

Document downloaded from the institutional repository of the University of Alcalá: <https://ebuah.uah.es/dspace/>

This is an accepted Manuscript version of the following article, accepted for publication in Bulletin of Environmental Contamination and Toxicology:

Alonso, A. and Camargo, J.A. (2004) 'Toxic Effects of Unionized Ammonia on Survival and Feeding Activity of the Freshwater Amphipod *Eulimnogammarus toletanus* (Gammaridae, Crustacea)', Bulletin of environmental contamination and toxicology, 72(5), pp. 1052–1058. doi:10.1007/s00128-004-0350-z

To link to this article: <https://doi.org/10.1007/s00128-004-0350-z>

It is deposited under the terms of the Creative Commons Attribution-Non-Commercial-NoDerivatives License: (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited, and is not altered, transformed, or built upon in any way.

More information: <https://www.springernature.com/gp/open-research/policies/accepted-manuscript-terms>



This work is licensed under a
Creative Commons Attribution-NonCommercial-NoDerivatives
4.0 International License.



(Article begins on next page)

Universidad de Alcalá



This work is licensed under a
Creative Commons Attribution-NonCommercial-NoDerivatives
4.0 International License.

Toxic Effects of Unionized Ammonia on Survival and Feeding Activity of the Freshwater Amphipod *Eulimnogammarus toletanus* (Gammaridae, Crustacea)

A. Alonso, J. A. Camargo

Department of Ecology, Faculty of Sciences, University of Alcalá, 28871 Alcalá de Henares, Madrid, Spain

Received: 7 July 2003/Accepted: 21 January 2004

High concentrations of ammonia may be present in freshwater ecosystems as a consequence of organic pollution and nutrient enrichment. The foremost anthropogenic sources of ammonia are urban and agricultural runoff, animal farming, industrial wastes and sewage effluents (Williams et al. 1986; Maltby 1995). In a freshwater solution, ammonia can be found as two different chemical species, the unionized form (NH_3) and the ionized form (NH_4^+), this chemical equilibrium depending on water temperature and pH (Emerson et al. 1975; Williams et al. 1986). The toxicity of ammonia is related to the unionized form (Alabaster and Lloyd 1982; Williams et al. 1986; Maltby 1995; Frías-Espericueta et al. 2000), since the ionized form has little toxicity (Alabaster and Lloyd 1982; Williams et al. 1986).

Unionized ammonia at relatively elevated concentrations may cause mortality and other toxic effects on aquatic animals (Alabaster and Lloyd 1982; Williams et al. 1986; Maltby 1995; Frías-Espericueta et al. 2000). In the case of aquatic invertebrates, the toxic effects of ammonia are mediated by damage to the respiratory surfaces and by changes in the haemolymph pH (Colt and Armstrong 1981). Additionally, as gills are involved in the excretion of ammonia, this process is also hindered by high external ammonia concentrations (Spicer and McMahon 1994; Maltby 1995).

Amphipods have been cited as representative shredders of many freshwater ecosystems that play an important role in detritus processing (Cummins 1979). However, there is a lack of knowledge on the effects of unionized ammonia on the feeding activity of these crustaceans. Therefore, the aim of this study is to assess the toxic effects of unionized ammonia on the survival and feeding activity, under laboratory conditions, of the freshwater amphipod *Eulimnogammarus toletanus* (Pinkster & Stock) (Gammaridae, Crustacea) during short-term exposures. This Palearctic species is relatively abundant in unpolluted calcareous streams of the Iberian Peninsula. The endpoint of feeding activity, measured either as egestion rate or as feeding rate, has been previously used to assess the effects of ammonia and other toxicants (e.g., pyrene, phenanthrene, cadmium, zinc) on several aquatic invertebrates (Lotufo and Fleeger 1996; Leppänen et al. 1998; Frías-Espericueta et al. 2000).

MATERIALS AND METHODS

Amphipods were collected from an unpolluted upper reach of the Henares river (Guadalajara, Central Spain). Physico-chemical characteristics of river water are presented in Table 1. In the laboratory, organisms were distributed into glass aquaria (gravid adults and precopulatory pairs were rejected) and acclimatized to test water (bottled drinking water without chlorine) conditions for a week prior to laboratory experiments (Table 1). During acclimatization, animals were fed with stream-conditioned poplar (*Populus* sp.) leaves.

A first short-term bioassay (4 d) was conducted in triplicate to assess the effects of unionized ammonia on the survival of *E. toletanus*. Twenty-one glass vessels, each containing 0.1 L of test water and 8 amphipods, were used. They were covered with a perforated plastic foil to prevent water evaporation. No aeration was supplied in order to avoid ammonia oxidation. A control and six nominal concentrations of unionized ammonia were used, in triplicate (0.55, 0.75, 0.96, 1.17, 1.37 and 1.78 ppm $\text{NH}_3\text{-N}$). The control had less than 0.01 ppm $\text{NH}_3\text{-N}$ (Table 1). Nominal concentrations were prepared by dissolving the required amounts of ammonium chloride (NH_4Cl , PANREAC, Spain, Lot No. 149959380, reported purity 99.5%) on pre-established volumes of test water. Mortality observations, water renovation and parameter measurements (pH, water temperature and ammonia concentrations) were carried out at 24 hr intervals. The concentrations of unionized ammonia as nitrogen were determined using aqueous ammonia equilibrium calculations on the basis of mean values of water temperature and pH (Emerson et al. 1975). The 24, 48, 72 and 96 hr $\text{LC}_{0.01}$, LC_{10} and LC_{50} values, and their respective 95% confidence limits, were calculated using multifactor probit analysis (MPA) software (US Environmental Protection Agency 1991). The 120 and 144 hr $\text{LC}_{0.01}$, LC_{10} and LC_{50} values were also calculated by extrapolation using the MPA software. The MPA methodology solves the concentration-time-response equation simultaneously via the iterative reweighed least squares technique (multiple linear regression). The dependent variable is the probit of the proportion responding to each concentration, and the independent variables are exposure time and toxicant concentration. Mean actual concentrations of unionized ammonia were used for calculating LC values (0.50, 0.73, 0.88, 1.06, 1.39 and 1.85 ppm $\text{NH}_3\text{-N}$). These concentrations were measured by spectrophotometry (detection limit = 0.01 ppm $\text{NH}_3\text{-N}$) in accordance with American Public Health Association (1995). In this study, 24, 48, 72, 96, 120 and 144 hr $\text{LC}_{0.01}$ values are considered as short-term safe concentrations of unionized ammonia for *E. toletanus*, since these calculated values refer to the concentration of unionized ammonia affecting 0.01% individuals of the test population. Amphipods were not fed during toxicity test. Dead animals were removed every day.

A second short-term bioassay (6 d) was conducted to assess the toxic effects of unionized ammonia on the feeding activity of *E. toletanus*. This bioassay was performed comparing a control and three nominal concentrations of unionized ammonia (0.06, 0.22 and 0.33 ppm $\text{NH}_3\text{-N}$). Six replicates per treatment and

control were used (glass vessels of 0.1 L). The control had less than 0.01 ppm NH₃-N (Table 1). Test solutions were prepared as previously indicated for the first short-term bioassay. The egestion rate (mg dry wt feces/mg dry wt amphipod/d) was used as a feeding activity endpoint on the basis of the methodology developed by Leppänen et al. (1998). Four 2.2 cm-diameter discs of poplar leaves were added to each vessel, so that the mean leaf dry weight per vessel was 88.5 mg. These discs were previously conditioned for 7 d in glass aquaria with stream water and oxygenation to allow the growth of fungi and bacteria on their surfaces. Water oxygenation was produced with airpumps and airstones. Fecal pellets, daily produced in each glass vessel, were collected with a Pasteur pipette and stored at 5°C in glass tubes. After 2 d, both the amphipods and their fecal pellets from three replicates were dried at 60°C for 72 hr. The remaining three replicates were subsequently processed in the same way after 4 d more. Thus, the egestion rate was estimated after 2 d and 6 d. Water renovation and measurements of pH, water temperature and ammonia concentrations were carried out at 24 hr intervals. The mean actual concentrations of unionized ammonia were 0.04, 0.18 and 0.30 ppm NH₃-N per respective treatment. Significant ($P<0.05$) differences in egestion rate between the control and each treatment were examined by means of an analysis of variance (ANOVA-Dunnnett test) (Zar 1984) for each exposure time (2 d and 6 d).

Table 1. Physicochemical characteristics of both Henares river water and toxicity test water.

	Toxicity tests	Henares river
Conductivity (μS)	392±7	536.4±13.6
pH	8.0±0.1	7.7±0.1
Calcium (ppm)	45.4±0.9	87.2±14.6
Dissolved oxygen (ppm)	6.4±0.5	8.7±0.7
Water temperature (°C)	15.5±0.7	11.3±1.8
NO ₃ -N (ppm)	0.6±0.08	1.6±1.3
NO ₂ -N (ppm)	<0.005	<0.005
NH ₃ -N (ppm)	<0.01	<0.01

Mean values ± standard deviations are presented for each parameter. Water analyses were performed following standard methods described by American Public Health Association (1995).

After the bioassays, the body length from antennal base to third uropod of amphipods was measured with an ocular micrometer. Differences in body length between control and NH₃-N treatments were assessed by means of an analysis of variance for the first short-term bioassay (ANOVA-Dunnnett test) (Zar 1984), and through a Kruskal-Wallis test for the second short-term bioassay due to the absence of normality of data (Zar 1984).

RESULTS AND DISCUSSION

In the first short-term bioassay, all unionized ammonia concentrations caused mortality on amphipods, this lethal effect being proportional to the unionized

ammonia concentration and the exposure time. No mortality was found in control vessels after 96 hr. Mean values of body length in control and the six NH₃-N treatments were 5.8±1.4, 5.8±1.2, 5.8±1.0, 6.2±1.1, 6.8±1.0, 5.9±0.9 and 5.9±1.1 mm, respectively. The control-treatment differences were not significant ($P>0.05$; Dunnett test).

The LC_{0.01}, LC₁₀ and LC₅₀ values of NH₃-N, and their 95% confidence limits, for *E. toletanus* are presented in Table 2. From the comparison of LC₅₀ values for *E. toletanus* with LC₅₀ values for other freshwater amphipods (Table 3), we can see that *E. toletanus* was more sensitive to NH₃-N toxicity than *Crangonyx pseudogracilis*, *Hyaella azteca* and *Gammarus pulex*, but more tolerant than *Paracalliope fluviatilis* (Table 3). The result of this comparison suggests that amphipods with a wide geographical range in freshwater ecosystems, *C. pseudogracilis*, *H. azteca* and *G. pulex*, have a relatively high tolerance to the toxicity of unionized ammonia, while amphipods with a narrow geographical range, the Iberian amphipod *E. toletanus* and the Australian amphipod *P. fluviatilis*, are more sensitive to the NH₃-N toxicity. This might explain the potential influence of unionized ammonia on the distribution of freshwater amphipods in organic polluted rivers. A similar NH₃-N influence was suggested for an exotic Spanish population of the aquatic snail *Potamopyrgus antipodarum* (Alonso and Camargo 2003).

Table 2. 24, 48, 72, 96, 120 and 144 hr LC_{0.01}, LC₁₀ and LC₅₀ values of NH₃-N (ppm) for *Eulimnogammarus toletanus*.

	LC _{0.01}	LC ₁₀	LC ₅₀
24 hr	0.29 (0.18-0.39)	0.75 (0.61-0.86)	1.23 (1.09-1.40)
48 hr	0.19 (0.12-0.26)	0.49 (0.41-0.55)	0.80 (0.75-0.86)
72 hr	0.17 (0.10-0.22)	0.43 (0.35-0.48)	0.70 (0.64-0.76)
96 hr	0.15 (0.09-0.21)	0.40 (0.32-0.46)	0.65 (0.59-0.72)
120 hr	0.15 (0.09-0.20)	0.38 (0.30-0.44)	0.62 (0.56-0.69)
144 hr	0.14 (0.09-0.20)	0.37 (0.29-0.43)	0.61 (0.54-0.68)

95% confidence limits are presented in parentheses.

The second short-term bioassay showed that unionized ammonia affected the egestion rate of *E. toletanus* (Figure 1). No mortality was found in control vessels after 2 d and 6 d. Mean values of body length were 6.0±1.2, 6.2±1.0, 6.0±0.9 and 6.0±0.9 mm for control and treatments. The control-treatments differences were not significant ($P>0.05$; Kruskal-Wallis Test). The egestion rates did not differ between control vessels and NH₃-N treatments after 2 d of exposure ($P>0.05$; Dunnett Test). However, the egestion rate tended to be higher than that in the control at 0.04 and 0.18 ppm NH₃-N (Figure 1). This suggests that the egestion rate in *E. toletanus* may be stimulated at low concentrations of unionized ammonia. Similar results were found by Lawrence and Poulter (1998) for ventilation response of *Gammarus duebeni* to copper, and by Crane et al. (1995) for feeding rate of *Gammarus pulex* exposed to malathion 60 (organophosphorus insecticide). In contrast, the egestion rate of amphipods at 0.30 ppm NH₃-N was significantly lower than that in control vessels after 6 d of exposure ($P<0.05$;

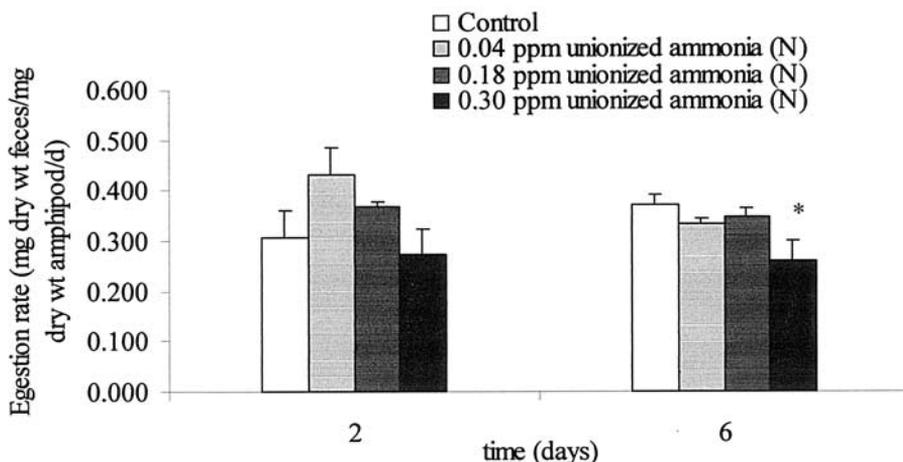


Figure 1. Egestion rate (mg dry wt feces/mg dry wt amphipod/d) of *E. toletanus* exposed to different levels of unionized ammonia during 2 and 6 d. Asterisk shows significant differences between control and treatment for each exposure time (ANOVA; Dunnett test; $P < 0.05$).

Dunnett Test). At 0.04 and 0.18 ppm $\text{NH}_3\text{-N}$, after 6 d of exposure, the egestion rate was not significantly different from that in control vessels ($P > 0.05$; Dunnett Test) (Figure 1). The 144 hr (6 d) $\text{LC}_{0.01}$ for *E. toletanus* was 0.14 (0.09-0.20) ppm $\text{NH}_3\text{-N}$ (Table 2). The no observed effect concentration (NOEC) for the second bioassay was 0.18 ppm $\text{NH}_3\text{-N}$ after 6 d of exposure, this value being within the 95% confidence limits of the calculated $\text{LC}_{0.01}$ value after 6 d. This confirms that the calculated safe concentration of 0.14 ppm $\text{NH}_3\text{-N}$ is a good limit to avoid mortality and reduction in feeding activity of *E. toletanus* exposed to unionized ammonia stress. However, changes in water hardness, velocity and substrate could either reduce or increase this safe level (Thomas et al. 1991; Ankley et al. 1995).

Table 3. LC_{50} values of $\text{NH}_3\text{-N}$ (ppm) for several species of freshwater amphipods.

	LC_{50} 24 hr	LC_{50} 48 hr	LC_{50} 96 hr	Reference
<i>Crangonyx</i>	-	-	1.63-5.63	Arthur et al.
<i>pseudogracilis</i>			(1.42-6.91)	1987
<i>Hyaella azteca</i>	-	-	2.14-5.38	Ankley et al.
(in hard water)			(1.68-6.04)	1995
<i>Gammarus pulex</i>	2.64	2.08	1.69	Williams et al.
				1986
<i>Eulimnogammarus</i>	1.23	0.80	0.65	Present study
<i>toletanus</i>	(1.09-1.40)	(0.75-0.86)	(0.59-0.72)	
<i>Paracalliope</i>	0.31	0.18	-	Hickey and
<i>fluviatilis</i>	(0.23-0.45)	(0.14-0.24)		Vickers 1994

95% confidence limits are presented in parentheses.

Overall, it is concluded that *E. toletanus* exhibited a relatively high sensitivity to

the short-term toxicity of NH₃-N. In order to protect this species against NH₃-N pollution, a safe level of a maximum NH₃-N concentration of 0.20 ppm for short-term exposures (6 d) is recommended.

Acknowledgments. Funds for this study came from Alcalá University and a research project (REN2001-1008) from Ministry of Science and Technology in Spain. A Alonso was supported by a predoctoral grant from the Council of Castilla-La Mancha Community. Our sincere gratitude to Pilar for correcting the English text.

REFERENCES

- Alabaster JS, Lloyd R (1982) Water quality criteria for freshwater fish, 2nd Edition. Butterworths, London
- Alonso A, Camargo JA (2003) Short-term toxicity of ammonia, nitrite and nitrate to the aquatic snail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca). Bull Environ Contam Toxicol 70: 1006-1012
- American Public Health Association (1995) Standard methods for the examination of water and wastewater, 19th Edition. APHA-AWWA-WPCF, Washington, DC
- Ankley GT, Shubauer-Berigan MK, Monson PD (1995) Influence of pH and hardness on toxicity of ammonia to the amphipod *Hyaella azteca*. Canadian J Fish Aquat Sci 52: 2078-2083
- Arthur JW, West CW, Allen KN, Hedtke SF (1987) Seasonal toxicity of ammonia to five fish and nine invertebrate species. Bull Environ Contam Toxicol 38: 324-331
- Colt JE, Armstrong DA (1981) Nitrogen toxicity to crustaceans, fish and molluscs. Bio-Engineering Symposium for Fish Culture (FCS Publ. 1), pp 34-47
- Crane M, Delaney P, Watson S, Parker P, Walker C (1995) The effect of malathion 60 on *Gammarus pulex* (L.) below watercress beds. Environ Toxicol Chem 14: 1181-1188
- Cummins KW (1979) Feeding ecology of stream invertebrates. Ann Rev Ecol Syst 10: 147-172
- Emerson K, Russo RC, Lund RE, Thurston RV (1975) Aqueous ammonia equilibrium calculations: effect of pH and temperature. J Fish Res Bd Canada 32: 2379-2383
- Frías-Espericueta MG, Harfush-Melendez M, Páez-Osuna F (2000) Effects of ammonia on mortality and feeding of postlarvae shrimp *Litopenaeus vannamei*. Bull Environ Contam Toxicol 65: 98-103
- Hickey CW, Vickers ML (1994) Toxicity of ammonia to nine native New Zealand freshwater invertebrate species. Arch Environ Contam Toxicol 26: 292-298
- Lawrence AJ, Poulter C (1998) Development of a sublethal pollution bioassay using the estuarine amphipod *Gammarus duebeni*. Water Res 32: 569-578
- Leppänen MT, Postma JF, Groenendijk D, Kukkonen JVK, Buckert-de Jong MC (1998) Feeding activity of midge larvae (*Chironomus riparius* Meigen) in metal-polluted river sediments. Ecotoxicol Environ Safety 41: 251-257

- Lotufo GR, Fleeger JW (1996) Toxicity of sediment-associated pyrene and phenanthrene to *Limnodrilus hoffmeisteri* (Oligochaeta: Tubificidae). *Environ Toxicol Chem* 15: 1508-1516
- Maltby L (1995) Sensitivity of the crustaceans *Gammarus pulex* (L.) and *Asellus aquaticus* (L.) to short-term exposure to hypoxia and unionized ammonia: observations and possible mechanisms. *Water Res* 29: 781-787
- Spicer JJ, McMahon BR (1994) Gill function in the amphipod *Megalorchestia (Orchestoidea) californiana* (Brandt, 1851) (Crustacea). *Canadian J Zool* 72: 1155-1158
- Thomas PC, Turner C, Pascoe D (1991) An assessment of field and laboratory methods for evaluating the toxicity of ammonia to *Gammarus pulex* (L.) – effects of water velocity. In: Jeffrey DW, Madden B (eds) *Bioindicators and Environmental Management*. Academic Press, London, pp 353-363
- US Environmental Protection Agency (1991) Multifactor probit analysis. Environmental Protection Agency, 600/X-91-101, Washington DC
- Williams KAW, Green DWJ, Pascoe D (1986) Studies on the acute toxicity of pollutants to freshwater macroinvertebrates: 3. Ammonia. *Arch Hydrobiol* 106: 61-70
- Zar JH (1984) *Biostatistical analysis*, 2nd edition. Prentice-Hall, New Jersey