AN EVALUATION OF COMMERCIAL DIETS FOR REARING *WYEOMYIA SMITHII*[1, 2]

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ABSTRACT. *Wyeomyia smithii* larvae were reared on 6 allotments of 7 larval diets. The allotments ranged from 0.125 to 1.5 mg of food/larva. Both the quantity and quality of the larval diet had a significant effect on larval development, adult fecundity and adult longevity. Larvae reared on Tetramin or a mixture of Tetramin and dog food developed faster and ultimately yielded adults that lived longer and produced more eggs. Fewer larvae developed to the pupal stage in the highest quantity of all diets tested but those surviving produced significantly more eggs as adults. Tetramin or a mixture of Tetramin and dog food at a rate of 0.5 to 0.75 mg/larva are recommended as diets for rearing *Wy. smithii* larvae.

The effects of carbohydrate ingestion on adult fecundity and longevity also were examined. Fecundity and longevity were significantly greater for adults given access to a 10% sugar solution.

INTRODUCTION

The pitcher plant mosquito, *Wyeomyia smithii* (Coq.), has been colonized on several occasions for laboratory study and a variety of diets have been used for rearing the larvae. Price (1958) first colonized the species using finely ground rabbit food in distilled water as the larval rearing medium. Others have used Gaines dog food pellets (Wallis and Frempong-Boadu 1967), Tetramin® E fish food (Istock et al. 1975), Biomin 66 fish food (Evans and Brust 1972), high protein hog chow pellets (Fish and Hall 1978), or liver powder (O'Meara et al. 1981). Still others have used mixtures of such materials as lactalbumin, brewer's yeast, and Purina® standard laboratory chow (Lang 1978); pulverized guinea pig chow, daphnia, and mosquito larvae (Bradshaw and Lounibos 1972); dried blood meal, blood fibrin, yeast extract, Tetramin E fish food, and Tetramin L fish food (Smith and Brust 1971); Tetramin fish food, dried daphnia, brewer's yeast, dried blood meal, and dog food (Evans and Brust 1972); or Tetramin fish flakes and Purina dog chow (Lillie et al. 1980).

The amount of food, composition, and specific feeding regimen are not always reported for tests with *Wy. smithii* but such factors may influence larval and adult development. Lang (1978) showed that the protein content of the larval diet could affect adult fecundity. There is also evidence that the quantity of food given to the larvae may affect larval development, adult fecundity, and adult longevity (Istock et al. 1975). Lea (1964) showed that the quantity and quality of the larval diet could affect autogenous egg maturation by *Aedes taeniorhynchus* (Wied.).

Northern *Wy. smithii* are obligatorily autogenous (Bradshaw 1980). Adult females utilize nutrient reserves in the fat body and possibly in larval muscle remnants to complete ovarian development (Smith and Brust 1971). The mouthparts of *Wy. smithii* are morphologically similar to biting species (Hudson 1970) but the adults have never been observed ingesting blood. They will, however, ingest honey (Bradshaw and Lounibos 1972), raisin juice (Price 1958), or a sugar solu-

[1] The opinions and assertions contained herein are those of the authors and are not to be construed as views, either official or unofficial, of the U.S. Air Force or the Department of Defense.

[2] Mention of a proprietary product in this paper does not constitute an endorsement of the product by the U.S. Air Force or the Department of Defense.

tion (O'Meara et al. 1981) in the laboratory. *Wyeomyia smithii* which feed on carbohydrates live longer (Smith and Brust 1971) and produce more eggs (Lang 1978).

Larvae of this species have been used as bioassay organisms to evaluate water quality following fish kill incidents and to determine the relative toxicity of various compounds (Lillie et al. 1980). The effects of different larval diets on larval and adult development should be known to maintain a laboratory colony for bioassay purposes.

MATERIALS AND METHODS

In this study, we evaluated 4 commercial products and 3 combinations of the 4 as diets for rearing *Wy. smithii* larvae. Protein, fat and fiber percentages for the 7 diets are listed in Table 1. Larval development time, number pupating, adult fecundity and adult longevity were used as criteria for comparing 6 concentrations of each diet. In addition, we examined the effects of carbohydrate ingestion on adult fecundity and longevity.

Table 1. Manufacturer's analysis from container label for the diets tested. A 1:1 mixture was used for diets composed of 2 products.

Diet	Crude protein (min)	Crude fat (min)	Crude fiber (max)
Tetramin[a]	46%	5%	8%
Tetramin and Catfish food	38	4	8
Tetramin and Dog food	36	7	6
Tetramin and Rabbit food	31	4	13
Catfish food[b]	30	2	7
Dog food[c]	25	9	4
Rabbit food[c]	16	2	18

[a] Tetra Werke, Melle, W. Germany.

[b] Master Mix® Catfish Grower, Central Soya and Subsidiaries, Fort Wayne, IN.

[c] Purina® Dog Chow and Purina Rabbit Chow Checkers® (G), Ralston Purina Company, St. Louis, MO.

A colony of *Wy. smithii* was established from larvae collected in July 1974 from *Sarracenia purpurea* L. growing in a bog near Massillon, Ohio. Procedures for rearing the colony are described elsewhere (Lillie et al. 1980). The larval feeding tests were conducted in plexiglass covered plastic pans (12.5 cm high × 29 × 17.5 cm) containing 2 liters of chlorinated tap water and 400 larvae (1–2 days old). The larvae were not fed prior to the tests. Four replicates of each concentration of each diet were evaluated. An initial allotment of 1.0 mg of a given diet/larva was added to each pan. Seven days later and every other day thereafter, either 0.125, 0.25, 0.5, 0.75, 1.0 or 1.5 mg of food/larva were added. Each allotment was based on 400 larvae/pan throughout the study. The amount of food was not reduced as larvae died or pupated. A 1:1 mixture was used for diets composed of 2 products (Table 1). The food was ground in a mortar and saturated with chlorinated tap water to cause it to sink when added to the pans. The larvae were transferred to clean pans with fresh chlorinated tap water when pupae were first observed. A final allotment of food was provided at that time. Pupae were removed and counted daily thereafter.

For the adult phase, 10 male pupae and 10 female pupae (only 5 males and 5 females for the 1.5 mg/larva treatments) were placed in a 120 ml container with 90 ml of chlorinated tap water. This container also served as an oviposition site for emerged adults. The container was attached to the base of a cage constructed from a 1 liter disposable beaker covered with nylon tulle. Three treatments were compared for each concentration of each larval diet. Vials with cotton wicks, saturated with water or a 10% sugar solution, were inserted through the walls of the cages for 2 treatments. The vials and wicks were changed every other day. There were no vials attached to the cages for the third treatment. The number of adult males and females surviving and the number of eggs oviposited were counted daily until all individuals had died. The

dead females were dissected to count the retained stage IV and V oocytes (Clements 1963). The total number of eggs oviposited in a given cage and the number of oocytes retained at death were added and averaged per female to determine fecudity. Two replicates were performed for all adult tests.

All larval and adult tests were conducted in an environmental chamber with a constant setting of 27±1°C, 70±5% relative humidity, and 16 h/8 h light/dark. Incandescent bulbs were used as the light source. The results were analyzed using an analysis of variance randomized complete block design (ANOVA) and Duncan's multiple range test (Steel and Torrie 1960). The ANOVA included analyses for 2-way and 3-way interaction between the type of larval food, the quantity of larval food and the type of adult diet. The level of acceptance was 0.01 for all ANOVA tests and 0.05 for all Duncan's tests.

When the data from the adult phase for individuals given access to water were compared statistically with the data for adults with no water or sugar, the difference was not significant. Individuals in both treatments had access to water in the oviposition container. Future reference to adults without access to a carbohydrate source refers to the combined data for these 2 treatments.

RESULTS

The quantities of food tested were found to range from an insufficient amount to an overabundance. Larvae fed the highest and lowest allotments of rabbit food developed to the pupal stage in 23.8 ± 0.5 days (mean ± standard error) and 23.6 ± 0.4 days respectively (Table 2). Only 19.7 ± 0.3 days were required in the intermediate allotments (0.5 and 0.75 mg/larva) of rabbit food. In contrast, larvae given a mixture of Tetramin and dog food developed to the pupal stage in less than 17 days at all concentrations.

The effects of food quantity and food quality on larval survival are also apparent (Table 3). Significantly fewer larvae developed to the pupal stage in the 1.5 mg/larva allotment than in all other levels. Only 8% of the larvae developed to the pupal stage on a diet of 1.5 mg of catfish food/larva. The greatest percentage (88%) of larvae pupated when a diet of 0.25 mg of Tetramin/larva was administered. The percentage of larvae developing to the pupal stage on a diet of Tetramin was not significantly different than the results for rabbit food, a mixture of Tetramin and rabbit food, or a mixture of Tetramin and dog food.

The test for interaction between the type of larval food and the quantity of larval food was significant when larval

Table 2. Average number of days required for larvae to develop to the pupal stage on 6 allotments of 7 larval diets.

	Food allotment (mg/larva)							
Larval diet	0.125	0.25	0.5	0.75	1.0	1.5	Mean*	±S.E.
Tetramin	16.8	16.3	17.1	16.1	16.6	17.0	16.7^{c}	±0.5
Tetramin and Catfish food	17.5	16.7	15.9	16.6	16.3	15.7	16.5^{c}	±0.3
Tetramin and Dog food	16.4	16.9	16.5	15.8	15.9	15.8	16.2^{c}	±0.2
Tetramin and Rabbit food	20.2	17.1	16.1	15.3	16.7	16.2	16.9^{bc}	±0.4
Catfish food	18.9	18.7	17.0	16.8	17.6	19.6	18.1^{b}	±0.3
Dog food	17.8	20.9	20.1	21.0	22.7	22.1	20.9^{a}	±0.8
Rabbit food	23.6	23.5	19.8	19.6	20.3	23.8	21.8^{a}	±0.5

* Means not followed by the same letter are significantly different (0.05 level).

Table 3. Percentage of larvae developing to the pupal stage on 6 allotments of 7 larval diets. The percentage for each allotment of each diet is based on a sample size of 1,600 larvae.

Larval diet	Food allotment (mg/larva)						% of 9,600*
	0.125	0.25	0.5	0.75	1.0	1.5	
Tetramin	83	88	83	72	75	18	70^{ab}
Tetramin and Catfish food	53	64	72	72	68	52	64^{b}
Tetramin and Dog food	73	82	84	77	82	51	75^{a}
Tetramin and Rabbit food	66	85	82	77	50	46	68^{ab}
Catfish	68	77	79	82	74	8	65^{b}
Dog food	58	70	71	67	51	14	55^{c}
Rabbit food	69	78	81	80	83	22	69^{ab}

* Values not followed by the same letter are significantly different (0.05 level) (based on comparison of the means before converting to percentages).

survival to the pupal stage was considered. Maximum larval survival was not achieved at the same allotment for all diets tested. For example, less Tetramin than rabbit food was required to maximize larval survival (Table 3). All other tests for interaction were not significant.

Larvae reared on different types and amounts of food yielded adults with significantly different numbers of eggs. Adults from larvae reared on 0.5 mg of Tetramin/larva and given access to a 10% sugar solution had the highest fecundity (103.4 ± 12.5 eggs/female) (Table 4). The lowest fecundity (10.5 ± 0.8 eggs/female) was observed when adults from larvae reared on a mixture of Tetramin and rabbit food at a rate of 0.125 mg/larva were not given access to a carbohydrate source (Table 5).

The quality and quantity of larval food as well as the presence or absence of a carbohydrate source influenced adult longevity. Adults given access to a carbohydrate source lived significantly longer than those without a carbohydrate source (Table 6).

DISCUSSION

Both the quality and quantity of the larval diet significantly affected all aspects of development evaluated. There is evi-

Table 4. Fecundity for carbohydrate fed females from larvae reared on 6 allotments of 7 larval diets. A 10% sugar solution was used as the carbohydrate source.

Larval diet	Food allotment (mg/larva)						Mean*	±S.E.
	0.125	0.25	0.5	0.75	1.0	1.5		
Tetramin	58.6	86.5	103.4	97.6	97.0	97.5	90.1^{a}	±6.0
Tetramin and Catfish food	53.1	80.8	92.6	87.1	84.5	89.1	81.2^{abc}	±5.8
Tetramin and Dog food	60.1	71.7	94.6	102.7	98.3	90.7	86.4^{ab}	±5.4
Tetramin and Rabbit food	46.1	49.8	77.5	94.4	88.7	93.6	75.0^{bc}	±6.7
Catfish food	45.8	62.6	79.8	93.9	74.4	69.6	71.0^{c}	±5.3
Dog food	66.9	72.3	75.4	80.5	90.1	73.2	76.4^{bc}	±2.7
Rabbit food	43.6	52.9	85.8	88.4	78.3	94.1	73.9^{c}	±5.9

* Means not followed by the same letter are significantly different (0.05 level).

Table 5. Fecundity for carbohydrate deprived females from larvae reared on 6 allotments of 7 larval diets.

Larval diet	Food allotment (mg/larva) 0.125	0.25	0.5	0.75	1.0	1.5	Mean*	±S.E.
Tetramin	25.0	41.4	44.9	42.5	44.1	59.2	42.8[a]	±3.0
Tetramin and Catfish food	24.0	43.0	46.6	37.3	49.7	53.4	42.3[a]	±3.0
Tetramin and Dog food	25.6	31.8	47.4	49.1	49.6	54.2	43.0[a]	±2.5
Tetramin and Rabbit food	10.5	34.0	43.1	45.1	48.9	53.8	39.2[a]	±3.4
Catfish food	11.1	33.5	51.1	48.6	50.7	44.4	39.9[a]	±3.1
Dog food	32.3	48.0	42.9	52.0	43.5	45.7	44.1[a]	±2.1
Rabbit food	11.0	24.9	30.6	37.2	31.0	45.9	30.1[b]	±3.0

* Means not followed by the same letter are significantly different (0.05 level).

dence that the protein content of the larval diet may influence larval development and adult fecundity. Larvae reared on the lowest protein diets, rabbit food and dog food (Table 1), required significantly more time to develop to the pupal stage than larvae reared on other diets (Table 2). Larval development on rabbit food was more rapid than previously reported for *Wy. smithii* larvae reared on the same diet at approximately the same temperature (Price 1958). The shorter development time which we observed may have resulted because the procedures used in maintaining the parent colony (Lillie et al. 1980) have selected for early developers. Carbohydrate deprived adults from larvae reared on a mixture of Tetramin and rabbit food had the lowest fecundity (Table 5). The effect of protein content on egg production is in agreement with that previously reported by Lang (1978).

Larvae did not survive as well in the highest allotment of all diets (Table 3). The lethal effect of an overabundance of food has been attributed to scum formation on the surface of the rearing medium for other mosquito species (Asahina 1964). In our study, scum did not appear to be a problem because *Wy. smithii* larvae

Table 6. Mean* longevity (days ± standard error) for adults given access to a 10% sugar solution or water. The adults were obtained from larvae reared on 6 allotments of 7 larval diets. The data were pooled by larval diet for all allotments.

Larval diet	Adult diet: Female Sugar	Female Water	Male Sugar	Male Water
Tetramin	29.3[a] ±0.2	4.4[a] ±0.1	32.6[a] ±0.6	3.7[a] ±0.1
Tetramin and Catfish food	20.8[bc] ±1.4	4.0[b] ±0.2	24.8[b] ±2.5	3.8[a] ±0.1
Tetramin and Dog food	19.5[bc] ±1.0	4.0[b] ±0.1	25.2[b] ±2.9	3.6[ab] ±0.1
Tetramin and Rabbit food	17.9[bc] ±1.1	3.8[b] ±0.2	20.6[bc] ±1.6	3.3[bc] ±0.2
Catfish food	16.2[c] ±0.6	4.0[b] ±0.2	17.8[c] ±1.0	3.2[c] ±0.1
Dog food	16.3[c] ±0.9	3.9[b] ±0.1	20.6[bc] ±1.6	3.6[ab] ±0.1
Rabbit food	22.0[b] ±1.6	3.7[b] ±0.2	22.7[bc] ±1.7	3.6[ab] ±0.2

* Means (in a given column) not followed by the same letter are significantly different (0.05 level).

remained on the bottom of the rearing pans during most of their development, a behavior also observed by Price (1958). Fourth instar larvae abandoned the bottom existence to hang suspended from the surface. Scum was reduced from the surface during the rearing procedure by transferring larvae to clean pans with fresh tap water when pupae were first observed. Larval mortality in the highest allotment was probably caused by something other than scum formation such as products of biodegradation.

The 1.5 mg/larva allotment had significantly fewer larvae develop to the pupal stage (Table 3) but yielded adults that produced significantly more eggs when a carbohydrate source was not provided (Table 5). When given access to a 10% sugar solution, the fecundities of adults from larvae reared on 0.5, 0.75, 1.0 and 1.5 mg/larva were not significantly different (Table 4). Adults had the lowest fecundity for all diets when larvae were reared on 0.125 mg/larva.

Larvae reared on the lowest concentration of rabbit food, catfish food, or a mixture of Tetramin and rabbit food produced less than 12 eggs/female when the adults were not given access to a carbohydrate source (Table 5). Istock et al. (1975) contend that a fertility rate of at least 25 eggs/female would have to be achieved for an autogenous mosquito population to maintain itself. Based on their contention, 0.125 mg of certain foods/larva would be insufficient for maintenance of a *Wy. smithii* field population.

The fecundity (Table 4) and longevity (Table 6) of carbohydrate fed adults were greater than that of adults without a carbohydrate source (Table 5). Lang (1978) reported a higher fecundity for adults given sucrose but stated that it may be due to increased longevity. By living longer, adults may be able to more fully utilize their protein reserves.

Future reports of laboratory studies with *Wy. smithii* should mention both the quality and quantity of the larval diet because of their influence on larval development, adult fecundity and adult longevity. Tetramin or a mixture of Tetramin and dog food administered in the manner reported here at a rate of 0.5 to 0.75 mg/larva are recommended as diets for rearing *Wy. smithii* larvae. Carbohydrate ingestion by adults will increase longevity.

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References Cited

Asahina, S. 1964. Food material and feeding requirements for mosquito larvae. Bull. W. H. O. 31:465–466.

Bradshaw, W. E. 1980. Blood-feeding and capacity for increase in the pitcher plant mosquito, *Wyeomyia smithii*. Environ. Entomol. 9:86–89.

Bradshaw, W. E. and L. P. Lounibos. 1972. Photoperiodic control of development in the pitcher plant mosquito *Wyeomyia smithii*. Can. J. Zool. 50:714–720.

Clements, A. N. 1963. The physiology of mosquitoes. Macmillan Co., N.Y. 393 pp.

Evans, K. W. and R. A. Brust. 1972. Induction and termination of diapause in *Wyeomyia smithii* (Diptera: Culicidae), and larval survival studies at low and subzero temperatures. Can. Entomol. 12:1937–1950.

Fish, D. and D. W. Hall. 1978. Succession and stratification of aquatic insects inhabiting the leaves of the insectivorous pitcher plant, *Sarracenia purpurea*. Am. Midland Nat. 99:172–183.

Hudson, A. 1970. Notes on the piercing mouthparts of three species of mosquitoes (Diptera: Culicidae) viewed with the scanning electron microscope. Can. Entomol. 102:501–509.

Istock, C. A., S. S. Wasserman and H. Zimmer. 1975. Ecology and evolution of the pitcher plant mosquito: 1. Population dynamics and laboratory responses to food and population density. Evolution 29:296–312.

Lang, J. T. 1978. Relationship of fecundity to the nutritional quality of larval and adult diets of *Wyeomyia smithii*. Mosq. News 38:396–403.

Lea, A. O. 1964. Studies on the dietary and endocrine regulation of autogenous reproduction in *Aedes taeniorhynchus*. J. Med. Entomol. 1:40–44.

Lillie, T. H., J. M. Campbell, C. E. Thalken and J. T. Lang. 1980. The pitcher plant mosquito, *Wyeomyia smithii*, a recent introduction to the bioassay laboratory. Proc. 14th Ann. Conf. Trace Subs. in Environ. Hlth. 14:383–389.

O'Meara, G. F., L. P. Lounibos and R. A. Brust. 1981. Repeated egg clutches without blood in the pitcher plant mosquito. Ann. Entomol. Soc. Am. 74:68–72.

Price, R. D. 1958. Notes on the biology and laboratory colonization of *Wyeomyia smithii* (Coquillett) (Diptera: Culicidae). Can. Entomol. 90:473–478.

Smith, S.M. and R. A. Brust. 1971. Photoperiodic control of maintenance and termination of larval diapause in *Wyeomyia smithii* (Coq.) (Diptera: Culicidae) with notes on oogenesis in the adult female. Can. J. Zool. 49:1065–1073.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and procedures of statistics, with special reference to the biological sciences. McGraw-Hill, N.Y. pp. 107–109.

Wallis, R. C. and J. Frempong-Boadu. 1967. Colonization of *Wyeomyia smithii* (Coq.) from Connecticut. Mosq. News 27:9–11.

MERMITHID PARASITISM IN *CULICOIDES VARIIPENNIS* (DIPTERA: CERATOPOGONIDAE) IN NEW YORK STATE

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ABSTRACT. Parasitism of late instar *Culicoides variipennis* larvae by the mermithid nematode, *Heleidomermis vivipara*, is reported. Parasites were found at 4 geographically distinct sites in central New York State. The nematodes emerged from the host as adults, mated, and the females gave birth to live parasites. The sex ratio was 41:59 (male:female), with generally either 1 female or 1 or 2 males emerging/host. During the 7-month study at 2 of the sites, average parasitism was 9.8% and 7.1%, but individual collections ranged from 0–54%.

Mermithid parasitism in ceratopogonids has been reported on numerous occasions, although proper parasite identifications are often lacking (see Wirth 1977 for review). The first report of mermithids attacking midges of the subgenus *Monoculicoides* was by Glukhova (1967), who found parasitized larvae of *Culicoides stigma* (Meigen), *C. nubeculosus* (Meigen) and *C. puncticollis* (Becker) in a silty, manure-contaminated habitat near a cattle watering trough in Karelia, USSR. The nematodes were later described by Rubtsov (1970, 1972) as *Heleidomermis vivipara*. Another species, *H. ovipara* (Rubtsov 1974a) was found developing primarily in late instar larvae (rarely pupae) of *C. helveticus* Callot, Kremer and Deduit, though the nematode also parasitized *C. pulicaris* L., *C. circumscriptus* Kieffer and *C. manchuriensis* Tokunaga. Very little is known of their biologies.

MATERIALS AND METHODS

We first discovered mermithid parasites emerging from 4th instar larvae of *Culicoides v. variipennis* (Coquillett) collected from Fanton Farm, near Belvidere, Allegheny Co., NY in May 1980. Parasitized midge larvae were sub-