

## EFFECTS OF LOW pH AND HIGH TEMPERATURE ON HATCHING AND SURVIVAL OF THE WATER MITE *UNIONICOLA FOILI* (ACARI: UNIONICOLIDAE)

**Dale D. Edwards:** Department of Biology, University of Evansville, Evansville, Indiana 47722 USA

**ABSTRACT.** Adult females, larvae, and eggs of the water mite *Unionicola foili* were removed from their host mussel *Utterbackia imbecillis*, and their tolerance to varying pH (4.1, 5.2, 7.0, and 7.8) and temperature (25°, 33°, and 38° C) was examined. Longevity of adult *U. foili* was significantly reduced at pH 4.1, whereas survival of larvae was significantly reduced at pH 5.2. Hatching of mite eggs was unaffected by exposure to low pH. Survival of adult mites was significantly reduced when exposed to increasing temperature treatments. Larval mites experienced a significant decrease in survivorship at 33° C, but exposure to higher temperature yielded no further changes in longevity. Egg hatching was not affected by exposure to increasing temperature. Although eggs of *U. foili* were comparatively more resistant to low pH and elevated temperature than adults or larvae, the reason for the observed differences remains to be tested. Overall, *U. foili* were fairly sensitive to low pH and elevated temperature. Active stages of *U. foili* appear to be more vulnerable to pH changes than their adult host mussels, making them useful biomonitors of acute exposures to acid-contaminated waters.

**Keywords:** *Unionicola foili*, water mite, hatching, survival, temperature, pH

Water mites of the genus *Unionicola* (Acari: Unionicolidae) are common symbionts of freshwater mussels of the family Unionidae. Their life cycle is complex and includes larvae that leave a host mussel and undergo a brief parasitic phase with chironomid dipterans. Following this association, larvae re-invade a host mussel and undergo developmental transformation that is typical of acariformes, eventually becoming sexually mature adults. The extent to which members of the genus depend on host mussels is variable. Some species are free-living as nymphs and adults, using mussels only as a site for oviposition and post-larval developmental transformations. Other species are obligate symbionts of their bivalve hosts.

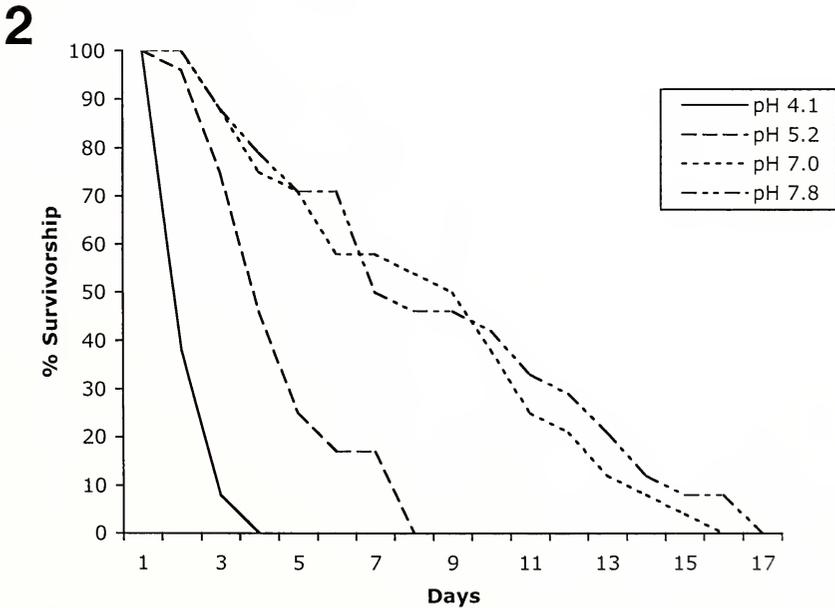
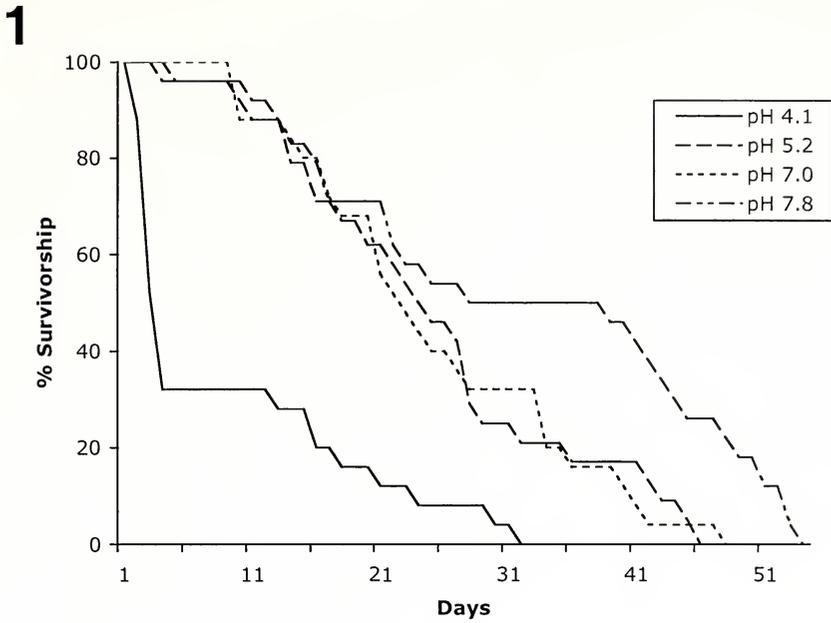
Although there is a substantial amount of information pertaining to the tolerance of their insect (Thornton & Wilhm 1974; Jernelöv et al. 1981; Pascoe et al. 1989) and mussel hosts (Holwerda & Veenhof 1984; Pynnönen 1990; Keller & Zam 1991) to a variety of environmental conditions, there is very little known about the effects of environmental stress on unionicolid water mites. With the exception of a few biological monitoring studies (Scullion & Edwards 1980; Kowalik & Biesiadka 1981;

Rundle 1990; Biesiadka & Kowalik 1991; Cicolani & Di Sabatino 1991) and a laboratory study by Rousch et al. (1997), the physiological ecology of water mites as a group is limited. The lack of information on the tolerance of water mites to changing environmental conditions is disconcerting because field studies suggest that water mites are sensitive to acid stress (Rundle 1990) and organic pollutants (Cicolani & Di Sabatino 1991) and thus may be useful as indicators of pollution.

The present study addresses the tolerance of various developmental stages of the water mite *Unionicola foili* to changes in pH and thermal stress. Adults of this species are common symbionts of freshwater mussels of the genus *Utterbackia* (Unionoida: Unionidae). Larval *U. foili* typically occur in parasitic association with midges (Diptera: Chironomidae). *Unionicola foili* was selected for study because of its broad distribution and high prevalence and abundance with mussels and chironomid midges in the southeastern and midwestern United States.

### METHODS

**Study animals.**—*Unionicola foili* was obtained from a population of *Utterbackia imbecillis* that was collected from Butler's Pond,



Figures 1, 2.—Survivorship curves of *Unionicola foili* at four values of pH. 1. Adult *U. foili*; 2. Larval *U. foili*. Percentage based on  $n = 24-25$ .

a 4 ha farm pond located in Perry County, Indiana (37°56'N, 86°43'W). Mussels were collected from May to July 2000 when mite larvae were emerging from host gill tissue. In the laboratory, adult *U. foili* were removed from their host mussels and were washed sev-

eral times in artificial pond water (APW) (Dietz & Alvarado 1970). Only females were isolated because they are substantially more abundant than males (Dimock 1985). Larvae and eggs were obtained by teasing apart hosts' gills in petri dishes containing APW and

Table 1.—The *in vitro* survivorship (mean  $\pm$  SE) of adult and larval *Unionicola foili* at four values of pH. *n* = sample size.

pH treatment	Adult mites			Larval mites		
	Longevity (days)	Range	<i>n</i>	Longevity (days)	Range	<i>n</i>
4.1	7.2 $\pm$ 1.9	1–31	25	1.4 $\pm$ 0.1	1–3	24
5.2	24.1 $\pm$ 2.3	4–44	24	3.8 $\pm$ 0.4	1–7	24
7.0	24.4 $\pm$ 2.2	9–49	25	7.6 $\pm$ 0.8	2–15	24
7.8	31.3 $\pm$ 3.2	5–52	24	8.4 $\pm$ 0.9	2–16	24

washing them several times in this medium. Adults, larvae, and eggs were kept in APW at 25° C for no longer than 24 h prior to use.

**pH tests.**—To test the sensitivity of *U. foili* to changes in pH, adult and larval mites were placed individually in wells of a flat bottom tissue-culture well plate (1.5 cm i.d.) containing 3 ml of APW adjusted to one of four pH levels: 4.0, 5.2, 7.0, 7.8. Each well was examined daily until it contained a dead mite. Larval and adult mites were defined as dead when they did not respond to tactile stimulation.

Treatment solutions were prepared and stored in 1000 ml Erlenmeyer flasks. To minimize pH drift, stock solutions were adjusted with a 1 mol solution of sulfuric acid or sodium hydroxide every 24 h. Preliminary tests with no animals indicated that pH levels in the well plates fluctuated between 0.2–0.8 pH units over a 48 h period. Test solutions in each of the wells were replaced with freshly adjusted APW every 24 h.

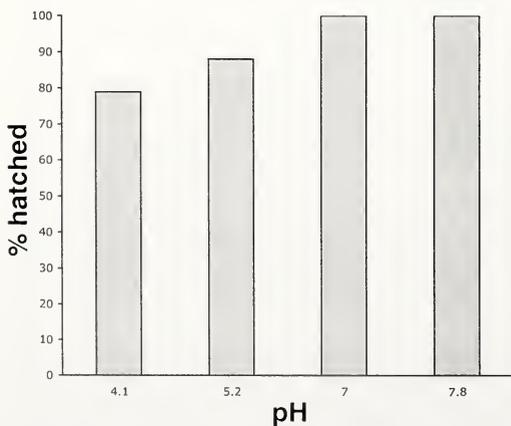
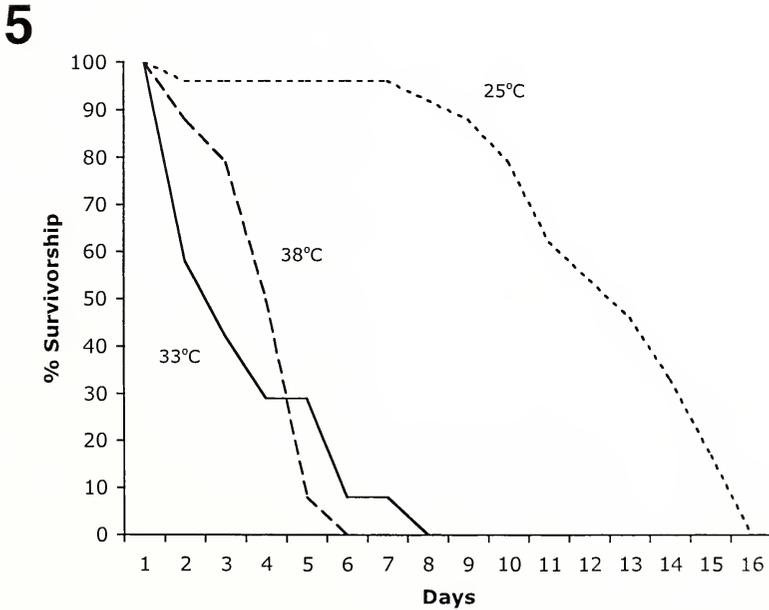
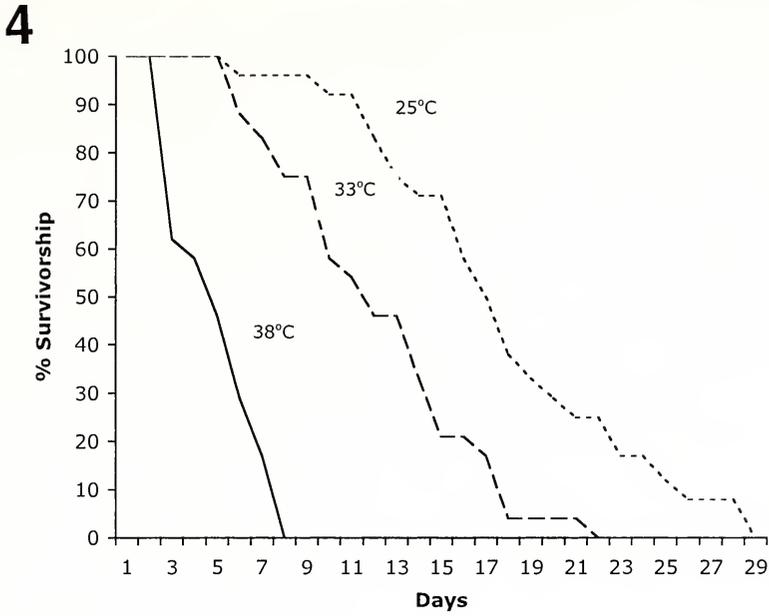


Figure 3.—Hatching of *Unionicola foili* eggs exposed to four values of pH. Percentage based on *n* = 24.

The longevity of 24–25 adult and larval *U. foili* was monitored in each test solution. In an effort to test females of similar ages, only those individuals with approximately the same body length (1.10–1.30 mm; mean = 1.18  $\pm$  0.01 SE, *n* = 96) were selected for study. Larvae were used in assays if they had metamorphosed from prelarvae within 24 h of their removal from host gill tissue.

The potential effect of different pH levels on the eggs of *U. foili* was assessed by monitoring the percentage of eggs that hatched at pH levels 4.0, 5.2, 7.0, and 7.8. Twenty-four eggs were placed individually into wells containing 3 ml of pH adjusted APW and each well was examined daily for the presence of free-swimming larval mites. Treatment solutions used for pH tests were prepared, adjusted, and replaced following the procedures outlined above for assays involving larval and adult mites.

**Thermal tests.**—To test the effects of thermal stress on *U. foili*, adult and larval mites were placed individually in 20  $\times$  30 mm open-ended plastic tubes fitted with 200  $\mu$ m Nitex<sup>TM</sup> mesh as a floor. The plastic tubes were fitted into a Plaskolite<sup>TM</sup> rack (12 tubes/rack) and immersed in a 22  $\times$  20  $\times$  14 cm clear acrylic plastic (“Plexiglas<sup>TM</sup>”) chamber containing approximately 4 l of APW held at 25° (control) 33°, or 38°  $\pm$  0.2° C. The plastic tubes were positioned in the rack so that the bottom half of the tubes was immersed in water. At the beginning of each assay, adult and larval mites were exposed to water at 25° C. Temperatures in the chamber were adjusted and maintained by a constant-temperature, circulating water bath (Neslab Model RTE-111). Equilibrated APW was pumped into the chamber using a peristaltic pump, and an air-stone was introduced into the chamber to provide continuous aeration. Experimental tem-



Figures 4, 5.—Survivorship curves for *Unionicola foili* at two experimental temperatures (35° and 38° C) and one control temperature (25° C). 4. Adult *U. foili*; 5. Larval *U. foili*. Percentage based on  $n = 24$ .

peratures were achieved within approximately 25 min of pumping equilibrated APW into the Plexiglas<sup>®</sup> chamber.

The contents of each tube were examined daily, using a stereoscopic microscope, for the presence of dead mites. Temperature of water in the plastic tube was held constant during

microscopic examination by placing the tube in a 50 ml glass vial containing APW from the Plexiglas<sup>®</sup> chamber. The longevity of 24 adult and larval *U. foili* was monitored at each temperature.

Tolerance of the eggs of *U. foili* to thermal stress was determined by monitoring the per-

Table 2.—The *in vitro* survivorship (mean  $\pm$  SE) of adult and larval *Unionicola foili* at two experimental and one control (25°C) temperature. *n* = sample size.

Temperature (°C)	Adult mites			Larval mites		
	Longevity (days)	Range	<i>n</i>	Longevity (days)	Range	<i>n</i>
25	17.0 $\pm$ 1.2	1–31	24	11.4 $\pm$ 0.7	1–15	24
33	11.3 $\pm$ 0.9	5–21	24	2.8 $\pm$ 0.4	1–7	24
38	4.1 $\pm$ 0.4	5–28	24	3.2 $\pm$ 0.2	1–5	24

centage of eggs that hatched in APW held at 20°, 33° and 38°  $\pm$  0.2° C. For each temperature, 24 eggs were placed individually in 20  $\times$  30 mm open-ended plastic tubes fitted with 200  $\mu$ m Nitex<sup>TM</sup> mesh as a floor. Following the same procedures used to monitor larvae and adults, tubes containing eggs were examined daily for the presence of free-swimming larval mites. Water in the Plexiglas<sup>TM</sup> chamber was equilibrated and aerated following the procedures outlined above.

**Data analysis.**—Analysis of variance (ANOVA) was used to compare the longevity of adult and larval *U. foili* exposed to the various pH and temperature treatments. Multiple comparison a posteriori tests were conducted using a Tukey's honestly significant difference (HSD) test (Zar 1999). The LT<sub>50</sub> and LC<sub>50</sub> values for *U. foili* for each exposure treatment were calculated using the Kaplan-Meier survivorship analysis (SPSS Inc. 1993). A Chi-square analysis was used to compare the hatching success of mite eggs in the pH and temperature treatments (Zar 1999).

## RESULTS

**pH tests.**—*In vitro* survivorship of adult and larval *U. foili* from different pH treatments is presented in Table 1. Adult mites lived significantly fewer days in pH 4.1 when compared to other pH treatments (ANOVA,  $F = 16.5$ ,  $df = 3$ ,  $P < 0.001$ , Tukey's HSD). Despite an apparent increase in survivorship at pH 7.8 (Fig. 1), there were no significant differences in the longevity of mites for this treatment when compared to pH 5.2 and 7.0. Exposure of adult *U. foili* to pH 4.1 had a median LC<sub>50</sub> of 3 days. Adult mites experienced 50% mortality at pH 5.2, 7.0, and 7.8 after 23.5, 22, and 32.5 days respectively.

The longevity of larval *U. foili* was significantly lower at pH 4.1 and 5.2 when compared to the other pH conditions (ANOVA,  $F = 23.4$ ,  $df = 3$ ,  $P < 0.001$ , Tukey's HSD; Fig.

2). Exposure of larval mites to pH 4.1 and 5.2 yielded 50% mortality within 1 and 3 days, respectively. Survivorship improved dramatically in the other treatments, with the median LC<sub>50</sub> occurring after 8.5 days in pH 7.0 and 6.5 days in pH 7.8.

The hatching success of *U. foili* eggs at different pH levels is illustrated in Fig. 3. Hatching of eggs was unaffected by pH ( $\chi^2 = 1.4$ ,  $df = 3$ ,  $0.50 < P < 0.75$ ).

**Thermal tests.**—The longevity of adult and larval *U. foili* at different temperatures is presented in Table 2. There were significant differences in the longevity of adult mites in the three temperature treatments (ANOVA,  $F = 49.5$ ,  $df = 2$ ,  $P < 0.001$ , Tukey's HSD). Overall, adult *U. foili* exhibited diminished survival when exposed to increasing temperature (Fig. 4). Exposure of adults to 25°, 33°, and 38° C resulted in 50% mortality within 16.5, 11, and 4 days, respectively.

The longevities of larval *U. foili* at the 33° and 38° C were significantly lower when compared to the 25° C exposure (ANOVA,  $F = 101.9$ ,  $df = 2$ ,  $P < 0.001$ , Tukey's HSD). Survivorship curves for larval mites indicated that the survival of *U. foili* was greatly reduced at 33° C, while exposure to 38° C had no apparent effect (Fig. 5). Larvae experienced 50% mortality at 25° C after 12 days. Exposure to 33° and 38° C resulted in an LT<sub>50</sub> of 2 and 4 days, respectively.

The hatching success of mite eggs at 25°, 33°, and 38° C was 100% (24 of 24), 96% (23 of 24), and 79% (19 of 24), respectively. Temperature had no effect on egg hatching ( $\chi^2 = 1.1$ ,  $df = 2$ ,  $0.50 < P < 0.75$ ).

## DISCUSSION

*Unionicola foili* were reasonably sensitive to low pH, but sensitivity did vary somewhat with stage of development. Eggs were unaffected by low pH, and mite larvae appeared to be more sensitive than adults. These results

differ somewhat from those of Rousch et al. (1997) who found that low pH had similar effects on the survival of larvae and adults of the water mite *Arrenurus manubriator*. The reason for the greater relative sensitivity of larval *U. foili* to low pH is uncertain. Rousch et al. (1997) reported that deutonymphs of *A. manubriator* were more sensitive than adults to low pH and suggested that their relative tolerances could be due to differences in the number of genital acetabula they possessed. Genital acetabula, which presumably function in osmoregulation among water mites (Alberti & Bader 1990), are considerably more numerous in adults than deutonymphs (Rousch et al. 1997) and therefore could provide them with greater osmoregulatory potential. Although female *U. foili* have a relatively large number ( $n > 30$ ) of acetabula in their genital region (pers. observ.), the larvae, which lack a genital opening, possess none. Most larval water mites do, however, possess Claparède organs at the base of legs I and II that presumably serve the same function as acetabula (Alberti & Bader 1990). Unfortunately, the extent to which either of these structures play a role in ion regulation among adult and larval *U. foili* has not been investigated, making it difficult to comment on their osmoregulatory potentials and their contributions to the tolerances of adult and larval mites to low pH.

The reason for the greater relative tolerance of *U. foili* eggs to low pH is also unknown. Rousch et al. (1997) found that hatching of eggs of *A. manubriator* was unaffected at pH 4, but significantly reduced at pH 3. In addition, Rousch et al. (1997) suggested that the gelatinous covering associated with *A. manubriator* egg masses may enhance their tolerance to low pH. This hypothesis was supported by the observation that the gelatinous coating on mite eggs became opaque and gummy when exposed to pH 3. Eggs of *U. foili* are different from those of *A. manubriator* in that they are deposited singly rather than in clusters. Whether they are covered in a gelatinous matrix reminiscent of *A. manubriator* and to what extent it may serve to protect them from increased exposure to  $H^+$  ions remains to be tested.

Thermal tolerance of adult *U. foili* followed classic patterns, with diminished survival accompanying exposure to increasing temperature. Interestingly, larval mites experienced a

significant decrease in survivorship at 33° C, with exposure to higher temperature yielding no further changes in longevity. Why larval *U. foili* were relatively more sensitive to increasing temperature than adults is presently unknown, but may reflect size-related differences in thermal tolerance. The overall size of adult female *U. foili* (body length = 1.36 mm  $\pm$  0.02 SE,  $n = 186$ ; body width = 0.80 mm  $\pm$  0.01 SE,  $n = 186$ ) (Edwards 1993) is substantially greater than that of newly emerged larvae (body length = 0.36 mm  $\pm$  0.01 SE,  $n = 20$ ; body width = 0.22 mm  $\pm$  0.01 SE,  $n = 20$ ) (Edwards & Dimock 1995). The effects of temperature on the longevity of adults or larvae have not been reported for any other species of water mite. An increase in temperature had no effect on hatching of *U. foili* eggs. Whether some type of encasement or coating on the eggs may contribute to their tolerance, as was suggested for the tolerance of eggs to low pH, remains to be tested.

Adult and larval *U. foili* appear to be fairly sensitive to low pH and elevated temperature, with larval mites exhibiting greater sensitivity than adults. Although there are no comparative data for exposure to temperature, the results of this study for pH are consistent with field studies indicating that water mites are sensitive to low pH (Scullion & Edwards 1980; Rundle 1990). Interestingly, larval and adult *U. foili* appear to be more vulnerable to pH changes than their adult host mussels (Pynnönen 1990; Machado et al. 1988) and are equally as sensitive as juvenile stages (Dimock & Wright 1993), making them useful biomonitors of acute exposures to acid-contaminated waters.

#### LITERATURE CITED

- Alberti, G. & C. Bader. 1990. Fine structure of external 'genital' papillae in the freshwater mite *Hydrovolzia placophora* (Hydrovolziidae, Actiniedida, Actinotrichida, Acari). *Experimental and Applied Acarology* 8:115–124.
- Biesiadka, E. & W. Kowalik. 1991. Water mites (Hydracarina) as indicators of trophy and pollution in lakes. Pp. 475–481. *In* *Modern Acarology*. Volume 1. (F. Dusbabek & V. Bukva, eds.). Academia, Prague, Czechoslovakia.
- Cicolani, B. & A. Di Sabatino. 1991. Sensitivity of water mites to water pollution. Pp. 455–474. *In* *Modern Acarology*. Volume 1. (F. Dusbabek & V. Bukva, eds.). Academia, Prague, Czechoslovakia.

- Dietz, T.H. & R.H. Alvarado. 1970. Ionic regulation in *Lumbricus*. *Biological Bulletin* 138:247–261.
- Dimock, R.V., Jr. 1985. Population dynamics of *Unionicola formosa* (Acari: Unionicolidae), a water mite with a harem. *American Midland Naturalist* 114:168–179.
- Dimock, R.V., Jr. & A.H. Wright. 1993. Sensitivity of juvenile freshwater mussels to hypoxic, thermal and acid stress. *Journal of the Elisha Mitchell Scientific Society* 109:183–192.
- Edwards, D.D. 1993. Host specificity and reproductive isolation: Experimental evidence from the symbiotic water mite *Unionicola formosa*. Ph. D. Dissertation. Wake Forest University, Winston-Salem, North Carolina. 153 pp.
- Edwards, D.D. & R.V. Dimock, Jr. 1995. Life history characteristics of larval *Unionicola* (Acari: Unionicolidae) parasitic on *Chironomus tentans* (Diptera: Chironomidae). *Journal of Natural History* 29:1197–1208.
- Holwerda, D.A. & P.R. Veenhof. 1984. Aspects of anaerobic metabolism in *Anodonta cygnea* L. *Comparative Biochemistry and Physiology* 78B: 707–711.
- Jernelöv, A., B. Nagell & A. Svenson. 1981. Adaptation to an acid environment in *Chironomus riparius* (Diptera, Chironomidae) from Smoking Hills, NWT, Canada. *Holarctic Ecology* 4:116–119.
- Keller, A.E. & S.G. Zam. 1991. The acute toxicity of selected metals to the freshwater mussel, *Anodonta imbecilis*. *Environmental Toxicology and Chemistry* 10:539–546.
- Kowlalik, W. & E. Biesiadka. 1981. Occurrence of water mites (Hydracarina) in the River Wieprz polluted with domestic-industry sewage. *Acta Hydrobiologia* 23:331–348.
- Machado, J.J. Coimbra, C. Sa & I. Cardoso. 1988. Shell thickening in *Anodonta cygnea* by induced acidosis. *Comparative Biochemistry and Physiology* 91A:645–651.
- Pascoe, D., K.A. Williams & D.W.J. Green. 1989. Chronic toxicity of cadmium to *Chironomus riparius* Meigen: Effects upon larval development and adult emergence. *Hydrobiologia* 175:109–115.
- Pynnönen, K. 1990. Physiological responses to severe acid stress in four species of freshwater clams (Unionidae). *Archives of Environmental Contamination and Toxicology* 19:471–478.
- Rousch, J.M., T.W. Simmons, B.L. Kerans & B.P. Smith. 1997. Relative acute effects of low pH and high iron on the hatching and survival of the water mite (*Arrenurus manubriator*) and the aquatic insect (*Chironomus riparius*). *Environmental Toxicology and Chemistry* 16:2144–2155.
- Rundle, S.D. 1990. Micro-arthropod seasonality in streams of varying pH. *Freshwater Biology* 24: 1–24.
- Scullion, J. & R.W. Edwards. 1980. The effects of coal industry pollutants on the macroinvertebrate fauna of a small river in South Wales coalfield. *Freshwater Biology* 10:141–162.
- SPSS Inc. 1993. SPSS for Windows. 6.0. SPSS Inc., Chicago, Illinois.
- Thorton, K. & J. Wilhm. 1974. The effects of pH, phenol, and sodium chloride on survival and caloric, lipid, and nitrogen content of a laboratory population of *Chironomus attenuatus* (Walk.). *Hydrobiologia* 45:261–280.
- Zar, J.H. 1999. *Biostatistical Analysis*. Fourth Edition. Prentice Hall, Upper Saddle River, New Jersey. 663 pp.

*Manuscript received 23 July 2003, revised 16 October 2003.*