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Effects of wastewater treatment plant effluents on freshwater mollusks in the upper Clinch River, Virginia, USA

Stephanie E. Goudreau [1], Richard J. Neves & Robert J. Sheehan [2]
Virginia Cooperative Fish and Wildlife Research Unit[3], U.S. Fish and Wildlife Service, Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061–0321, USA; [1] Current address: North Carolina Wildlife Resources Commission, 320 South Garden Street, Marion, NC 28752, USA; [2] Current address: Fisheries Research Laboratory and Department of Zoology, Southern Illinois University, Carbondale, IL 62901, USA; [3] The Unit is jointly supported by the U.S. Fish and Wildlife Service, Virginia Department of Game and Inland Fisheries, The Wildlife Management Institute and Virginia Polytechnic Institute and State University.

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Abstract

Field and laboratory studies were conducted to determine mollusk distributions in proximity to wastewater treatment plants (WTP's) in the upper Clinch River and to test the tolerance of two mollusk species to monochloramine and unionized ammonia, the major toxicants in domestic effluent. River reaches up to 3.7 km downstream of WTP's were devoid of freshwater mussels (Unionidae), and tolerance to effluents varied among snails, sphaeriid clams, and the asian clam *Corbicula fluminea*. Residential communities with septic systems had no measurable impact on mollusk assemblages downstream.

Laboratory bioassays with glochidia of *Villosa iris* yielded the following results: 24 h EC_{50} and LC_{50} values of 0.042 mg l^{-1} and 0.084 mg l^{-1} monochloramine, respectively; and 24 h EC_{50} and LC_{50} of 0.237 mg l^{-1} and 0.284 mg l^{-1} unionized ammonia, respectively. Glochidia rank among the most sensitive invertebrates in their tolerance to these toxicants. The snail *Pleurocera unciale unciale* was moderately sensitive, with 96 h LC_{50} values of 0.252 mg l^{-1} monochloramine and 0.742 mg l^{-1} unionized ammonia. Monitoring of monochloramine and unionized ammonia concentrations 0.1 km below WTP outfalls indicated that monochloramine was the toxicant likely inhibiting mollusk recovery below these plants.

Introduction

The toxicity of chlorinated effluents from wastewater treatment plants (WTP's) to biota has been well documented (Brungs, 1973, 1976; Tsai, 1975; Bellanca & Baily, 1977; USEPA, 1985a). Chlorine, added to effluent in the form of free chlorine, reacts with effluent ammonia to form chloramines. The pH, temperature, and chlorine to ammonia nitrogen ratio in most WTP effluents ensure that most total residual chlorine (TRC) is in the form of monochloramine (MCA), which is less toxic and more stable than free chlorine, and consequently persists longer in the aquatic environment (Zillich, 1972; Brungs, 1976; Johnson, 1978; Thomas *et al.*, 1980; White, 1986).

Ammonia nitrogen (NH_3-N) may be in natural water as a product of organic decomposition at 0.05–0.40 mg l^{-1} (Tsai, 1973; White, 1986). However, very high NH_3-N levels (up to 40 mg l^{-1}) may occur in WTP effluent because of decomposition of protein (White, 1986). Ammonia is in equilibrium between the toxic unionized form (NH_3) and the relatively nontoxic ammonium ion (NH_4^+), which is determined by pH and temperature (Emerson *et al.*, 1975; Thurston *et al.*, 1981; Broderius *et al.*, 1985; Sheehan & Lewis, 1986). Nitrifying bacteria convert ammonia to nitrite and then to nitrate, which is used by aquatic flora (Wetzel, 1975; Lewis *et al.*, 1981). Because of the influence of pH and temperature on the conversion of ammonia to nitrate (Lewis *et al.*, 1981), the toxicity of ammonia from WTP effluent to receiving streams is variable.

Many studies assessed the effects of WTP effluent on fishes, but few focused on freshwater mollusks. Freshwater mussels (Unionidae) have been considered one of the most sensitive faunal groups to organic enrichment, and their presence below a sewage outfall has been used as an indication that full biological recovery has occurred (Simmons & Reed, 1973). Horne & McIntosh (1979) attributed the reduced number of mussels below a secondary WTP to ammonia because no residual chlorine was detected. Mussel shells with stained nacre downstream of sewage and fertilizer plants can have abnormally low calcium concentrations and contain increased amounts of silicon, sulfur, iron, magnesium, phosphorus, and chlorine (Rosenberg & Henschen, 1986).

The tolerance of snails to WTP effluent seems to be related to the mode of gaseous exchange. Pulmonate snails are often associated with organic pollution, but gilled snails, such as pleurocerids, are less tolerant of organic enrichment and low dissolved oxygen (Mason *et al.*, 1970). Stansbery & Stein (1976) observed that both pleurocerids and unionids were eliminated downstream of WTP's in the Clinch River. They stated furthermore that towns with septic tank systems, rather than WTP's, maintained mollusk fauna downstream.

Presently, no freshwater bivalves are routinely used in bioassays for setting limitations on effluent discharges. Because they can avoid some toxicants by closing their valves for extended periods of time (ASTM, 1980a), adult unionids in bioassays are often ineffective and their use is time-intensive. Glochidia, the parasitic larval stage of unionids, have not been commonly used in bioassay, possibly because of difficulties in obtaining and handling them. Because chlorine residual is harmful to oyster larvae, it may also adversely affect glochidia (Roberts & Gleeson, 1978; Roberts, 1980; Roberts & Casey, 1985). Bioassays have been conducted to determine tolerance of some gastropods to chlorine derivatives and ammonia (Gregg, 1974; Arthur *et al.*, 1975; West, 1985, as cited in USEPA, 1985b; Brooks & Szmania, 1989), but testing of additional species is needed to assess the range of their sensitivities.

The objectives of this study were to 1) compare water quality and the molluscan fauna upstream (above) and downstream (below) of selected WTP's and of towns using septic tank systems in the upper Clinch River, and 2) determine the toxicity of monochloramine and unionized ammonia to glochidia of *Villosa iris* and the snail *Pleurocera unciale unciale*.

Study area

The Clinch River, a headwater tributary of the Tennessee River, originates near Bluefield, Virginia, and flows 380 river miles (RM) southwesterly through Tazewell, Russell, Wise, and Scott counties before entering Tennessee at Norris Reservoir. Ahlstedt (1984) recently collected 47 species of mussels from the river, eight of which are listed as endangered. Seven WTP's, treating principally sewage, serve small towns along the Clinch River in Virginia. Wastewater treatment plants at Tazewell (RM 346) and Richlands (RM 317) and two communities with septic systems, Cliffield (RM 336) and Pounding Mill (RM 330), were selected for study. The Tazewell WTP processes 0.70 million gallons of wastewater per day (MGD), comprising 5.8% of the average river flow. It is a secondary treatment plant that chlo-

rinates its effluent to 1–2 mg l^{-1} TRC. The Richlands WTP is a primary treatment plant, built to handle 0.8 MGD but treating up to 1 MGD (1.1% of the average river flow). It also chlorinates effluent to 1–2 mg l^{-1} TRC. All sampling sites were in Tazewell County, Virginia, although some sampling was done in Russell County, downstream of the Richlands WTP.

Materials and methods

Field surveys

Mollusk surveys were conducted upstream and downstream of the Tazewell and Richlands WTP's and Pounding Mill and Cliffield, in July and August 1985 with a 0.5 m^2 quadrat sampler to identify and quantify mollusks at selected sites. One location above each WTP and town was sampled as a reference site. Four sites were sampled below the Tazewell WTP and three sites below the Richlands WTP. One site below Cliffield and another below Pounding Mill also were examined. Ten randomly selected quadrat samples at each site were collected to a depth of 15 cm in comparable substrata. Mollusks (mussels, clams, snails) were identified, counted, and returned to the stream. An additional mussel survey with waterscopes was conducted in September 1985 at each site because mussels often have a clustered distribution that may be misrepresented in quadrat sampling (Kovalak *et al.*, 1986). Sampling time (about 70 min per site) was recorded to calculate the collected number per unit effort (CPUE). Scoping also was done farther downstream of the two WTP's to better determine the length of stream reach without mollusks.

One way ANOVA and least squares methods were used to test for differences in mollusk densities among sites at each of the four towns in Tazewell County (SAS, 1985). Sites near each town (upstream, downstream) were compared for significant differences among sites. To eliminate differences in densities by chance, significance levels were used according to the Bonferroni Inequality Rule (Miller, 1966), and set at 0.005 for Tazewell, and 0.008 for Richlands.

Water quality was monitored at all sites during low flow conditions in summer (July–September) 1985 to identify the worst-case effect of WTP effluent and septic systems on water quality. Water chemistry parameters that were measured monthly during low flow at each site were TRC, total ammonia, pH, temperature, dissolved oxygen (DO), conductivity, and alkalinity. Chlorine residuals were measured on site with a Fischer-Porter portable chlorine amperometric titrator (model 17T2012). Detection levels were 0.01 mg l^{-1} for TRC and 0.001 mg l^{-1} unionized ammonia. Water chemistry values were recorded as follows: temperature (°C thermometer), pH (Fisher portable meter model 640), conductivity and dissolved oxygen (YSI meters). A 500 ml water sample was collected at each site, and half of it was preserved with concentrated sulfuric acid (Paller *et al.*, 1983) and returned to the laboratory for ammonia analysis with a Fisher Accumet pH meter (model 825MP) and a Corning ammonia combination electrode. Unionized ammonia (NH_3) concentrations were calculated by the method of Emerson *et al.*, (1975). The remainder of the water sample was measured for alkalinity; both measurements were obtained within 48 hours of collection.

The same water quality values were monitored monthly from October 1985 through June 1986 at the upstream site and the first downstream site of Tazewell and Richlands WTP's to determine annual variation in water quality near WTP's. Hardness also was measured within 48 hours of collection with Hach chemicals from a 100 ml water sample. Because of high water conditions, no sampling was conducted in May 1986.

Laboratory testing

Twenty-four hour static-renewal bioassays were conducted with glochidia of *Villosa iris* to calculate the effective median concentration (EC_{50}) and lethal median concentration (LC_{50}) of MCA and NH_3, recognized toxicants in WTP effluent. Preliminary bioassays were conducted with glochidia of *V. iris* in May 1986 to determine the

range of concentrations for use in subsequent definitive bioassays. For each bioassay, three gravid female mussels were collected from the Clinch River at Cliffield, Virginia, and transported to the laboratory. Mussels were acclimated to laboratory conditions for 4 days and were not fed during this time (ASTM, 1980a). Because WTP effluents have their greatest effect on water quality during periods of low flow and high temperature (Hubbs, 1933; Brinley, 1943), bioassays were conducted in a walk-in environmental chamber with temperature and photoperiod controls to allow simulation of summer conditions. Temperature was set at 22 ± 1 °C, and photoperiod was 16 hours light: 8 hours dark (ASTM, 1980a). A dechlorinator was constructed with activated charcoal to remove residual chlorine from dilution water; no residual chlorine was detected in water samples tested during the study. After passing through the dechlorinator, dilution water was held in reservoirs and aerated (ASTM, 1980a).

The bioassay setup consisted of twelve 4 l glass test chambers and Masterflex pumps, with twelve different toxicant concentrations in 19 l glass carboys. The solutions were pumped through tubing from the carboys to corresponding test chambers with a turnover time of 4 hours. Replicates of five geometrically increasing toxicant levels and a control (same pH but no toxicants) were tested during each bioassay (ASTM, 1980a). Toxicant solutions were buffered to a pH of 8.0–8.2 with sodium phosphate and potassium phosphate to approximate Clinch River water (Sheehan & Lewis, 1986). A free chlorine stock solution was made from aqueous sodium hypochlorite and the ammonia stock solution from ammonium chloride. A monochloramine stock solution was produced from a 1:1 molar ratio of sodium hypochlorite to ammonium chloride in dilution water with a pH of 8.5 (Brooks & Bartos, 1984).

Glochidia were removed from the female's marsupia with a 1 ml water-filled syringe (Waller *et al.*, 1985). The needle was inserted into the gill, and water was slowly injected to propel glochidia out through the excurrent aperture and into a beaker. Glochidia from three adults were combined, and viability of a subsample of roughly 100 glochidia was confirmed under a compound microscope by observing their response to 1% sodium chloride solution (Zale & Neves, 1982a). Baskets of 30-micron mesh nettting were fashioned to hold several hundred glochidia per basket in the test chambers. Each basket was immersed in a 300 ml beaker of dilution water, and 5 ml of glochidia were added. After 30 minutes, baskets were moved to the test chambers and suspended in the toxicant.

Water chemistry measurements consisted of DO, conductivity, alkalinity, hardness, and nitrites and were recorded at the start and end of each bioassay test in one control replicate and one replicate of the highest toxicant concentration. Dissolved oxygen was measured by Winkler titration, and nitrite level was determined with a LaMotte chemical kit. The other water quality values were measured as described previously. Temperature, MCA, and pH were measured four times in each test chamber during MCA tests. Ammonia levels were measured in the control and highest toxicant concentration replicates at the start and finish of MCA tests. During ammonia bioassay, NH_3, pH, and temperature were measured four times in each test chamber. All measurements were made as described previously except pH, which was measured with a Fisher Accumet pH meter (model 825MP) and Fisher pH electrodes. Each set of measurements was taken at least 4 h apart during bioassays to allow a complete turnover of toxicant volume in each 4 l test chamber.

At the end of 24 h, baskets were removed from the test chambers and returned to beakers of dilution water. A 1 ml sample of glochidia was removed sequentially by pipet from each basket, placed on a Sedgewick-Rafter cell, and viewed under a compound microscope ($10 \times$). The number of glochidia that were open and alive (open with a healthy adductor muscle), closed and alive (normal appearance except for being closed), and dead (open or closed with abnormal or missing adductor muscle) was recorded. One percent NaCl solution was added to the slide with a 1 ml syringe to confirm that all glochidia recorded as open-and-alive snapped shut. Individuals that did

not respond were tested a second time. Glochidia that did not respond in the second test (functionally dead) were subtracted from the open-and-live category and added to the dead group. The responses of about 100 glochidia were examined from each test chamber and often necessitated repeating the process up to three times per basket; glochidia then were discarded. After about 100 glochidia were examined from all 12 baskets, the baskets remained in the beakers of dilution water for 24 additional hours, and then more glochidia from each basket were examined in the same manner. Because we noted in preliminary studies that glochidia reopened when a stimulus was removed, the purpose of the 24 h post-exposure period was to allow glochidia that closed from irritation by the toxicants to reopen if they had not been killed (Hansen & Kawatski, 1976; Maciorowski & Clarke, 1980; Buikema *et al.*, 1982).

Glochidia, dead or closed after a 24 h test, were considered to be affected, and were used to calculate the 24 h EC_{50}. At the end of the 24 h post-exposure period, closed and dead glochidia were pooled to calculate a 24 h LC_{50}. Preliminary longevity studies indicated that mortality of glochidia increased after 48 h out of the marsupia. Therefore, glochidia remaining closed after 48 h would likely die rather than reopen and attach to a host fish.

The snail *Pleurocera unciale unciale* was used in 96 h static renewal bioassay to calculate LC_{50} values of MCA and NH_3. Preliminary bioassays (as described previously) were conducted to determine the range of toxicants to use in final bioassays. Adult snails were collected in October and November, 1986, from the Clinch River at Cliffield, Virginia, transported in the same manner as described for mussels, acclimated for 6 days, and fed algae up to 24 h before a test (ASTM, 1980a). The laboratory set-up was identical to that described for glochidia bioassays, except that 500 ml beakers with a turnover time of 40 min were used for the test chambers; no baskets were used. Ten snails were placed in each test chamber, and netting was placed over each beaker to prevent escapement. Snails were examined every 12 h, and all dead snails were removed. If a snail could not be distinguished as living or dead by observation, its body was gently prodded with a dissecting needle to assess its status.

Analyses of water chemistry, as described for glochidia bioassay, were performed at the start and end of each bioassay. Temperature, MCA or NH_3, and pH were recorded at the start and every 12 h in each test chamber until the conclusion of the test. At that time, we examined the snails and recorded the number dead and alive. Snails that had withdrawn into their shells were placed in dilution water and observed until their viability could be confirmed.

Data analysis

Because as many as 50% of the glochidia in bioassay were closed before the start of each bioassay, the relativized product index (RPI) was calculated to assess the response of glochidia to the control and toxicant treatments with the following equation (Farris *et al.*, 1988):

$$\text{RPI}\% = \{1 + (\text{treatment response-original response})/\text{original response}\}\{100\}$$

The original response was the number of glochidia alive at the start of the bioassay; treatment response was the number affected or killed after exposure. The RPI measured percent unaffected in each test. The value 100-RPI was calculated for the two control treatments in each bioassay and results were averaged to provide a mean mortality for the control in that bioassay. If the mean exceeded 2%, the treatment responses were corrected for the mean level of control mortality with the equation (ASTM, 1980b).

$$\text{Net treatment mortality } (\%) = \{1\text{-(number of surviving or unaffected larvae per treatment/mean number of surviving control larvae)}\}\{100\}$$

This equation was used to calculate control mortality for both LC_{50} and EC_{50} trials. Tests in which control mortality exceeded 10% for snails

(ASTM, 1980a; Buikema *et al.*, 1982) and 30% for glochidia were rejected (ASTM, 1980b).

Results from all bioassays were analyzed by probit analysis (Bliss, 1934; Finney, 1971; Buikema *et al.*, 1982; SAS Institute, 1985). To determine whether the log-probit model fit these data, Chi-square tests were conducted. If the test was significant, as in the glochidia bioassay, the bioassay was repeated. Results from glochidia bioassays continued to depart significantly from a normal distribution; therefore, results of the glochidia bioassays were analyzed with the non-parametric Spearman-Karber method (Stephan, 1977).

Results

Only two mussel species, *V. iris* and *F. barnesiana*, were collected upstream of the Tazewell WTP (Table 1). The mean density of *V. iris* declined from 5.4 mussels m^{-2} at the reference site (T1) to 0.6 m^{-2} at T2. This species was not collected again until T5, 4.25 km below the WTP. The mean density of *F. barnesiana* declined from 1.0 m^2 at T1 to 0.2 m^2 at T5, and none was collected at sites between T1 and T5. The reference site above the Tazewell WTP had a significantly higher mean density of *V. iris* than at all sites sampled below the Tazewell outfall (<0.001). The mean density of *F. barnesiana* at the reference site also was significantly higher than at T2, T3 and T4 ($p = 0.0023$), but not at T5 ($p = 0.013$).

No additional mussel species were collected during surveys by waterscope above and below the Tazewell WTP (Table 2). At T1 and T5, *F. barnesiana* was the most commonly collected species (CPUE of 5.45 and 3.49 mussels h^{-1}, respectively), but *V. iris* also was common at both T1 and T5 (CPUE of 3.41 and 2.79, respectively). Although three young *V. iris* (one to three years old) previously were found in quadrat sampling at T2, no mussels were collected at T3 or T4. During a qualitative survey of the entire river reach from T1 to T5, the first mussel (*V. iris*) was collected about 3.75 km downstream of the Tazewell WTP. Results of both quadrat sampling and mussel surveys in suitable habitats indicated,

Table 1. Mean densities (no. m^{-2}) of mollusks at thirteen sites, based on ten 0.5 m^2 quadrats per site, in the Clinch River, Tazewell County, 1985.

	Location												
	Tazewell					Cliffield		Pounding Mill		Richlands			
	T1	T2	T3	T4	T5	C1	C2	P1	P2	R1	R2	R3	R4
Unionidae													
Villosa iris	5.4	0.6	0	0	0.4	3.4	3.8	0.4	0.8	0.4	0	0	0
Fusconaia barnesiana[1]	1.0	0	0	0	0.2	0	2.6	0.2	0	0	0	0	0
Medionidus conradicus	0	0	0	0	0	0.4	1.8	0	0	0	0	0	0
Lampsilis fasciola	0	0	0	0	0	0.2	0	0	0	0	0	0	0
Pleuroceridae													
Pleurocera u. unciale	80.8	30.8	0.2	0	1.2	102.8	56.8	0	1.2	2.0	3.6	7.2	2.2
Elimia simplex	34.2	7.4	20.0	120.4	0.6	18.2	7.2	1.6	6.2	0.8	0	0	0
Anculosa subglobosa	0.2					92.4	119.2	18.8	62.8	1.6	0.2	0	0
Corbiculidae													
Corbicula fluminea	55.8	0.8	0.8	0.6	0.4	0	0	0	0	2.2	0.6	2.4	0.4
Sphaeriidae	211.4	12.8	0	0.2	7.6	7.8	153.8	1.0	0.2	0	9.0	0.4	0.4

[1] Most specimens were *F; barnesiana*, although some *Pleurobema oviforme* may have been included.

Table 2. Mussels collected per hour of sampling (CPUE) at sites in the Clinch River, Tazewell County, 1985.

Species	Tazewell					Cliffield		Pounding Mill		Richlands			
	T1	T2	T3	T4	T5	C1	C2	P1	P2	R1	R2	R3	R4
Villosa iris	3.41	0	0	0	2.79	9.09	4.96	9.31	3.46	1.50	0	0	0
Fusconaia barnesiana	5.45	0	0	0	3.49	2.73	5.45	3.10	0	0.75	0	0	0
Medionidus conradicus	0	0	0	0	0	0.91	12.89	0	1.15	0	0	0	0
Lampsilis fasciola	0	0	0	0	0	0	0.50	1.03	1.15	0	0	0	0
Ptychobranchus fasciolaris	0	0	0	0	0	0	0	0	0	2.25	0	0	0
Ptychobranchus subtentum	0	0	0	0	0	0	0	0	0	0.75	0	0	0
Lasmigona costata	0	0	0	0	0	0	0	0	0	0.75	0	0	0
Total CPUE	8.86	0	0	0	6.28	12.73	23.80	13.44	5.76	6.00	0	0	0
Total species	2	0	0	0	2	3	4	3	3	5	0	0	0

therefore, that the number of mussels below the Tazewell WTP was greatly reduced from upstream levels and was zero at some sites.

Quadrat sampling and mussel surveys also indicated that the river reach from the Richlands WTP to about 5.25 km downstream was depauperate of mussels. During quadrat sampling at the Richlands reference site (R1), only *V. iris* was found and at a low mean density (0.4 m^{-2}) not significantly different from no mussels at downstream sites ($p = 0.048$). Four other species were found at R1 during the mussel survey. In addition to *V. iris*, specimens of *F. barnesiana*, *Ptychobranchus subtentum*, *P. fasciolaris*, and *Lasmigona costata* were collected at R1, although in low numbers (CPUE of 0.75 to 2.25). No mussels were collected at three sites below the outfall to a distance of 3.25 km downstream (R4). A qualitative survey of the entire river reach from R1 to R4 also failed to locate mussels below the outfall. When the mussel survey was continued below the Raven-Doran WTP, the next WTP downstream of Richlands, we collected six species at a site 3.90 km below the Raven-Doran WTP or 8.00 km below the Richlands outfall. Quadrat sampling and mussel surveys suggested that effluent from one or both WTP's may limit mussels in this river reach.

Three species of mussels were collected in quadrat sampling at sites above (C1) at Cliffield. Specimens of *V. iris* and *Medionidus conradicus* were collected in quadrats at both sites; *Lampsilis fasciola* also was taken at C1 and *F. barnesiana* at C2. Mean densities did not differ significantly between sites for any mussel species except *M. conradicus*, which was present in significantly higher numbers at C2 ($p = 0.0035$). During the mussel surveys, three of these species again were collected at C1, and all four were collected below Cliffield. The CPUE at C2 was nearly double the CPUE at the sites above Cliffield. Mussel numbers below Cliffield did not seem to be reduced in comparison to numbers above this residential community. Fewer mussels were collected above (P1) and below (P2) at Pounding Mill during quadrat sampling than at Cliffield. Both *V. iris* and *F. barnesiana* were found at P1, whereas only *V. iris* was found at P2; all were at low mean densities (0.2 to 0.8 m^{-2}). No significant difference in mean densities was detected between the two sites for either mussel species ($p = 0.33$, $p = 0.51$).

Snail abundance

Three species of pleurocerid snails, *Pleurocera unciale unciale*, *Elimia (Goniobasis) simplex*, and *Anculosa subglobosa*, were collected in Tazewell County (Table 1). At the Tazewell WTP, mean density of *P. u. unciale* was highest at T1 and significantly lower ($P < 0.001$) at sites below the outfall, but mean densities did not differ among the lower sites ($p = 0.01$–0.99). The total number of

collected snails at T2 was relatively high, but unlike quadrats sampled on the opposite side of the river channel, most quadrats sampled near or in the effluent plume had few or no snails.

The snail *E. simplex* reached its greatest mean density at T4 (120.4 m^{-2}), which was significantly higher than all other sites ($p < 0.001$) and nearly four times the density recorded at T1 (34.2 m^{-2}). Mean density at T1 was not significantly different from T2 ($p = 0.016$) or T3 ($p = 0.45$) but was significantly higher than at T5 ($p = 0.003$). Although WTP effluent may have decreased numbers at T2, the great increase in numbers at T4 and the dramatic decrease again at T5 indicated that factors other than the WTP affected *E. implex* distribution downstream.

Anculosa subglobosa was rare at Tazewell sites. A mean density of only 0.2 m^{-2} was recorded at T1, while none were collected below the outfall. Since members of this genus prefer rocky riffles (Stansbery, 1970), it is likely that sampling sites were not optimum habitat for this species.

The same three species of snails at Tazewell also were collected near the Richlands WTP, although none were common. *Pleurocera u. unciale* was found at all sites at low mean densities not significantly different among sites ($p = 0.08$ to 0.94). *E. simplex* was collected only at R1 in low density, whereas *A. subglobosa* was collected at low mean densities at both R1 and R2.

Pleurocera u. unciale and *A. subglobosa* were very common above and below Cliffield, and mean densities did not differ significantly between the two sites for either species ($p = 0.16$, $p = 0.41$). *Elimia simplex* was less common at both sites, but again the difference in numbers between sites was not significant ($p = 0.18$). At Pounding Mill, *A. subglobosa* was the most common snail, followed by *P. u. unciale* and *E. simplex*. Collections indicated that snail numbers were not reduced downstream of Cliffield or Pounding Mill.

Other bivalves

Corbicula fluminea occurred at its highest mean density of 55.8 m^{-2} at T1 above the Tazewell WTP (Table 1). This density was significantly higher than at other sites below the outfall ($p < 0.001$). No specimens of *C. fluminea* were collected at Cliffield or Pounding Mill, despite their presence upstream at Tazewell and downstream at Richlands. In general, *C. fluminea* occurred in low densities at most sample sites in Tazewell County.

Fingernail clams (sphaeriids) also reached their greatest mean density at site T1 (211.4 m^{-2}) where it was significantly higher than at all four sample sites below the Tazewell WTP ($p < 0.001$). Mean densities did not differ significantly among sites T2 to T5 ($p = 0.51$–0.99). No sphaeriids were collected at R1 above the Richlands WTP, but a mean density of 9.0 m^{-2} was recorded at R2. Only two of the ten quadrats contained sphaeriids, both on the side of the river opposite the effluent plume. Despite slight variations, mean densities of sphaeriids among sites were not significantly different at Richlands ($p = 0.16$ to 1.00).

The mean density of sphaeriids at Cliffield site C2 (153.8 m^{-2}) was significantly higher than at C1, 7.8 m^{-2} ($p = 0.0018$). While mean numbers of sphaeriids were not reduced below Cliffield, their distribution appeared to be highly clustered. Sphaeriids were rare above and below Pounding Mill, and collected numbers were not significantly different between sites P1 and P2 ($p = 0.18$).

Water quality

Total residual chlorine was detected only at sites T2 and R2, 0.10 km below each WTP outfall, in July, August, and September, 1985; none was detected farther downstream (Table 3). From July 1985 to June 1986, monthly levels of TRC at T2 on the plume side of the river channel were highest in July (0.25 mg l^{-1}), September (0.13 mg l^{-1}), October (0.15 mg l^{-1}), and November (0.18 mg l^{-1}), whereas samples in January to March and June had the lowest concentrations ≤ 0.05 mg l^{-1}. The highest TRC concentrations were measured at 0.10 km below the Richlands WTP on the plume side in July

Table 3. Concentrations (mg l^{-1}) of total residual chlorine (TRC) and unionized ammonia (NH_3) at sites 0.1 km below WTP's at Tazewell (T2) and Richlands (R2), Virginia.

Month	TRC		NH_3	
	T2	R2	T2	R2
1985				
July	0.25	0.18	0.052	0.039
August	0.01	0	0.125	0.066
September	0.13	0	0.812	0.107
October	0.15	0	0.112	0.020
November	0.18	0.10	0.120	0.037
December	0.09	0.05	0.036	0.014
1986				
January	0.05	0.07	0.020	0.021
February	0.05	0	0.010	0.006
March	0.04	0	0.028	0.029
April	0.10	0.09	0.127	0.030
May[1]				
June	0	0.02	0.189	0.145

[1] No sample.

(0.18 mg l^{-1}), November (0.10 mg l^{-1}), and April (0.09 mg l^{-1}). Total residual chlorine was not detected at R2 in 5 months of the year. During many of these months, little or no TRC was detected in the effluent entering the river. According to the plant manager, the Richlands WTP was treating sewage above its capacity at this time, and one indication of this may be lower than normal TRC concentrations in the effluent and subsequently in the river. These data indicate that relatively short river reaches had TRC present during the summer.

Unionized ammonia concentrations ranged from 0.001 to 0.02 mg l^{-1} at the reference sites above the outfalls at Tazewell and Richlands. Concentrations were highest in the plume at T2 and R2 and gradually decreased toward the opposite bank. High values of 0.145 mg l^{-1} in the plume at R2 (June 1986) and 0.812 mg l^{-1} in the plume at T2 (September 1985) were recorded (Table 3). From July to September 1985, NH_3 concentrations remained elevated at T3 and T4. At T5, levels (0.006–0.014 mg l^{-1}) were no higher than at the reference site. The NH_3 levels (0.006–0.028 mg l^{-1}) at R3 and R4 were slightly higher than NH_3 levels at R1.

Unionized ammonia concentrations remained very low downstream of Cliffield and Pounding Mill. Concentrations at Cliffield were low from July to September 1985 and ranged from 0.004 to 0.013 mg l^{-1} at C1 and C2. Unionized ammonia levels at Pounding Mill also were low (0.008 to 0.019 mg l^{-1}). Conductivity, DO, alkalinity, pH, and temperature were not appreciably affected by the effluent of either WTP and were very similar above and below Cliffield and Pounding Mill.

Mollusk bioassay

Glochidia of *V. iris* were more sensitive to monochloramine (MCA) than NH_3 in laboratory bioassay (Table 4). The 24 h EC_{50} for MCA was 0.042 mg/l^{-1} and lower than the 24 h EC_{50} of 0.237 mg l^{-1} for NH_3. The 24 h LC_{50} for MCA was 0.084 mg l^{-1} and lower than the 24 h LC_{50} of 0.284 for NH_3. Although NH_3 affected glochidia at low levels, glochidia were three to five times more sensitive to MCA.

Bioassay results suggest that concentrations of TRC downstream of the Tazewell and Richlands

Table 4. Twenty-four hour EC_{50} and LC_{50} values for glochidia of *Villosa iris* and 96-hour LC_{50} values for adults of the snail *Pleurocera u. unicale* exposed to monochloramine (MCA) and unionized ammonia (NH_3) at 22 ± 1 °C.

	MCA (mg l^{-1})	NH_3 (mg l^{-1})
	Glochidia	
24-h EC_{50}	0.042	0.237
95% Fiducial limits	0.039–0.046	0.219–0.256
Control mortality	15%	0%
24-h EC_{50}	0.084	0.284
95% Fiducial limits	0.078–0.092	0.264–0.306
Control mortality	21%	8%
	Snail	
96-h LC_{50}	0.252	0.742
95% Fiducial limits	0.172–0.547	0.587–0.856
Control mortality	0%	0%

STP's may have a more severe effect on glochidia than NH_3. Total residual chlorine concentrations in nine of the eleven monthly samples at 0.10 km below the WTP outfall at Tazewell (T2) were above the lower 95% confidence limit for the 24 h EC_{50} of MCA, calculated for glochidia. Similarly, five of eleven monthly samples were above this limit at 0.10 km below the Richlands outfall (R2). Total residual chlorine concentrations were within the 95% confidence interval for the 24 h LC_{50} of MCA, calculated for glochidia at T2 during six months and R2 during three months. Only one of eleven monthly concentrations of NH_3 measured at T2 was within the 95% confidence interval for 24 h EC_{50} and LC_{50} values of NH_3 for glochidia. Concentrations of NH_3 measured at R2 never reached the lowest limit of the 95% confidence interval on sampling days. These data suggest that TRC in the effluent may be a major factor affecting survival of glochidia below WTP outfalls in some months.

Examination of subsamples before bioassay revealed that up to nearly 50% of *V. iris* glochidia may be alive but closed at the start of bioassay, possibly because of the method of their removal from the marsupia. Between 20.6 and 49.8% of the glochidia were observed as closed before bioassay began, but very few (1.2 to 3.9%) were dead upon removal from the marsupia. Nearly all glochidia used in bioassay were viable, although many had closed before testing began. The mean level of control mortality ranged from 0 to 21% after standardization by the relativized product index (Farris *et al.*, 1988), and was always below the allowed maximum of 30% for molluscan larvae (ASTM, 1980b).

Monochloramine was significantly more toxic than NH_3 to the snail *P. u. unciale* (Table 4). The 96 h LC_{50} for MCA was 0.252 mg l^{-1} and lower than 0.742 mg l^{-1} for NH_3. This snail is much more tolerant of both MCA and NH_3 than glochidia of *V. iris*. Fifty percent of the snails were killed in 96 hours by more than three times the concentrations of MCA and NH_3 needed to kill 50% of the glochidia in just 24 hours. Total residual chlorine concentrations and NH_3 concentrations at 0.10 km below the WTP's during monthly sampling occasionally reached the 96 h LC_{50} values of MCA and NH_3 calculated for *P. u. unciale*. Total residual chlorine concentrations in two of eleven monthly samples from below the Tazewell outfall and one of eleven samples from below the Richlands outfall were above the lower 95% confidence limit for the 96 h LC_{50} of MCA. One sample of NH_3 below the Tazewell WTP was above this limit for the 96 h LC_{50} of NH_3, whereas no samples during eleven months at R2 reached the lower limit of the confidence interval. Although toxicant levels at 0.10 km below the WTP's may be tolerable to *P. u. unciale* during most of the year, results suggest that MCA and NH_3 concentrations reached levels lethal to *P. u. unciale* during at least one or two months in 1985–1986.

Discussion

Effluent effects on mussels

The mussel fauna below at least two WTP outfalls in the Clinch River in Tazewell County, Virginia, has been depleted. The presence of mussels immediately upstream of the Tazewell and Richlands WTP's and their presence farther downstream suggest that mussels once populated river reaches below the WTP's and probably the entire upper Clinch River (Ortmann, 1918; Stansbery *et al.*, 1986). Previous researchers identified unionids as cleanwater organisms that are readily eliminated by environmental degradation, and have used the presence of mussels below domestic WTP's and power plants to indicate full biological recovery (Ortmann, 1909; Ingram, 1957; Simmons & Reed, 1973; Horne & McIntosh, 1979; Havlik & Marking, 1987). Mackenthun (1966) noted that long-lived, benthic invertebrates are valuable to indicate past and present water quality, and Ortmann (1909) considered unionids to be the most reliable indicator of stream pollution. If unionids are used as indicators of water quality in Tazewell County, then river reaches below the Tazewell and Richlands WTP outfalls have been severely impacted.

Because of their relative immobility, unionids are extremely vulnerable to toxic effluents (Ellis, 1931; Horne & McIntosh, 1979; Weber, 1981; Sheehan *et al.*, 1989). Unlike fish, which can move out of effluents quickly to avoid toxicants, mussels can only respond by closure. Horne & McIntosh (1979) reported that mussel species most tolerant of low oxygen and high NH_3 levels closed their valves tightly when exposed to these conditions, whereas less tolerant species continued siphoning or had their mantles exposed. Adult eastern oysters (*Crassostrea virginica*) can respond to TRC concentrations as low as 0.01 mg/l^{-1}, closing promptly to avoid exposure (Blogoslawski, 1980). Although the closure response may not normally be effective below WTP's that continually release effluent, mussels along the bank opposite the plume below the Tazewell WTP may be able to survive intermittent doses of toxicants simply by shutting their valves tightly until water quality improves. Because these specimens were young (one to three years old), mussels along the right bank may periodically be killed by high or prolonged exposures.

The effects of residual chlorine on marine bivalve mollusks have been studied intensively because chlorine is used in the shellfish industry to protect larvae from pathogens and to cleanse contaminated adult shellfish of bacteria at depuration stations (Blogoslawski, 1980). Khalanski & Bordet (1980) noted that chlorination has at least six effects on marine bivalve mollusks: (1) mortality, (2) pathology, (3) reduction of filtration rate, (4) reduction of growth, (5) reduction in settlement, and (6) detachment of settled larvae. The latter two are not pertinent to glochidia, but may be analogous to reduction in the number of glochidia parasitizing host fish. Chlorination may affect unionids in much the same manner as marine bivalves.

The large number of unionids at the reference site above the Tazewell WTP outfall suggests that glochidia are available to eventually repopulate the sites below the outfall. However, the nature of the unionid life cycle makes the reproductive stages particularly vulnerable to pollution (Ingram, 1957; Stein, 1971; Fuller, 1974; Gardner *et al.*, 1976). Sperm are fully exposed to toxicants upon release by males (Ingram, 1957; Stein, 1971; Fuller, 1974), as are glochidia released by the female to parasitize host fishes. Although no information is available on the sensitivity of unionid sperm cells to WTP effluent, bioassay results indicate that glochidia of *V. iris* are exposed to harmful TRC and NH_3 concentrations for at least 0.10 km below the Tazewell and Richlands STP's during nine months of the year. Glochidia that are not killed upon exposure to these toxicants may close their valves to avoid the effluent and therefore be functionally dead. Bioassay data indicated that most glochidia that closed during 24 h of exposure to MCA or NH_3 did not reopen after being placed in clean water for another 24 h, and EC_{50} values approximated LC_{50} values. Our results suggest that a certain level of exposure to MCA or NH_3 may prevent most glochidia from infesting fish, even if exposure to these toxicants is sublethal.

In addition to toxicants in WTP effluent, the reproductive stages of unionids may be affected by bacteria and protozoans often present below WTP outfalls. Fuller (1974) noted that fertilized ova in the marsupia of a female mussel are vulnerable to attack by both bacteria and protozoans, as are glochidia (van der Schalie, 1938). This may be especially pertinent to the conditions below the Richlands WTP, which was being used beyond its capacity to treat sewage. In addition, the Richlands WTP only treats at the primary level, which may lead to an abundance of undesirable bacteria and protozoans below the outfall. Although this possible problem was not examined below the WTP's, other threats to mussel reproduction below WTP outfalls are likely.

Water quality in summer 1985 was excellent between 1.50 and 3.75 km below the Tazewell WTP and 1.75 to 3.25 km below the Richlands WTP. However, sampling was limited to once monthly, so the worst conditions likely were missed (Hubbs, 1933; Gaufin & Tarzwell, 1952; Horne & McIntosh, 1979). Even if water quality was usually excellent at these sites, pulses of TRC or high NH_3 concentrations may be sufficient to periodically eliminate mussels and prevent recolo-

nization. For example, TRC was detected as far as 1.94 km below the Tazewell WTP in 1967 (Wollitz, 1985).

An abundance of large (old) specimens of *F. barnesiana* and *V. iris* was collected above the Tazewell WTP, but the few collected specimens 4.25 km below the outfall were much smaller (younger). This distribution may indicate that mussels at this site once were eliminated, but conditions have improved such that recolonization has begun. Greater restrictions on toxicant concentrations in effluents released into Virginia's rivers in recent years may have resulted in a shorter septic zone below the Tazewell WTP. Results from previous studies indicate that despite recolonization of other aquatic species in the affected areas, unionids are slow to recolonize a river reach after toxic spills (Stansbery *et al.*, 1986), cessation of toxic effluent (Ahlstedt, 1979; Sheehan *et al.*, 1989), or after a dredging operation (Stansbery, 1970). The legacy of poor water quality in past years, partly attributable to weaknesses in environmental regulations, may be observable today in the distribution of mussels in proximity to WTP outfalls (Gaufin & Tarzwell, 1952; Tarzwell & Gaufin, 1953; Mackenthun, 1966).

Effluent effects on snails

Effluent from the Tazewell WTP seemed to reduce densities of *E. simplex* and *P. unciale unciale* at 0.10 km below the outfall. It appeared that *E. simplex* was more tolerant of the WTP effluent than *P. u. unciale* because the former species was not completely eliminated in the effluent plume. However, high variability in numbers of *E. simplex* at downstream sites indicate that this snail's distribution is being influenced by factors other than WTP effluent. Few conclusions can be reached on this species' tolerance to WTP effluent. Because snail species were uncommon at the Richlands reference site, no conclusions can be reached about their tolerance to the Richlands WTP effluent.

Sinclair (1969) noted that the pleurocerid fauna in the Tennessee River system has been altered by sewage and other disturbances. Stansbery & Stein (1976) reported that pleurocerids were disappearing below sewage outfalls in the Clinch River and cited the Tazewell WTP as an example. However, researchers present differing opinions on the tolerance of pleurocerids to pollution. Ortmann (1909) noted that *Pleurocera, Goniobasis (Elimia)*, and *Anculosa* were usually eliminated in polluted rivers but were found in areas of the Allegheny River where unionids and fishes were eliminated. Other researchers reported that *Goniobasis* and *Pleurocera* were less tolerant to WTP effluent than sphaeriid clams and pulmonate snails (Baker, 1926; Beck, 1954), and Goodrich (1945) used the presence of *G. livescens* to indicate good water quality. Mason *et al.* (1968) referred to *Pleurocera* and *Goniobasis* as extremely eurytopic organisms. Harman (1974) concluded that pulmonate snails (*Physa, Helisoma*) seem to be more resistant to organic pollution than gilled snails, but that almost every common snail species has been found in polluted environments. Apparently the nature of the pollutant determines the tolerance of pleurocerids to the disturbance, and that tolerance may vary among species. For example, WTP effluent with little or no TRC (*i.e.* Richlands WTP) may have little effect on mollusks if other factors below the outfall (NH_3 concentration, habitat) are suitable. Conversely, the Tazewell WTP effluent with frequently higher TRC levels may have a major effect on some pleurocerids. In Tazewell County, the effluent from the Tazewell outfall seems to reduce *E. simplex* and especially *P. u. unciale* 0.10 km downstream.

Effects on other mollusks

A concern of malacologists, once unionids are eliminated from a river reach, is the possible proliferation of the asian clam, *Corbicula fluminea* (Fuller & Imlay, 1976; Jenkinson, 1979; Kraemer, 1979). In our study, the greatest number of *C. fluminea* was collected at the Tazewell reference site, where seemingly healthy unionid populations also resided. Jenkinson (1979) also found

large numbers of *C. fluminea* and unionids occupying the same areas of Uchee Creek, Alabama. At sites below the Tazewell outfall where no unionids were collected, *C. fluminea* was present, but only at low densities. The notion that *C. fluminea* invades only disturbed systems is therefore not supported by observations in the upper Clinch River.

Researchers have indicated that *C. fluminea* has a competitive advantage over unionids because it is monoecious, incubatory, attains sexual maturity in less than one year (Gardner *et al.*, 1976), and because the adults are environmentally tolerant (Kraemer, 1979). Jenkinson (1979) reported that *C. fluminea* was able to live and reproduce in a stream receiving textile mill and WTP effluents where mussels had been eliminated. Therefore the WTP effluent probably did not limit *C. fluminea* numbers as far as 4.25 km below the outfall where unionids had recovered. However, we believe that *C. fluminea* is still invading the headwaters of the Clinch River and that its abundance has not yet reached an equilibrium level.

The distribution of sphaeriid clams around WTP's followed no obvious pattern. The greatest density was observed at the Tazewell reference site, but none was collected from the Richlands reference site. Sphaeriids were collected at some sites below each WTP outfall where no mussels were collected, but only in low numbers. Other researchers reported fingernail clams to be generally more tolerant of pollution than unionids (Allen, 1923; Baker, 1926; Ingram, 1957; Fuller, 1974). Fuller (1974) noted that some sphaeriids may even increase in number below WTP's. Baker (1926) observed that pollution from sewage and industries eliminated many mollusks from the Illinois River, but that at least four species of sphaeriids remained. Beck (1954), Wurtz (1956), and Ingram (1957) placed sphaeriids in the group of mollusks most tolerant of polluted areas and often indicative of a degraded river reach. Because sphaeriids were found to be greatly reduced below the outfall at Tazewell and relatively uncommon at all Richlands sites, the same conclusions cannot be drawn from our study.

Effluent from the Tazewell WTP comprised about 5.8% of the average flow in the Clinch River at Tazewell, whereas the maximum amount of effluent treated at the Richlands WTP comprised only 1.1% of the average river flow below the Richlands outfall (Fred Wyatt, Virginia Water Control Board, pers. comm.). Nevertheless, the Richlands WTP, in combination with the Raven-Doran WTP, seemed to impair downstream fauna more severely than the Tazewell WTP. As judged by bioassay results, toxicants (TRC and NH_3) seemed to be major limiting factors downstream of the Tazewell WTP, whereas both unionized ammonia and physical degradation seemed to limit mollusk populations below the Richlands outfall. Siltation, sludge deposits, abundant fungal and algal growths, and increased turbidity downstream of the Richlands WTP likely contributed to the lack of mussels there. Paller *et al.* (1983) noted that an overloaded WTP, like the Richlands WTP, may considerably impair downstream fauna irrespective of chlorination. Unchlorinated secondary WTP effluent was significantly less toxic to the golden shiner *Notemigonus crysoleucas*, stickleback *Gasterosteus* spp., and rainbow trout *Oncorhynchus mykiss* than unchlorinated primary effluent (Tsai, 1975). Goodfellow *et al.* (1989) found primary effluent to be as much as four times as toxic to *Ceriodaphnia dubia* than secondary effluent. Our results concur with these observations; namely, primary WTP effluent seemed to have more of an impact on mollusks than secondary effluent, even in the absence of TRC.

Ambient toxicant levels

Toxicant levels below the Tazewell outfall may be harmful to *P. u. unciale* only periodically because TRC concentrations at 0.10 km below the two STP's were above the lower 95% confidence limits for the 96 h LC_{50} values of NH_3 and MCA calculated for *P. u. unciale* during only one or two months. However, TRC and NH_3 concentrations may be above the lower 95% confidence limits for the 96 h LC_{50} values more often than these lim-

ited data indicate. Measurement of water quality in our study was limited to only one day per month at each WTP. Such limited sampling did not allow the detection of extremes in toxicant concentrations; consequently, concentrations of toxicants probably reached the EC_{50} and LC_{50} values for glochidia and snails more often than reported. The concentration of TRC measured at 0.10 km below the Tazewell WTP in July 1985, and the concentration of NH_3 in September 1985 shows to what extent these toxicants may vary below outfalls.

Glochidia may be more affected than snails by TRC and NH_3 concentrations measured below the WTP outfalls because toxicants reached the lower 95% confidence limits of the EC_{50} and LC_{50} values for glochidia in nine months of the year, and toxicant levels below the outfalls may be at their highest during crucial periods in the reproduction of mussels. Total residual chlorine concentrations were highest during July, September, October, and November at Tazewell, and July and November at Richlands. Unionized ammonia concentrations were highest in September at Tazewell and in June at Richlands. As expected, toxicant concentrations were at their highest during summer and fall months because of seasonal low river flow in Virginia. Unfortunately, these months are usually critical times in mussel reproduction. Glochidia of long-term brooders, such as *V. iris* and *L. fasciola*, are released from April to August (Zale & Neves, 1982b). Short-term brooders such as *F. barnesiana* also release glochidia during summer months (Kitchel, 1985). Because these months may have the highest toxicant concentrations below WTP outfalls, many glochidia likely are affected by toxicants and unable to successfully parasitize a host fish and contribute to recruitment. In addition, long-term brooders typically spawn in late summer, and sperm is also vulnerable to high toxicant concentrations. Zale & Neves (1982b) noted that the release of sperm by males during low-flow conditions in summer and fall may increase the likelihood of fertilization under normal conditions. However, if toxicant concentrations reach high levels during this time, fertilization rates may decrease. Indeed, worst case conditions below WTP outfalls overlap with the critical reproductive stages in the life cycle of unionids in the upper Clinch River.

Sensitivity to unionized ammonia

The snail *P. u. unciale* and glochidia of *V. iris* were more sensitive to NH_3 than other mollusks that have been tested in bioassay (Table 5). The 96 h LC_{50} for *P. u. unciale*, and the 24 h EC_{50} and LC_{50} values for glochidia were lower than 96 h LC_{50} values calculated for the fingernail clam *Musculium transversum*, and the snails *Helisoma trivolis* and *Physa gyrina* (Arthur *et al.*, 1987). Juveniles of the marine bivalves *Crassostrea virginica* (eastern oyster) and *Mercenaria mercenaria* (hard clam) were much more tolerant to NH_3 than *P. u. unciale* and glochidia (Epifanio & Srna, 1975). Although glochidia of *V. iris* and *P. u. unciale* seem to be more sensitive to NH_3 than other mollusks, too few bioassays have been conducted with mollusks to assess differences among taxa
or between freshwater and saltwater species (USEPA, 1985b).

Our test species also were more sensitive to NH_3 than most crustaceans and aquatic insects, with the exception of the cladoceran *Daphnia magna* (Russo *et al.*, 1985). Both glochidia and *P. u. unciale* were more sensitive than the amphipod *Crangonyx pseudogracilis* (Arthur *et al.*, 1987), the crayfish *Orconectes nais* (Evans, 1979), and the mayfly *Ephemerella grandis* (Thurston *et al.*, 1984). Glochidia, and to a lesser extent *P. u. unciale*, rank among the most sensitive to NH_3 of those invertebrate species tested. Glochidia also are more sensitive to NH_3 than most fish species (USEPA, 1985b).

Sensitivity to total residual chlorine

Comparison of sensitivities to residual chlorine among aquatic organisms is difficult because researchers may report LC_{50} values as TRC, which

Table 5. The EC_{50} and LC_{50} values of unionized ammonia (NH3) reported for aquatic invertebrates.

Species	Duration (hours)	Effect	Concentration ($mg\,l^{-1}$)	Reference
Mollusks				
Villosa iris				
glochidia	24	EC_{50}	0.237	This study
glochidia	24	LC_{50}	0.2847	This study
Pleurocera unciale unciale	96	LC_{50}	0.742	This study
Musculium transversum	96	LC_{50}	0.93–1.29	Arthur *et al.*, 1987
Helisoma trivolis	96	LC_{50}	2.04–2.76	Arthur *et al.*, 1987
Physa gyrina	96	LC_{50}	1.59–2.49	Arthur *et al.*, 1987
Crassostrea virginica				
juveniles	96	LC_{50}	8.3–13	Epifanio & Srna 1975
Mercenaria mercenaria				
juveniles	96	LC_{50}	4.6–7.2	Epifanio & Srna 1975
Other invertebrates				
Daphnia magna	48	LC_{50}	0.53–2.77	Russo *et al.*, 1985
Simocephalus vetulus	96	LC_{50}	1.27–2.29	Arthur *et al.*, 1987
Crangonyx pseudogracilis	96	LC_{50}	1.63–5.63	Arthur *et al.*, 1987
Mysidopsis bahia	96	LC_{50}	0.50–2.87	Miller *et al.*, 1990
Asellus racovitzai	96	LC_{50}	4.95–5.09	Arthur *et al.*, 1987
Orconectes nais	96	LC_{50}	3.15	Evans 1979
Orconectes immunis	96	LC_{50}	14.72–33.84	Arthur *et al.*, 1987
Ephemerella grandis	96	LC_{50}	3.86–5.88	Thurston *et al.*, 1984
Philartcus quaeris	96	LC_{50}	10.07–10.17	Arthur *et al.*, 1987
Callibaetis skokianus	96	LC_{50}	3.15–4.82	Arthur *et al.*, 1987

may contain free chlorine, monochloramine, and dichloramine (Table 6). Thomas *et al.* (1980) reported free chlorine as the most toxic, whereas Heath (1977) and Mattice & Tsai (1983) reported dichloramine as the most toxic. However, previous studies agree that monochloramine is significantly less toxic to fish than the other two fractions. Researchers may report LC_{50} values as chlorine-produced oxidants (CPO) for marine organisms, which may consist of the previously mentioned components and hypobromous acid and bromamines (Jolley & Carpenter, 1983).

The most sensitive mollusk to TRC is the oyster, *C. virginica*, with a 48 h LC_{50} of $0.026\,mg\,l^{-1}$ CPO for larvae and a 96 h LC_{50} of $0.023\,mg\,l^{-1}$ CPO for juveniles (Roberts & Gleeson, 1978). The 24 h LC_{50} value of monochloramine for glochidia was slightly higher ($0.084\,mg\,l^{-1}$). However, if exposure duration was increased from 24 hours to 48 or 96 hours for glochidia, the LC_{50} would decrease, possibly into the range determined for *C. virginica*. Larvae of the asian clam are more tolerant to TRC than glochidia; the 72 h LC_{50} for *C. fluminea* was higher than the 24 h LC_{50} calculated for glochidia (Sickel, 1976). Adult asian clams were much more tolerant to TRC than glochidia or snails (Cairns & Cherry, 1983).

Pleurocera u. unciale is much more tolerant to MCA than another pleurocerid, *Goniobasis livescens*. Brooks & Szmania (1989) calculated a 96 h LC_{50} of $0.045\,mg\,l^{-1}$ MCA for *G. livescens*, compared with $0.252\,mg\,l^{-1}$ for *P. u. unciale*. Gregg (1974) also tested two pleurocerids, *G. virginica* and *Nitocris carinata*, but bioassays were conducted with the majority of TRC as free chlorine, which is more than twice as toxic as MCA (Thomas *et al.*, 1980). Arthur *et al.* (1975) reported that 96 h LC_{50} values for the pulmonate snails *Physa integra* and *Campeloma decisum* were greater than $0.810\,mg\,l^{-1}$ TRC in chlorinated secondary WTP effluent. Total residual chlorine in typical domestic WTP effluent is largely MCA

Table 6. The EC_{50} and LC_{50} values of chlorine derivatives reported for aquatic invertebrates.

Species	Duration (h)	Effect	Concentration ($mg\ l^{-1}$)	Form	Reference
Mollusks					
Crassostrea virginica					
larvae	48	LC_{50}	0.026	CPO[1]	Roberts & Gleeson 1978
juveniles	96	LC_{50}	0.023	CPO	Roberts & Gleeson 1978
Villosa iris					
glochidia	24	EC_{50}	0.042	MCA	This study
glochidia	24	LC_{50}	0.084	MCA	This study
Corbicula fluminea					
larvae	48	LC_{66}	1.2	TRC	Sickel 1976
larvae	72	LC_{50}	0.1	TRC	Sickel 1976
adults	240	LC_{50}	0.69	TRC	Cairns & Cherry 1983
Goniobasis virginica	96	LC_{50}	0.044	free chlorine	Gregg 1974
Goniobasis livescens	96	LC_{50}	0.045	MCA	Brooks & Szmania 1989
Nitocris carinata	96	LC_{50}	0.086	free chlorine	Gregg 1974
Pleurocera unciale unciale	96	LC_{50}	0.252	MCA	This study
Physa heterostropha	96	LC_{50}	0.258	free chlorine	Gregg 1974
Physa integra	96	LC_{50}	>0.810	MCA	Arthur *et al.*, 1975
Campeloma decisum	96	LC_{50}	>0.810	MCA	Arthur *et al.*, 1975
Other invertebrates					
Daphnia magna					
<24 hours old	48	LC_{50}	0.017	MCA	Ward *et al.*, 1976
Gammarus pseudolimnaeus	96	LC_{50}	0.220	MCA	Arthur & Eaton 1971
Pteronarcys sp.	96	LC_{50}	0.400	MCA	Arthur *et al.*, 1975
Orconectes nais	96	LC_{50}	0.673	TRC	USEPA 1985a

[1] Chlorine-produced oxidants.

(Johnson, 1978); therefore, *P. u. unciale* was much more sensitive to MCA than *P. integra* and *C. decisum*. Comparison of the sensitivity of the snails tested by Gregg (1974) with the sensitivity of *P. u. unciale* is difficult because of differences in the toxicities of free chlorine and MCA. Some indication of the toxicity difference between free chlorine and MCA is revealed from the vastly different 96 h LC_{50} values of TRC calculated for *P. heterostropha* (Gregg, 1974) and *P. integra* (Arthur *et al.*, 1975). *Pleurocera u. unciale* is much less tolerant of MCA than some pulmonate snails, but it may be one of the more tolerant species of pleurocerids.

Glochidia of *V. iris* rank with those species of crustaceans and aquatic insects that are most sensitive to MCA. Ward *et al.* (1976) calculated the 48 h LC_{50} for *D. magna* (<24 h old) as $0.017\ mg\ l^{-1}$, and a 48 h bioassay with glochidia would likely put them closer to this value. Species more tolerant than *P. u. unciale* included the stonefly *Pteronarcys* sp. (Arthur *et al.*, 1975) and the crayfish *O. nais* (USEPA, 1985a). In general, *P. u. unciale* is a moderately sensitive species in comparison with other invertebrates.

Use of glochidia in bioassay

A problem in our bioassay was the difficulty in ascertaining the death of a glochidium. Staining is often used to distinguish live and dead oyster larvae and other bivalve larvae, but our attempt to stain glochidia with neutral red and trypan blue provided inconsistent results. Zale & Neves (1982a) observed that normal, active glochidia snap shut when exposed to a weak saline solution. This response was used as a criterion to

check gaping glochidia for viability, but glochidia that were already closed before the addition of saline solution could not respond. Closed glochidia with damaged or missing adductor muscles were functionally dead, but those that were closed and looked normal were problematic. Difficulty in determining a point of death is common with invertebrate bioassay, and the use of a 24 h post-exposure period has been recommended to determine recovery and latent mortality (Maciorowski & Clarke, 1980).

Another difficulty was the removal of glochidia from the marsupium of an adult. Waller *et al.* (1985) used a 1 ml syringe to remove glochidia from the federally endangered *L. higginsi* to avoid sacrificing adults, and this method was used to remove glochidia from *V. iris*. However, glochidia that have been gently excised from marsupia, after an adult has been sacrificed, are typically open and may be in better condition for bioassay. A more effective, non-lethal means of removing glochidia is required so that standardization (RPI) is eliminated.

Glochidia offer advantages and disadvantages as bioassay organisms. First, larval forms are usually more sensitive than adults of the same taxon and so are likely to be more affected by toxicants (ASTM, 1980a; Buikema *et al.*, 1982). If bioassay results are to be used in establishing water quality criteria, then the most sensitive (critical) stage in an animal's life history should be tested. Unionids are intolerant of poor water quality and other perturbations, and a taxon that is among the most sensitive to toxicants should be tested when the entire stream community is to be protected. Adult mussels are seldom used in bioassay because of their ability to close for long periods of time to escape some toxicants (ASTM, 1980a). However, thousands of glochidia are available from one gravid mussel to provide sufficient test organisms.

Disadvantages of using glochidia as test organisms in bioassay are the lack of standardized procedures, seasonal availability, and the inability to maintain cultures in the laboratory. Some fishes (e.g., *Oncorhynchus mykiss*, *Pimephales promelas*) and macroinvertebrates (e.g., *Daphnia magna*) have been used so extensively in bioassay that standardized procedures and comparative data are available. Varanka (1977, 1978, 1979) and others have conducted bioassay with glochidia, using inhibition of the tryptamine-induced rhytmic activity of the adductor muscle as an indicator of pesticide effects, but such bioassays are rare. A standard protocol is needed, therefore, to facilitate the use of glochidia or juvenile freshwater mussels as bioassay organisms.

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