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SHORT COMMUNICATIONS

AMMONIA TOXICITY TO LARVAE OF *ERYTHROMMA NAJAS* (HANSEMANN), *LESTES SPONSA* (HANSEMANN) AND *SYMPETRUM FLAVEOLUM* (LINNAEUS) (ZYGOPTERA: COENAGRIONIDAE, LESTIDAE; ANISOPTERA: LIBELLULIDAE)

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Three different types of toxicological test were conducted, viz. the standard toxicological test at varying pH, a test with starved larvae and a test with different ionic composition of the water. For the larvae of *L. sponsa*, ammonia toxicity was examined only in the standard test at one pH value and in the test with varying ionic composition of the medium. Total ammonia was more toxic at elevated than at low pH to both *S. flaveolum* and *E. najas* larvae. In contrast, toxicity based on the un-ionized form appeared to increase with decrease in pH value. In general, larvae of all spp. have a high ammonia tolerance when compared to other aquatic animals. Tests with starved larvae showed that the ammonia tolerance of starved larvae of *S. flaveolum* was 3.7 times greater than that for the fed ones; for *E. najas*, this difference was only 1.2 times, explanations of this effect are discussed. Tests in varying ionic composition of the water illustrated that the absence of sodium ions accounts for a considerable increase in ammonia toxicity. It is interesting that a similar trend was found for fishes and crustaceans. Mechanistic explanations, which may differ from that for other groups, are proposed. Odon. larvae seem to be unsuitable for the bioindication of ammonia pollution.

INTRODUCTION

Ammonia is one of the most common pollutants in fresh waters in the world. Total ammonia in aqueous solution consists of two principal forms, ammonium ions (NH_4^+) and un-ionized ammonia (NH_3). The relative concentrations of these two forms are pH and temperature dependent. Un-ionized ammonia is more toxic for most of aquatic animals when compared to ammonium ions, although this latter form also contributes

to toxicity.

Toxicity of ammonia has been well studied for many fishes, crustaceans, some oligochaets and aquatic insects. As far as the Odonata are concerned, no tests have been conducted to the best of our knowledge before our first experiments (BEKETOV, 2001).

Ammonia is a waste product of protein metabolism of most aquatic animals including dragonfly larvae. Tolerance for ammonia in the external medium is bound up with excretion and ionic regulation. Studies carried out on aquatic insects illustrate that both the regulatory capacities and the nature of the regulatory system vary widely, not only amongst animals habitually living in different environmental media, but also across the various orders with aquatic representatives. This is not surprising, since these different orders have evolved aquatic representatives independently, and variation, rather than conformity, might be expected amongst the aquatic insects (NICHOLLS, 1983). It would, therefore, be of interest to examine the tolerance of odonate larvae for ammonia.

The general aims of the present investigation are to estimate the acute toxicity of ammonia for larvae of dragonflies, and therefore to consider the possibility of using the larvae as indicators of ammonia pollution, also to estimate toxicity under different conditions, and consequently to make suppositions for the mechanism(s) of tolerance. Three different types of test were conducted. These are standard toxicological tests at varying pH values, tests with starved larvae and tests with varying ionic composition of the medium.

MATERIAL AND METHODS

Mixed age *E. najas* and *L. sponsa* larvae were obtained from a small lake (Karasevo Lake) near Novosibirsk. *S. flaveolum* larvae were captured in a small swampy pond near Novosibirsk. These were in approx. 8th-10th (last) instar. All the larvae were collected just before each test.

Analysis of the lake water showed that ammonia was not found with a minimum detection limit of 0.08 mg/l ($N\text{-}NH_4^+$), and the pH level was 7.45. As to the swamped pond water, measurement showed that ammonia concentration was 0.14 mg/l ($N\text{-}NH_4^+$), and the pH value was 7.77.

All animals were fed daily, except for tests with starved larvae. *S. flaveolum* were fed on *Chironomus* sp.; zygopteran larvae were fed on *Cyclops* sp.

Table I
Ionic composition of water in the standard toxicity test

Ions	Concentration mg/L
Ca^{2+}	50
Mg^{2+}	30
Na^+	30
K^+	30
Cl^-	134
HCO_3^-	198

STANDARD TOXICOLOGICAL TEST AT VARYING pH VALUES. – Ammonia toxicity was estimated in standard, acute 96-h toxicity tests (U.S.EPA, 1993). Seven solutions contained ammonia and one did not (control). Each solution was prepared from de-ionized water and mineral salts. Ammonia chloride was added in a logarithmic concentration series. Concentrations of sodium, potassium, calcium and magnesium were constant in each solution. The amount of all ions present are given in Table I. All the solutions were renewed every 24 hours in every test. Available pH values were obtained by means of potentiometric titration. All the tests were conducted in glass cans immersed in a water bath. The temperature of the bath water, and consequently the solutions, was regulated by an electric thermoregulator. The measured temperature was 25° ± 1°C. The criterion for an acceptable test was =80% control survival (U.S.EPA, 1994). Median lethal concentrations (LC50) were calculated using the Spearman-Karber

technique (HAMILTON et al., 1977) and were based either on total ammonia or un-ionized ammonia.

TEST WITH STARVED LARVAE. – Toxicity of ammonia to starved larvae was estimated for *S. flaveolum* and *E. najas*. All conditions were identical to the previous test at pH = 7.5. Larvae were not fed during the test and for 24 hours before the test.

TEST WITH DIFFERENT IONIC COMPOSITION OF WATER. – Ammonia toxicity in different media was investigated using eight solutions (Tab. III). Values of ammonia concentrations were equal to the LC50 values which were obtained in the previous tests at appropriate pH levels. Concentrations of ions present in the solutions were equal to the values used in the usual toxicity tests. All solutions did not differ in the presence of anions excepting solution number 2. Ionic composition of solution number 1 is equal to the medium that was used in the previous tests and therefore it is used as a control. In addition, special control tests were conducted with ammonia free solutions for all the species.

RESULTS

Values of pH in the individual tests remained relatively constant and close to the nominal values over the course of the 96-h exposure (Tab. II). Ammonia concentrations were not measured, but all the solutions were renewed every 24 hours as described above. Ammonia concentration decreases by approximately 10% every 24 hours in a static system (ANKLEY et al., 1995). Thus, LC50 values were calculated on the basis of nominal concentrations which were obtained as arithmetic means between the concentrations at the beginning and the end of the 24 hours period of time.

In fed larvae the LC50 for total ammonia decreased with increase in pH for both *S. flaveolum* and *E. najas* (Tab. II). However, for *S. flaveolum* at least the LC50 for un-ionized ammonia increased with increase in pH for the range 7.0 to 8.2. For *E. najas* there was an increase from pH 7.5 to 8.7, but it fall below the pH 8.7 level at pH 9.2 (Tab. II).

At pH 7.5 the LC50 for total ammonia was lowest in *S. flaveolum* (192.75) and highest in *E. najas* (589.0), with that for *L. sponsa* intermediate (412.2). For un-ionized

Table II

Toxicity of ammonia to *Sympetrum flaveolum*, *Erythromma najas* and *Lestes sponsa* larvae in standard 96-h tests at varying pH levels, and tests with starved larvae

Test organism	Nominal pH	Measured pH (± SD)	LC50(96 h) N-mg/l (95% Confidence Intervals) Total ammonia LC50 (95% C.I.)	Un-ionized ammonia LC50 (95% C.I.)
E. najas	7.5	7.52 (± 0.11)	589.0 (436.30-795.15)	10.42 (7.72-14.07)
	8.7	8.70 (± 0.14)	168.0 (120.00-235.20)	37.80 (27.00-52.92)
	9.2	9.14 (± 0.14)	49.2 (36.44- 66.42)	22.14 (16.40-29.89)
Starved	7.5	7.43 (± 0.27)	703.4 (521.04-949.59)	12.45 (9.22-16.81)
L. sponsa	7.5	7.54 (± 0.12)	412.2 (343.50-494.64)	7.30 (6.08- 8.76)
S. flaveolum	7.0	6.96 (± 0.15)	304.49 (209.99-441.51)	1.72 (1.19- 2.49)
	7.5	7.44 (± 0.24)	192.75 (142.78-260.21)	3.41 (2.53- 4.60)
	8.2	8.22 (± 0.18)	76.31 (52.63-110.65)	6.11 (4.21- 8.86)
Starved	7.5	7.42 (± 0.15)	709.42 (525.50-957.72)	12.56 (9.30-16.96)

ammonia the LC50 at pH 7.5 was highest in *E. najas* (10.42) and lowest in *S. flaveolum* (3.41) with that for *L. sponsa* intermediate (7.30) (Tab. II).

In both *S. flaveolum* and *E. najas* the LC50 values for total ammonia at pH 7.5 were higher in the starved larvae compared to those that had been fed (Tab. II).

LC50 values based on un-ionized ammonia were calculated using standard ammonia tables, at 25°C (THURSTON et al., 1979). For the purpose of calculating the LC50 values, pH was determined by interpolation between mean pH levels at concentrations surrounding the LC50. All concentrations reported for both total and un-ionized ammonia are expressed as N (nitrogen).

Tests with different ionic compositions of the water showed that the absence of sodium caused an increase in toxicity for *S. faveolum*, *E. najas* and *L. sponsa*. Ammonia concentrations which were equal to the LC50 values accounted for 90-100% mortality in certain solutions (Tab. III). LC50 values were not calculated for these conditions.

DISCUSSION

The relative concentrations of two forms of ammonia are pH and temperature dependent. Concentration of the un-ionized form and pH value tend to be positively correlated. The ratio of un-ionized ammonia to ammonium ion increases by 10-fold for each unit rise in pH and by about 2-fold for each 10°C rise in temperature over the 0-30° C range (THURSTON et al., 1979).

Early studies of the ammonia toxicity to fishes indicated that total ammonia is more toxic at elevated than at low pH (CHIPMAN, 1934). This finding prompted the suggestion that the toxicity of ammonia was due solely to NH_3 rather than NH_4^+. Later investigations with fishes (THURSTON et al., 1981), crustaceans (ANKLEY et al.,

Table III

Ionic composition of water and mean mortality percentage in tests with different ionic composition of the water

Cation / Test organism	Numbers of solutions and presence of the cations / Mortality percentage (± SE)							
	1	2	3	4	5	6	7	8
Na^+	+	-	-	+	+	+	-	+
K^+	+	-	+	-	+	+	-	+
Ca^{2+}	+	-	+	+	-	+	+	-
Mg^{2+}	+	-	+	+	+	-	+	-
S. flaveolum	40%	100%	90%	40%	50%	40%	80%	50%
pH=7.5	(± 8.94)		(± 5.48)	(± 8.94)	(± 9.13)	(± 8.94)	(± 7.30)	(± 9.13)
E. najas	60%	100%	100%	60%	60%	50%	100%	70%
pH=7.5	(± 15.5)			(± 15.5)	(± 15.5)	(± 15.8)		(± 15.5)
L. sponsa	50%	100%	100%	80%	70%	50%	100%	70%
pH=7.5	(± 15.8)			(± 12.6)	(± 15.5)	(± 15.8)		(± 15.5)

1995; ARMSTRONG et al., 1978; LANDAU & SANCHEZ, 1991) and other invertebrates (SCHUBAUER-BERIGAN et al., 1995) demonstrated the same dependence for total ammonia, but ammonia toxicity, expressed on the basis of the un-ionized form, was not constant over a range of pH; toxicity of un-ionized ammonia appeared to increase at low pH values. The joint toxicity model for NH_3 and NH_4^+ was proposed as an explanation of this effect (ERICKSON, 1985). According to this hypothesis, at low pH values NH_4^+ exerts toxicity that is not apparent at higher pH values where comparatively greater concentrations of the more potent NH_3 are present.

The concept of the joint toxicity model for NH_3 and NH_4^+ was supported by investigations of ammonia excretion mechanisms of fishes and crustaceans. Blood ammonia concentrations are controlled through a combination of passive diffusion of NH_3 (and, to a lesser degree, NH_4^+) and active exchange of NH_4^+ from gills for Na^+ or Ca^{2+} from the external media (EVANS & CAMERON, 1986; VINOGRADOV, 1988; VINOGRADOV et al., 1983). These ionic exchanges cause the tolerance of fishes and crustaceans for ammonium ion. Experiments with sodium and calcium free media or soft waters showed that the absence, or just low concentrations of these cations accounted for the increase in the toxicity of NH_4^+. In these conditions toxicity, expressed on the basis of total ammonia, was almost constant across pH (ANKLEY et al., 1995). Thus, ammonium ion exerts toxicity and the tolerance for this form of ammonia is dependent on the cations in the external environmental.

As to aquatic insects, influence of pH on the toxicity has been well studied only for *Chironomus tentans* (Diptera) (SCHUBAUER-BERIGAN et al., 1995). This investigation demonstrated that total ammonia toxicity increased with increasing pH, toxicity of un-ionized form respectively decreased under these conditions. This trend in ammonia toxicity with pH is similar to the above described results obtained for fishes and invertebrates. However, the degree of pH dependence of toxicity was less pronounced for *C. tentans* than for fishes, but it was more similar to the crustaceans.

Results of the present investigation indicated the same dependence. However, LC50 (NH_3) obtained for *E. najas* at pH 9.2 is lower than this value at pH 8.7. This discrepancy can logically be explained as a result of some toxic effect of the very high pH level. Larvae of *E. najas* in wild conditions inhabit waters at pH values 4.5-8.0 (MULLER, 1986). The ammonium ion is less toxic than the un-ionized form for odonate larvae as well as for other aquatic animals. It is possible that the mechanisms of the tolerance for NH_4^+ of the larvae may differ from those of other groups.

It is very interesting that tests with different ionic compositions of water showed that absence of sodium causes the increase of toxicity. This type of response is similar to the observed trends in ammonia toxicity for other aquatic animals. The ionic exchange mechanisms in the hindgut may be proposed as an explanation of this finding by analogy with fishes and crustaceans. Moreover, active sodium uptake has been demonstrated for the hindgut of dragonfly larvae (LEADER & GREEN, 1978) but it was not connected with ammonia in these experiments. Unfortunately, results of the present experiments cannot provide evidence for ionic exchanges because the absence of sodium impedes

fluid secretion by the Malpighian tubules for the first days of adaptation (NICHOLLS, 1985) and therefore it may just reduce ammonia excretion. Unlike the majority of insects, dragonflies have a primitive sodium-reach haemolymph which resembles that of non-insect mandibulate arthropods (e. g. the millipede *Glomeris marginata* (FARQUARSON, 1974)). The fluid flow in the Malpighian tubules of Odonata is driven by active transport of sodium ions. In contrast, other insects use potassium to generate fluid flow. Unfortunately, mechanisms of ammonia excretion of Odonata have not been studied and so it is very difficult to formulate a mechanistic explanation concerning the sensitivity of the larvae to ammonia in sodium free medium.

Tests with starved larvae showed unusual results. There was a considerable difference between LC50 values for starved and fed *S. flaveolum*. In *E. najas*, this difference was rather less pronounced. The logical explanation for the high tolerance of the starved larvae is low haemolymph ammonia concentration because of the slow metabolic rate. Fed larvae of course could also take the toxicant with food. In addition, they could drink the medium during eating, and besides, the food could contain ammonia.

Median lethal concentrations (LC50) obtained for *E. najas* larvae are unusually high; they could be determined by inactive feeding. Unlike *E. najas*, *S. flaveolum* fed very actively, therefore the distinction of the toxicity for fed and unfed organisms was more pronounced in the latter species. The amount of food taken was not measured in the tests, therefore there is no strong evidence for this correlation. Consequently, it is very difficult to make suppositions for undangerous ammonia concentrations for Odonata.

Values of LC50 for starved *S. flaveolum* and *E. najas* are similar and the rate of protein metabolism of these larvae could also be considered quite similar. Permeability of the body wall in these tests seemed to be the main factor which determines the tolerance. Thus, the similarity of the above LC50 values bears out the suggestion (MOENS, 1967; NICHOLLS, 1983) that, although the general body wall cuticle in Anisoptera is less permeable than that in the Zygoptera, the presence of a large surface area of presumably permeable cuticle of the rectal gills in the former suborder results in an overall increased permeability.

Table IV

LC50 values based on un-ionized ammonia for different aquatic invertebrates

Species		pH	LC50 N-NH_3 mg/l	Reference
Chironomus tentans	(Diptera)	7.0	1.58	SCHUBAUER-BERIGAN et al., 1995
Chironomus riparis	(Diptera)	7.0	1.36	WILLIAMS et al., 1986
Limnodrilus hoffmeisteri	(Oligochaeta)	7.0	1.58	WILLIAMS et al., 1986
Dendrocoelum lacteum	(Turbellaria)	8.2	1.4	STAMMER, 1953
Tubifix tubifex	(Oligochaeta)	8.2	2.2	STAMMER, 1953
Lumbriculus variegatus	(Oligochaeta)	8.2	1.1	SCHUBAUER-BERIGAN et al., 1995
Hyalella azteca	(Amphipoda)	7.5	0.83	ANKLEY et al., 1995

Results of this investigation illustrate the high ammonia tolerance of dragonfly larvae when compared to other aquatic invertebrates (Tab. IV). It is logical to suppose that, in ammonia polluted water bodies, odonates will be affected indirectly when the toxicant depletes their prey. Using the larvae as indicators of ammonia pollution seems to be unsuitable, but final conclusions are being addressed in chronic toxicity tests and field research. Subsequent investigations focused on the physiological basis of the tolerance would be of interest for insect physiology in general.

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