

Interactions between *Metriocnemus knabi* (Diptera: Chironomidae)  
and *Wyeomia smithii* (Diptera: Culicidae) in the  
purple pitcher plant (*Sarracenia purpurea*)

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## Interactions between *Metriocnemus knabi* and *Wyeomia smithii*

### ABSTRACT

Larval competition between *Wyeomia smithii* (Culicidae) and *Metriocnemus knabi* (Chironomidae) was examined to test whether intraspecific and/or interspecific interactions influenced growth and development of either insect while they coexisted in the purple pitcher plant, *Sarracenia purpurea*. Constant density was maintained in a replacement series experiment with seven different ratios of population densities. Response variables used to measure competitive interactions were biomass, percent pupation, and percent mortality. High densities of conspecifics of both species resulted in both increased biomass and percent pupation while the density of the other species was low. Nutrition, in conjunction with the presence of both the other species and conspecifics, also had a strong influence on biomass. In monocultures, intraspecific competition caused decreases in pupation success and in biomass. Replacement diagrams based on biomass demonstrate that *M. knabi* outcompetes *W. smithii*.

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### INTRODUCTION

The carnivorous purple pitcher plant, *Sarracenia purpurea*, serves as the residence of three insects: a mosquito, *Wyeomyia smithii* (Culicidae), a midge, *Metriocnemus knabi* (Chironomidae), and a sarcophagid fly, *Blaesoxipha fletcheri* (Sarcophagidae). The plant has hollow pitcher-shaped leaves protecting an inner environment of phytotelmata in which these insects coexist with bacteria, protozoa, rotifers, nematodes, copepods, and mites (Fish and Hall, 1978). The objective of this study was to examine the interactions associated with this living arrangement. Life cycles of the three insects enable them to live in a potentially deadly environment. By selecting only young leaves in which to deposit their eggs, female mosquitoes coordinate their offspring's development with the stage of leaf development (Bradshaw and Holzapfel, 1991). The plant provides a food-rich and predator-free environment for all three insect larvae to develop. In addition, the relationship enables the insects to survive during the harsh northern winters.

Because there are three insects developing in the pitcher plant, investigating the interactions is crucial in understanding the optimal conditions for growth of the three species. Many experiments illustrating the relationships of insects living in a confined environment have been studied to examine the effects living conditions have on the insect life histories. Novak et. al. (1993) recently studied the effects of this type of relationship between *Aedes albopictus* and *Aedes triseriatus*. Most of their results were based upon larval competition and the effects on growth and development in differing density treatments of the two species. In the present study, inter- and intraspecific relationships were elucidated using a variety of variables including larval biomass, percent pupation and percent mortality. These variables were compared to determine the type of competition, if any, that is occurring within the pitcher plant. In many such mutual relationships, optimal conditions would not occur when the insect is living alone. This is because

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the organisms benefit each other via direct and indirect feeding interactions (Berg and Lodge, 1993). However, certain density ratios of the two species are more favorable to the other than other density ratios.

### MATERIALS AND METHODS

Insect larvae living in pitcher plants were collected from the bog area surrounding Tuesday Lake on the property of UNDERC (University of Notre Dame Environmental Research Center). Larvae of *Blaesoxipha fletcheri* were not present during the late May sampling period because of life history patterns. *Wyeomia smithii* and *Metriocnemus knabi* were collected from the plants with a suction apparatus by using negative pressure to withdraw the insect larvae along with the phytotelmata. The latter was used to fulfill nutritional and other living requirements for the insects. To ensure that the pitcher plant was not negatively affected by the removal of phytotelmata, water from Lake Tuesday was used to replace the fluid that was extracted. The basis of the suction apparatus is a modified version of a siphoning pump attached to a negative pressure chamber (plastic flask, with clear rubber tubing and a two-holed rubber stopper).

Upon collection of the organisms and phytotelmata, they were transported back to the laboratory and separated into cups in one of seven density ratios while keeping the overall density constant (Table 1). Five replicates of each density ratio were established and provided with a constant food supply, liver powder, and a small amount of phytotelmata obtained from the pitcher plant. Tap water was used to keep the total fluid level constant across all ratios and throughout the duration of the experiment. Insect head capsule widths were measured every two weeks using a grid under a dissecting microscope. Throughout the experiment, numbers of pupating and dying insects were recorded from each cup. At the completion of the experiment, the insects were placed in 95% ethanol for later biomass determination upon returning to Notre Dame. Prior to weighing, insects were dried in an oven at 60° C for 70 h. Biomass

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estimates were obtained for all insects.

All statistical analyses were performed using Systat software. The response variables used to measure species interactions were larval growth (head capsule width and biomass), percent pupation and percent mortality (Table 2).

### RESULTS

Response variables were examined using Tukey's multiple comparison test. In comparisons with no variability in one of the response variables, Tukey's could not be calculated.

#### HEAD CAPSULE SIZE vs. BIOMASS

Statistical analysis on head capsule sizes of *M. knabi* at the completion of the experiment could not be performed because sizes were uniform. *W. smithii*, on the other hand, displayed sufficient variability in head capsule size (0.7 - 0.95mm) and in biomass (0.2 - 0.6mg). Head capsule size vs. biomass were graphed for *W. smithii*, but there was no significant correlation between the two variables. (Figure 1).

#### TREATMENT vs. BIOMASS

Biomass of *W. smithii* was directly related to the number of conspecifics in all treatments except the monoculture (20:0) (Figure 2). Biomass of *M. knabi* increased from the 8 midge (12:8) treatment to the 16 midge (4:16) treatment and then decreased in the treatment containing only midges (0:20) (Figure 3).

#### TREATMENT vs. PERCENT PUPATION

The percent pupation of *W. smithii* was low (55%) in the 4:16 treatment but there was a similar measurement around 70% for the other 5 treatments containing both midges and mosquitoes (Figure 4). The percentage of *M. knabi* that pupated was low (10 - 25%) in the treatments containing both midges and mosquitoes and increased dramatically to 60% in the treatment containing only midges (Figure 5).

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### BIOMASS vs. PERCENT PUPATION

Pupation rates of *W. smithii* remained around 70% for weights above 0.36 mg. Below 0.36 mg there was a decline to 55% pupation at 0.34 mg. (Figure 6). Percent pupation of *M. knabi* was relatively constant at all weights below 0.32 mg, however increased at 0.23 mg to 58% (Figure 7).

### REPLACEMENT SERIES

The relationship between treatments and biomass were examined using a de Wit replacement series graph to evaluate the type of competition occurring (Figure 8). Another measure of competition, the relative crowding coefficient (RCC), also was used to examine species interactions (Harper, 1977). The RCC is calculated by choosing a variable indicative of competition (e.g. biomass) and comparing biomass differences of species without competitors to those with competitors at a ratio of 1:1. The RCC was calculated according to the formula:

$$\frac{(\text{mean biomass } W. \text{ smithii at 1:1}) / (\text{mean biomass } M. \text{ knabi at 1:1})}{((\text{mean biomass } W. \text{ smithii at 0:1}) / \text{mean biomass } M. \text{ knabi at 0:1})}$$

$$\text{RCC} = 0.9375$$

With this equation R can be any number greater than 0. According to Harper (1977), an  $\text{RCC} < 1$  indicates the second species is outcompeting the first. Therefore in the present study *M. knabi* is outcompeting *W. smithii* according to Harper's values, however 0.9375 is very close to a value of 1.0 which corresponds to neither of the species outcompeting the other. Because of this, it appears that *M. knabi* only slightly outcompetes *W. smithii*.

### **DISCUSSION**

Data from treatments containing different ratios of mosquitoes and midges suggest that intraspecific and

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interspecific relationships occur between *W. smithii* and *M. knabi* in *Sarracenia purpurea*. This is demonstrated by analyzing the development and growth of each species in each treatment.

Head capsule width of both *W. smithii* and *M. knabi* did not vary between treatments suggesting that different treatments had no effect on the larval growth of either species. From this, one can conclude that head capsule size is not a good indicator of biomass in either *W. smithii* or *M. knabi*.

Biomass of *W. smithii* increased with the number of mosquitoes, and concurrently with a decrease in midges. In the monoculture, however, biomass decreases from its optimal value of .464 mg at 16 mosquitoes to .420 mg. This suggests that mosquitoes benefit from the presence of other conspecifics and small numbers of midges. Even in the monoculture, the biomass of the mosquito is high in comparison to low density levels of mosquitoes and high density levels of midges. This phenomenon could result from ingestion of nutritional fecal pellets of other mosquitoes. As the number of mosquitoes increases, the amount of fecal material increases and therefore more bacteria is available for consumption.

In *M. knabi*, as the number of *W. smithii* decreased in a treatment, the biomass of the midge subsequently increased. The exception occurred when the midges were in a monoculture where the biomass decreased. As food (liver powder) was added to the experimental cups, it could be ingested first by *W. smithii*, which can inhabit all areas of the pitcher plant. *M. knabi*, which inhabits the bottom of the plant suffers from nutrient limitation when large numbers *W. smithii* are present. As the numbers of *W. smithii* are decreased in a treatment, the biomass of *M. knabi* subsequently increases. However, once the midge is in monoculture, biomass decreases. This shows that either there is some benefit that the midge receives from having a small number of mosquitoes in the pitcher plant or there is some negative effect of so many conspecifics. Perhaps it is the nutritional value of bacteria from the waste of *W. smithii*. This decrease in biomass also could be due to intraspecific competition between midges, perhaps for space. Because

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*M. knabi* can occupy only the bottom of the plant, competition for space would increase with each additional midge.

The pupation of *W. smithii* is relatively low at low densities. This is due to the mortality rate of *W. smithii* in the low density treatment having a large effect on percent pupation. At this treatment level (4 mosquitoes: 16 midges), the percent of pupating *M. knabi* was high. This was due to increased intraspecific interactions occurring between the midges. As previously noted, the spatial configurations of the midges has the effect of decreased biomass. Another effect that spatial constraints may cause is to increase the rate of pupation to alleviate the stress. This can be called an evolutionary push because in order to survive, the insect must take the initiative to leave the high stress environment and pupate to a less competitive environment. Although unlikely, if this is the reason for increased pupation, the early pupated midges would most likely be less developed and have a lesser probability of fecundity. The pupation rates of *W. smithii* remained relatively high at all treatments except for the treatment of 4 mosquitoes: 16 midges and the pupation rates of *M. knabi* remained relatively low at all treatments besides the monoculture. This could also be related to the nutritional status of the larvae as *W. smithii* is probably ingesting higher quality food than *M. knabi* due to the mosquitoes ability to inhabit any area in the plant. There was a direct relationship between biomass and percent pupation of *W. smithii*. The rate of pupation of *M. knabi* did not increase until biomass reached high levels. These results suggest that pupation of both species is partially dependent on biomass.

In viewing the replacement series graph, it appears that there is interspecific competition occurring between *W. smithii* and *M. knabi*. This conclusion is based on the concavity of the curve for each species. This means that biomass of one species was inversely proportional to the number of interspecific competitors. Explanations for this observation are similar to those used to explain the effects of treatment on biomass

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which dealt primarily with nutrient limitations. The RCC was used to determine whether either insect was outcompeting the other. An  $RCC < 1$  suggested that *M. knabi* was outcompeting *W. smithii* with respect to the biomass response variable. This is due to the fact that *M. knabi* had a much higher relative biomass at its optimal treatment of 16 mosquitoes to 4 midges compared to the highest biomass found in *W. smithii* at its optimal treatment at 16 mosquitoes to 4 midges.

To test to see if intraspecific competition had an effect on biomass and percent pupation, a similar replacement series with differing densities should be conducted. This could determine whether the observations found in the intraspecific treatments (20:0 and 0:20) were significant or whether the density chosen was too small. Also, future experiments could be conducted at differing density levels to test the effect of higher densities on the response variables.

### ACKNOWLEDGEMENT

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### FIGURE CAPTIONS

FIGURE 1. Biomass vs. Head Capsule Width for *W. smithii*

FIGURE 2. Biomass vs. Treatment for *W. smithii*

FIGURE 3. Biomass vs. Treatment for *M. knabi*

FIGURE 4. Percent Pupation vs. Treatment for *W. smithii*

FIGURE 5. Percent Pupation vs. Treatment for *M. knabi*

FIGURE 6. Percent Pupation vs. Biomass for *W. smithii*

FIGURE 7. Percent Pupation vs. Biomass for *M. knabi*

FIGURE 8. Replacement Series Diagram for biomass of *W. smithii* and *M. knabi*

FIGURE 1

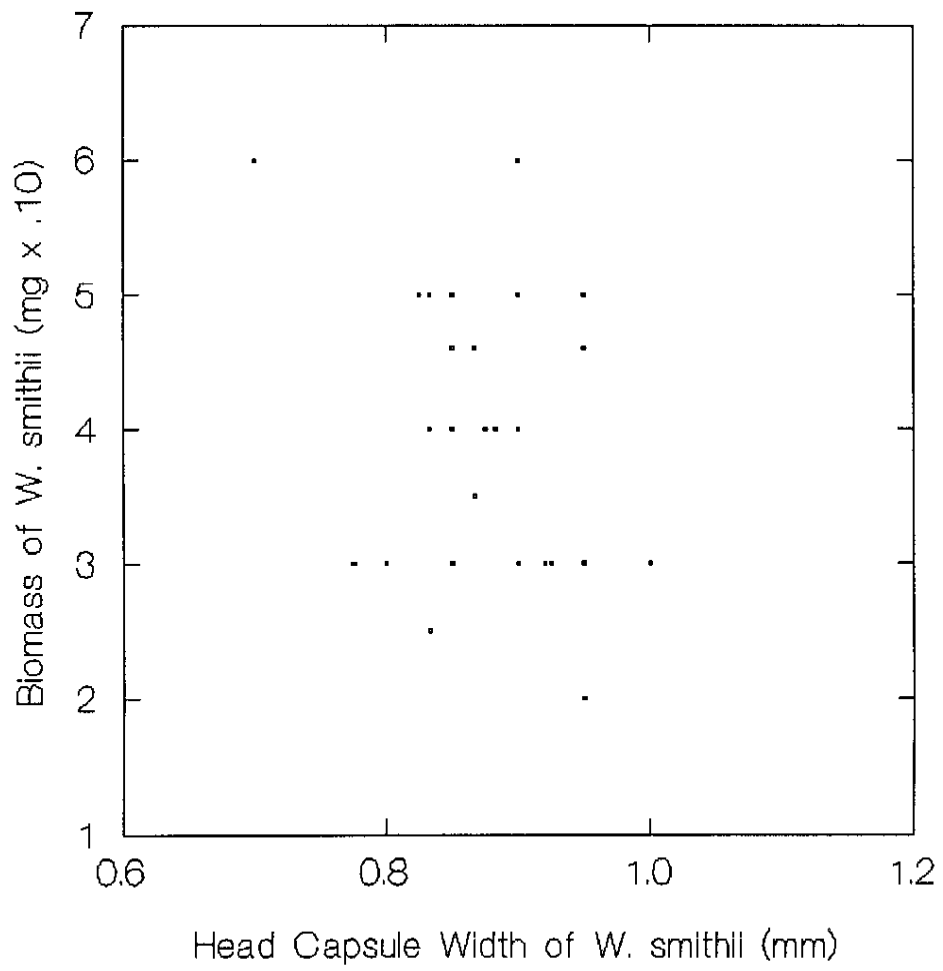


FIGURE 2

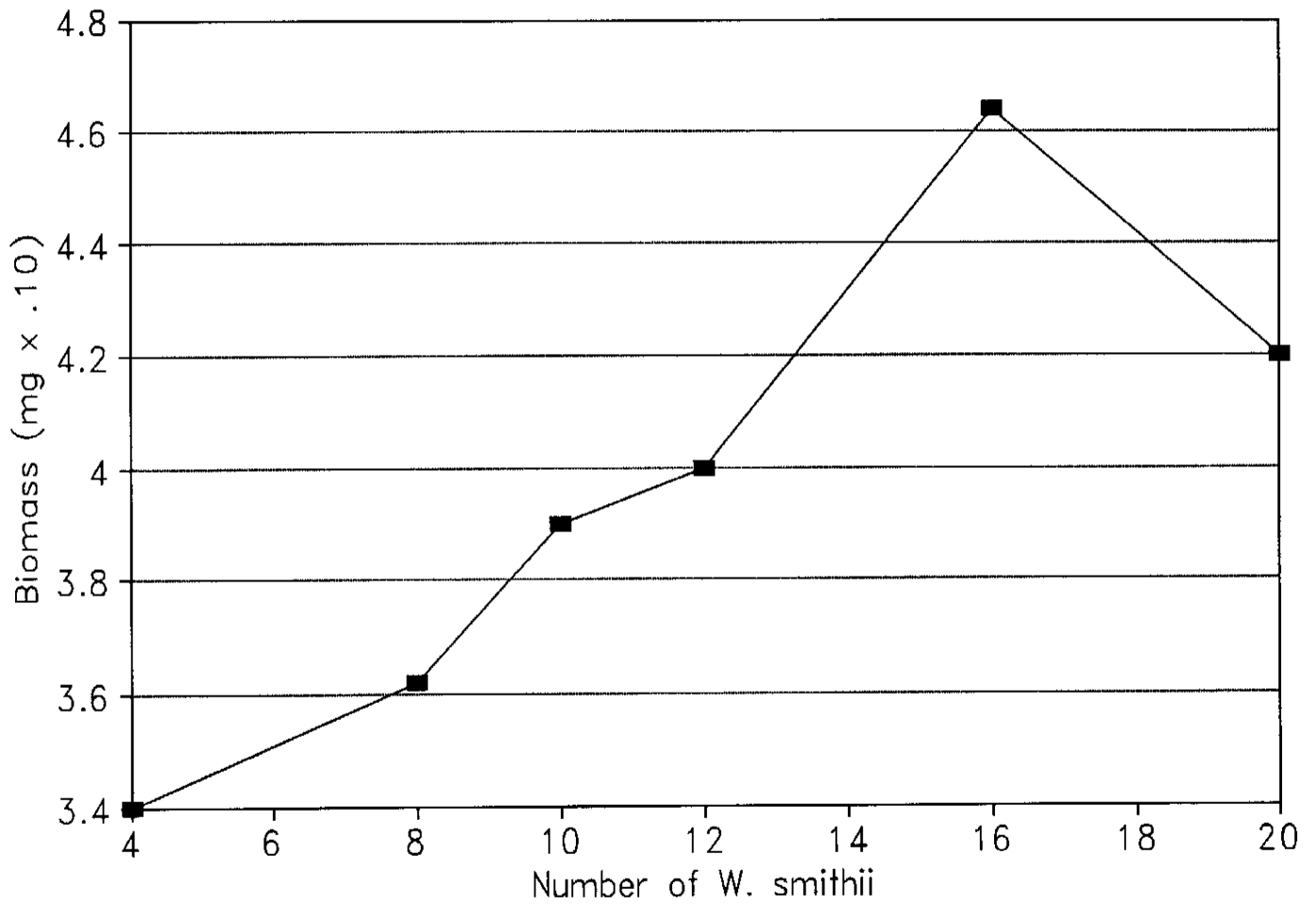


FIGURE 3

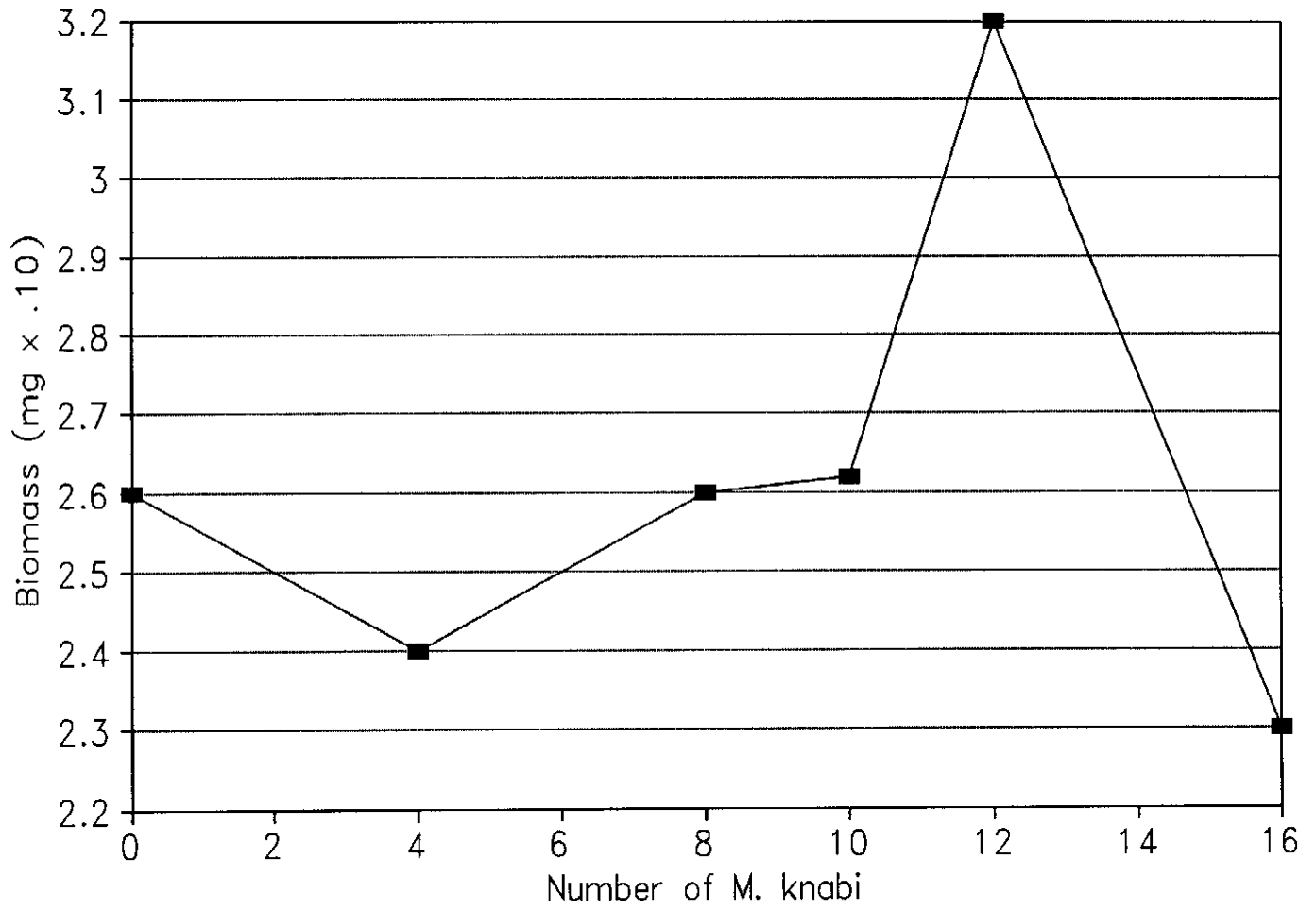


FIGURE 4

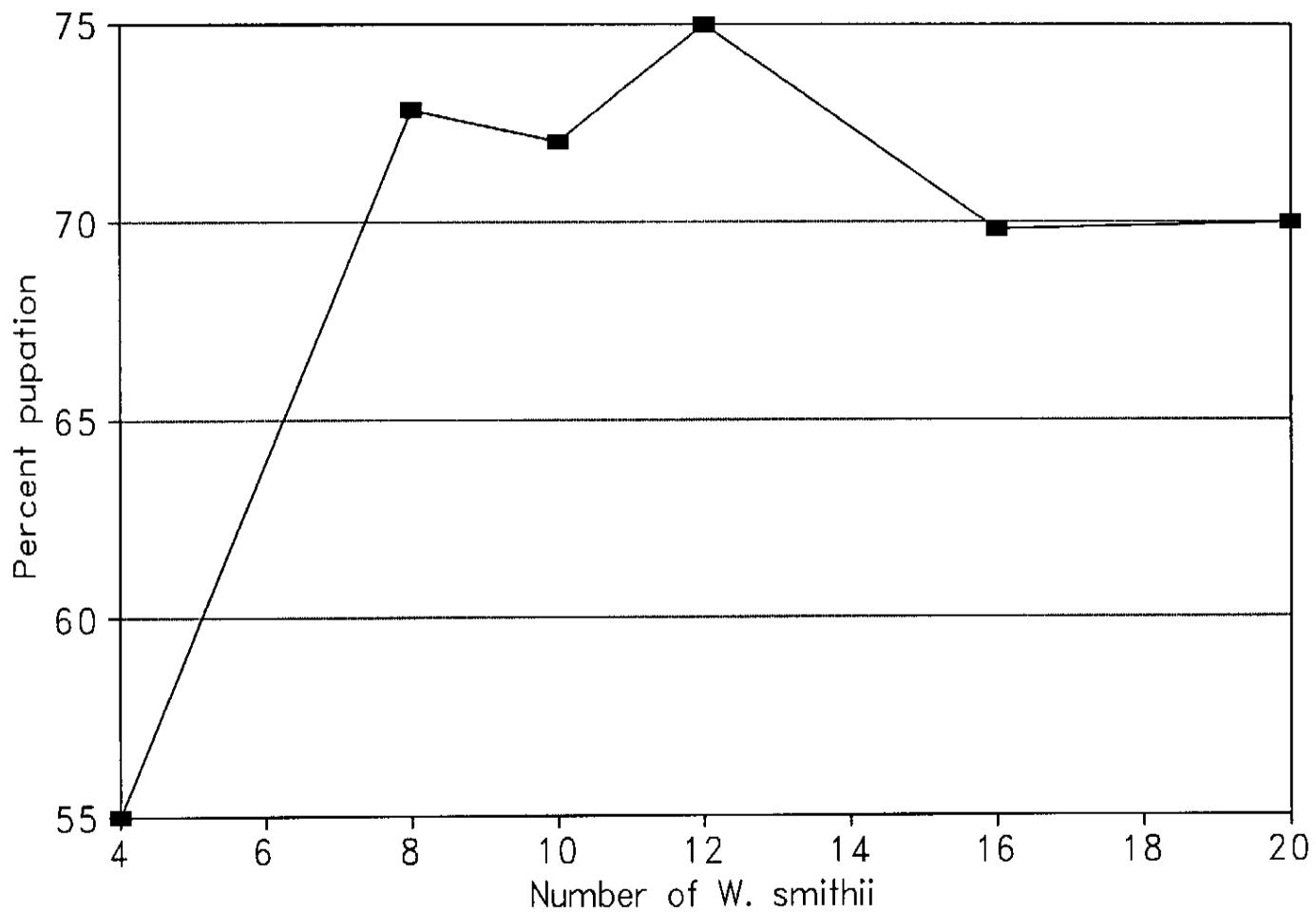


FIGURE 5

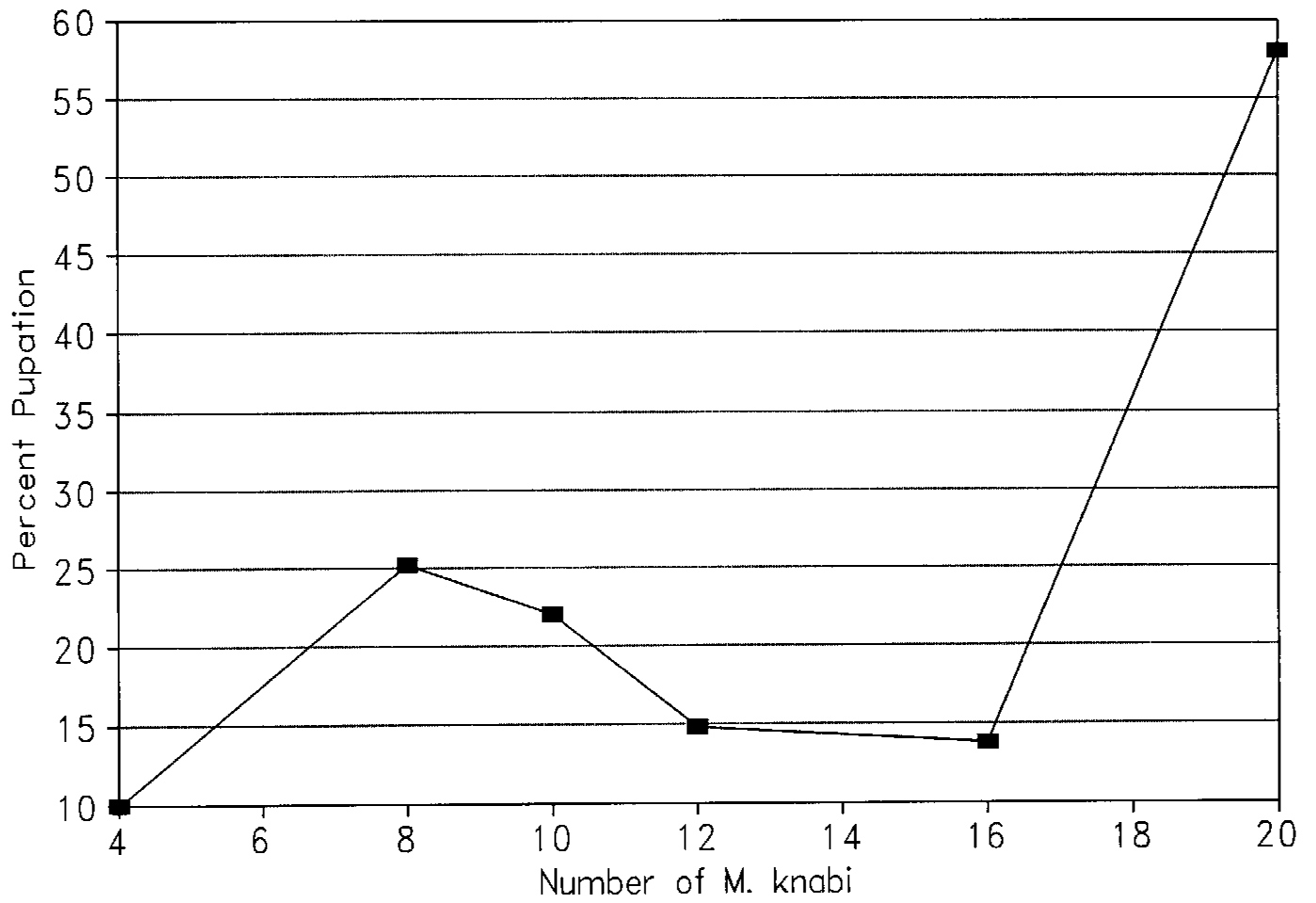
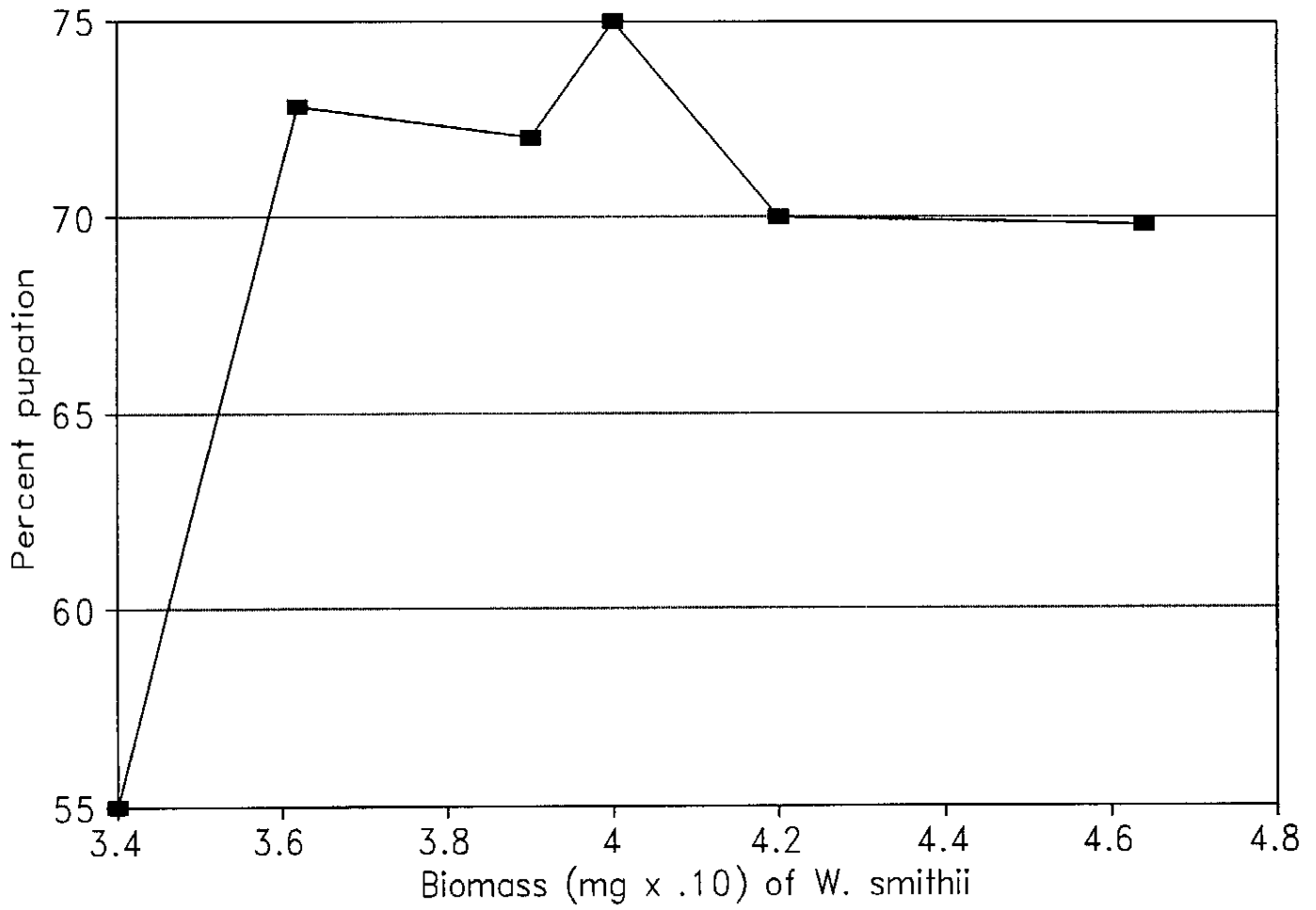
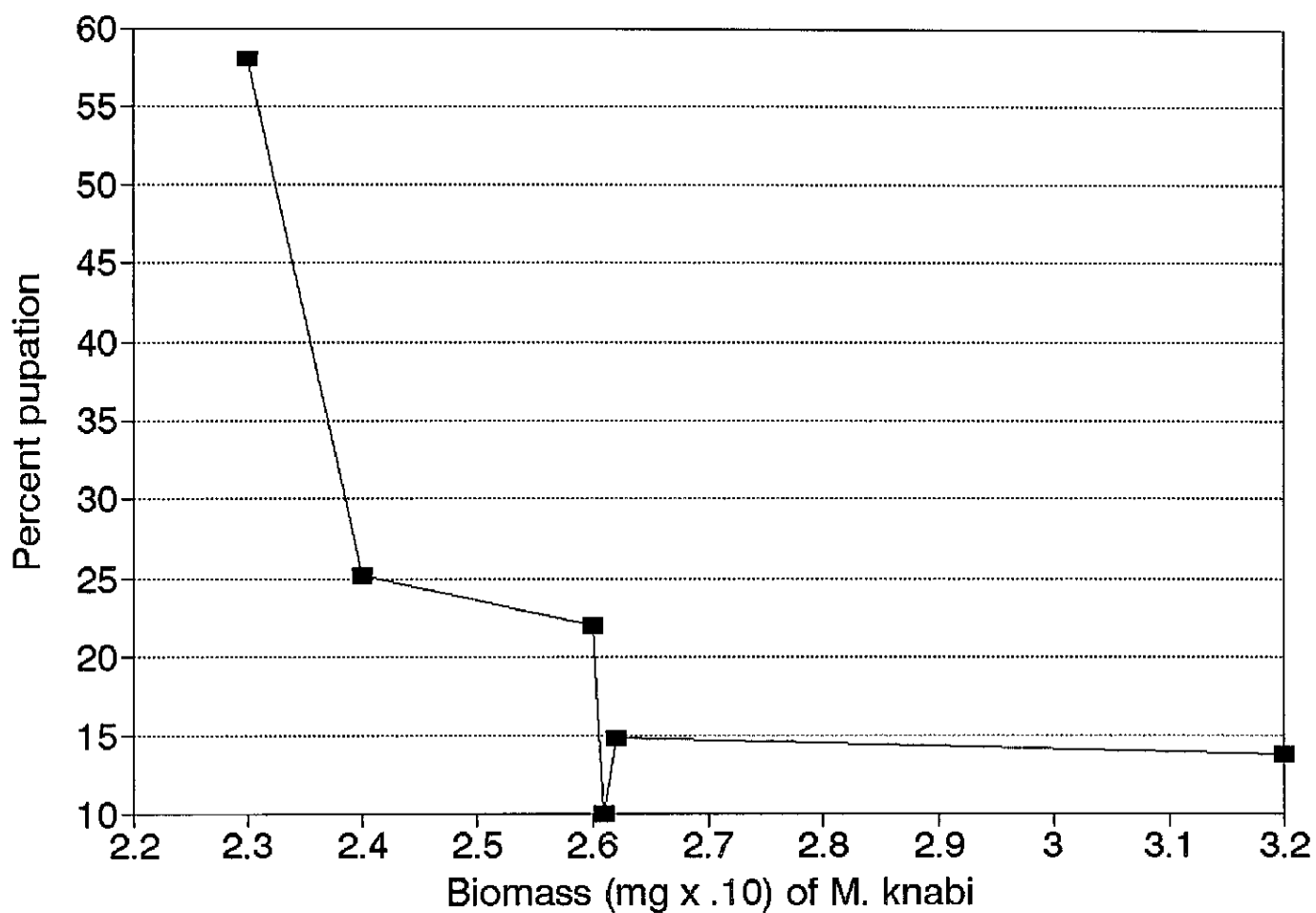


FIGURE 6



# FIGURE 7



# FIGURE 8

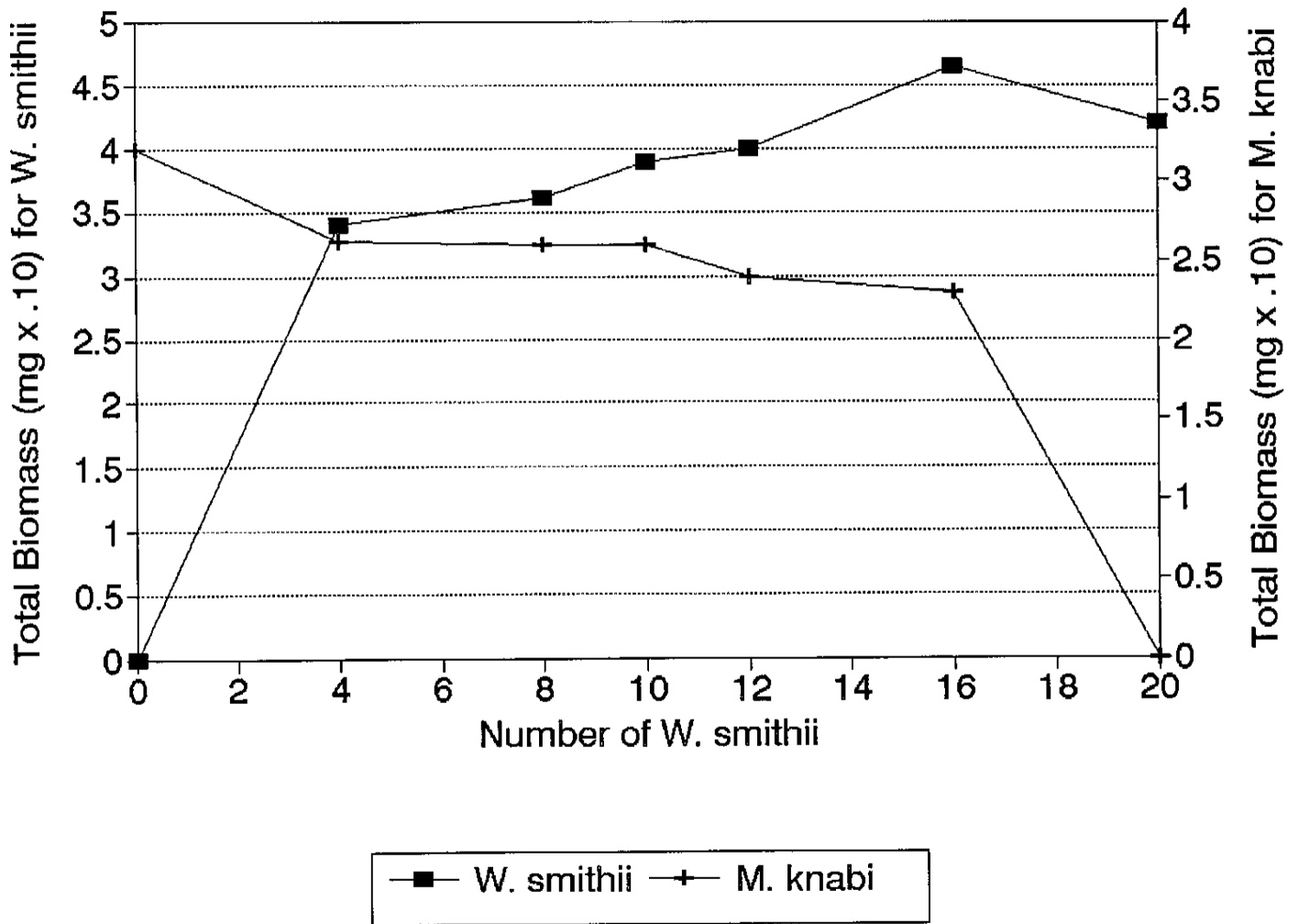


TABLE 1. Treatment set-up for *W. smithii* and *M. knabi*

Cup Number	Midge Density	Mosquito Density
1	20	0
2	16	4
3	12	8
4	10	10
5	8	12
6	4	16
7	<u>0</u>	<u>20</u>
<b>TOTAL</b>	70	70

Table 2. Mean values for each variable at each treatment

TREATMENT	INSECT	HEAD CAPS	BIOMASS	PUP%	MORT%	MOSQ#	MIDGE#	ASSRTPU	ASSRTM
20:0	1	0.843	4.2	70	3	20	0	0.89	0.078
16:4	1	0.848	4.64	69.8	16.4	16	4	0.886	0.266
12:8	1	0.872	4	75	1.8	12	8	0.923	0.057
10:10	1	0.902	3.9	72	6	10	10	0.949	0.153
8:12	1	0.833	3.62	72.6	12.6	8	12	0.906	0.273
4:16	1	0.899	3.4	65	5	4	16	0.72	0.101
0:20	2	0.3	2.3	58	0	0	20	0.792	0
4:16	2	0.3	3.2	13.8	0	4	16	0.305	0
8:12	2	0.295	2.62	14.6	3.4	8	12	0.315	0.083
10:10	2	0.3	2.6	22	8	10	10	0.366	0.128
8:12	2	0.3	2.4	25.2	5	12	8	0.438	0.101
4:16	2	0.28	2.6	10	60	16	4	0.201	0.798

LEGEND:

Insect 1= *W. smithii*

Insect 2= *M. knabi*

HEAD CAPS= Head capsule width

BIOMASS= mg x .10

PUP%= percent pupation

MORT%= percent mortality

NOMOSQ= number of *W. smithii* in treatment

NOMIDGE= number of *M. knabi* in treatment

ASSRTPUP= arcsin square root of percent pupation

ASSRTMORT= arcsin square root of mortality