# REARING *HYDROMETRA MARTINI* (HETEROPTERA: HYDROMETRIDAE): FOOD AND SUBSTRATE EFFECTS

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ABSTRACT

*Hydrometra martini* Kirkaldy was reared using two food treatments (*Sminthurides malmgreni* [Tullberg] or *Drosophila melanogaster* Meigen) and two substrate treatments (filter paper or duckweed) to investigate the effects of differing food and substrate on stadium and survivorship. Food, substrate, and the interaction of food and substrate affected survivorship and stadium lengths, but effects varied among instars. To maximize laboratory survivorship, the data indicate that the more effective food was *Sminthurides* on a filter paper substrate and *Drosophila* on a duckweed substrate.

Key Words: *Hydrometra martini*, laboratory rearing, survivorship, stadium

RESUMEN

*Hydrometra martini* Kirkaldy fue criada empleando dos fuentes de alimento (*Sminthurides malmgreni* [Tullberg] o *Drosophila melanogaster* Meigen) y dos sustratos (papel filtro o "duckweed"). El objetivo del estudio fue determinar los posibles efectos de distintas fuentes de alimento y diferentes sustratos en la duración y supervivencia de cada estadío. La fuente de alimento, sustrato y la interacción de fuente de alimento y sustrato afectaron la supervivencia y duración de cada estadío, pero los efectos variaron entre instars. Los resultados indicaron que las mejores fuentes de alimento fueron *Sminthurides* sobre sustrato de papel filtro y *Drosophila* sobre sustrato de "duckweed".

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Our knowledge of hydrometrid biology and ecology in North America is based primarily on Sprague's (1956) monographic study of the biology and morphology of *Hydrometra martini* Kirkaldy, Lanciani's (1971, 1975, 1991, 1995) studies of *Hydrometra australis* Say and its relationship with water mites, and Wood and McPherson's (1995) life history study of *Hydrometra hungerfordi* Torre-Bueno.

Lanciani (1991) compared *H. australis* reared on springtails (Collembola: Sminthuridae: *Sminthurides* sp.) with those he had reared earlier (Lanciani 1975 [as *Hydrometra myrae* Torre-Bueno]) on fruit flies (Diptera: Drosophilidae: *Drosophila melanogaster* Meigen). He found that hydrometrid stadia were shorter and survivorship was higher when springtails were used as food. He suggested that aquatic Collembola may provide important nutrients that are lacking in fruit flies. Lanciani reared the animals under identical temperature and photoperiod regimes, but the two studies were conducted using differing substrates (paper in 1975, duckweed in 1991). Lanciani (1991), using a duckweed substrate, successfully reared *H. australis* through 2nd instar on collembolans, with subsequent instars receiving *Drosophila* as

food, while a control group reared exclusively on *Drosophila* failed to reach adults. The survivorship differences related to food seem plausible, but we felt that some affect could be attributed to the rearing substrates. We investigated this possibility by rearing *H. martini* under both of the food and substrate conditions employed by Lanciani (1975, 1991). Food and survivorship were then examined as factors influencing survivorship and stadia.

Lanciani (1975, 1991) used *H. australis* in his studies, but the widespread occurrence of character states intermediate between those of *H. australis* and *H. martini* (Bennett & Cook 1981; Gonsoulin 1973; S. J. Taylor, unpublished data) suggests that these two species are synonymous (Polhemus & Chapman 1979; J. T. Polhemus 1996, Colorado Entomological Museum, Englewood, personal communication). Thus, our findings should be comparable to Lanciani's (1975, 1991) work. Following Smith (1988), we treat Illinois specimens as *H. martini*.

## Materials and Methods

Forty-nine micropterous (*sensu* Polhemus & Polhemus 1987) adult *H. martini* were collected from Crab Orchard Lake (Williamson County, Illinois) on September 6, 1991, within 2 m of the shoreline from shallow (<0.3 m), still, unshaded water with abundant floating and emergent vegetation. The specimens (32 ♀♀, 17 ♂♂) were divided randomly into four screen-covered, one-quart mason jars, each with about 3 cm of dechlorinated tap water and three floating plastic disks. Disks were 4 cm in diameter, each with five 5.5 mm diameter holes. The disks provided a dry retreat and oviposition substrate. Frozen *D. melanogaster* were provided *ad libitum* to each container. Each day, fruit flies were replaced and the disks rinsed, dried and returned to the jars. Oviposition containers were incubated at a constant photoperiod (12L:12D) and temperature (28 ± 1°C), which was the same temperature and photoperiod used by Lanciani (1975, 1991). Three "daylight" fluorescent lamps provided approximately 2800 lux. Eggs from the ovipositional containers were removed daily and distributed in equal numbers into each of four experimental groups described below. Sufficient eggs were collected in four days to rear 40 individuals in each of four treatments.

Screw top, straight-sided plastic containers (4.25 cm tall and 4.8 cm inside diameter) were used to test duckweed substrate and filter paper substrate treatments. The duckweed substrate treatment consisted of 80 containers filled approximately half full (2 cm) of dechlorinated tap water that was covered with a dense layer of *Lemna minor* L., *Spirodela polyrhiza* (L.), and *Wolffia papulifera* Thompson (Lemnaceae), collected from a pond in Carbondale, Illinois. These species are widespread in Illinois (Weik & Mohlenbrock 1968). One *H. martini* egg was placed in the center of each container on a large *S. polyrhiza* leaf. Containers were covered with a piece of fiberglass screening (1 $mm^2$ mesh) secured with a rubber band. The screen top duplicated conditions of Lanciani (1991).

The filter paper substrate treatment consisted of 80 containers with a 4.8 cm diameter circular piece of Eaton-Dikeman (Mt. Holly Springs, PA) grade 617 filter paper placed in the bottom of each container. These containers were tilted at an 8° angle and moistened with dechlorinated tap water to a maximum depth of 3 to 5 mm to approximate Lanciani's (1975) rearing conditions. Each of these containers received one *H. martini* egg, placed in the center of the filter paper, out of the pooled water. These containers were loosely covered with a screw cap to prevent the filter paper from drying out. This also duplicated conditions of Lanciani (1975).

Containers were checked daily, and water was added to maintain appropriate levels described above for each substrate treatment. As eggs hatched, each treatment

group (duckweed and filter paper substrates) was further divided into two food treatments: fruit fly and springtail. Each day, containers in the fruit fly treatment received two frozen *D. melanogaster*, and those in the springtail treatment about 20 live *Sminthurides malmgreni* (Tullberg) (Collembola: Sminthuridae), approximating Lanciani's (1975, 1991) feeding regimes. Dead fruit flies were removed daily; dead springtails were removed if they did not appear fresh, and new food items were provided as needed to maintain numbers of individuals.

Fruit fly cultures were maintained in the laboratory on a commercially available culture medium (Ward's Natural Science, Rochester, New York) and were killed by freezing no more than two days prior to their use. Preliminary work indicated that flies frozen for prolonged periods became dehydrated. Springtails were obtained by repeatedly passing a plastic box slowly over the surface of a duckweed covered pond, as described by Lanciani (1991). Springtail colonies were maintained at 28 ± 1°C in quart jars, following the methods of Purrington et al. (1991). Additional springtails were collected and added to the colonies every 1-3 days.

Statistical analyses were carried out using SAS procedures (SAS Institute 1988). Survivorship (percent of individuals molting to the next instar) was examined using a general linear model (PROC GLM) with data coded as 0 or 1. Stadia and transformed stadia for nymphal instars were not normally distributed. We used the two way ANOVA's to compare stadia among treatments for first through fourth instars. This procedure is fairly robust to deviations from normality (Glass et al. 1972, Srivastava 1959, Tiku 1971, Zar 1984). Post hoc comparisons, stadia of fifth instars, and total length of development were examined using t-tests. Numbers of eggs hatching and the proportion of the stadia available for calculation in the two substrate treatments were examined using chi-square tests. The significance level was 0.05 for all tests.

Voucher specimens are housed in the Southern Illinois University at Carbondale Entomology Collection and the collections of the authors.

## Results

First through fifth instar *H. martini* nymphs in both duckweed food treatments were observed on several occasions feeding on arthropods other than *Sminthurides* and *Drosophila*, most often upon emerging or recently (<24 h) emerged adult Chironomidae (Diptera). Several early anisopteran instars (Odonata), larval Chironomidae and one small larval Dytiscidae (Coleoptera) were removed from rearing containers in the course of the experiment, but we found no evidence that these insects were preying upon *H. martini*.

During rearing, molting occasionally was overlooked, particularly in early instars of the two duckweed treatments where exuviae were difficult to find. This resulted in some stadium data being lost (Table 1). Missed molts were discovered later when individuals molted to later instars; the animals were then assigned to the correct instar. To examine the effect of substrate on our ability to detect molts, we compared the sample sizes for numbers of individuals surviving each stage with the number of individuals of each stage for which stadia could be calculated. The proportion of the stadia available for calculation was greater on filter paper (98%, 327 of 334 stadia) than on duckweed (72%, 219 of 303 stadia) ($\chi^2$ = 83.1, df = 1, $P < 0.001$), indicating that our ability to detect exuviae was influenced by the substrate.

Survivorship for each treatment decreased at nearly every instar (Table 1). Survivorship from egg to adult differed among treatments (F = 11.28, df = 3,156, $P$ = 0.0001): substrate, food, and the interaction of food and substrate were all significant ($P$ = 0.007, 0.0459, and 0.0001, respectively). Survivorship from egg to adult differed

TABLE 1. STADIUM (DAYS) AND SURVIVORSHIP OF *H. MARTINI* NYMPHS REARED ON TWO SUBSTRATES AND UNDER TWO FEEDING REGIMES AT 28 ± 1°C.

| | | | Stadium Length | | | Survivorship | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Number | | Percent[b] |
| Substrate | Food | Stage | N[a] | $\bar{X}$ ± SE | Range | $N_1$ | $N_2$ | % |
| Filter Paper | | Egg | 73 | 5.6 ± 0.1 | 5-6 | 80 | 76 | 95.00 |
| | *Sminthurides* | 1st instar | 37 | 2.0 ± 0.0 | 1-2 | 39 | 37 | 94.87 |
| | | 2nd instar | 36 | 1.0 ± 0.0 | 1-2 | 37 | 36 | 97.30 |
| | | 3rd instar | 34 | 1.4 ± 0.1 | 1-2 | 36 | 34 | 94.44 |
| | | 4th instar | 27 | 1.9 ± 0.2 | 1-4 | 34 | 28 | 82.35 |
| | | 5th instar | 16 | 3.8 ± 0.3 | 2-5 | 28 | 17 | 60.71 |
| | | Total of 1st through 5th | 17 | 9.9 ± 0.2 | 8-10 | 39 | 17 | 43.59 |
| | | Total of egg through 5th | 17 | 15.4 ± 0.2 | 14-16 | 40 | 17 | 42.50 |
| | *Drosophila* | 1st instar | 35 | 2.1 ± 0.1 | 1-4 | 37 | 37 | 100.00 |
| | | 2nd instar | 32 | 1.9 ± 0.1 | 1-4 | 37 | 32 | 86.49 |
| | | 3rd instar | 24 | 2.2 ± 0.2 | 1-4 | 32 | 24 | 75.00 |
| | | 4th instar | 13 | 2.8 ± 0.2 | 2-4 | 24 | 13 | 54.17 |
| | | 5th instar | 0 | | | 13 | 0 | 0.00 |
| | | Total of 1st through 5th | 0 | | | 37 | 0 | 0.00 |
| | | Total of egg through 5th | 0 | | | 40 | 0 | 0.00 |

[a]Includes only individuals for which stadium length could be calculated.
[b]Percent entering stage ($N_1$) that survived to enter next ($N_2$); includes individuals lacking stadium data.

TABLE 1. (CONTINUED) STADIUM (DAYS) AND SURVIVORSHIP OF *H. MARTINI* NYMPHS REARED ON TWO SUBSTRATES AND UNDER TWO FEEDING REGIMES AT 28 ± 1°C.

| | | | Stadium Length | | | Survivorship | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Number | | Percent[b] |
| Substrate | Food | Stage | N[a] | $\bar{X}$ ± SE | Range | $N_1$ | $N_2$ | % |
| Duckweed | | Egg | 63 | 6.6 ± 0.1 | 5-9 | 80 | 72 | 90.00 |
| | *Sminthurides* | 1st instar | 15 | 2.1 ± 0.2 | 1-3 | 32 | 27 | 84.38 |
| | | 2nd instar | 10 | 1.1 ± 0.1 | 1-2 | 27 | 26 | 96.30 |
| | | 3rd instar | 16 | 1.4 ± 0.2 | 1-3 | 26 | 24 | 92.31 |
| | | 4th instar | 17 | 1.8 ± 0.2 | 1-3 | 24 | 21 | 87.50 |
| | | 5th instar | 14 | 2.9 ± 0.1 | 2-4 | 21 | 15 | 71.43 |
| | | Total of 1st through 5th | 14 | 9.0 ± 0.2 | 8-10 | 32 | 15 | 46.88 |
| | | Total of egg through 5th | 15 | 15.7 ± 0.3 | 13-17 | 40 | 15 | 37.50 |
| | *Drosophila* | 1st instar | 18 | 2.2 ± 0.2 | 1-3 | 33 | 25 | 75.76 |
| | | 2nd instar | 13 | 1.6 ± 0.2 | 1-3 | 25 | 25 | 100.00 |
| | | 3rd instar | 15 | 1.7 ± 0.1 | 1-2 | 25 | 24 | 96.00 |
| | | 4th instar | 18 | 1.8 ± 0.1 | 1-3 | 24 | 23 | 95.83 |
| | | 5th instar | 20 | 2.5 ± 0.1 | 2-3 | 23 | 21 | 91.30 |
| | | Total of 1st through 5th | 21 | 9.3 ± 0.2 | 8-11 | 33 | 21 | 63.64 |
| | | Total of egg through 5th | 21 | 15.8 ± 0.2 | 14-17 | 40 | 21 | 52.50 |

[a]Includes only individuals for which stadium length could be calculated.
[b]Percent entering stage ($N_1$) that survived to enter next ($N_2$); includes individuals lacking stadium data.

between food treatments within the filter paper treatment (42.5% versus 0% survivorship; F = 28.8, df = 1,78, $P = 0.0001$) but not in the duckweed treatment (37.5% versus 52.5% survivorship; F = 1.8, df = 1,78, $P = 0.182$), and between substrate treatments in the fruit fly treatment (52.5% versus 0% survivorship; F = 43.1, df = 1,78, $P = 0.0001$) but not in the springtail treatment (37.5% versus 42.5% survivorship; F = 0.20, df = 1,78, $P = 0.653$).

Egg stadia (Table 1) were 15% shorter on filter paper than on duckweed (t = 8.24, df = 91.5, $P < 0.001$), but hatching success did not differ ($\chi^2 = 1.441$, df = 1, $P = 0.230$).

The first stadium did not differ by either food or substrate treatments (Table 2). The second and third stadia were 40% and 28% shorter, respectively, for individuals reared on springtails. The interaction of food and substrate was significant for the third stadium; *post hoc* comparisons revealed a substrate difference in stadia of fruit fly treatments (2.2 versus 1.7 days; t = -2.56, df = 37, $P = 0.015$) but not in stadia of springtail treatments (1.4 versus 1.4 days; t = 0.16, df = 48, $P = 0.877$). Both food and substrate affected the length of the fourth stadium, and the interaction term was also significant; *post hoc* comparisons revealed a substrate difference in stadium lengths of fruit fly treatments (2.8 versus 1.8 days; t = -4.67, df = 29, $P < 0.001$) but not in springtail treatments (1.9 versus 1.8 days; t = -0.53, df = 42, $P = 0.596$), and a food difference in stadia on filter paper (2.8 versus 1.9 days; t = -3.51, df = 38, $P = 0.001$) but not on duckweed (1.8 versus 1.8 days; t = -0.343, df = 33, P = 0.734).

No individuals in the filter paper/fruit fly treatment reached adults. Therefore, stadia for fifth instars and total length of development (with and without egg stage) were tested separately, using only the duckweed treatments to examine food effects and only the springtail treatments to examine substrate effects.

The fifth stadium in the springtail treatments was 24% shorter on duckweed than on filter paper (t = -2.83, df = 22.0, $P = 0.010$). No difference in food treatments was detected for fifth stadium on duckweed (t = 1.96, df = 32, $P = 0.058$), perhaps because of the small sample size for the springtail fed individuals.

The length of total development (egg through fifth instar) did not differ between substrates in springtail treatments (t = 1.18, df = 30, $P = 0.247$) or by food in duckweed treatments (t = -0.08, df = 34, $P = 0.936$). Total length of nymphal development (first through fifth instars) was 9% shorter on duckweed than on filter paper in springtail treatments (t = -3.84, df = 29, $P = 0.001$), but did not differ between foods in duckweed treatments (t = -1.18, df = 33, $P = 0.247$).

## Discussion

Differences between treatment groups in survivorship from egg to adult were affected by the interaction between food and substrate. To maximize survival of laboratory colonies, our survivorship data suggest that when filter paper was used as a substrate, springtails were a more effective food source, and when fruit flies were used as a food source, duckweed was the more effective rearing substrate. When stadia differences between springtail and fruit fly treatments were detected (in the third stadium and on filter paper in the fourth stadium), the springtail treatments had shorter stadia, corroborating Lanciani's (1991) observations for springtail treatments. However, differences between substrate treatments also were found in our study. When stadium differences between substrate treatments were detected (in third and fourth instar fruit fly treatments, and in springtail treatments in the fifth instar and total nymphal development), stadia were shorter on duckweed. These data indicate that the shorter stadia Lanciani (1991) found for springtail reared samples in comparison to fruit fly reared samples (Lanciani 1975) may actually reflect a difference in rearing substrates in his two studies.

TABLE 2. TWO-WAY ANOVAS (UNBALANCED DESIGN) OF STADIA WITHIN INSTARS OF *H. MARTINI* REARED ON TWO SUBSTRATES AND UNDER TWO FEEDING REGIMES AT 28 ± 1°C[a].

| Instar | Source | df | F | $P > F$ |
|---|---|---|---|---|
| First | Substrate | 1 | 0.43 | 0.512 |
| | Food | 1 | 1.18 | 0.279 |
| | Substrate*Food | 1 | 0.03 | 0.853 |
| | Error | 101 | | |
| Second | Substrate | 1 | 0.93 | 0.338 |
| | Food | 1 | 30.17 | <0.001 |
| | Substrate*Food | 1 | 2.31 | 0.132 |
| | Error | 87 | | |
| Third | Substrate | 1 | 3.83 | 0.054 |
| | Food | 1 | 15.16 | <0.001 |
| | Substrate*Food | 1 | 4.63 | 0.034 |
| | Error | 85 | | |
| Fourth | Substrate | 1 | 10.74 | 0.002 |
| | Food | 1 | 8.61 | 0.005 |
| | Substrate*Food | 1 | 6.30 | 0.014 |
| | Error | 71 | | |

[a]Fifth instar not included because no individuals in filter paper/*Drosophila* treatment reached adult.

Only the egg stadium was shorter on filter paper than on duckweed. Some Heteroptera have eggs that develop on damp ground or in water (e.g., *Notonecta triguttata* Say, *Gerris lacustris latiabdominalis* Miyamoto, and *Gerris gracilicornis gracilicornis* Horvath). Mori (1986) noted that the egg stadium increased when water uptake was experimentally reduced and emphasized the apparent importance of water absorption in the embryonic development of these taxa. We suspect that the waxy coating on the duckweeds created a substrate condition that inhibited water absorption, whereas the moist filter paper facilitated water uptake by the eggs.

We found that filter paper was clearly a more suitable choice for rearing *H. martini* eggs than was duckweed. The choices were less clear for the nymphal instars. Nymphal stada often were shorter on duckweed, but potential contamination of duckweed with a wide array (Rathke 1979) of other organisms, and the greater difficulty with which exuviae were detected, may make this substrate less suitable for some laboratory studies. Our data were not consistent with a general trend of nutritional augmentation due the presence of other taxa in duckweed treatments; the effect of these taxa appeared to be negligible. However, it is possible to rear more nearly sterile monocultures of duckweed (Landolt & Kandeler 1987).

The sometimes shorter stadia observed in springtail reared *H. martini* may not be sufficient reason to reject fruit flies as food organisms. Cultures of *Drosophila melanogaster* are readily available and easy to maintain, allowing more repeatable experiments. Collembola are more difficult to obtain and culture, and collecting the same species as used in previous studies may be difficult. Additionally, the assumption that

shorter stadia are indicative of healthier or more natural development in *Hydrometra* has not been demonstrated.

We have shown that food and substrate affect the stadia and survival of *H. martini*. The advantages of an artificial substrate (cleanliness, repeatability, control, ease of observation) should be weighed against the advantages of duckweed (more closely replicating field conditions and, in some cases, shorter stadia) before decisions regarding rearing conditions are made. *Drosophila* may be more effective for laboratory rearing than springtails for *Hydrometra* even though, as suggested by Lanciani (1991), there may be nutritional differences between these two food species.

Average total developmental time for *H. martini* in the laboratory was shorter than for several other North American gerromorphans. Another hydrometrid, *H. hungerfordi*, took 25.6 d at 28°C (Wood & McPherson 1995). *Mesovelia cryptophila* Hungerford, a mesoveliid, required 28.6 d at 26.7°C to complete development (Taylor & McPherson 1998). The veliid *Microvelia pulchella* Westwood, took 34.1 d (at 23.3°C) (Taylor & McPherson 1999). *Gerris argenticollis* Parshley had a longer developmental period, 58.3 d at 21°C (Korch & McPherson 1987).

Detailed life history studies have been conducted only for a small portion of the North American heteropteran fauna (Schaeffer 1990, Spence & Andersen 1994). We are fortunate to know more about the biology of *H. australis/H. martini* than is known for most Heteroptera.

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