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Acute and Chronic Toxicity of Ammonium Chloride to the Cladoceran *Daphnia pulex* Leydig in Relation to Algal Food Density

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Cladocerans are generally a dominant group in freshwater zooplankton where they may contribute up to 80% of the secondary production. In natural waterbodies they are subjected to strong variations in the physical and chemical variables. Among abiotic factors, temperature and pH are known to influence the density and dynamics of cladocerans (Downing and Rigler 1984). Nitrogen is a macronutrient directly controlling the phytoplankton densities, which in turn influence the biomass of zooplankton (Hutchinson 1967). Among nitrogenous compounds, free-ammonia has a strong and negative influence on both survival and reproduction of cladocerans. Dissolved ammonia is also toxic to zooplankton but at much higher concentrations (Manning et al. 1996). However, dissolved ammonia is also an important source of nitrogen for phytoplankton, which they could directly absorb from the ambient (Pales-Espinosa and Moreau 1997). Therefore, the toxicity of ammonium compounds to zooplankton in general and cladocerans in particular, could be influenced by the seasonal variations in the density of phytoplankton.

Much of the information on the toxic effects of ammonia to cladoceran zooplankton is concerned with deriving median lethal tests or chronic tests at one food level (Andersen and Buckley 1998) or field-observations where several factors simultaneously interact (Arthur et al. 1987). Since variations in the algal densities naturally occur, it is of considerable interest to know the influence of phytoplankton on the toxicity of ammonia to cladoceran zooplankton under both acute and chronic test conditions.

The aim of this work was to study the effect of low and high algal food concentrations on the toxicity of ammonium chloride to the survival and population growth of the cladoceran *Daphnia pulex*.

MATERIALS AND METHODS

The test organism, *Daphnia pulex* Leydig, 1860 was isolated from the dam Manuel Avila Camacho in the State of Puebla (Mexico) and mass cultured using

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the green alga *Chlorella vulgaris* as the exclusive food. Conditions that favoured good population growth in mass culture tanks were, pH 7.5; temperature 24°C, continuous fluorescent illumination and *Chlorella* at $1.5X10^6$ cells/mL and change of culture media every alternate day. In the mass culture tanks the average density of *D. pulex* was maintained at about 2 ind./mL. *Chlorella* was mass cultured in 2L transparent bottles using Bold's basal medium (Borowitzka and Borowitzka 1988). For routine cultures as well as for experiments we used reconstituted moderate hard water (the EPA medium, Anon. 1985). The EPA medium was prepared by dissolving 96 mg $NaHCO_3$, 60 mg $CaSO_4$, 60 mg $MgSO_4$ and 4 mg KCl in one litre of distilled water.

For acute toxicity tests, we used neonates (24 ± 2 h age) of *D. pulex*. We selected three densities of *Chlorella vulgaris* (resuspended alga) viz. 0 (no food), $0.5X10^6$ and $1.5X10^6$ cells/mL. A stock solution of 500 mg/L ammonium chloride was prepared using distilled water. Bioassays were conducted for 24 h using eight ammonia levels ranging from 0 (control) to 300 mg/L. For each of the ammonia concentrations and algal levels, we used three replicates and each replicate with 10 individuals. We counted the number of live cladocerans from each replicate and the data were used to derive median lethal concentration (LC50) following probit method (Finney 1971).

Based on the data of median lethal concentration, chronic toxicity tests were conducted in a static-renewal system using the population growth of *D. pulex* at five concentrations of ammonium chloride viz., 0 (control), 20, 40, 80 and 120 mg/L under two food levels (low: $0.5X10^6$ and high: $1.5X10^6$ cells/mL). For the growth experiments we used a mixed population of *D. pulex* obtained from mass culture tanks in the exponential phase. The experimental design consisted a total of 30 test jars (5 ammonia concentrations X 2 algal food levels X 3 replicates) of 200 mL capacity, each containing 50 mL medium with specified algal food-ammonia combination. Into each test jar, we introduced 10 individuals of *D. pulex* using a Pasteur pipette under stereomicroscope at 20X. Following inoculation, every day we counted the number of test cladocerans alive in each replicate and medium was changed appropriately with alga-ammonia combinations. The population growth experiments were discontinued after 4 weeks by which time most replicates began to decline. Based on the data collected, we evaluated statistically using the analysis of variance the combined effects of ammonia concentrations and the algal food levels on the peak cladoceran density, day of maximal population density and the rate of population density (r) per day. The r values were derived using the exponential equation:
$r = (\ln N_t - \ln N_0)/t$, where N_0 and N_t are the initial and final population densities respectively, and t is time in days. We used varying data points along the growth curve to calculate the mean growth rate per replicate. In general we took 4-6 data points during the exponential phase of the population for each replicate following Dumont et al. (1995).

RESULTS AND DISCUSSION

The 24 hr median lethal concentration values of ammonium chloride for *D. pulex* in relation to algal food density are presented in Table 1. There was a distinct difference between with and without inclusion of food in the acute toxicity tests. When the tests were conducted without food, the concentration of ammonia required to produce a 50% mortality of neonate *D. pulex* was much lower than when food was supplied. Data on the population growth of *D. pulex* in relation to ammonia concentrations and *Chlorella* levels are presented in Figure 1. Regardless of ammonia concentration, an increase in *Chlorella* level from $0.5X10^6$ to $1.5X10^6$ cells/mL, there was an increase in the population growth. However, at any food level, an increase in ammonia concentrations resulted in a decrease of population growth of *D. pulex*. At any given ammonia level, an increase in algal food density resulted in a higher population abundance (Figure 2) or growth rate (Figure 3). Statistically significant ($p<0.001$, ANOVA, F-test) effects of ammonia on the peak population density, day of maximal abundance and the rate of population were observed. Similarly, food density also significantly influenced these variables. The interaction of food level X ammonia concentration was also significant ($p<0.001$) except for rate of population increase ($p>0.12$).

Table 1. LC50 values (mean ±standard error) of ammonium chloride for *D. pulex* in relation to different levels of *Chlorella*. Bioassays were performed at 24h.

Food density (X 10^6 cells/mL)	Median lethal concentration (mg/L)
0.0 (no food)	247.12±8.29
0.5	271.66±1.39
1.5	277.10±0.42

The fact that the toxicity of ammonia could be reduced by the presence of living algae is evident in both acute and chronic test conditions. The algal food density chosen here was based on the literature where *Chlorella* generally higher than $1.5X10^6$ cells/mL could cause feeding inhibition and the resultant reduction in population growth rates (Nandini and Sarma 2000). In controls an increase in algal concentration resulting in an increase mean population density observed here is known among cladocerans (Lampert and Sommer 1997). Thus, the maximal population density (7.6±0.4 ind./mL) observed at $1.5X10^6$ cells/mL of *Chlorella* in controls was not uncommon even for *Daphnia*. For example *D. carinata* reached up to 20 ind./mL when fed *Microcystis* (Nandini and Rao 1998). Similarly, the rate of population increase per day as observed here (positive values ranging from 0.02 to 0.12) (Figure 3) were earlier observed for other daphnids (Hietala et al. 1997, Lürling and Van Donk 1997).

In most toxicity tests using dissolved ammonia, the mortality of test individuals were also attributed to the free ammonia associated with the former (Manning et al. 1996). In our study, such a possibility exists, but this could decrease with increasing algal abundance. Thus, when we measured the ammonia levels using

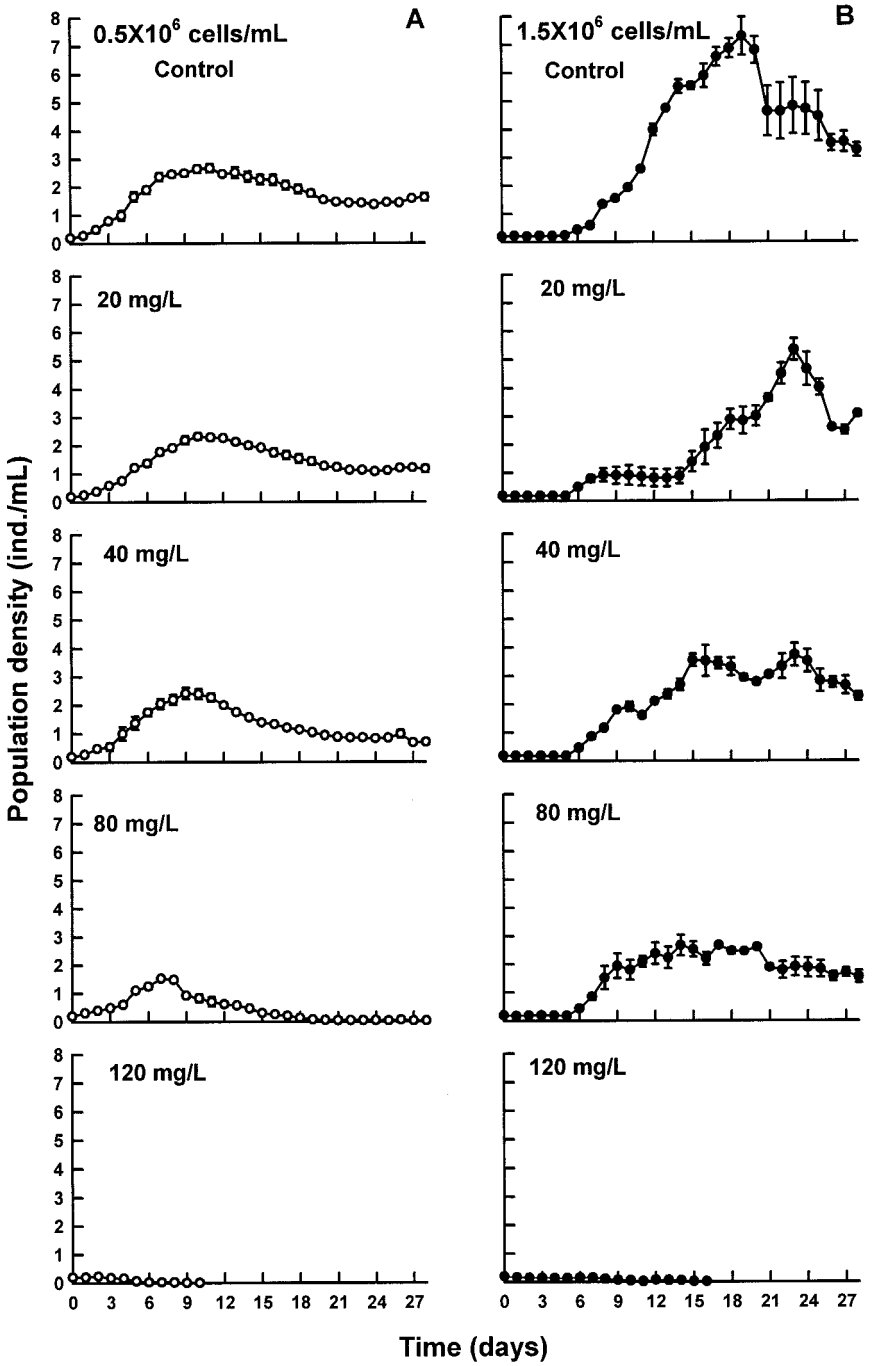

Figure 1. Population growth of *Daphnia pulex* in relation to different concentrations of ammonium chloride under low (column A, $0.5X10^6$) and high (column B, $1.5X10^6$ cells/mL) *Chlorella* densities. Shown are mean ± standard error values.

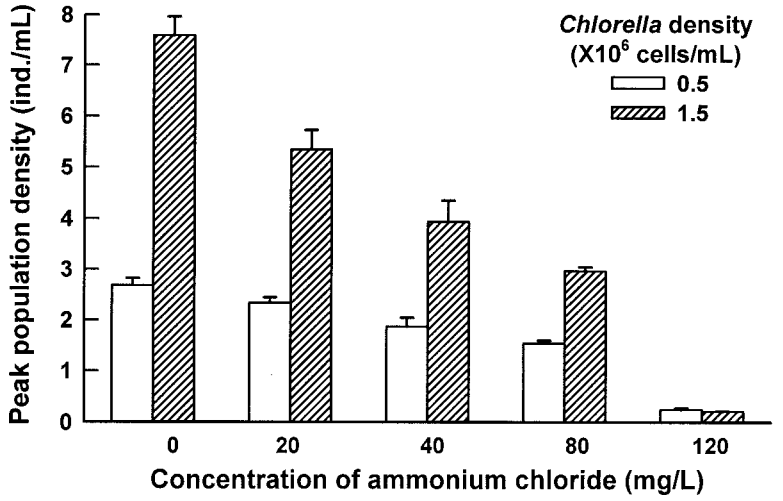

Figure 2. Maximum population density of *D. pulex* in relation to different concentrations of ammonium chloride at low ($0.5X10^6$) and high ($1.5X10^6$ cells/mL) *Chlorella* levels. Shown are mean±standard error values.

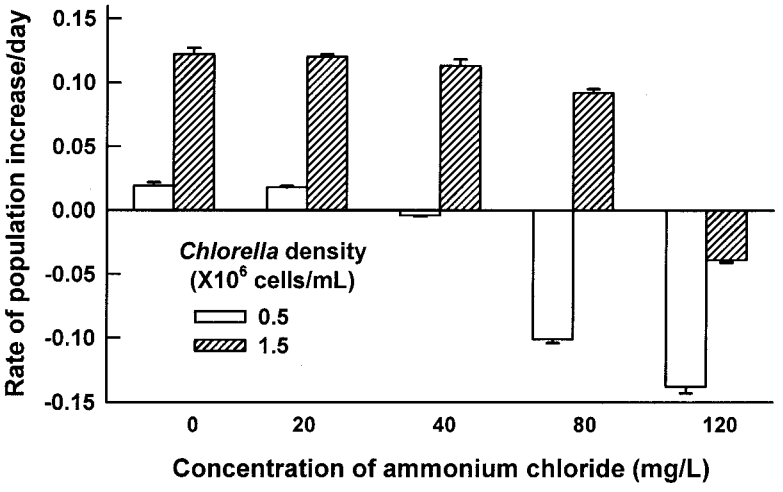

Figure 3. Rate of population increase per day of *D. pulex* in relation to different concentrations of ammonium chloride at low ($0.5X10^6$) and high ($1.5X10^6$ cells/mL) *Chlorella* levels. Shown are mean±standard error values.

various concentrations of *Chlorella* alone (without the presence of cladocerans), and after 24 h, there was a reduction in dissolved ammonia levels (and thus the free ammonia). This condition however, cannot be also directly attributed to the experimental containers because the algal density could vary due to grazing depending on the density of *D. pulex* in each container (Downing and Rigler 1984) and the excretion of ammonia by the cladocerans (Arner and Koivisto 1993).

Based on the published data, dissolved ammonia in general is less toxic and cladocerans can tolerate more than 100 mg/L without apparent ill-effects (Manning et al. 1996; Andersen and Buckley 1998). Our observations in acute toxicity bioassays support this. However, due to prolonged exposure *D. pulex* became more sensitive to even much less ammonia concentrations. In conclusion our study emphasizes the importance of chronic toxicity tests instead of relying exclusively on the results from the acute bioassays since the final effect of a toxicant depends not only on its concentration but also the duration of exposure. This study also demonstrates the effectiveness of algae in mitigating adverse effects of ammonia to *D. pulex*.

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REFERENCES

Andersen HB, Buckley JA (1998) Acute toxicity of ammonia to *Ceriodaphnia dubia* and a procedure to improve control survival. Bull Environ Contam Toxicol 61: 116-122

Anonymous (1985) Methods of measuring the acute toxicity of effluents to freshwater and marine organisms. US Environment Protection Agency EPA/600/4-85/013

Arner M, Koivisto S (1993) Effects of salinity on metabolism and life history characteristics of *Daphnia magna*. Hydrobiologia 259: 69-77

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

Borowitzka MA, Borowitzka LJ (1988) Micro-algal biotechnology. Cambridge University Press, London

Downing JA, Rigler FH (eds) (1984) A manual on the methods for the assessment of secondary productivity in fresh waters. IBP Handbook 17, Blackwell Scientific Publ. London

Dumont HJ, Sarma SSS, Ali AJ (1995) Laboratory studies on the population dynamics of *Anuraeopsis fissa* (Rotifera) in relation to food density. Freshwater Biol. 33: 39-46

Finney DJ (1971) Probit analysis. Cambridge University Press, 3rd edn, London

Hietala J, Lauren-Määttä C, Walls M (1997) Life history responses of *Daphnia* clones to toxic *Microcystis* at different food levels. J Plankton Res 19: 917-926.
Hutchinson GE (1967) A treatise on limnology. 2. Introduction to lake biology and limnoplankton. John Wiley, New York
Lampert W, Sommer U (1997) Limnoecology. The ecology of lakes and streams. Oxford University Press, New York
Lürling M, Van. Donk E (1997) Life history consequences for *Daphnia pulex* feeding on nutrient-limited phytoplankton. Freshwater Biol 38: 693–709
Manning TM, Wilson SP, Chapman JC (1996) Toxicity of chlorine and other chlorinated compounds to some Australian aquatic organisms. Bull Environ Contam Toxicol 56: 971-976
Nandini S, Rao TR (1998) Somatic and population growth in selected cladoceran and rotifer species offered the cyanobacterium *Microcystis aeruginosa* as food. Aquat Ecol 31: 283-298
Nandini S, Sarma SSS (2000) Lifetable demography of four cladoceran species in relation to algal food (*Chlorella vulgaris*) density. Hydrobiologia 435: 117-126
Pales-Espinosa E, Moreau C (1997) Effect of ammonium nitrogen on the biodiversity of natural phytoplanktonic communities from Bourgneuf Bay (France). Determination of optimal and toxic amounts for the growth of tested diatoms. J Rech Oceanogr 22: 117-124.