. 1994). The larvae of three dipteran species, the flesh fly *Blaesoxipha fletcheri* Aldrich

(Sarcophagidae), the midge *Metriocnemus knabi* Coquillet (Chironomidae) and the mosquito *Wyeomyia smithii* Coquillett (Culicidae) often co-habitate the fluid of the leaves, feeding on organic material derived from the decomposing insects captured by the plant (Nastase et al 1991, Harvey and Miller, 1996).

It has been hypothesized that these three dipteran species equally partiton the available water column resources both spatially and temporally, effectively creating separate niches with minimal overlap (Fish and Hall 1978, Giberson and Hardwick 1999; Figure 1). *Metriocnemus knabi* inhabits the lower portion of the water column and feeds on the dead invertebrates that sink to the bottom of the pitcher (Harvey and Miller 1996). *Wyeomyia smithii* is a filter feeder that procures nourishment from the invertebrate particles provided by *M. knabi* and the bacteria that colonize the decomposing insects (Addicott 1974, Heard 1994a). *Blaesoxipha fletcheri* restricts its feeding activity to the surface of the water column and rarely interacts with the two other dipteran species (Fish and Hall 1978). Although all three species can often be found cohabiting the same leaf, it has been demonstrated that each species oviposits in the leaf water column at different leaf ages, further paritioning resources on a temporal scale (Fish and Hall 1978).

Research and experimentation on the inquiline community within *Sarracenia purpurea* has generally focused on *Wyeomyia smithii* for several resons. *W. smithii* has evolved a close and dependant relationship with *Sarracenia purpurea*. The larvae of *W. smithii* are found only in the leaves of *S. purpurea* and the adult mosquitoes are very weak fliers, rarely leaving the peatland areas where these pitcher plants are found (Bradshaw and Holzapfel 1991). Evidence indicates that the distribution of *W. smithii* closely follows that of its host plant (Armbruster et al. 1997). It has been suggested that this close association has allowed *W. smithii* to inhabit much cooler and more variable climates than the other members of its tropically distributed genus (Bradshaw and Holzapfel 1991).

Multiple interactions have been observed between *Wyeomyia smithii* and *Metriocnemus knabi* in the water column of *Sarracenia purpurea*. Intraspecific and interspecific competiton of *W. smithii* in addition to a commensalism between *W. smithii* and *M. knabi* has been documented in both the field and the laboratory (Bradshaw 1983, Miller et al 1994, Heard 1994a). The documentation of both competition and commensalism within the leaves of *S. purpurea* can be partially explained by variation in local abiotic factors and the partial overlap of spatial and temporal niches (Fish and Hall 1978, Kingsolver 1979, Heard 1994b).

The purpose of this experiment was to further investigate the inquiline relationships between the populations of *Wyeomyia smithii* and *Metriocnemus knabi* in the *Sarracenia purpurea* population present in a northern Michigan Fen. Differences in the mean larval lengths per unit volume of both *W. smithii* and *M. knabi* when found occuring alone and in cohabitation would provide evidence for either a commensalism or interspecific competion between the two species. Additionally, the mean larval length of each species when found occuring alone was compared to average larval densitiy. Significant correlations between these two variables would provide evidence for intraspecific competition within species. Assuming that the dipteran species partition inquiline resources so as to minimize niche overlap both spatially and temporally, it is expected that intraspecific competition will exist within the populations of both *W.*

smithii and *M. knabi* and that a commensalism will exist between the populations of *W. smithii* and *M. knabi*.

Methods:

Waldron Fen, located in Emmet County, Michigan (45°23'N, 84°46'W) is an intermediate fen with an open water pH of approximately 5 (Greenwood, 1998). The vegetation of Waldron Fen is dominated by *Sphagnum* mosses, sedges (e.g. *Carex oligosperma*, *Carex lasiocarpa*), black spruce (*Picea muriana*), and ericaceous shrubs including *Andromeda glaucophylla, Kalmia polifolia* and *Chamaedaphne calyculata.* Near the open pools of water the pitcher plant *Sarracenia purpurea* is especially prominent.

A transect was drawn on the West side of the fen. The transect extended thirty meters laterally and forty meters longitudinally from the open water near the center of the peatland. The transect was partitioned into four 10 m x 30 m quadrats. In each of these partitions, all pitcher plants were individually marked and numbered with surveyor's flags to ensure that no plant would be sampled more than once.

The youngest leaf of each marked plant was identified using the age determination system outlined by Fish and Hall (1978) (Fig. 2). The height of each leaf was measured in millimeters. The mean height for all samples was 8.44 mm (standard error ± 0.18) and the range was 4.0-14.0 mm. The leaf contents were extracted using an eye-dropper and transferred to a 30 ml graduated cylinder. The mean volume of the leaf contents for all samples was 3.36 ml (standard error ±0.22) and the volume range was 0.0-12.5 ml. After the volume of the leaf contents was recorded, pH of the contents was measured in the graduated cylinder using a standardized pH probe. The mean pH of all samples was 5.1 (standard error ± 0.10) and the pH range was 2.6-7.8. The leaf contents were then transferred to a labeled 6 dram vial. The graduated cylinder was rinsed thoroughly with distilled water and transferred to the vial to ensure that no larvae remained in the cylinder.

In the lab all living fly larvae present in each sample were identified and counted. The type of larval association (i.e midges alone, midges in the presence of mosquitoes, mosquitoes alone, flesh flies alone, etc.) was noted. The length of all larvae were measured in mm and averaged for each species in each vial. Determinations of mean larva length per leaf volume were made for each sample. These data were then averaged by association type. Mean larva length per leaf volume was used as a measure of larva size in order to eliminate the error associated with volume dependant larval growth. It is likely that larvae grown in larger volumes will be larger than larvae grown in smaller volumes. Dividing the mean larva length by leaf content volume effectively removes this error.

Mann-Whitney U tests for non-parametric data were utilized to determine if these averages were statistically different. The strengths of correlations between variables were quantified using Spearman's rank correlation. Data was compiled and analyzed using SYSTAT (SPSS, Chicago, IL) and Excel 7.0 (Microsoft, Redmond, WA).

Results:

There was a large discrepancy in the frequency of occurrence of both the three dipteran taxa and their larval associations. *Wyeomyia smithii* occurred with the greatest frequency, followed by *Metriocnemus knabi* and *Blaesoxipha fletcheri* (Figure 3). The most frequent larval association found was the cohabitation of *W. smithii and M. knabi* (Figure 4). The occurrence of *W. smithii* alone and the absence of inquiline larvae exhibited similar frequencies (Figure 4).

A series of correlations between variables were investigated using Spearman's rank correlation (Table 1). The mean larval lengths per unit volume for *W. smithii* found alone, *W. smithii* found in cohabitation, *M. knabi* found alone and *M. knabi* found in cohabitation were all positively correlated with volume. The mean larval length of *W. smithii* when found alone was negatively correlated with mean *W. smithii* density. The correlation between the mean larval length of *M. knabi* when found alone and mean *M. knabi* density was not significant (Table 1).

Table 1: Spearman's σ correlations for volume, mean larval length per unit volume, mean larval length and larval density (* indicates a significant correlation)

Variables	**Correlation**	**n**	**Spearman's σ**
Volume and mean larval length per unit volume (by association)	Volume/*W. smithii* alone*	44	0.481
	Volume/*W. smithii* in cohabitation*	107	0.364
	Volume/*M. knabi* alone*	16	0.537
	Volume/*M. knabi* in cohabitation*	81	0.307
Mean larval length and mean larval density (singular species occurrences)	Mean *W. smithii* length /mean density*	44	-0.302
	Mean *M. knabi* length / mean density	16	-0.383

Mann-Whitney U tests for non-parametric data were utilized to compare mean larval density (larval number / plant content volume (ml)) across species association types. The mean larval density for *Wyeomyia smithii* occurring alone was not statistically different from the mean larval density for *W. smithii* occurring in the presence of *Metriocnemus knabi* (1.19 ±0.41 vs. 1.47 ±0.16, p=0.364, α=0.05; Figure 5). Similarly, the mean larval density for *M. knabi* occurring alone was not statistically different from the mean larval density of *M. knabi* occurring in the presence of *W. smithii* (1.19 ±0.34 vs. 1.03 ±0.12, p=0.882, α=0.05; Figure 5).

Mann-Whitney U tests for non-parametric data were also utilized to compare average larval length per plant content volume across species association types. The mean larval length per plant content volume for *Wyeomyia smithii* occurring alone was not statistically different from the mean larval length per plant content volume for *W. smithii* occurring in the presence of *Metriocnemus knabi* (1.24 ±0.16 vs. 1.07 ±0.15, p=0.209, α=0.05; Figure 6). However, the average larval length per plant content volume for *M. knabi* occurring alone was statistically greater than the average larval length per plant content volume for *M. knabi* occurring in the presence of *W. smithii* (2.64 ±0.40 vs. 1.35 ±0.15, $p < 0.05$, α=0.05; Figure 6).

Discussion:

Based on these data, it appears that there is both intraspecific and interspecific interaction within and between the populations of *Metriocnemus knabi* and *Wyeomyia smithii.* These results suggest that interspecific competition rather than commensalism exists between *M. knabi* and *W. smithii* when the two species are found in cohabitation. Assuming that mean larval length is an accurate indication of fitness, *W. smithii* detrimentally effects *M. knabi* when both species are present in the same leaf. This is indicated by the statistical decrease in mean *M. knabi* larval length per plant volume when both species are present (Figure 6).

Rather than exhibiting interspecific competition, it is also possible that when these two species are found in cohabitation, they exhibit mutual interference. In this form of competition, one species reduces either the other's feeding time or amount of food that each individual of the other species obtains (Allaby 1998). Additionally, if interspecific competition exists, the two species are likely weak competitors, since the highest frequency of larval associations was the co-habitation of *W. smithii* and *M. knabi* (Figure 4). It would be expected that if the two species were strong competitors, they would be found in less frequent association, according to the competitive exclusion principle (Stiling 1999).

The findings that densities were not statistically different for either species when compared occuring alone and in co-habitation eliminates one possible data interpretation (Figure 5). Based on these statistics, one cannot argue that the discrepancy between mean co-habitational *M. knabi* larval length per unit volume and mean singly occurring *M. knabi* larval length per unit volume can be accounted for by differing larvae densities. If the density of *M. knabi* larvae were greater in the co-inhabited leaves, intraspecific competition within the population of *M. knabi* could explain the differences in mean larval length per unit volume.

However, after these experiments were completed, a flaw in sampling methods was discovered. Nastase et al. (1991) indicated that the use of an eye-dropper to remove *Metriocnemus knabi* larvae was even more ineffective than the use of a specially designed suction apparatus, which only collected 32.2% of all *M. knabi* present. They concluded that the complete removal and dissection of *Sarracenia purpurea* leaves was the only way to accurately sample *M. knabi* larvae numbers.

Thus it is possible that the apparrent competition between *Metriocnemus knabi* and *Wyeomyia smithii* could simply be the occurrence of intraspecific competition within the population of *M. knabi.* Although not significant, mean larval length of *M. knabi* when found alone was negatively correlated with mean *M. knabi* density, indicating that intraspecific competition within the population of *M. knabi* may in fact be present (Table 1). This finding would decrease the disparity between this experiment's findings and those of Heard (1994a). To test this hypothesis, this experiment would need to be replicated using the more accurate larval removal method outlined by Nastase et al (1991).

The negative correlation between increasing *Wyeomyia smithii* density and *W. smithii* average length provides evidence for the existence of intraspecific competition within the population of *W. smithii* when no other species larvae are present (Table 1). These results are in agreement with the findings of Miller et al. (1994) who also documented *W. smithii* intraspecific competition by correlating initial numbers of individuals with final size of small mosquitoes. The study found similar negative correlations in both the lab and in the field. However, neither this study nor that of Miller et al. (1994) documented intraspecific competition within the population of *W. smithii* in the presence of the larvae of other species.

Volume was relatively well correlated with mean larval length per unit volume for all four species combinations evaluated (30-53% σ correlation), indicating that the mean larval length per unit volume tends to increase as volume increases (Table 1). This lends support to the hypothesis that intraspecific competition may be present within both species populations, since larvae competing in closer proximity would tend to be smaller than larvae competing in greater volumes of fluid. If no intraspecific competition was present, it would be expected that volume would have no effect on mean larval length per unit volume. These statements assume that food availability is not correlated with volume (i.e. that all leaves capture the same amount of prey, regardless of leaf volume).

Although his experiments indicated that intraspecific competition may exist within the population of *Metriocnemus knabi*, the results of this study are contradictory to those of Heard (1994a) who clearly documented a commensalism between the two species. However, Heard (1994a) conducted his experiments using artificial "pitchers" and supplemented these pitchers with freeze-dried ants over the course of the experiment. Additionally, Heard (1994a) filled these "pitchers" with 15 ml of distilled water and included only two drops of natural pitcher food as a bacterial, rotifer and protozoan inoculum. It is quite possible that, with such a limited amount of consumable organic material, *Metriocnemus knabi* did indeed create a greater food supply for *Wyeomyia smithii*, resulting in larger mosquitoes when the two species were in cohabitation.

Additionally, Fish and Hall (1978) indicated that pH, mean insect capture rates and number of larvae present within the leaf are all functions of leaf age. The larvae of all species of interest do not reach their maximum mean number in a leaf until the leaf is 30 days old, a leaf age well past the maximum mean leaf insect capture rates which occur around day 12 (Fish and Hall, 1978). Thus, the bacterial, rotifer and protozoan communities upon which the *W. smithii* larvae feed are well established as the number of *W. smithii* in the leaf reach their maximum. Thus food supply is not a limiting factor in natural settings, while this factor was surely a limiting factor in the experiments of Heard (1994a). Kingsolver (1979) also found that the thermal and hydric (principally daily water loss) components of environmental variation effect the fitness of *W. smithii* larvae. It is likely that these components of environmental variation were altered in Heard's (1994a) experiments, possibly effecting more homogenous results.

This study found evidence for multiple interactions within and between the larval populations of *Metriocnemus knabi* and *Wyeomyia smithii* contained in the leaves of *Sarracenia purpurea* present at Waldron Fen, Emmet County, Michigan. The data indicated that both interspecific competition and intraspecific competition may be present in the populations of *M. knabi* and *W. smithii* contained in the leaves of *Sarracenia purpurea* present at Waldron Fen, Emmet County, Michigan. The co-habitation of *M. knabi* and *W. smithii* resulted in a decreased *M. knabi* mean larval length per unit volume, indicating the presence of interspecific competition. A Spearman's σ correlation indicated that singly-occurring *W. smithii* average length decreased with increasing density, indicating the presence of intraspecific competition within the population of *W. smithii*. More intensive studies, such as experimental manipulations of biotic and abiotic factors that influence the life histories of both dipteran populations (i.e. pH, amount and intensity of sunlight, temperature, insect capture rates, inquiline dissolved oxygen, etc.), would surely lead to a more definitive understanding of the community population interactions present within the leaves of *S. purpurea*.

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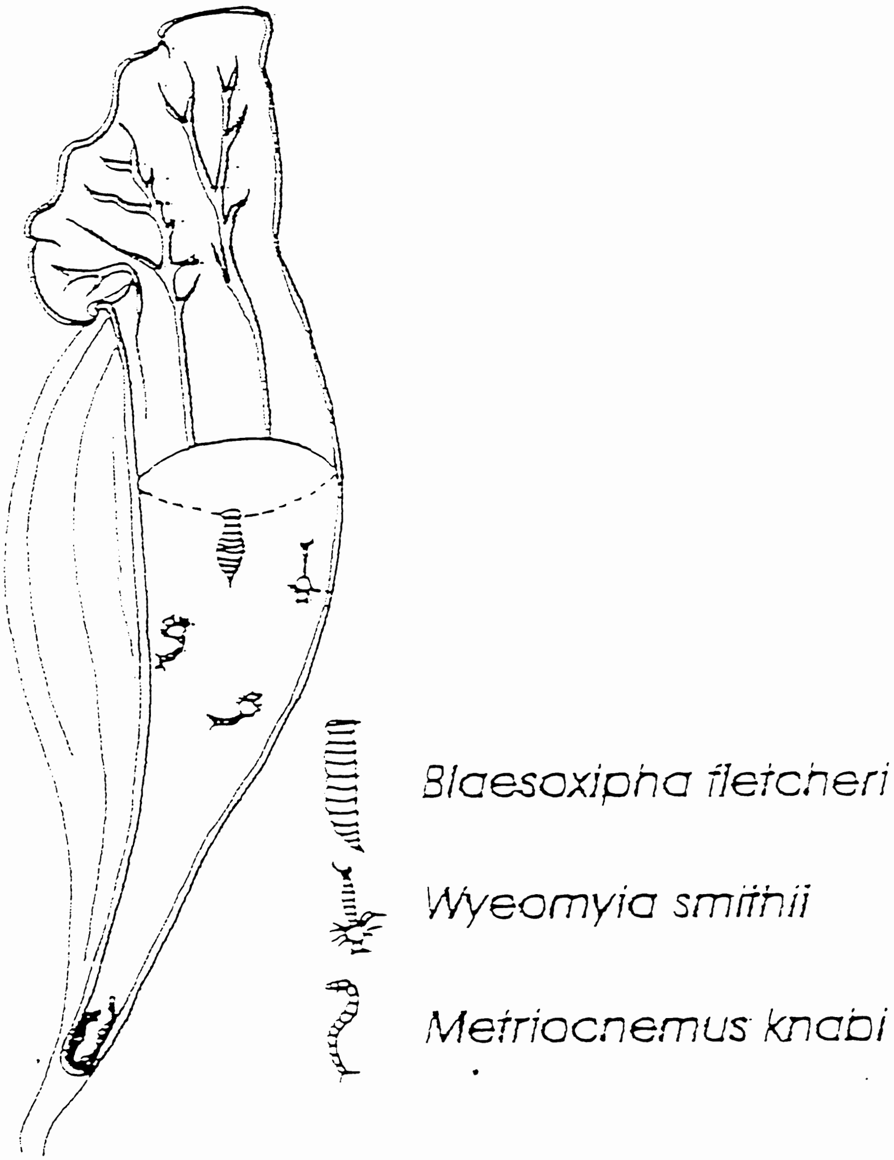

Figure 1: The spatial distribution of the three inquiline dipteran species within the water column of *Sarrencia purpurea* are indicated. (adapted from Giberson and Hardwick 1999)

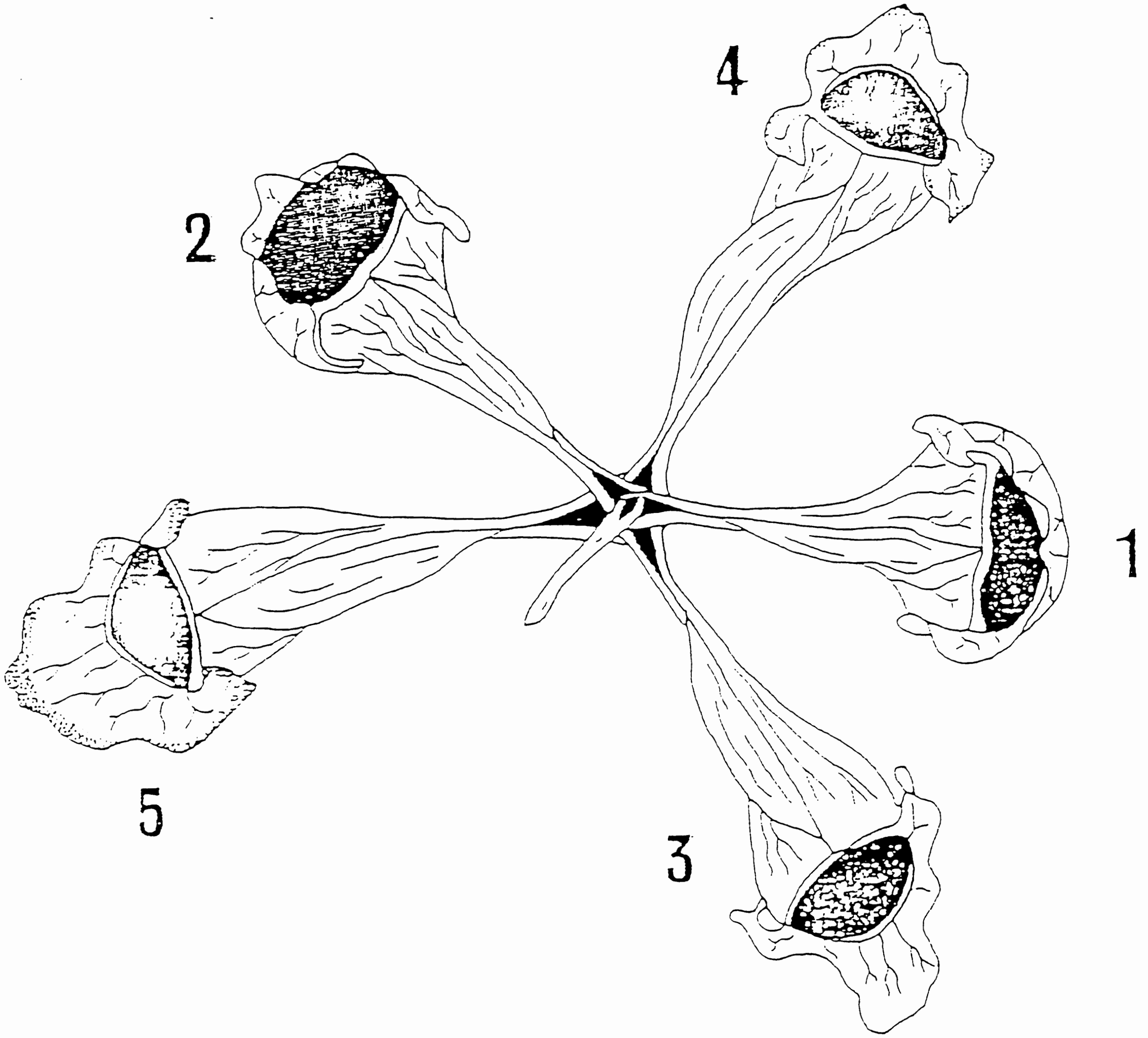

Figure 2: Leaf arrangement of *Sarrencia purpurea*. The relative ages of the leaves are indicated, with the youngest leaf numbered "1". Rising from the center of the whorl, the youngest leaf is nearly surrounded by the petioles of the older leaves. For this experiment, the youngest leaf of each plant was sampled. (adapted from Fish and Hall 1978)

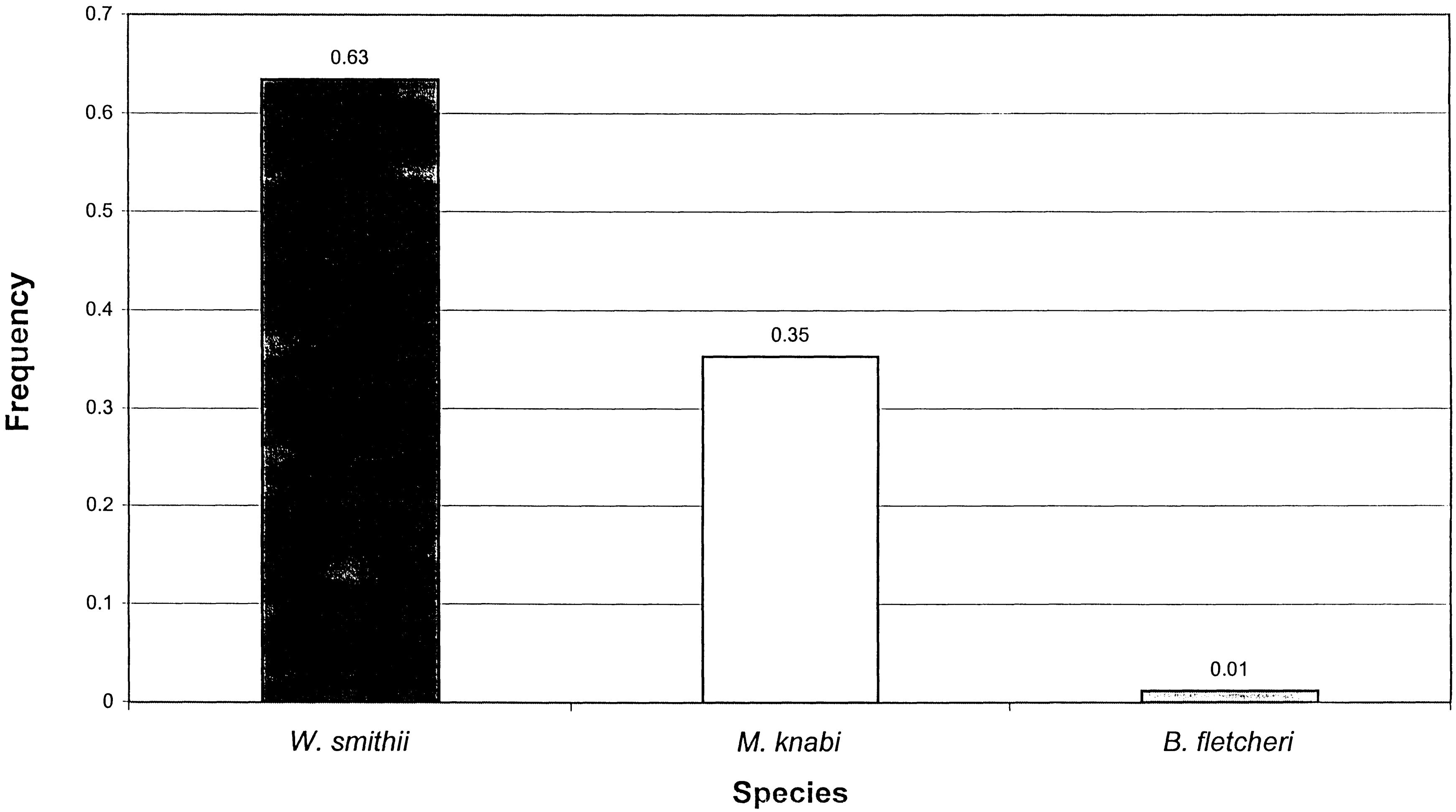

Figure 3: Frequency (n=846) of larval fly species. The larvae of *Wyeomyia smithii, Metriocnemus knabi* and *Blaesoxipha fletcheri* were counted. The frequency value appears above each respective species name.

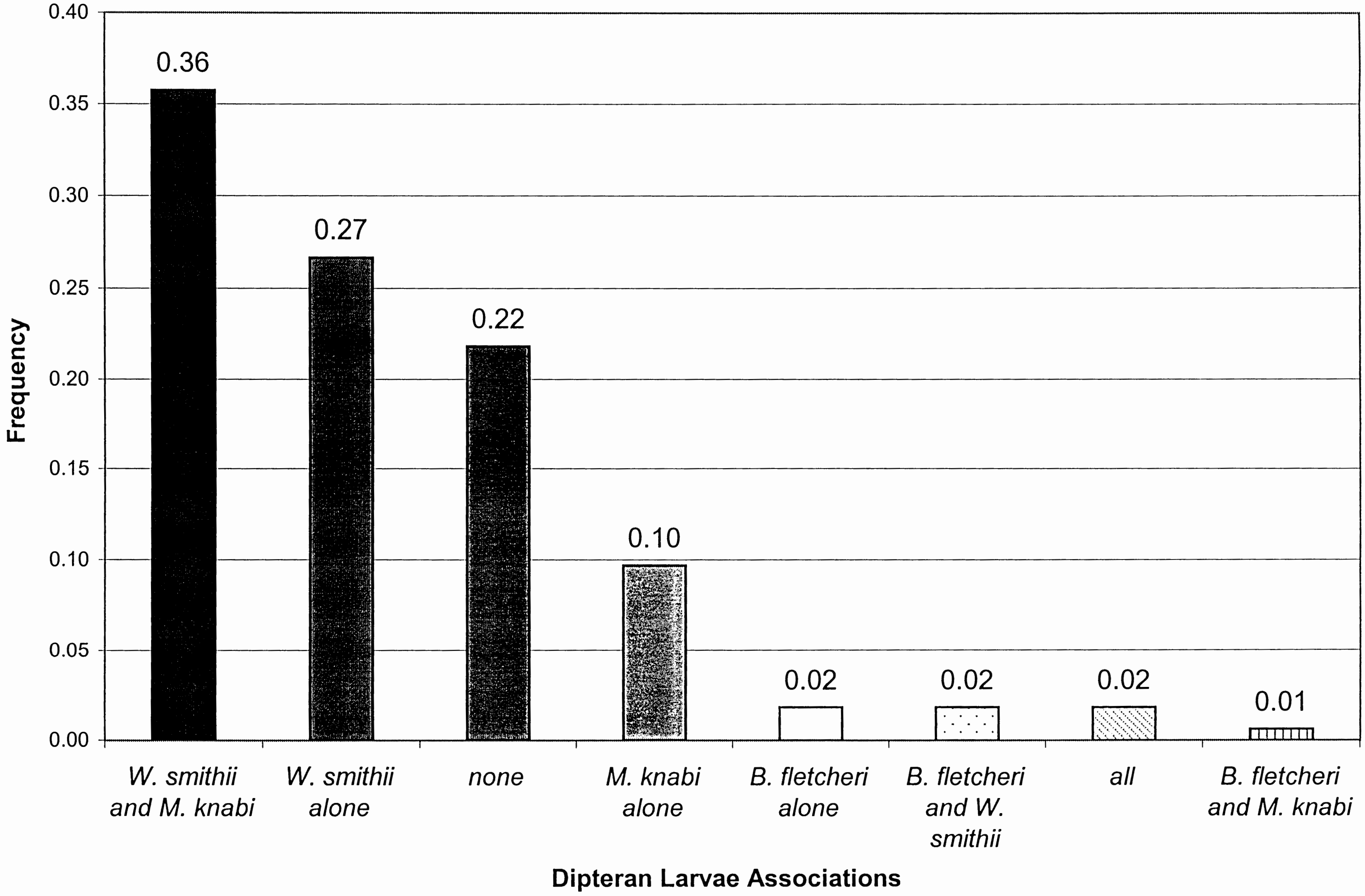

Figure 4: Frequency (n=165) of inquiline dipteran larvae associations in *Sarracenia purpurea*. Frequencies are indicated above their respective associations. The association type "all" indicates that all three fly species were found within the leaf. The association type "none" indicates that no larvae of any fly species were found within the leaf.

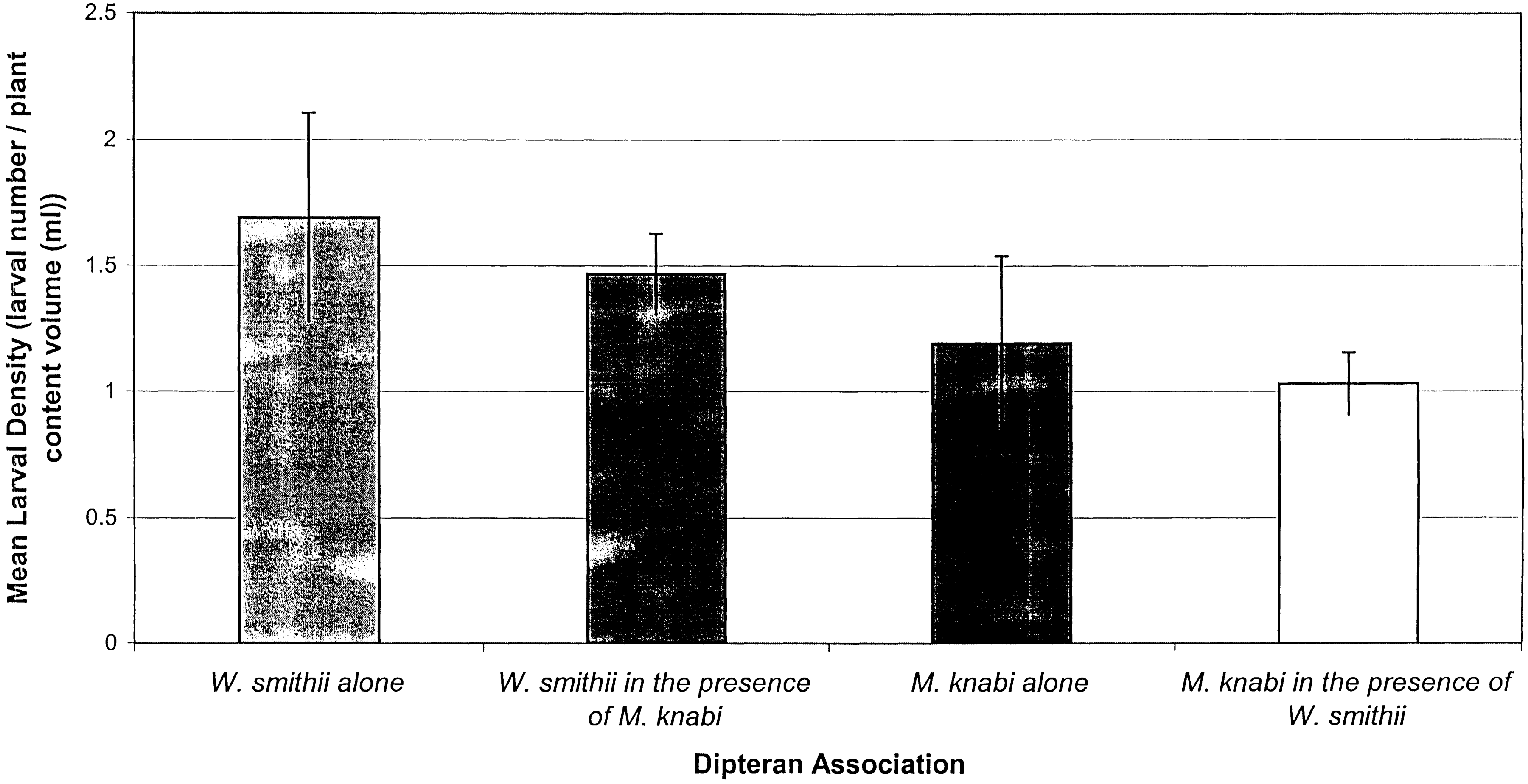

Figure 5: Mean larval density by association type. A Mann-Whitney U test indicated that the difference between the means of *W. smithii* alone (n=59) and *W. smithii* in the presence of *M. knabi* (n=44) was not statistically significant. Similarly, a Mann-Whitney U test indicated that the difference between the means of *M. knabi* alone (n=16) and *M. knabi* in the presence of *W. smithii* (n=59) was not statistically significant.

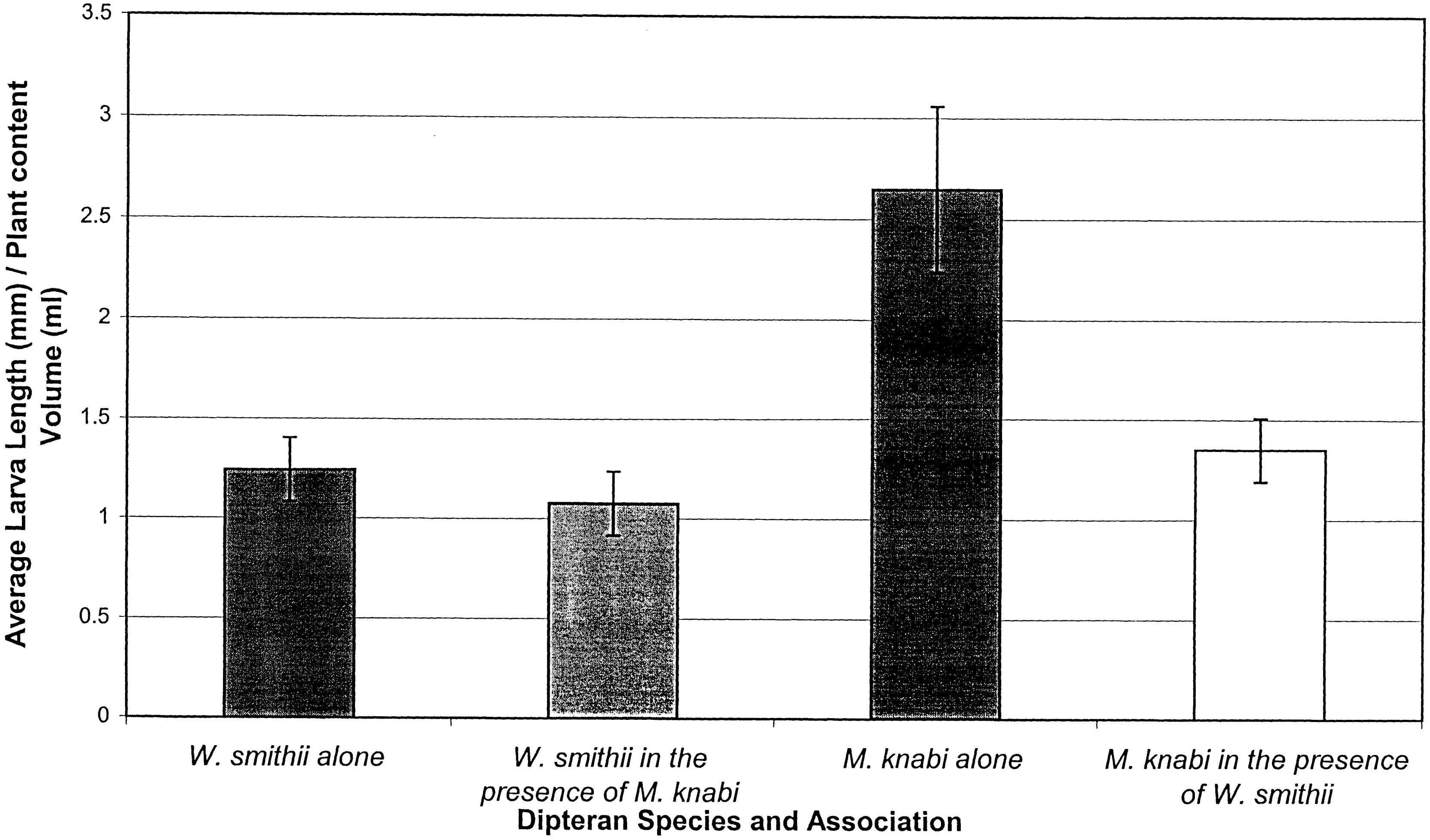

Figure 6: **Average larva length (mm) per leaf content volume (ml) of *Sarrecenia purpurea*.** A Mann-Whitney U test indicated that the difference between the means of *W. smithii* alone (n=44) and *W. smithii* in the presence of *M. knabi* (n=59) was not statistically different. A Mann-Whitney U test indicated that the difference between the means of *M. knabi* alone (n=16) and *M. knabi* in the presence of *W. smithii* (n=59) was significant.